SECTION: **Chemistry**

SUB SECTION: **Roche Cobas Procedure**

SUBJECT: **Anti-SARS-CoV-2**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

Anti-SARS-CoV-2

This immunoassay is for the *in vitro* qualitative detection of total antibodies to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in human serum and plasma. The test is intended for use as an aid in identifying patients with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long the antibodies persist following infection and if the presence of antibodies confers protective immunity. The Elecsys Anti-SARS-CoV-2 assay should not be used to diagnose acute SARS-CoV-2 infection. Results are for the detection of SARS-CoV-2 antibody. Antibodies to the SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Patients may have detectable virus present for several weeks following seroconversion.

The sensitivity of the Elecsys Anti-SARS-CoV-2 assay early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for the Elecsys Anti-SARS-CoV-2 assay may occur due to cross reactivity from pre-existing antibodies or other possible causes.

CLINICAL SIGNIFICANCE

SARS-CoV-2 is an enveloped, single-stranded RNA virus of the family Coronaviridae, genus Betacoronavirus. All coronaviruses share similarities in the organization and expression of their genome, which encodes 16 nonstructural proteins and the 4 structural protein: spike (S), envelope (E), membrane (M), and nucleocapsid (N). Viruses of this family are of zoonotic origin. They cause disease with symptoms ranging from those of a mild common cold to more severe ones such as Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS) and Corona Disease 2019 (COVID-19). Other coronaviruses known to infect people include 229E, NL63, OC42 and HKU1. The latter are ubiquitous and infections typically cause common cold or flu-like symptoms. The Elecsys Anti-SARS-CoV-2 Assay uses a recombinant protein representing the nucleocapsid (N) antigen for the determination of antibodies against SARS-CoV-2.

TEST PRINCIPLE / PURPOSE

The electrochemiluminescence immunoassay “ECLIA” is intended for use on the **cobas e** immunoassay analyzers.

Sandwich principle. Total duration of assay: 18 minutes.

I. 1st incubation: 20 µL of sample cobas e601/e602, biotinylated SARS-CoV-2-specific recombinant antigens and SARS-CoV-2-specific recombinant antigens labeled with a ruthenium complex[a)] form a sandwich complex.
II. 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin
III. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
IV. Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

SPECIMEN TYPE(S)

For specimen collection and preparation, only use suitable tubes or collection containers. When processing samples in primary tubes, follow the instructions of the tube manufacturer. Only the specimens listed below were tested and found acceptable.

I. Li-heparin, K_2-EDTA and K_3-EDTA
II. Stable for 3 days at 15-25 °C, 7 days at 2-8 °C, and 28 days at -20 °C (± 5 °C). The samples may be frozen once.
III. Centrifuge samples containing precipitates and thawed samples before performing assay
IV. Do not use heat inactivated samples.
V. Do not use samples and controls stabilized with azide.

REQUIRED REAGENT(S)

I. The reagent rackpack (M, R1, R2) is labeled as **ACOV2**.

M= Streptavidin-coated microparticles (transparent cap), 1 bottle: Streptavidin-coated microparticles ; preservative

R1= SARS-CoV-2-Ag~biotin, (gray cap), 1 bottle: biotinylated SARS-CoV-2-specific recombinant antigen (*E.coli*); preservative

R2= SARS-CoV-2-Ag~($Ru(bpy)_3^{2+}$ (black cap), 1 bottle: SARS-CoV-2-specific recombinant antigen labeled with ruthenium complex; preservative.

II. Calibrators
ACOV2 Cal1 - Negative calibrator (white cap), 1 bottle of human serum, non-reactive for Anti-SARS-CoV-2 antibodies

ACOV2 Cal2 - Positive calibrator (black cap), 1 bottle of human serum, reactive for Anti-SARS-CoV-2 antibodies; preservative.

III. Stability of the reagent rackpack:
Unopened at 2-8 °C: up to the stated expiration date
After opening at 2-8 °C: 14 days
On the e601/e602 analyzers: 14 days

CALIBRATION

I. No international standard is available for Anti-SARS-CoV2.

II. Calibration frequency: Calibration must be performed once per reagent lot using ACOV2 Cal1/ ACOV2 Cal 2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
A. After 3 days when using the same reagent lot
B. After 3 days (when using the same reagent kit on the analyzer.
C. As required: e.g. quality control findings outside the defined limits.

III. Calibration may be extended based on acceptable verification of calibration by the laboratory.

QUALITY CONTROL

I. For quality control, use PreciControl Anti-SARS-CoV-2 Level 1 and 2. At least two control levels are run in the same manner as patient specimens. Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

II. Values obtained should fall within the laboratory's defined control intervals and limits. If all controls values are within the posted tolerance limits for the assay, patient results may be reported. If one control is outside of tolerance rerun the control; if the rerun control is within tolerance patient results may be reported.

III. If the rerun control is still out or if two controls exceed set limits corrective action may include but is not limited to the following (order depends on assay):
A. Replace control material
B. Recalibrate the assay and repeat all controls.
C. Prepare fresh reagent and/or recalibrate and repeat all controls.
D. Check analyzer for proper function.

IV. Controls may also be prepared according to the manufacturer's protocol:
A. **Negative Control:** Determine the COI of ACOV2 Cal1 by measuring it as a routine sample. Pool serum samples with a COI of ≤150% compared to the COI result of ACOV2 Cal1 (pooling of ≥5 non-reactive samples in this range is recommended). Mix carefully, avoiding foam formation. Prepare aliquots of at least 250µL from this sample pool and store frozen at -20˚C (±5˚C) or colder. Use these aliquots to perform regular quality control. **The negative control has a target value of COI<0.8 (qualitative assay result "non-reactive")**

B. **Positive Control:** Determine the COI of ACOV2 Cal2 by measuring it as a routine sample. Pool serum samples with a COI that is higher than the COI result of ACOV2 Cal2 (pooling of ≥3 reactive samples in this range is recommended). Dilute the sample pool by adding pooled negative serum (for pooling criterion, see negative control) or Diluent MultiAssay to obtain a COI between 3 and 15. Mix carefully, avoiding foam formation. It is recommended to confirm reactivity after dilution by a measurement. Prepare aliquots of at least 250µL from this sample pool and store frozen at -20˚C (±5˚C) or colder. Use these aliquots to perform

regular control. Upon first use of this control, determine the COI of the control by measurement of the control in triplicate and using a freshly opened and calibrated reagent **cobas e** pack.

C. **The obtained median of these measurements serves as target values for this positive control. Subsequent measurements of all aliquots of this control must match this target value ±45% (3SD=45%, 1 SD=15%; qualitative assay result "reactive".** The target value of the positive control is lot- specific and target value assessment as described above has to be performed for every assay lot.

D. Aliquots may be re-used if tightly sealed and stored immediately at 2-8˚C for a maximum of 3 days.

E. Controls for both concentration ranges (negative and positive) will be run at least every 24 hours when the test is in use, once per reagent kit/**cobas e** pack, and following calibration.

F. The controls will be run as external controls. All values and ranges will be entered manually. Only one target value and range for each control level will be entered in the analyzer. The reagent lot-specific target values will be re-entered each time a specific reagent lot with different control target values and ranges is used. Two reagent lots with different control target values and ranges cannot be used in parallel in the same run.

V. In addition, other suitable control material may be used

CALCULATIONS

The analyzer automatically calculates the cutoff based on the measurement of ACOV2 Cal1 and ACOV2 Cal2. The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI; signal sample/cutoff).

REFERENCE INTERVALS/ INTERPRETATION OF RESULTS

Results obtained withe Elecsys Anti-SARS-CoV2 assay can be interpreted as follows:

Numeric Result	Result message	Interpretation
COI<1.0	Non-reactive	Negative for anti-SARS-CoV2 antibodies
COI≥1.0	Reactive	Positive for anti-SARS-CoV2 antibodies

The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present in the sample. The individual immune response follwoing SARS-COV-2 infection varies considerably and might give different results with assays from different manufacturers. Results of assays from different manufacturers should not be used interchangeably.

INTERFERENCES

I. The effect of the pharmaceutical compound, biotin, on assay performance was tested. This assay has no biotin interference in serum concentrations up to 1200 ng/mL (4912nmol/L).

II. Potential endogenous interferences e.g., hemolysis, bilirubin, rheumatoid factors and pharmaceutical compounds other than biotin have not been tested and an interference cannot be excluded.

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DEFINITIONS **N/A**

ATTACHMENTS **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	5/18/2020	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES **N/A**

EFFECTIVE 05/18/2020

REVISED **See Review/Revise Sign off Page**

REVIEWED **See Review/Revise Sign off Page**

SECTION: **Chemistry**

SUB SECTION: **Roche Cobas Procedure**

SUBJECT: **Anti-SARS-CoV-2-S**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

Anti-SARS-CoV-2-S

The Elecsys Anti-SARS-CoV-2 S is an electrochemiluminescence immunoassay intended for qualitative and quantitative detection of antibodies to SARS-CoV-2 spike (S) protein receptor binding domain (RBD) in human serum and plasma. The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** immunoassay analyzers and is intended as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The Elecsys Anti-SARS-CoV-2 S assay should not be used to diagnose acute SARS-CoV-2 infection.

Results are for the detection of SARS-COV-2 antibodies. Antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time the antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

The sensitivity of the Elecsys Anti-SARS-CoV-2 S early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for Elecsys Anti-SARS-CoV-2 assay may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.

This test should not be used to determine SARS-CoV-2 infection in donated blood units or be used for blood donor screening.

CLINICAL SIGNIFICANCE

SARS-CoV-2, the causative agent of Coronavirus Disease 2019 (COVID-19), is an enveloped, single-stranded RNA Betacoronavirus. 7 coronaviruses have been identified as agents of human infection, causing disease ranging from mild common cold to severe respiratory failure.

SARS-COV-2 is transmitted primarily from person-to-person through respiratory droplets and aerosols. The incubation period from infection to detectable viral load in the host commonly ranges from 2 to 14 days. Detection of viral load can be associated with the onset of clinical signs and symptoms, although a considerable proptortion of individuals remain asymptomatic or mildly symptomatic. The interval during which an individual with COVID-19 is infectious has not yet been clearly established, however, transmission from symptomatic to asymptomatic and pre-symptomatic indicviduals has been well described. Coronavirus genomes encode 4 main structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N). The S protein is a very large transmembrane protein that assembles into trimers to form the distinctive surface spikes of coronaviruses. Each S monomer consisits of a N-terminal S1 domain and a membrane-proximal S2 domain. The virus gains entry into the host cell through binding of the S protein to the angiotesnin-converting enzyme 2 (ACE2), which is enzymatically active on the surface of numerous cell types inlcuding alveolar type II cells of the lung and epithelical cells of the oral mucosa. Mechanicalistically, ACE2 is engaged by the receptor-binding domain (RBD) on the S1 subunit.
Upon infection with SARS-CoV-2, the host mounts an immune response against the virus, typically including production of specific antibodies against viral antigens. IgM and IgG antibodies to SARS-CoV-2 appear to rise nearly simultanaeously in blood. There is significant inter-individual difference in the levels and chronological appearance of antibodies in COVID-19 patients, but median serocovnersion has been observed at approximately 2 weeks

Serologoc assays can play an important role in understadning viral epidemiology in the general population. The Elecsys Anti-SARS-CoV-2 S assay uses a recombinant protein reporesenting the RBD of the S antigen in the double-antigen sandwhich assay format

TEST PRINCIPLE / PURPOSE

Double-antigen sandwich principle. The antigens within the reagent captures predominantly anti-SARS-CoV-2 IgG, but also anti-SARS-CoV-2 IgA and IgM. Total duration of assay: 18 minutes.

I. 1st incubation: 20 µL of sample cobas e601/e602, biotinylated SARS-CoV-2-specific recombinant antigens and SARS-CoV-2-S-RBD-specific recombinant antigen and SARS-COV-2 S-specific recombinant antigens labeled with a ruthenium complex[a)] form a sandwich complex.
II. 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin
III. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
IV. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.
    A. Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

SPECIMEN TYPE(S)

For specimen collection and preparation, only use suitable tubes or collection containers. Only the specimens listed below were tested and found acceptable:

I. Serum collected using standard sampling tubes or tubes containig separating gel
II. Li-heparin, dipotassium EDTA (K_2-EDTA), tripotassium EDTA (K_3-EDTA), and sodium citrate plasma. Plasma tubes containing separating gels can be used.
III. Stable for 3 days at 15-25 °C, 7 days at 2-8 °C, and 28 days at -20 °C (± 5 °C). The samples may be frozen once.
IV. Centrifuge samples containing precipitates and thawed samples before performing assay
V. Do not use heat inactivated samples.
VI. Ensure the samples and calibrators are at 20-25˚C before peforming the assay.

REQUIRED REAGENT(S)

I. The reagent rackpack is labeled as **ACOV2S**.

M= Streptavidin-coated microparticles (transparent cap), 1 bottle, 12mL: Streptavidin-coated Microparticles 0.72mg/ml; preservative

R1= SARS-CoV-2 S Ag~biotin, (gray cap), 1 bottle, 16mL: biotinylated RBD domain of SARS CoV-2 S as recombinant antigen <0.4 mg/L; HEPES buffer 50mmol/L, pH 7.4; preservative.

R2= SARS-CoV-2 S-Ag~(Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 16mL: RBD domain of SARS-CoV-2 S as recombinant antigen labeled with ruthenium complex<4.0 mg/L;HEPES bufffer 50mmol/L, pH7.4; preservative.

II. Calibrators
Anti-SARS-COV-2 positive serum in two concentration ranges:
ACOV2S Cal1 and ACOV2S Cal2.

III. Stability of the reagent rackpack:
Unopened at 2-8 °C: up to the stated expiration date
After opening at 2-8 ºC: 28 days
After opening at 2-8 °C, on cobas e 602: 14 days

CALIBRATION

I. This method has been standardized against internal Roche standard for Anti-SARS-CoV-2-S. No international standard is currently avaliable for the Anti-SARS-CoV-2 S assay

II. Calibration frequency: Calibration must be performed once per reagent lot using ACOV2S Cal1/ ACOV2S Cal 2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows the cobas e602 analyzers:
   A. After 31 days when using the same reagent lot
   B. After 14 days when using the same reagent kit on the analyzer
   C. As required: e.g. quality control findings outside the defined limits.
   D. Calibration may be extended based on acceptable verification of calibration by the laboratory.

QUALITY CONTROL

I. For quality control, use PreciControl Anti-SARS-CoV-2 S, Level 1 and 2. Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

II. Other commercially available quality control material can be used that covers at least two levels of analyte.

III. Values obtained should fall within the laboratory's defined control intervals and limits. If all controls values are within the posted tolerance limits for the assay, patient results may be reported. If one control is outside of tolerance rerun the control; if the rerun control is within tolerance patient results may be reported.

IV. If the rerun control is still out or if two controls exceed set limits corrective action may include but is not limited to the following (order depends on assay):
   A. Replace control material.
   B. Recalibrate the assay and repeat all controls.

C. Prepare fresh reagent and/or recalibrate and repeat all controls.
D. Check analyzer for proper function.

CALCULATIONS

The analyzer automatically calculates the analyte concentration of each sample in U/mL.

LIMITS

The analytical measuring interval is 0.4-250 U/mL. Specimens with an initial value >250U/mL will repeat for an automatic 1:10 dilution on the analyzer. Values above the measuring range after dilution are reported as >2500U/mL.

REFERENCE INTERVALS AND INTERPRETATION OF RESULTS

Results obtained with the Elecsys Anti-SARS-CoV2 S assay can be interpreted as follows:

Numeric Result	Interpretation
<0.80 U/mL	Negative for anti-SARS-CoV2-S
≥0.80 U/mL	Positive for anti-SARS-CoV-S

Note: Due to the diversity of the antibodies, the measured anti-SARS-CoV-2-S value can vary depending on the testing procedure used and the applied standard. Results obtained from a single sample using tests from different manufacturers can therefore differ.

INTERFERENCES/LIMITATIONS

I. Drug interferences are based on recommendations given in the CLSA (Clinical and Laboratory Standards Institute) guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

II. In rare cases, interference due to extremely high titers of antibodies to analyte specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

III. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for a second, but different, serology test to confirm an immune response. A negative result indicates the absence of detectable anti-SARS-CoV-2 antibodies or that these antibodies are not present during the stage of the disease in which the sample was collected.

IV. The clinical applicability of semi-quantitative results is currently unknown and cannot be interpreted as an indication or degree of immunity nor protection from re-infection, nor compared to other SARS-CoV-2 antibody assays.

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DEFINITIONS **N/A**

ATTACHMENTS **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	3/2/2021	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES **N/A**

EFFECTIVE

REVISED **See Review/Revise Sign off Page**

REVIEWED **See Review/Revise Sign off Page**

SECTION: **Hematology**

SUB SECTION:

SUBJECT: **Streck Diesse MINI-Cube ESR Instrument**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

Streck Diesse MINI-CUBE ESR Instrument

TEST PRINCIPLE / PURPOSE

The basic principle of the erythrocyte sedimentation rate (ESR) test is that anticoagulated blood is placed in a vertical column that remains undisturbed for a specified amount of time. At the conclusion of the test, the distance the red cells have settled is measured in mm per hour (Westergren method).

The MINI-CUBE is an automated ESR analyzer incorporating new technology that accurately and precisely measures the sedimentation rate of erythrocytes in standard 13 x 75 mm K_2EDTA blood collection tubes with Hemogard™ or conventional stoppers, or K_2EDTA Microtainer® tubes (BD). The results are recorded as mm per hour in only 20 minutes. The instrument compensates for temperatures above 18 °C according to Manley's Nomogram and offers the ability to adjust for patient Hematocrit values <40%.

CLINICAL SIGNIFICANCE

Red cell sedimentation is accelerated by an increase in the plasma concentration of "acute phase proteins," which are increased in acute tissue damage, chronic inflammation, chronic infection, and pregnancy. The ESR reflects both the increase in certain accelerating proteins, such as fibrinogen and gamma globulins, and the decrease in retarding proteins, such as albumin. Conditions that promote the formation of rouleaux produce an elevated ESR result.

SPECIMEN TYPE(S)

13 x 75 mm standard K_2EDTA blood collection tubes;
Acceptable sample volume: 2.0 mL – 4.0 mL.

BD Microtainer K_2EDTA blood collection tubes; Minimum sample volume: 500 µL

The MINI-CUBE can accept EDTA sample tubes with a maximum of **one** secondary patient label adhered as close to the lavender cap as possible and with a label-free gap on one side of the tube.

The test should be performed within 4 hours of collection with the sample at room temperature (18 – 25 °C). The test may be performed on blood samples stored at 2 – 8 °C for a maximum period of 24 hours. Ensure that the sample is at room temperature for 15 minutes and well-mixed prior to inserting into the instrument and performing the test.

REQUIRED REAGENT(S)

I. Transponder MINI-CUBE (Streck part numbers 240403, 240404); holds executable tests memory (1 transponder for 1000 tests)

Loading a Transponder

1. From the HOME screen, select the SETTINGS icon.
2. Navigate to page 2 in SETTINGS and select REFILL to load more tests on the MINI-CUBE.
3. A window reading “Insert CHECK DEVICE and press CHECK key” will open.
4. Gently lay the instrument down with the display screen facing up and insert the transponder tube with the cap to the left.
5. Place the instrument upright, press CHECK and follow the instructions on the screen.
6. The number of remaining tests will update in the upper left corner of the screen.

II. Streck ESR-Chex Plus L1 and L2

CALIBRATION **N/A**

QUALITY CONTROL

Registering a New QC Lot

Only one control lot may be registered in the QC file at a time. Registering a new control lot will delete the registration of the previous lot. Control data from the previous lot will be saved in the QC Archive.

1. From the HOME screen, select the SETTINGS icon.
2. Navigate to page 2 in SETTINGS and Select QC to open the QC SETTINGS screen.
3. Scan the barcode on each level of ESR-Chex Plus to auto-populate the barcode, lot number, expiration date and control range fields. Alternatively, manually enter the information listed on the control assay into these fields.
4. Press the RETURN arrow on the lower left corner of the screen to exit QC SETTINGS.
5. Press YES to save the new settings, or NO to discard the new settings.

Running QC Samples

1. From the HOME screen, select the START icon.
2. Scan the barcode on the vial from the registered lot of ESR-Chex Plus.
3. A window titled “Inserted QC Code” that contains the registered QC lot data will open.
4. Press YES to run the tube as a QC sample, or NO to run the tube as a patient sample.
5. Insert the QC vial into any available well with the label gap facing the dot on the right side of the well.
6. The control result and the expected range will automatically print when the test is complete. If the result is outside the expected range, the result on the MINI-CUBE screen will be highlighted in red.

If the result is within the expected range, the result on the MINI-CUBE screen will be highlighted in green.

Viewing QC Results
The QC Archive stores up to 5,000 QC results per level. Lot-specific statistical reports can be generated from these archive files. When the QC Archive reaches capacity, the oldest value will be deleted and the newest value will be added.

1. From the HOME screen, select the ARCHIVE icon.
2. From the ARCHIVE menu, select the QC icon to view all available QC data in the archive list.
3. To reprint QC results:
    a. Select an individual result in the QC Archive list.
    b. Press the DOWNLOAD icon and select PRINT to reprint the individual result, or PRINT LIST to print the entire QC archive.
4. To view details for an individual QC result:
    a. Press and hold an individual result in the QC Archive list.
    b. Press the CHART icon to view the Levey-Jennings chart and statistical data about the entire lot.
    c. Press the DOWNLOAD icon to reprint the result.
5. To view details for a specific QC lot:
    a. Select an individual QC result from the desired lot in the QC Archive list.
    b. Press the CHART icon to view the Levey-Jennings chart and statistical data about the entire lot.
    c. From the Levey-Jennings Chart, press the DOWNLOAD icon to print the collection of data in the chart.
6. To delete a QC result:
    a. Select an individual result in the QC Archive list.
    b. Press the DOWNLOAD icon and select DELETE to permanently remove an individual QC result. **NOTE:** QC results must be deleted individually.

RUNNING PATIENT SAMPLES

The mixing procedure is critical for accurate results. Before starting a test, EDTA patient samples must be prepared as follows:

1. Ensure that the sample is at room temperature for 15 minutes prior to analysis.
2. Immediately before starting the analysis, **gently** and completely invert the EDTA tube end-over-end 10 to 12 times to resuspend the sample. Do not shake or agitate the sample vigorously, as this could cause bubbling or hemolysis.
3. Examine the sample to ensure bubbling is not present at the meniscus as this could interfere with the sample reading.

Entering a Sample ID Code
Scanning a Sample ID:

1. From the START screen, scan the patient barcode with the barcode scanner.
2. A window titled “New Sample” will open with the barcode information displayed in the Sample ID field.
3. Insert the well-mixed patient sample into any available well with the label gap facing the dot on the right side of the well.
4. A full-color image of the tube containing a barcode image will appear on the screen and testing will automatically initiate.

Manually Entering a Sample ID:

1. From the START screen, insert the well-mixed patient sample into any available well with the label gap facing the dot on the right side of the well.
2. A full-color image of the tube without a barcode image will appear on the screen and testing will automatically initiate.
3. Press the tube image on the screen. A window titled “Position” will open.
4. Press the “Sample ID” field to open a keyboard and manually enter the patient ID.
5. Press the ENTER arrow to close the keyboard.
6. Press OK to close the “Position” window.
7. A barcode image will appear on the tube indicating that it contains a Sample ID.

.**PROCEDURAL STEPS** **N/A**

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS

It is good laboratory practice to visually correlate the level of sedimentation in the sample tube to the printed result. The MINI-CUBE automatically prints the result on the printer and data output port and displays an image of the sample tested on the screen.

I. Limitation of Procedure:

A. Reportable range: 13 x 75 mm EDTA tubes (2.0 mL – 4.0 mL): 0 – 140 mm/hr
Samples with a result greater than 140 mm/hr should be reported as “>140 mm/hr.”

B. BD Microtainer EDTA tubes (500 µL): 0 – 60 mm/hr
Samples with a result greater than 60 mm/hr should be reported as “>60 mm/hr.”

C. If the result is below the normal threshold programmed in the SETTINGS menu, the result on the MINI-CUBE screen will be highlighted in green. If the result is equal to or higher than the normal threshold, the result on the MINI-CUBE screen will be highlighted in red.

D. Samples run in BD Microtainer tubes will display an image of a small tube on the screen and “PEDIATRIC” will be indicated on the sample printout. In the RESULTS Archive, a subscript “P” will be displayed immediately to the right of the ESR result.

The RESULTS Archive stores up to 5,000 patient results. When the RESULTS Archive reaches capacity, the oldest value will be deleted and the newest value will be added.

Hematocrit Adjustment

The MINI-CUBE contains the option to apply an adjustment to ESR results for patient hematocrit (HCT) values <40%.

1. From the HOME screen, select the ARCHIVE icon.
2. From the ARCHIVE menu, select the RESULTS icon to view all available patient data in the archive list.

3. Press and hold the desired patient result to open a detailed record of the sample result.
4. Press the HCT field to open a keypad and manually enter the HCT value.
5. Press the ENTER arrow to close the keypad.
6. Press the RETURN arrow on the lower left corner of the screen to save the HCT adjustment.
7. The adjusted ESR result will override the original ESR result, and a superscript “H” will be displayed immediately to the right of the ESR result.
8. The HCT value may be deleted to display the original ESR result.

II. Expected Results:

A. Normal ranges are as follows:
   1. Females 0-20 mm/hr
   2. Males 0-10 mm/hr
   3. Children 0-15 mm/hr

III. Computer Resulting:

A. Standard “Results Entry”

B. Enter patient’s specimen number

C. Place cursor in result column adjacent to the test identifier

D. Enter numeric results

E. Press F12, spacebar, and enter for verification (release)

PREVENTIVE MAINTENANCE

Always turn off the MINI-CUBE and disconnect it from the power source before performing any type of maintenance. Do not open or remove the MINI-CUBE cover.

1. Regularly verify that the print head on the MINI-CUBE printer is free from dust.
2. Regularly clean the instrument using a soft, damp cloth or paper.
3. In the event of biological material leakage, wipe the outer surface of the instrument with 70% isopropyl alcohol and immediately contact Streck Technical Services at 800-843-0912 or technicalservices@streck.com for further instruction.

REFERENCES

1. MINI-CUBE User Manual, www.Streck.com
2. Clinical and Laboratory Standards Institute, H02, Procedures for the erythrocyte sedimentation rate test. Approved Standard – Fifth Edition.

DEFINITIONS **N/A**

ATTACHMENTS **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES **N/A**

EFFECTIVE **_/__/2020**

REVISED **See Review/Revise Sign off Page**

REVIEWED **See Review/Revise Sign off Page**

SECTION: **Hematology**

SUBJECT: **Quality of Water**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

Quality of Water

TEST PRINCIPLE / PURPOSE

Laboratory function requires a quality source for deionized water. Facilities Management supplies the laboratory with deionized water which is further polished in the Clinical Laboratory for applications requiring type I water.

CLINICAL SIGNIFICANCE

Quality water is essential for laboratory testing.

SPECIMEN TYPE(S) **N/A**

REQUIRED REAGENT(S) **N/A**

CALIBRATION **N/A**

QUALITY CONTROL **N/A**

PROCEDURAL STEPS

I. Water types and usage

A. Deionized water (reagent grade type 2): Deionized water is distributed throughout the laboratory from Facilities Management via the whitewater taps. Criteria for quality is resistance of greater than 2.0 mega ohms.

B. Deionized water (reagent grade type 1) is provided by the euro 100 water polishing unit supplied by General Water Technologies (GWT). When in use, the meter should display a resistance 2.0 – 18.2 ohms. All maintenance and repairs of the polishing unit is contracted with GWT.

II. Sterility check of water: Sample water from GWT spigot is collected and cultured annually. Less than 10 colony forming bacteria per milliliter are acceptable.

A. If more than 10 colonies of bacteria are cultured, the test will be repeated.

B. If the repeat is again found to contain more than 10 colonies of bacteria, the water system must be evaluated by either Facilities Management – type 2 water source and / or GWT type 1 water source.

III. Procedure for Establishing the Quality of Water

A. Resistance measurements:
1. The conductance of the Type 2 water is measured constantly by a light indicator located in Chemistry. Daily record of observance of this light will be maintained by day shift Chemistry personnel. The light sensor is manufactured to detect water resistance of 2.0 ohms or greater.
2. The delivery pressure of the water is in the range of 7.5-47 PSI. Daily record of observance of this measurement will be recorded by day shift Chemistry personnel.
3. Conductivity of Hematology type 1 water is checked weekly to be between 10.1 and 18.2

B. Sterility checks – see section II.

IV. Remedial action of non-compliance

A. Unacceptable Resistance:
1. Type 2 water issue – call Facilities Management (x00037) to notify failed resistivity measurement. Use only type 1 water from the euro 100 water polishers until repairs can be made.
2. Type 1 water issue – The affected unit is taken off line until repair has been completed by GWT (888)647.5990 and type 1 water from Chemistry is to be used in the meantime

B. Unacceptable colony counts – repeat sterility check and if still unacceptable, contact Facilities Management or GWT.

V. Uses of water

A. Type 2 uses include but are not limited to:
1. Rinsing of washed glassware, pipettes etc.
2. Preparation of bacteriologic media
3. Preparation of bacteriologic reagents

B. Type 1 uses include but are not limited to:

1. All reagents prepared for use in Hematology
2. Feed water for the Sysmex RU unit and SP-50 stain rinse
3. Control reconstitution
4. CAP survey specimen reconstitution

VI. Annual Maintenance of the euro100 water polisher is completed by GWT. Results of verification of operation and testing for conductivity, resistivity, Sio2 and CFU/ml is supplied.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS **N/A**

REFERENCES

I. General Water Technologies

II. euro 100 Reverse Osmosis System Manual

DEFINITIONS **N/A**

ATTACHMENTS **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES **N/A**

EFFECTIVE April 2021

REVISED **See Review/Revise Sign off Page**

REVIEWED **See Review/Revise Sign off Page**

SECTION **HISTOLOGY**

SUB SECTION: **SPECIAL STAIN**

SUBJECT: **Alcian Blue - Periodic acid–Schiff (PAS) STAIN**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

Alcian Blue / PAS Stain

All laboratory personnel are expected to follow this procedure for Alcian Blue / PAS stain. Any deviations or modification is prohibited without approval of a supervisor, histology technologist-in- charge or pathologist.

TEST PRINCIPLE / PURPOSE

The purpose: is to differentiate between neutral and acidic mucosubstances.
Principle: Acid mucosubstances are stained with Alcian blue technique and neutral mucosubstances are stained by PAS reaction.

PROCEDURE

I. Deparaffinize in 2 changes of histoclear for 5 minutes each.
II. Hydrate in three changes of 100% alcohol for 1 minute each.
III. Hydrate in two 95% alcohol for 1 minute each.
IV. Place in distilled water.
V. Place the slides in 3% Acetic acid solution A for 3 minutes.
VI. Stain sections in Alcian Blue PH 2.5 solution B for 20 minutes.
VII. Rinse sections in running DI water.
VIII. Place the slides in 0.5% Periodic Acid Solution C for 5 minutes
IX. Wash the sections in DI water.
X. Place slides in Schiff Reagent Solution D for 15 minutes
XI. Wash the slides in Tap water for 5-10 minutes.
XII. Stain the slides with Hematoxylin stain in solution E for 1 minute.
XIII. Rinse in running Tap water for 1 minute then with DI water.
XIV. Dehydrate sections with 2 changes of 95% alcohol for 1 minute each.
XV. Dehydrate sections with 3 changes of 100% alcohol for 1 minute each.
XVI. Clear sections with two changes of Histoclear for 1 minute each.
XVII. Mount with Shandon Synthetic.

CLINICAL SIGNIFICANCE **N/A**

SPECIMEN TYPE(S)

I. Cut sections at 4-5 microns.
II. 10% neutral buffered formalin is preferred or Zenker Solution.

REQUIRED REAGENT(S)

I. Solution A, Acetic acid 3% aqueous.
II. Solution B, Alcian Blue Stain 1%.
III. Solution C, Periodic Acid 5% aqueous.
IV. Solution D, Schiff ‘s Reagent
V. Solution E, Hematoxylin Stain, Myer Modified.
VI. 95% Alcohol
VII. 100% Alcohol
VIII. Histoclear
IX. Store 95%alcohol, 100%alcohol and histoclear in flammable safety cabinet. Store all other reagents at room temperature
X. Equipment and Materials

A. Coplin jars
B. Filter paper
C. Pipettes
D. Staining rack
E. Alcian Blue / PAS Stain Kit includes (solution A: Acetic acid 3%, Solution B Alcian Blue stain PH 2.5, Solution C: Periodic acid 0.5%, Solution D: Schiff ‘s reagent, and Solution E Hematoxylin stain Myer Modified.)

CALIBRATION **N/A**

QUALITY CONTROL

Depending on the diagnostic tissue, Colon, small intestine, or Cervix tissue including exo- and endo-cervix is a good control.

*Any new special stain reagent lot must be ran using the manufacturer’s special stain procedure on a positive control slide. A comparison of the old lot versus the new lot on a positive control slide will be performed. The two slides will be reviewed by the Surgical Pathologist. Results will be documented in the Histology Special Stain Reagent Kit Quality Assessment Log.

PROCEDURAL STEPS **N/A**

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTREPRETATION OF RESULTS

I. Reporting result

A.	Acid Mucosubstances	Blue color
B.	Neutral Polysaccarides	Magenta
C.	Nuclei	Pale Blue
D.	Other substances colored with both Alcian Blue and PAS Purple	

REFERENCES

I. Bancroft JD and Stevens A. Theory and Practice of Histology Techniques. Churchill Livingstone, New York, NY.1977.

II. Sheehan DC and Hrapchak BB. Theory and Practice of Histology. 2nd Edition, Mosby St. Louis, MO 1980

III. Freida L Carson, PhD. Histotechnology A Self Instructional Text. 4th Edition

DEFINITIONS **N/A**

ATTACHMENTS **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	3/1/2021	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES **N/A**

EFFECTIVE **03-01-21**

REVISED **See Review/Revise Sign off Page**

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SECTION: **HISTOLOGY**

SUB SECTION: **SPECIAL STAIN**

SUBJECT: **Gram stain**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

All laboratory personal are expected to follow this procedure for Gram Stain. Any deviation or modification is prohibited without approval of supervisor, histologist in charge, and pathologist.

TEST PRINCIPLE/ PURPOSE

Purpose:

The demonstration of Gram negative (–ve) and Gram positive (+ve) bacteria in the tissue.

Principle:

Both Gram -ve and Gram +ve bacteria get stained with crystal violet, followed by gram iodine mordent which form a dye lake (Large molecular complex of crystal violet and iodine).
Gram +ve bacteria have Thick cell wall of peptidoglycan so the big molecule of crystal violet and iodine cannot be easily washed out in the decolorization step using the acetone- alcohol, but the acetone-alcohol disrupt the outer lipoprotein layer of Gram -ve bacteria , washing out the crystal violet molecule, and get stained red with using the safranin as a counter stain.

PROCEDURE:

I. Deparafanize slides in 2 changes of histoclear for 5 minutes each, dehydrate in 3 changes of 100% alcohol for 1 minutes each and 2 changes of 95% alcohol 1 minute each. Then rinse in distilled water.
II. Stain slides with crystal violet for 1 minute, then rinse with DI water
III. Stain the slides with Gram Iodine for 30 Second, then rinse in DI water
IV. Decolorize the slides with acetone alcohol, and rinse in DI water
V. Counter stain using Safranin for 1 minute, then rinse in DI water
VI. Air dry the slides followed by 3 changes in histoclear 1 minute each and Mount

CLINICAL SIGNIFICANCE N/A

SPECIMEN TYPE(S)

I. Cut paraffin sections 4 Micrometer each
II. 10% neutral buffered formalin for fixation.

REQUIRED REAGENT(S)

I. Gram Crystal violet
II. Gram Iodine
III. Gram Safranin
IV. Acetone-Alcohol
V. 50% Acetone- 50% Alcohol (100%)
VI. 100% Alcohol
VII. 95% Alcohol
VIII. Histoclear, Mount

Equipment/ Material

I. Coplin jars
II. Pipettes
III. Staining racks

CALIBRATION **N/A**

QUALITY CONTROL

Control tissues have both Gram +ve and Gram -ve bacteria should be used.

CALCULATIONS: **N/A**

REFERENCE INTERVALS: **N/A**

INTERPRETATION OF RESULTS:

Gram +ve Bacteria	Blue-Purple
Gram -ve Bacteria	Red
Back ground	Red
Nuclei	Light- Red

Definitions: N/A

REFERENCES:

I. Bancroft JD and Stevens A. Theory and Practice of Histology Techniques. Churchill Livingstone, New York, NY.1977.
II. Sheehan DC and Hrapchak BB. Theory and Practice of Histology. 2nd Edition, Mosby St. Louis, MO 1980.
III. Freida L Carson, PhD. Histotechnology A Self Instructional Text. 4th Edition

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	3/1/2021	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES: **N/A**

EFFECTIVE: **03-01-2021**

REVISED **See Review/Revise Sign off Page**

REVIEWED **See Review/Revise Sign off Page**

SECTION **HISTOLOGY**

SUB SECTION: **SPECIAL STAIN**

SUBJECT: **Mucin, Myer Mucicarmine STAIN**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

Mucicarmine Stain
All laboratory personnel are expected to follow this procedure for Mucicarmine stain. Any deviations or modification is prohibited without approval of a supervisor, histology technologist-in- charge or pathologist.

TEST PRINCIPLE / PURPOSE

The purpose: is Staining of epithelial mucin in tissue sections.
Principle: Mucicarmine stains carboxylated and sulfonated mucins but not the neutral mucin. Aluminum is believed to form achelation complex with the carmine, resulting compound has a net of positive charge which attached to the acid group of mucins

PROCEDURE

I. Deparaffinize in 2 changes of histoclear for 5 minutes each.
II. Hydrate in three changes of 100% alcohol for 1 minute each.
III. Hydrate in two 95% alcohol for 1 minute each.
IV. Place in distilled water.
V. Stain sections in fresh weighert Iron Hematoxylin fresh working solution for 7 minutes.
VI. Rinse sections in running Tap water for 10 minutes.
VII. Stain slides in fresh Myer Mucicarmine Working solution for 1 hour
VIII. Wash the sections in Tap water.
IX. Counter stain in Solution D: Metanil Yellow Stain for 1 minute.
X. Dehydrate sections with 2 changes of 95% alcohol for 1 minute each.
XI. Dehydrate sections with 3 changes of 100% alcohol for 1 minute each.
XII. Clear sections with two changes of Histoclear for 1 minute each.
XIII. Mount with Shandon Synthetic.

CLINICAL SIGNIFICANCE N/A

SPECIMEN TYPE(S)

I. Cut sections at 4-5 microns.
II. 10% neutral buffered formalin is preferred.

REQUIRED REAGENT(S)

I. Mucicarmine working solution (10 ml Solution C, and 30 ml Tap Water)
II. Fresh Weigert Iron Hematoxylin Working solution (20 ml Solution A and 20 ml Solution B)
III. Metanil Yellow Solution
IV. 95% Alcohol
V. 100% Alcohol
VI. Histoclear

A. Store 95%alcohol, 100%alcohol and histoclear in flammable safety cabinet. Store all other reagents at room temperature

VII. Equipment and Materials

A. Coplin jars
B. Filter paper
C. Pipettes
D. Staining rack
E. Mucin, Myer Mucicarmine Kit includes (solution A: Ferric Chloride acidified, Solution B: Hematoxylin1% alcoholic, Solution C: Mucicarmine stock stain, and Solution D: Metanil Yellow Stain, Aqueous.)

CALIBRATION **N/A**

QUALITY CONTROL

A section of unautolyzed Colon, small intestine, or appendix.

*Any new special stain reagent lot must be ran using the manufacturer's special stain procedure on a positive control slide. A comparison of the old lot versus the new lot on a positive control slide will be performed. The two slides will be reviewed by the Surgical Pathologist. Results will be documented in the Histology Special Stain Reagent Kit Quality Assessment Log.

PROCEDURAL STEPS **N/A**

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS

I. Reporting result

A.	Acid epithelial mucin	Deep rose to Red
B.	Capsule of Cryptococcus neoformans	Deep rose to Red
C.	Nuclei	Black
D.	Other tissue elements	Yellow

REFERENCES

I. Bancroft JD and Stevens A. Theory and Practice of Histology Techniques. Churchill Livingstone, New York, NY.1977.

II. Sheehan DC and Hrapchak BB. Theory and Practice of Histology. 2nd Edition, Mosby St. Louis, MO 1980

III. Freida L Carson, PhD. Histotechnology A Self Instructional Text. 4th Edition

DEFINITIONS **N/A**

ATTACHMENTS **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	3/1/2021	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES **N/A**

EFFECTIVE **03-01-21**

REVISED **See Review/Revise Sign off Page**

REVIEWED **See Review/Revise Sign off Page**

SECTION: **Pathology**

SUB SECTION: **Equipment**

SUBJECT: **Leica CV 5030 Robotic Coverslipper**

APPROVED BY: _____
Laboratory Medical Director or Designee

POLICY :

The Leica CV5030 is a robotic coverslipper for coverslipping tissue sections.

All laboratory personnel designated to operate this instrument must read these instructions and be familiar with all technical features of the instrument before attempting to operate it.

CLINICAL SIGNIFICANCE: **N/A**

SPECIMEN TYPE(S): **N/A**

REQUIRED REAGENT(S):

Histoclear / Micromount mounting medium

CALIBRATION: **N/A**

QUALITY CONTROL: **N/A**

PROCEDURES:

I. Press the main switch on the front side of the instrument.
II. The instrument will start to initialize. The “READY” LED is illuminated in red.
III. “CHECK BATH” is the prompt to insert the loading bath. Open the loading door, pull out drawer, and remove cover on histoclear loading bath.
IV. Check the level of histoclear in the loading bath. Top off histoclear to the top of the circle on the insert.
V. Remove dispenser brush from histoclear loading bath, place brush in holder, push down lid until it clicks and place in position in front of pick & place module (cover slip mount).
VI. Push the loading drawer back and close the loading door.

VII. The message “PRIME POSITION” appears. Press the PRIME button (the 3rd button on the bottom) once and the message “ACTIVATING” appears. Pressure is generated and the message “PRIME” appears. Press the PRIME button a second time and hold the button until the mountant comes out of the dispenser without bubbles. When sufficient mountant has been dispensed, the message “DISP. POSITION” appears. Move the dispenser into working position. The message “READY” appears in the display and instrument is ready for use.

VIII. The “READY” LED is illuminated in green and the coverslip operation can be started by pressing the “START” button.

IX. Place a loaded specimen slide holder into histoclear bath, close drawer and press “START” button. The specimen slides are processed sequentially, coverslipped and pushed into successive positions in the output magazine (from top to bottom). The note “FINISHED” is displayed accompanied by a signal tone. The “READY” LED is illuminated in red.

X. Press the LIFT button to remove the output magazine and unload manually.

XI. Place the empty output magazines in the output station and remove the empty specimen slide holder from the loading bath.

XII. At the end of each day, complete the daily cleaning and maintenance. Remove brush from holder and place in histoclear bath. Move the dispenser to the resting position. Press the main switch on the front side of the instrument to turn off.

MAINTENANCE

I. DAILY CLEANING AND MAINTENANCE

A. Remove brush from brush holder and place in histoclear bath.
B. Clean slide transport arms, wipe off coverslip collection tray and clean gripper arms with a histoclear moistened gauze.
C. Clean suction cups and coverslip sensor to remove dust particles with an alcohol moistened gauze.
D. When mountant is added to bottle, wipe the inside lip of the bottle top as well as the outside of the bottle with a histoclear moistened gauze to remove residual mountant.
E. Pour mountant gently to minimize bubbles and allow to de-gas overnight before using.
F. Check primer container and top off daily.
G. Inspect the surfaces and remove any spilled mountant with histoclear moistened gauze.
H. Empty coverslip tray if there is any broken glass.

II. WEEKLY/BI-WEEKLY CLEANING AND MAINTENANCE

A. Empty and clean the prime container at least once a week.
B. Change the histoclear in the histoclear loading bath every two weeks or as needed.
C. Remove bath inserts and clean the bottom of the bath.
D. Change the charcoal filter as required up to a maximum usage of 4 months.
E. Clean the container of the brush holder to avoid deposits of released mountant.

REFERENCES: N/A

DEFINITIONS: N/A

ATTACHMENTS: N/A

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	4/7/2020	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES: **N/A**

EFFECTIVE: **02-24-20**

REVISED **See Review/Revise Sign off Page**

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SECTION: **Microbiology**

SUBSECTION: **Policies**

SUBJECT: **Antimicrobial Reporting Policy**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

Antimicrobial Reporting Policy

PRINCIPLE / PURPOSE

This policy has been developed based on ARMC antibiotics formulary, Microscan's antibiotic reporting guide and Clinical Laboratory Standards Institute (CLSI) recommendations.

The purpose of this policy is to ensure that only antimicrobial agents appropriate for the organism and body site are routinely reported.

CLINICAL SIGNIFICANCE

Selective reporting of antimicrobial agents should help improve the clinical relevance of antimicrobial reporting and help minimize overuse of broad-spectrum agents that might result in selection of multi-drug-resistant organisms. In addition, optimizing the use of antibiotics is critical to effectively treat infections and protect patients from harms caused by unnecessary antibiotic use.

The policy is also used in reporting proficiency testing susceptibility results, particularly for isolates from cerebrospinal fluid and urine.

SPECIMEN TYPE(S) **N/A**

REQUIRED REAGENT(S) **N/A**

CALIBRATION **N/A**

QUALITY CONTROL **N/A**

PROCEDURES

Available antimicrobial Agents	Abbr.	*Entrobacteriaceae*	*Salmonella spp*	*Shigella spp*	*P. aeruginosa*	*Acinetobacter Spp.*	*Burkholderia cepaciae*	*Stentrop. Multophilia*	Other Non fermenters
Amikacin	Ak	√	N/R	N/R	√	√			√
Amoxicillin/K Clavulanate [ORAL]*	Aug	H							
Ampicillin	Am	√	√ **S**	√ **S**					
Ampicillin/ Sulbactam	A/S	√	√	√		√			
Aztreonam	Azt	H	√	√	√				√
Cefazolin*	Cfz	√	N/R	N/R					
Cefepime	Cpe	√	√	√	√	√			√
Cefotaxime	Cft	H	√			H			H
Cefotaxime/ K Clavulanate	Cft/ CA	ESBL PHENOTYPIC-CONFIRMATION TEST							
Cefotaxime-ESβL		ESBL SCREENING TEST							
Cefoxitin*	Cfx	H	N/R	N/R					
Ceftazidime	Caz	H	√	√	√	√	√	√	√
Ceftazidime/ Avibactam	CZA	H			H				
Ceftazidime/K Clavulanate	Caz/ CA	ESBL PHENOTYPIC-CONFIRMATION TEST							
Ceftolozane/ Tazobactam	C/T	H			H				
Ceftriaxone	Cax	√	√			H			H
Cefuroxime*	Crm	√	N/R	N/R					
Ciprofloxacin*	Cp	√		√**S**	√	√			√
Ciprofloxacin-S	Cp-S		√[1]**S**						
Doripenem	Dor	H			H	H			
Ertapenem	Etp	H			N/R				
Gentamicin	Gm	√	N/R	N/R	√	√			√
Imipenem	Imp	√			√	√			H
Levofloxacin*	Lvx	√	√ **S**	√ **S**	√	√	√	√	√
Meropenem	Mer	√			√	√	√		√
Minocycline	Min					H	H	H	
Moxifloxacin	Mxf								
Nitrofurantoin	Fd	**UTI ONLY**							
Piperacillin/ Tazobactam	PT	√			√	√			√
Tetracycline*	Te	H	√			**UTI ONLY**			**UTI ONLY**
Tigecycline	Tgc	H							
Tobramycin	To	√	N/R	N/R	√	√			√
Trimethoprim/ Sulfamethoxazole	T/S	√	√ **S**	√ **S**		√	√	√	√

I. Antibiotics for Routine/Selective Susceptibility Testing and Reporting from Microscan Gram Negative Panel, Neg. MIC 53, by Major Bacterial Groups

***** INAPPROPRIATE TO REPORT FOR CSF ISOLATES FROM LUMBAR OR SHUNT FLUID **[**Aug, Cfz, Cfx, Crm, Cp, Lvx and Te]

√= ROUTINELY REPORTED BLANK = NOT REPORTED [1] Report on *Salmonella typhi*
H= SELECTIVELY REPORTED N/R = INAPPROPRIATE TO REPORT
S = REPORT ONLY THESE AGENTS ON ISOLATE FROM STOOL

II. Antibiotics for Routine/Selective Susceptibility Testing and Reporting from Microscan Gram Positive Panel, Pos. MIC 38, by Major Bacterial Groups

Available antimicrobial Agents	**ABBR.**	***Staphylo-coccus Spp.***	***Enterococcus Spp.***	***Strep Spp. Beta Hemol. Group B***	***S. bovis*** GROUP	***Listeria monocytog***
Amoxicillin/K Clavulanate [ORAL]*	Aug					
Ampicillin	Am		√	√	√	√
Ampicillin/Sulbactam	A/S					
Azithromycin*	Azi	H[2]				N/R
Cefazolin*	Cfz	√	N/R			
Cefepime	Cpe		N/R	H	H	
Cefotaxime	Cft		N/R	H	H	
Cefoxitin Screen	MRSA SCREENING TEST FOR SA & SLUG					
Ceftaroline	Cpt	H	N/R			N/R
Ceftriaxone	Cax	H	N/R	H	H	
Cephalothin*	Cf		N/R			
Ciprofloxacin*	Cp	H	**UTI ONLY**			
Clindamycin*	Cd	√[2]	N/R	√[2,4]	H[2]	
Daptomycin	Dap	H	H	H		
Erythromycin*	E	√[2]	N/R	√[2,4]	H[2]	N/R
Gentamicin	Gm		N/R	N/R		
Gentamicin Synergy Screen			H[3]			
Imipenem	Imp					
Inducible Clindamycin		AS APPLICABLE		AS APPLICABLE		
Levofloxacin*	Lvx	H	**UTI ONLY**	H		
Linezolid	Lzd	H	H	H	H	
Meropenem	Mer					
Nitrofurantoin	Fd	**UTI ONLY**	**UTI ONLY**			
Oxacillin	Ox	√				
Penicillin	P	√	√	√	√	√
Piperacillin/Tazobactam	P/T					N/R
Rifampin	Rif	H				
Streptomycin Synergy Screen			H[3]			
Synercid	Syn		N/R			
Tetracycline*	Te	√	**UTI ONLY**			
Tigecycline	Tgc					N/R
Trimethoprim/ Sulfamethoxazole	T/S	√	N/R			√
Vancomycin	Va	√	√			N/R

* INAPPROPRIATE TO REPORT FOR CSF ISOLATES FROM LUMBAR OR SHUNT FLUID [Aug, Azi, Cfz, Cf, Cp, Cd, E, Lvx, and Te]

[2] DO NOT REPORT ON ORGANISMS FROM URINARY TRACT [Azi, Cd, E]

[3] High-level resistance testing only (disease such as Endocarditis) Report on Isolates from Blood and Wound culture

[4] For Penicillin-allergic pregnant women, test E, Cd and inducible clindamycin and report only Cd

√ **= ROUTINELY REPORTED** **BLANK = NOT REPORTED**

H = SELECTIVELY REPORTED **N/R = INAPPROPRIATE TO REPORT**

III. Antibiotics for Routine Susceptibility Testing and Reporting by Major Bacterial Group from Microscan MICroSTREP *plus*, Type 1 Panel

Available antimicrobial Agents	Abbr.	*S. pneumoniae* (meningitis)*	*S. pneumoniae* (non-meningitis)	Large Colony β-hemolytic streptococci, except Group B	Viridans streptococci[1]
Amoxicillin/K Clavulanate [ORAL]	Aug	N/R			
Ampicillin	Am			√	√
Azithromycin[2]	Azi				
Cefaclor	Cfr				
Cefepime	Cpe		√	H	H
Cefotaxime	Cft	√	√**	H	H
Ceftriaxone	Cax	√	√**	H	H
Cefuroxime sodium (parenteral)	Crm				
Gatifloxin	Crm				
Chloramphenicol[2]	C			√	
Clindamycin[2]	Cd	N/R	√	√	
Erythromycin[2]	E	N/R	√	√	
Levofloxacin	Lvx	N/R	√		
Meropenem	Mer	√	√		
Penicillin	P	√	√**	√	√
Tetracycline	Te	N/R	√		
Trimethoprim/ Sulfamethoxazole	T/S		√		
Vancomycin	Va	√	√	H	

[1]Run MICroSTREP *plus 1 panel* from CSF, blood, fluids, or on special request. Viridans group also includes small colony-forming beta hemolytic strains A, C, F, or G (Strep. anginosus group).

[2]DO NOT REPORT ON ORGANISMS FROM URINARY TRACT

*REPORT ONLY MENINGITIS INTERPRETATION. ENTER CANNED MESSAGE 'PNCSF'

**REPORT BOTH MENINGITIS AND NON-MENINGITIS MIC AND INTERPRETATIONS ON NON-CSF SPECIMENS. ENTER CANNED MESSAGE 'PNNCSF'

All Pneumococcus: Perform Oxacillin KB Disk diffusion test in addition and report Penicillin susceptibility

√ = ROUTINELY REPORTED BLANK = NOT REPORTED

H = SELECTIVELY REPORTED N/R = INAPPROPRIATE TO REPORT

IV. Antibiotics for Routine/Selective Susceptibility Testing and Reporting from Gram Negative Kirby Bauer Disk Diffusion Test by Major Bacterial Groups

Available antimicrobial Agents	**Code**	***Entrobacteriaceae spp.***	***P. aeruginosa***	***Acinetobacter Spp.***	***Burkholderia cepaciae***	***Stentrop. Multophilia***	***Aeromonas Spp. P.shigeloid.***
Amikacin	AN-30	√	√	√			H
Ampicillin	AM-10	√					
Ampicillin/ Sulbactam	SAM-20	√		√			H
Aztreonam	ATM-30	H	√				H
Cefepime	FEP-30	√	√	√			√
Cefotaxime	CTX-30	H		H			√
Ceftazidime	CAZ-30	H	√	√	√	N/R	√
Ceftazidime/ Avibactam	CZA-50	H	H				
Ceftolozane/ Tazobactam	C/T-40	H	H				

Ceftriaxone	CRO-30	H		H			√
Cefuroxime*	CXM-30	√					
Ciprofloxacin*	CIP-5	√	√	√			√
Ertapenem	ETP-10	H	N/R	H			H
Gentamicin	GM-10	√	√	√			H
Imipenem	IPM-10	H	√	√			H
Meropenem	MEM-10	H	√	√	√		H
Nitrofurantoin	F/M-300	**UTI ONLY**					
Piperacillin/ Tazobactam	TZP-110	√	√	√			H
Tetracycline*	Te-30	H		**UTI ONLY**			H
Tobramycin	NN-10	√	√	√			
Trimethoprim/ Sulfamethoxazole	SXT	√		√	√	√	√

For All Other Non-fermenters, Disk diffusion testing is not recommended. INTERPRETATION IS AVAILABLE ONLY IN MIC, PER FDA/CLSI

* INAPPROPRIATE TO REPORT FOR CSF ISOLATES FROM LUMBAR OR SHUNT FLUID

√= ROUTINELY REPORTED
BLANK = NOT REPORTED
H= SELECTIVELY REPORTED
N/R = INAPPROPRIATE TO REPORT OR DISC DIFFUSION TEST UNRELIABLE

V. Antibiotics for Routine/Selective Susceptibility Testing and Reporting from Gram Positive Kirby Bauer Disk Diffusion Test by Major Bacterial Groups

Available antimicrobial Agents	**Code**	***Staphylococcus spp.***	***Enterococcus spp.***	***Streptococcus Viridans spp.[1,2]***	***Streptococcus B-Hemolytic Group[3,4]***	***Listeria spp.***	***Streptococcus pneumoniae***
Ampicillin	AM-10		√	*1*	√	√	
Cefotaxime	CTX-30		N/R	√			
Chloramphenicol	C-30	H[5]	H[5]	√[5]	H		
Clindamycin*	CC-2	√[5]	N/R	√[5]	√[4, 5]		
Ciprofloxacin*	CIP-5	H	**UTI ONLY**				
Erythromycin*	EE-15	√[5]	N/R	√[5]	√[4, 5]		
Oxacillin[6]	OX-1	√					√
Penicillin	P-10	√	√	*1*	√	√	
Tetracycline*	Te-30	√	**UTI ONLY**	H			
Trimethoprim/ Sulfamethoxazole	SXT	√	N/R				
Vancomycin	Va-30	MIC ONLY	√	√	√	N/R	
Nitrofurantoin	F/M-300	**UTI ONLY**	**UTI ONLY**				

√= ROUTINELY REPORTED BLANK = NOT REPORTED
H= SELECTIVELY REPORTED N/R = INAPPROPRIATE TO REPORT

*INAPPROPRIATE TO REPORT FOR CSF ISOLATES FROM LUMBAR OR SHUNT FLUID

[1] PERFORM MICroSTREP *plus*, Type 1 Panel ON ISOLATES FROM NORMALLY STERILE SITES (CSF, BLOOD, BONE, BODY FLUIDES). No interpretative categories & zone diameter breakpoints available from CLSI

[2] INCLUDES mutans group, salvarious group, bovis group, anginosus group (previously S. milleri group) and mitis group

[3] PERFORM GRAM POS. MIC 38 PANEL ON GROUP B STEREP SPP.

[4] INCLUDES GROUP A (S. pyogenes), C, OR G. SMALL COLONY FORMING B-HEMOLYTIC GROUP A, C, F, OR G ANTIGENS ARE CONSIDERED PART OF THE VIRIDANS GROUP

[5] DO NOT REPORT ON ORGANISMS FROM URINARY TRACT

[6] Disk diffusion test for *S.aureus* and *S.lugdunensis* not reliable. DO NOT REPORT

VI. Antibiotics for routine/selective susceptibility testing and reporting from Gram Negative Fastidious Organisms Kirby Bauer Disk Diffusion Test by major bacterial groups

Available antimicrobial Agents	**Code**	**Pasteurella spp.**	**Hemophilus and Parainfluenzae spp.**	**Neisseria meningitidis**	**Moraxella catarrhalis**	**Neisseria gonorrhoeae**
Ampicillin	AM-10	√	√ [1]			
Ampicillin/ Sulbactam	SAM-20		√			
Azithromycin				√	H	
Cefotaxime	CTX-30		√[1]	√		
Ceftriaxone	CRO-30	√	√[1]	√		
Cefuroxime	CXM-30		√		√	
Chloramphenicol	C-30	√	√ [1]	√		
Ciprofloxacin	CIP-5		√	√		
Erythromycin	EE-15	√			H	
Meropenem	MEM-10		√ [1]	√		
Penicillin	P-10	√				
Tetracycline	Te-30	√			H	
Trimethoprim/ Sulfamethoxazole	SXT	√		√	√	
Cefinase test[2]			√			√

√ = ROUTINELY REPORTED BLANK = NOT REPORTED
H = SELECTIVELY REPORTED N/R = INAPPROPRIATE TO REPORT
[1] REPORT ON CSF

[2] Cefinase tests for beta-lactamase activity. A positive result predicts resistance to P, AM, AND AMOXACILLIN. Enter a canned message, "BLAC"

VII. Antibiotics for Routine Susceptibility Testing and Reporting on Anaerobic Organisms from E-Test MIC by Major Bacterial Groups

Available antimicrobial Agents	**Code**	***Bacteroides fragilis*[1] Group and Other Gram-Negative Anaerobes**	**Gram-Positive Anaerobes**
Penicillin	PG		√
Clindamycin	CM	√	√
Imipenem	IP	√	√
Metronidazole	MZ	√	√
Ampicillin/Sulbactam	AB	√	√
Piperacillin/Tazobactam	TZP	√	√

√ = ROUTINELY REPORTED
[1] *Bacteroides Spp.* Resistant to Penicillin

VIII. Antibiotics for Routine Susceptibility Testing and Reporting on *Campylobacter spp*. Kirby Bauer Disk Diffusion Test

Available antimicrobial Agents	**Code**	**KB Disk Diffusion**
Erythromycin	**E**	√

√ = ROUTINELY REPORTED

IX. Fastidious Organisms for which Susceptibility Test (Broth Micro Dilution or Agar Dilution) Method is not available in-house

The following organisms should not be tested by the disk diffusion method because the results cannot be interpreted reliably. Isolates will be sent out to reference laboratory for susceptibility testing when requested by a provider and approved by infectious diseases doctor and laboratory director or designee.

A. *Abiotrophia spp. and Granulicatella Spp.*
B. *Bacillus Spp.*
C. *Corynebacterium Spp.*
D. *Erysipelothrix rhusiopathiae*
E. *HACEK Group*
F. *Helicobacter pylori*
G. *Lactobacillus, Pediococcus, Leuconostoc spp., Micrococcus spp. Rothia spp, Aerococcus spp.*
H. *Oligella spp.* and *Roseomonas spp.,*

XI. Potential Bacterial Agents of Bioterrorism

Report Cefinase result on *Aggregatibacter spp., Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens*, and *Kingella spp.* Cefinase tests for the production of beta-lactamase. A positive result predicts resistance to penicillin, ampicillin and amoxicillin. A negative result does not rule out resistance due to other mechanisms. Enter a canned message, “BLAC”, to state the above statement.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS **N/A**

REFERENCES:

I. Isenberg, Henry D. Clinical Microbiology Procedures Handbook. Second Edition, Volume 2, ASM Press. Washington D.C. 2004.

II. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guidelines-Second Edition. Clinical and Laboratory Standards Institute (CLSI), M45-A2, Vol.30, No.18, 09/06/2012.

III. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. Clinical and Laboratory Standards Institute (CLSI), M100 S24, Vol. 34 No.1, 2014.

IV. Performance Standards for Antimicrobial Susceptibility Testing, Clinical and Laboratory Standards Institute (CLSI), M100, 29th Edition, 02/05/2019.

DEFINITIONS: **N/A**

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES: Policy No. 800.27

EFFECTIVE: **July 21, 2020**

REVISED **See Review/Revise Sign off Page**

REVIEWED **See Review/Revise Sign off Page**

SECTION: **Bacteriology**

SUB SECTION: **Procedures**

SUBJECT: **Microscan Antibiotic Susceptibility Test**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

N/A

TEST PRINCIPLE / PURPOSE

This procedure refers to Microscan Dried Gram Negative (Neg. MIC 53) and Gram Positive (Pos. MIC 38) Antibiotic Susceptibility Tests on Microscan Walkaway 96 Plus (W/A) Instrument.

The antimicrobial susceptibility tests are miniaturizations of the broth dilution susceptibility test which have been dehydrated. Various antimicrobial agents are diluted in Mueller-Hinton broth supplemented with calcium and magnesium to concentrations bridging the range of clinical interest. After inoculation and rehydration with a standardized suspension of organism and incubation at 35°C for a minimum of 16 hours, the minimum inhibitory concentration (MIC) for the test organism is determined by observing the lowest antimicrobial concentration showing inhibition of growth.

On a Negative MIC 53 panel, Trimethoprim, and trimethoprim/sulfamethoxazole broth contain thymidine phosphorylase to reduce thymidine levels in the medium. Panels are also used to screen for presence of *Escherichia coli*, *Klebsiella oxytoca*, *K. pneumoniae* or *Proteus mirabilis* strains suspected of producing extended-spectrum beta-lactamases (ESBLs). The confirmation test is a ≥3 twofold dilution decrease in MICs of suspected organisms to either ceftazidime or cefotaxime in the presence of a fixed concentration of clavulanic acid, versus its MIC when tested alone.

On a Positive MIC panel, Oxacillin broth is supplemented with sodium chloride. The synergy screens utilize dextrose phosphate broth. The MicroScan Inducible Clindamycin test is intended to detect inducible resistance for staphylococci with the antimicrobial agent clindamycin. The MicroScan Cefoxitin Screen well is intended to determine the susceptibility of *S. aureus* and *S. lugdunensis* to the penicillinase-stable beta-lactams. The Cefoxitin Screen well uses the 16-20 hour result from a well containing cefoxitin at 4 µg/mL and growth media, labeled CfxS, and the oxacillin MIC at 16-20 hours.

CLINICAL SIGNIFICANCE

Susceptibility testing is important for prompt treatment with appropriate antibiotics, thereby decreasing patient morbidity and length of hospital admissions.

The Microscan Negative panels are designed for use in determining the antimicrobial agent susceptibility of rapidly growing aerobic and facultative anaerobic gram-negative bacilli.

The MicroScan Positive panels are designed for use in determining antimicrobial agent susceptibility of rapidly growing aerobic and facultative gram-positive cocci, some fastidious aerobic gram-positive cocci and *Listeria monocytogenes*.

SPECIMEN TYPE(S)

I. Gram-positive organisms from a single isolate or pure culture must be isolated on blood agar for 18-24 hours in CO_2 at 35°C prior to use.

II. Gram-negative organisms from a single isolate or pure culture must be isolated on McConkey agar and incubated 18-24 hours in non CO_2 at 35° C. Organisms that don't grow on McConkey should be isolated to blood agar.

REQUIRED REAGENT(S)

All reagents must be stored as recommended by manufacturer and used before manufacturer's stated expiration date unless noted otherwise below.

I. 0.5 McFarland Turbidity Standard
II. 100 uL pipette with disposable sterile tips
III. Cover trays
IV. Inoculator-D set for MIC panels
V. Inoculum water with pluronic stored at 15-30° C.
VI. MIC panels stored at 2-25° C
VII. Quality control organisms
VIII. Prompt Inoculation System-D
IX. Renok - Rehydrator/inoculator
X. Sterile autoclaved inoculum water
XI. Turbidity meter
XII. Vortex
XIII. W/A/LABPRO bar code label paper

CALIBRATION N/A

QUALITY CONTROL

Run susceptibility quality control using the following organisms, each week of use. (Refer to criteria for once-a-week QC susceptibility testing on Microscan panel's validation study binder and specific IQCP).

I. Gram Neg. MIC Panel 53

A. *Klebsiella pneumonia* (ATCC 700603)
B. *Escherichia coli* (ATCC 35218)
C. *Pseudomonas aeruginosa* (ATCC 27853)

D. *Escherichia coli* (ATCC 25922)

II. Gram Pos. MIC Panel 38

A. *Staphylococcus aureus (*BAA-977)
B. *Staphylococcus aureus (*ATCC 43300)
C. *Escherichia coli* (ATCC 35218)
D. *Staphylococcus aureus* (ATCC 29213)
E. *Enterococcus faecalis* (ATCC 29212)
F. *Enterococcus faecalis* (ATCC 51299)

Report all out of range results to the Microbiolgy Supervisor for corrective action.

PROCEDURAL STEPS

I. Allow panels and broths to reach room temperature.

II. Batch specimens that are to be run in W/A corresponding to panel types and then number the plates.

III. Pull needed panels of each panel type.

Note: panels should not be used if desiccant is not present or if the integrity of the packaging is compromised.

IV. Order specimens for W/A.
A. Meditech - Download specimens in batches by panel type; e.g., NM53, or PM38.
B. LABPRO - Order specimens that cannot be downloaded using patient order entry in LABPRO.

V. Print bar codes.
A. Check bar code printer alignment.
B. Print bar codes and number bar codes to match plate numbers.
C. Place bar codes on panels, centering the bar code.

VI. Weekly QC:
A. Order QC panels in QC order entry, print and label panels.
B. Inoculate panels and test along with patient specimens following the same procedures.
C. Check inoculum density by doing colony count. Inoculate 1 ul of *E. coli* (ATCC 25922) on to Blood Agar Plate from the Prompt suspension and determine CFU after 18 – 24 incubation. The expected colony count should closely approximate 5 X 10^5/mL.

VII. Panel Set-up:
A. Inoculum Preparation
1. Preparation of bacterial suspension using Prompt Inoculation System-D
a. Remove the required number of Prompt inoculation bottles from the box.
b. Remove an inoculation wand from the box.
c. Holding the wand tip *perpendicular* to the agar surface, touch 3 isolated colonies as large as or larger than the tip. *Do not* penetrate the agar. *Do not* scrape or drag the tip across the colonies.

NOTE: For very small, pin-point colonies, continue incubation of the primary plate until they reach the diameter of the wand tip. If the colony diameter is not likely to

reach this size (for example, some streptococci), an alternate method for inoculum preparation should be used.

d. Holding the wand by the handle with one hand, grasp the collar with the other hand and pull firmly to break the connection between the collar and wand shaft. Do not twist or bend the collar. Keep the tip pointed toward the floor, away from yourself. Slide the collar slowly down and off the wand shaft and discard the collar.
e. While holding the inoculation wand with one hand, pick up a Prompt inoculation bottle.
f. Bend the cap of the bottle sideways until it snaps off.
g. Place the inoculation wand into the bottle and press down with a twisting motion to assure a tight seal.
h. Shake the bottle vigorously 8 to 10 times to release the bacteria from the wand tip. If the organism is not released from the wand, let the solution sit for 5 minutes and shake again.
i. The bacterial suspension should be used within four hours of preparation. If not used immediately after preparation, shake vigorously to resuspend the bacteria just prior to use.

2. Turbidity Standard Technique – *Primary Inoculum Method*
   a. Using a sterile swab, touch the surface of 4-5 large or 5-10 small morphologically similar, well-isolated colonies from an 18-24 hour blood agar or McConkey agar plate.
   b. Emulsify in 3 mL of inoculum water (autoclaved sterile water). To insure an even distribution of the bacterial growth in the water, rub the swab on the bottom or side of the tube. This will reduce "clumping" and will aid in emulsification of mucoid growth.
   c. Cap tightly and Vortex the suspension for 2 – 3 seconds. The final turbidity should be equivalent to that of a 0.5 McFarland Turbidity Standard. An equivalent turbidity can be achieved by using a Microscan Turbidity Meter with the range of 0.06 - 0.10.
   d. Pipette 0.1 mL (100 ul) of the standardized suspension into 25 ml of inoculum water with Pluronic. Cap tightly, invert 8-10 times to mix.

B. Panel Rehydration/Inoculation
1. Rehydration and inoculation is performed using the MicroScan RENOK re-hydrator /inoculator system with inoculators.
2. With PROMPT system, remove the inoculation wand from the bottle and discard.
3. Remove the transfer lid from the inoculator set.
4. Pour the suspension into the seed tray by gently squeezing the bottle with a pumping action
5. With Pluronic water system, pour the entire 25 ml inoculated water with pluronic into the trough.
6. Replace the transfer lid. Tap the 4 corners of the lid to dislodge air bubbles at the bottom of the tray. Let equilibrate 20 seconds and attach the RENOK.
7. Transfer 115 uL of inoculum to each well of the MIC plate using the RENOK. (see below)

NOTE: The inoculated Prompt bottle, trays, wand and collar should be considered potential pathogen carriers. Handle and discard appropriately.

C. Renok Inoculation Procedure
1. Place the seed trough on a level, flat work surface. Pour the entire 25 mL PLURONIC water or PROMPT Suspension into inoculation tray.
2. Pick up the RENOK unit from its stand by depressing pick-up levers using thumb and fingers, and place RENOK unit on top of the transfer lid. Note: If a partitioned seed trough is used, make note of its orientation to the RENOK unit transfer lid. Ensure that it will correspond to the MicroScan panel's wells - failure to do so may result in incorrect inoculation of panels.
3. Release the pickup levers.
4. Fully lift the center lever of the RENOK inoculum into the transfer lid, then release hand pressure: the mechanical catch will hold the center lever in place.

5. Pick up the RENOK unit with the transfer attached, holding it level (shaking or rocking may cause spillage), then position the transfer lid onto a MicroScan panel. Note: If inoculating from a partitioned seed trough, insure that the RENOK unit and transfer lids are positioned on the MicroScan panel to dispense inoculum according to the appropriate panel procedural manual.
6. Depress the center release button of the RENOK unit to inoculate panel. There should be a smooth, even release of the inoculum without hesitation or sticking.
7. Dispose of the used transfer lid by positioning the RENOK unit, with the transfer lid attached, over a biohazard waste container. Depress the pickup levers using thumb and fingers to release the transfer lid from the RENOK unit. The seed trough may also be disposed of at this time. Alternatively the transfer lid may be released onto the seed trough and both may be disposed of simultaneously.
8. Place the RENOK unit on its stand for protection whether or not in use.
9. Stack plates for loading.

D. Load Panels into W/A
   1. In LABPRO, click on a time, e.g. 5 minutes, and click on access.
   2. Or for quick access, press unlock on walk away. This gives 5 minutes access time.
   3. When W/A door unlocks, open door and load panels with tray lids flat on top of panel, bar code label to the inside. Make sure the lids are not chipped or cracked.
   4. When one tower is full, close door and press "advance" button to go to next tower.
   5. When all panels have been loaded, close W/A door and press ESC. Do not load more than 24 panels in one session.
   6. Listen for bar code scan, watch for "Panel data now being uploaded", wait for screen to return to W/A monitor window with panels highlighted.

E. Review/print load status map
   1. Select print on W/A monitor window.
   2. Click on Load/Status map and then print OK.

F. Bar Code Read Errors
   1. If bar code read errors occur, check monitor for location of panels with errors.
   2. Select Unlock on W/A. Remove panels with bar code read errors and inspect the bar code. If bar code appears OK, then reposition or if not OK then reprint bar code and place over old label and return panel to W/A.

VIII. Maintenance

A. Daily Maintenance
   1. Print QC Diagnostics: On LABPRO computer, select W/A monitor, then select Print and then QC Diagnostics. This generates a printed copy of the daily maintenance check and calibration of the W/A. Check that all self-tests are OK. If failed, reboot W/A by turning off for 30 seconds, then on and wait for W/A to recalibrate then print QC Diagnostics. Use Maintenance List to record performance of daily maintenance. Check stage values 1-7. They should be 125 + 10 and remain constant over time. At the bottom of the report there is an array of eight rows and twelve columns of five digit signed integers. These readings should remain fairly constant. Out of control values will be underlined on the report. A drop of 500 or greater in individual calibration values between reports or underlined values indicates dirty shields or a dirty aperture plate. If the inconsistency remains after cleaning, a component failure could exist. Any power failure or fluctuations are printed as a second page to this report. Sign and file in Daily Microscan QC binder

2. Date and Time: Select maintenance on the LABPRO W/A monitor screen. Choose 30 minutes and click on Access. Check time on the front control panel of the W/A and compare it to the date and time of the LABPRO. If the date and time are incorrect on the W/A, correct it on the LABPRO.
3. Temperature: Check temperature on display and thermometer. Record (35 ± 1.0°C). Log in Daily QC Log.
4. Water Reservoir: Check water reservoir indicator light and if lit, fill with sterile distilled or sterile deionized water.
5. Shield maintenance: With the Service Hatch closed, advance to Tower 8 (Note: Tower 8 rack remains removed unless needed for panels) Select [click on], position for Shield Cleaning, from the Maintenance Menu. Open the Service Hatch and locate the glass photodiode shield. Use lens cleaner only, dry with lens paper, and be sure the shield is thoroughly dry before replacing it in the same position prior to removal. Close the hatch
6. Reference Disk: With the Service Hatch closed, select [Click On] Position for Reference Cleaning, from the Maintenance Menu. This allows you to access the reference disk. The reference disk is a glass disk recessed within the grabber bar. Use a cotton tipped applicator dipped in lens cleaner to clean the disk. Do this by lightly rubbing the surface of the disk in a circular motion with the wet applicator. Immediately dry the disk surface using a dry cotton-tipped applicator.
7. Note: Always dry the disk surface, never let the lens cleaner dry by itself on the disk.

B. Weekly Maintenance
   1. Photodiode Shields: With the service hatch-closed select (Click On) Position for Shield Cleaning, from the Maintenance Menu. Open the Service Hatch and locate the photodiode shield. This shield is a glass plate located beneath the photodiodes, which should be inspected daily and cleaned as needed. Rotate the right side of the photodiode shield forward and the shield to the right side to remove it. Clean with lens paper. If more extensive cleaning is required, use lens cleaner, or a mild solution of non-abrasive soap and water. Let the photodiode shield dry thoroughly then replace. Minor scratches on the surface of the shield have little to no effect on instrument performance. If, however, large scratches or other obstructions occur, it will be necessary to replace the photodiode shield.
   2. Diffuser Plate: With the Service Hatch closed select (Click On) Position for Shield Cleaning, from the Maintenance Menu. Open the Service Hatch and remove the front tower to locate the diffuser plate. This plate is a glass plate located above the fiber optic ends. Slide the plate forward until it is released from the block and clean in the same manner as the photodiode shield. Insert the diffuser plate square corner to the front right position of with smooth side up.
   3. Grasp the filter drawer cover and pull it out and away from the front of the instrument. Examine the filter for discoloration if necessary; hold it in front of a light to check for transparency. If dirty, clean with hot water using a mild detergent by forcing the water through in the opposite direction of airflow across the filter. After the filter has thoroughly dried, replace it. If the filter appears clogged, it must be replaced. Replace the filter cover.

C. Monthly Maintenance
   1. Run data base optimizer the first Monday of each month.
   2. Check the dispense volume of the RENOK as follows:
   3. The RENOK unit should also be checked any time if low fills are suspected. Check the fill volume (see steps 1-3 below) once per month and record in QC book.
   4. Required Tools
      -Several transfer lids, standard seed troughs, and cover trays.
      -Gravimetric scale (at least 0.1 Gram accuracy)
      -Small Phillips screwdriver

-Several vials of MicroScan Inoculum water with Pluronic-D

Step 1: Place one cover tray in the gravimetric scale, weigh the tray, and "zero" the scale.
Step 2: With the cover tray on the gravimetric scale, follow the dispensing procedures presented in the operating Instructions section of this manual, but inoculate the tray with Pluronic water.
Step 3: Record the final weight. If the final weight of the inoculated cover tray is not between 9.9 and 11.8 grams, retest the RENOK using another transfer lid and trough, preferably from another box. If the volume is still not within the above limits, try replacing the rubber seal (see section on Replacing the Rubber Seal). Finally, if the volume is still not within the above limits, contact MicroScan for a replacement.

IX. Routine Daily Task

1. Resolve Exceptions and print reports
   a. Print exception report for no growths etc. and give to appropriate station.
   b. Click on Exception Status.
   c. Click on first specimen and take appropriate action, e.g. print alerts, check for skipped wells, organism, name, etc.
   d. Print final reports and merge results to LIS:
   e. Print completed reports
   f. Select printer icon.
   g. Select completed reports.
   h. Select from T-1 thru T.
   i. Select Print; OK on Printer window.

2. Merge completed reports
   a. Select Interface Monitor Icon.
   b. Select Send Computer Icon.
   c. Select Test Group Status Complete.
   d. Select 1 Day Prior to Transmission Date.
   e. Select Send Computer Transmit.

CALCULATIONS N/A

REFERENCE INTERVALS N/A

INTERPRETATION OF RESULTS

Susceptibility is determined by comparing the MIC of an organism to the attainable blood or urine level of the antimicrobial agent. At the site(s) of infections this level should be at least 2-4 times the MIC and preferably greater before an organism can be considered sensitive. The following tables list interpretive criteria as recommended by CLSI. Some of these differ from the manufacturer's interpretative breakpoints listed in the Physicians' Desk Reference.

* **Microscan Antibiotics Reporting Guide: Gram Negative Panel, Neg. MIC 53**

Interpretive Breakpoints*

Antimicrobial Agents	Abbr.	Susceptible	Intermediate	Resistant
Amikacin	Ak	≤16	32	≥64
Amoxicillin/K Clavulanate - Enterobacteriaceae	Aug	≤8/4	16/8	≥32/16
Ampicillin[2] - Enterobacteriaceae and *V. cholerae*	Am	≤8	16	≥32
Ampicillin/Sulbactam - Enterobacteriaceae and *Acinetobacter* spp.	A/S	≤8/4	16/8	≥32/16
Aztreonam[3] - Enterobacteriaceae (CLSI M100-S19) and Non-Enterobacteriaceae	Azt	≤8	16	≥32
Cefazolin - Enterobacteriaceae (CLSI M100-S19)	Cfz	≤8	16	≥32
Cefepime[11]	Cpe	≤8	16	≥32
Cefotaxime[3] - Enterobacteriaceae (CLSI M100-S19) and Non-Enterobacteriaceae other than *P. aeruginosa*	Cft	≤8	16-32	≥64
Cefotaxime/K Clavulanate[6,7]	Cft/CA	--	--	--
Cefoxitin - Enterobacteriaceae	Cfx	≤8	16	≥32
Ceftazidime[2,3] - Enterobacteriaceae (CLSI M100-S19), Non-Enterobacteriaceae and *B. pseudomallei*	Caz	≤8	16	≥32
Ceftazidime/Avibactam	CZA			
Enterobacteriaceae: *C. freundii* complex, *C. koseri, E. aerogenes, E. cloacae, E. coli, K. oxytoca, K. pneumoniae, M. morganii, P. mirabilis, P. rettgeri, P. stuartii, S. marcescens*		≤8/4	--	≥16/4
P. aeruginosa		≤8/4	--	≥16/4
Ceftazidime/K Clavulanate[6,7]	Caz/CA	--	--	--
Ceftolozane/Tazobactam	C/T			
Enterobacteriaceae: *E. cloacae, E. coli, K. oxytoca, K. pneumoniae, P. mirabilis, C. koseri, M. morganii, P. vulgaris, P. rettgeri, P. stuartii, S. liquefaciens, S. marcescens*		≤2/4	4/4	≥8/4
P. aeruginosa		≤4/4	8/4	≥16/4
Ceftriaxone	Cax			
Enterobacteriaceae[9]		≤1	2	≥4
Non-Enterobacteriaceae other than *P. aeruginosa*		≤8	16-32	≥64
Ceftriaxone[3] - Enterobacteriaceae (CLSI M100-S19) and Non-Enterobacteriaceae other than *P. aeruginosa*	Cax	≤8	16-32	≥64
Cefuroxime axetil (oral) - Enterobacteriaceae	Crm	≤4	8-16	≥32
Cefuroxime sodium (parenteral) - Enterobacteriaceae	Crm	≤8	16	≥32
Ciprofloxacin[2] – Enterobacteriaceae[12] *Y. pestis* (CLSI M100-S17) and Non-Enterobacteriaceae	Cp	≤1	2	≥4
Ciprofloxacin-S *Salmonella* ser. Typhi	Cp-S	≤0.06	0.12-0.5	≥1
Doripenem[1]	Dor			
Enterobacteriaceae		≤0.5	--	--
A. baumannii		≤1	--	--
P. aeruginosa		≤2	--	--
Ertapenem – Enterobacteriaceae[10]	Etp	≤0.5	1	≥2
Ertapenem - Enterobacteriaceae (CLSI M100-S20)	Etp	≤2	4	≥8

Antimicrobial Agents	Abbr.	Susceptible	Intermediate	Resistant
Gentamicin[2] - Enterobacteriaceae, *Y. pestis* and Non-Enterobacteriaceae	Gm	≤4	8	≥16
Imipenem	Imp			
Enterobacteriaceae: *Citrobacter* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *M. morganii*, *P. vulgaris*, and *P. rettgeri*		≤1	2	≥4
P. aeruginosa		≤2	4	≥8
Acinetobacter spp.		≤2	4	≥8
Levofloxacin[8]	Lvx	≤2	4	≥8
Meropenem	Mer			
Enterobacteriaceae (CLSI M100-S20)		≤4	8	≥16
P. aeruginosa (CLSI M100-S21)		≤4	8	≥16
Non-Enterobacteriaceae		≤4	8	≥16
Minocycline	Min			
Enterobacteriaceae		≤4	8	≤16
Acinetobacter spp.		≤4	8	≤16
Moxifloxacin[1,4] - Enterobacteriaceae	Mxf	≤2	4	≥8
Nitrofurantoin[5] - Enterobacteriaceae	Fd	≤32	64	≥128
Piperacillin/Tazobactam	PT			
Enterobacteriaceae and Non-Enterobacteriaceae		≤16/4	32/4-64/4	≥128/4
P. aeruginosa (CLSI M100-S21)		≤64/4	--	≥128/4
Tetracycline[2] - Enterobacteriaceae, *V. cholerae*, *Y. pestis, B. pseudomallei,* and Non-Enterobacteriaceae other than *P. aeruginosa*	Te	≤4	8	≥16
Tigecycline[1] - Enterobacteriaceae	Tgc	≤2	4	≥8
Tobramycin	To	≤4	8	≥16
Trimethoprim/Sulfamethoxazole[2] - Enterobacteriaceae, *V. cholerae*, *Y. pestis, B. pseudomallei,* and Non-Enterobacteriaceae other than *P. aeruginosa*	T/S	≤2/38	--	≥4/76

*Based on Interpretive Breakpoints as indicated in CLSI Document M100-27th ed. or M45-A2. The antimicrobials included in this panel are not proven to be safe and effective in treating clinical infections for all organisms tested. For reporting of antimicrobial results which have shown to be active against organism groups *in vitro* or in clinical infections refer to CLSI M100, Tables 1 and 2 or the pharmaceutical package insert.

1. Based on manufacturer's breakpoints.
2. Interpretations for *V. cholerae*, *Y. pestis* or *B. pseudomallei* exist only for the antimicrobics designated.
3. Clinical isolates of *Klebsiella oxytoca*, *K. pneumoniae*, and *Escherichia coli* with increased MICs (≥2 µg/mL) of ceftazidime, aztreonam, cefotaxime, ceftriaxone, or MICs of ≥2 or ≥8 µg/mL (depending on panel type) of cefpodoxime should be suspected of harboring an extended-spectrum beta-lactamase. For *Proteus mirabilis* strains, only ceftazidime, cefotaxime, and cefpodoxime can be used for ESBL screening purposes.
4. Only systemic therapy will be reported.
5. Only urine therapy will be reported (based on CLSI Document M100-S20 for cephalothin).
6. No breakpoints exist for this test.
7. Clinical isolates of *Klebsiella oxytoca*, *K. pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* with a ≥3 two-fold dilution drop (i.e. a 3 well decrease) in an MIC value for the antibiotic tested with clavulanic acid as compared to the MIC value of that antibiotic tested alone, are considered ESBL phenotypic-confirmation positive (CLSI).[23] For further details see the LabPro Operator's Guide.
8. Interpretations for *Salmonella* species are based on CLSI M100-S21.
9. Updated Enterobacteria. Breakpoints are only implemented on panel types which minimally contain dilutions 1 and 2 µg/mL.

10. Updated Enterobacteri breakpoints are only implemented on panel types which minimally contain dilutions 0.5 and 1 µg/mL.
11. Interpretations for Enterobacteriaceae are based on CLSI M100-S23.
12. Interpretations for *Salmonella* species (other than *Salmonella* ser. Typhi) are based on CLSI M100-S21. Refer to Cp-S for reporting of *Salmonella* ser. Typhi.

* Microscan Antibiotics Reporting Guide: Gram Positive Panel, Pos. MIC 38

Interpretive Breakpoints*

Antimicrobial Agents	Abbr.	Susceptible	Intermediate	Resistant
Amoxicillin/K Clavulanate[5,6] – Staphylococci (CLSI M100-S22)	Aug	≤4/2	-	≥8/4
Ampicillin	Am			
Staphylococci (CLSI M100-S22)		≤0.25	-	≥0.5
L. monocytogenes[2] (CLSI M45-A2)		≤2	-	-
Enterococci		≤8	-	≥16
β-hemolytic Streptococci (*S. agalactiae*, Group B)[2]		≤0.25	-	-
Viridans Group Streptococci (*S. bovis* group)		≤0.25	0.5-4	≥8
Ampicillin/Sulbactam[5,6] – Staphylococci (CLSI M100-S22)	A/S	≤8/4	16/8	≥32/16
Azithromycin[3] - Staphylococci	Azi	≤2	4	≥8
Cefazolin[5] – Staphylococci (CLSI M100-S22)	Cfz	≤8	16	≥32
Cefepime[5] – Staphylococci (CLSI M100-S22)	Cpe	≤8	16	≥32
Cefotaxime[5] – Staphylococci (CLSI M100-S22)	Cft	≤8	16-32	≥64
Ceftaroline[1] – *S. aureus*[7]	Cpt	≤1	2	≥4
Ceftriaxone[5] – Staphylococci (CLSI M100-S22)	Cax	≤8	16-32	≥64
Cefuroxime axetil (oral) – Staphylococci (CLSI M100-S22)	Crm	≤4	8-16	≥32
Cefuroxime sodium (parenteral) – Staphylococci (CLSI M100-S22)	Crm	≤8	16	≥32
Cephalothin[5] – Staphylococci (CLSI M100-S22)	Cf	≤8	16	≥32
Ciprofloxacin - Staphylococci and Enterococci	Cp	≤1	2	≥4
Clindamycin[3]	Cd			
Staphylococci		≤0.5	1-2	≥4
β-hemolytic Streptococci (*S. agalactiae*, Group B)		≤0.25	0.5	≥1
Viridans Group Streptococci (*S. bovis* group)		≤0.25	0.5	≥1
Daptomycin[2]	Dap			
Staphylococci		≤1	-	-
Enterococci		≤4	-	-
β-hemolytic Streptococci (*S. agalactiae*, Group B)		≤1	-	-
Viridans Group Streptococci (*S. bovis* group)		≤1	-	-
Erythromycin[3]				
Staphylococci and Enterococci	E	≤0.5	1-4	≥8
β-hemolytic Streptococci (*S. agalactiae*, Group B)		≤0.25	0.5	≥1
Gentamicin - Staphylococci	Gm	≤4	8	≥16
Imipenem[5] – Staphylococci (CLSI M100-S22)	Imp	≤4	8	≥16
Levofloxacin	Lvx			
Staphylococci (CLSI M100-S14) and Enterococci		≤2	4	≥8
β-hemolytic Streptococci (*S. agalactiae*, Group B)		≤2	4	≥8
Viridans Group Streptococci (*S. bovis* group)		≤2	4	≥8
Linezolid	Lzd			
Staphylococci[2] (CLSI M100-S19)		≤4	-	-

Enterococci		≤2	4	≥8
β-hemolytic Streptococci (*S. agalactiae*, Group B)[2]		≤2	-	-
Viridans Group Streptococci (*S. bovis* group)[2]		≤2	-	-
Meropenem[3,5] – Staphylococci (CLSI M100-S22)	Mer	≤4	8	≥16
Nitrofurantoin[4] - Staphylococci and Enterococci	Fd	≤32	64	≥128
Oxacilline	Ox			
Coagulase - négative Staphylococcie		≤0.25	-	≥0.5
S. aureus/S. lugdunensis		≤2	-	≥4
Penicillin G	P			
Staphylococci		≤0.12	-	≥0.25
L. monocytogenes[2] (CLSI M45-A2)		≤2	-	-
Enterococci		≤8	-	≥16
β-hemolytic Streptococci (*S. agalactiae*, Group B)[2]		≤0.12	-	-
Viridans Group Streptococci (*S. bovis* group)		≤0.12	0.25-2	≥4
Piperacillin/Tazobactam[6] – Staphylococci (CLSI M100-S22)	P/T	≤8/4	-	≥16/4
Rifampin - Staphylococci and Enterococci	Rif	≤1	2	≥4
Synercid - Staphylococci	Syn	≤1	2	≥4
Tetracycline	Te			
Staphylococci and Enterococci		≤4	8	≥16
β-hemolytic Streptococci (*S. agalactiae*, Group B)		≤2	4	≥8
Viridans Group Streptococci (*S. bovis* group)		≤2	4	≥8
Tigecycline[1,8] – *S. aureus* and *S. epidermidis*[9]	Tgc	≤0.5	-	-
E. faecalis (Vancomycin-susceptible isolates)		≤0.25	-	-
Trimethoprim/Sulfamethoxazole - Staphylococci	T/S	≤2/38	-	≥4/76
Vancomycin	Va			
Staphylococci and Enterococci		≤4	8-16	≥32
S. aureus		≤2	4-8	≥16

*Based on Interpretative Breakpoints as indicated in CLSI Document M100, 27th ed. There are antimicrobials included in this panel that are not proven to be safe and effective in treating clinical infections for all organisms tested. For reporting of antimicrobial results which have shown to be active against organism groups *in vitro* or in clinical infections refer to CLSI M100, Tables 1 and 2 or the pharmaceutical package insert.

1. Based on manufacturer's breakpoints.
2. The absence of resistant strains precludes the CLSI from defining any result categories other than "Susceptible" at this time. Strains yielding results suggestive of a "non-susceptible" category should be submitted to a reference laboratory for further testing.
3. Only systemic therapy will be reported.
4. Only urine therapy will be reported.
5. For streptococci, refer to the penicillin result.
6. For beta-lactamase negative enterococci, refer to the penicillin result.
7. Ceftaroline should be used for methicillin-susceptible and methicillin-resistant *S. aureus* isolates from skin and skin structure infections and methicillin-susceptible *S. aureus* isolates from community acquired bacterial pneumonia.
8. The current absence of resistant isolates precludes defining any category other than Susceptible. Isolates yielding MIC results suggestive of a Nonsusceptible category should be subjected to additional testing.
9. There are no CLSI/FDA Interpretive Breakpoints for *S. epidermidis*. Due to the lack of CLSI/FDA interpretive breakpoints for *S. epidermidis*, overall performance of tigecycline was determined using *S. aureus* breakpoints for *S. epidermidis*.

Cefoxitin Screen Interpretive Criteria

Test	Negative	Positive
Cefoxitin Screen Well	≤4	>4

The MicroScan Cefoxitin Screen Well is intended to determine the susceptibility of *S. aureus* and *S. lugdunensis* to the penicillinase-stable beta-lactams (e.g. oxacillin), using the Cefoxitin Screen Well (CfxS) and the oxacillin MIC result at 16-20 hours. The CfxS result and oxacillin MIC are read independently at 16-20 hours and then processed through the LabPro software or interpreted manually to determine the final interpretation to oxacillin. The interpretation rules are shown in the following table:

		Final Oxacillin Interpretation
CfxS Result	Oxacillin MIC	*S. aureus* or *S. lugdunensis*
≤ 4 µg/mL Negative	≤0.25	S
	0.5	S
	1 or 2	S
	>2	R
> 4 µg/mL Positive	≤0.25	R*
	0.5	R*
	1 or 2	R*
	>2	R

Interpretations of R* are used by the LabPro software when the Cefoxitin Screen Well result changes the interpretation of the oxacillin MIC result. These criteria should also be followed when interpreting the results manually; however, the asterisk is not required.

Inducible Clindamycin

The MicroScan Inducible Clindamycin test is intended to detect inducible clindamycin resistance in staphylococci resistant or intermediate to erythromycin and susceptible or intermediate to clindamycin. Expression of resistance due to the erm gene may require induction by erythromycin. Results of ICd are equivalent to the D-zone disk approximation test. The interpretive criteria are shown in the following table:

Antimicrobial Test	**Negative**	**Positive**
Inducible Clindamycin Test - Staphylococci	≤4/0.5 µg/mL	> 4/0.5 µg/mL

When erythromycin is I or R and clindamycin is S or I, and the ICd test is positive, clindamycin should be reported as resistant.

Streptomycin and Gentamicin Synergy Screen

In enterococcal endocarditis the use of penicillin or ampicillin alone results in frequent treatment failures. When enterococci are susceptible in vitro to high levels of streptomycin or gentamicin, the addition of this antimicrobic to penicillin or ampicillin is synergistic and correlates clinically with an improved cure rate.[23,26] According to CLSI Document M07-A9, the recommended method for detection of high level aminoglycoside resistance (HLAR) for broth microdilution is as follows:

Antimicrobic Concentration	**Medium**	**Incubation**
Gentamicin 500 µg/mL	BHI*	24 hours
Streptomycin 1000 µg/mL	BHI*	24 - 48 hours

* Comparable results have been shown in limited testing with dextrose phosphate broth.

The performance of Gentamicin and Streptomycin Synergy Screens on the MicroScan Panels was compared to the microbroth reference methods recommended by the CLSI. Any evidence of turbidity should be considered growth or reincubated to confirm results. The results obtained with Gentamicin Synergy after 18 hours incubation were comparable to those obtained at 24 hours with the reference method. For best detection of resistance with Streptomycin Synergy Screen, MicroScan Panels should be incubated for 24 to 48 hours.

BACKUP PROCEDURE FOR ANTIBIOTIC SUSEPTIBLITY TEST

Whenever Microscan is out of function, and repair could not be performed on the same day, set up panels manually and read them using TouchScan (Light BOX). The TouchSCAN-SR panel reader is an instrument for reading MicroScan panels. It is designed to facilitate manually entering the determination of minimal inhibitory concentrations (MIC) to a battery of antimicrobics. Information is then processed by the LABPRO, which computes antimicrobic MIC results based upon the antimicrobic MIC endpoints.

The manual method is labor intensive and time consuming. It is perhaps not practical to perform manual antibiotic susceptibility tests on all positive cultures. Therefore, set-up manual tests according to the following priority:

1st CSF cultures

2nd Blood cultures (non-contaminated)

3rd Wounds culture from sterile sites such as body fluids or tissues

4th Endotracheal Aspirate (ETA)/Broncho Alveolar Lavage (BAL)

5th Urine cultures from inpatients/ Complicated UTI

Panel preparation, inoculum preparation and panel rehydration/inoculation for manual set-ups are performed in the same way as described in the previous sections.

Order appropriate panels in MediTech as described in the previous sections.

∗ Manual processing of Dried Gram Negative Panels

1. Incubation
   I. To ensure even thermal distribution during incubation, stack the panels in groups of 3-5.
   II. Place a clean Cover Tray on top of each group of panels to prevent evaporation. Cover Trays may be reused. Do not decontaminate Cover Trays with alcohol. They may be cleaned with soap and water. Rinse well and allow to air dry.
   III. Incubate the panels for a minimum of 16 hours at 35°C in a non-CO_2 incubator

2. Reading the Panels
   Panels can be read manually using the TouchScan Microdilution Viewer and results recorded on a Manual Panel Worksheet
   Following 16-20 hours incubation, remove the panels from the incubator.
   A. Wipe off the bottom of the panel with a lint-free tissue to remove any condensation or debris that may be present.
   B. Read the panels only if the growth well is turbid. Do not read the antimicrobics if the control well is turbid, or if there is no growth in the growth well. Growth in the antimicrobic wells appears as turbidity, which may take the form of a white haze throughout the well, a white button in the center of the well, or a fine granular growth throughout the well. Inadequate or no growth is defined as a slight whiteness in the well or the broth is clear.
   C. If results are read manually, record the results on the appropriate worksheet
   D. Reading Antimicrobic Susceptibilities
      1. Read all antimicrobics and CET against a black (indirectly lighted) background.
      2. Record MIC results as follows:

a. Following 16-20 hours incubation, record the MIC as the lowest antimicrobial concentration showing inhibition of growth.
b. When growth occurs in all concentrations of an antimicrobic, the MIC is recorded as greater than (>) the highest concentration.
c. When no growth occurs in any of the concentrations of the antimicrobics, the MIC is recorded as less than or equal to (≤) the lowest concentration.
d. A clear well in a series of growth wells, e.g., growth at 1, 2 and 8 µg/mL, but not at 4 µg/mL is called a skipped well and should be ignored.
e. Spot growth in isolated wells indicates contamination. The test should be repeated.
f. A "trailing effect" may be observed in some drug/organism combinations such as *Proteus* with cefuroxime (Crm) and imipenem (Imp), *Serratia* with beta-lactam antibiotics (e.g. imipenem (Imp) and piperacillin/tazobactam (P/T)), and *B. cepacia* and *B. pseudomallei* with ceftazidime (Caz) and piperacillin (Pi).
Trailing may also be observed with trimethoprim/sulfamethoxazole (T/S) and trimethoprim (T) with the use of the RENOK® Rehydrating/Inoculating System due to the inoculum concentration. The endpoint should be read as the lowest concentration which when compared to the growth well shows:

1. Approximately 80% reduction of growth (T/S,
2. A white button which is less than 2 mm in diameter, or
3. A white button which is semi-translucent.

* Manual processing of Dried Gram Positive
Panel preparation to incubation is done the same way as gram negative panels

A. Reading the Panels

1.. Reading Antimicrobial Susceptibilities (MICs)

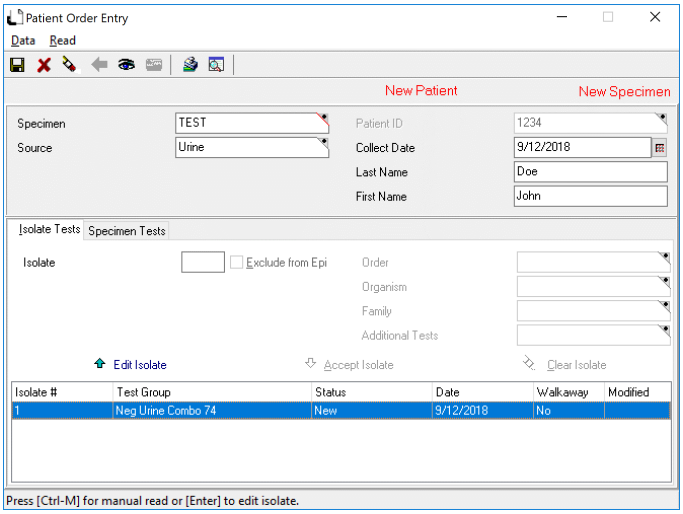
a. Read all antimicrobics, CV, MS, NOV, OPT, NACL, and BAC, against a black (indirectly lighted) background.
b. Perform β-lactamase test for staphylococci with Penicillin MIC of 0.06 µg/ml or 0.12 µg/ml. A nitrocefin-based, β-lactamase test is the preferred method.
c. Record Susceptibility Breakpoint results as follows:

1. MIC Results
   a. Following 16-20 hours incubation, record the MIC as the lowest antimicrobial concentration showing inhibition of growth.
   b. When growth occurs in all concentrations of an antimicrobic the MIC is recorded as greater than (>) the highest concentration.
   c. When no growth occurs in any of the concentrations of the antimicrobics, the MIC is recorded as less than or equal to (≤) the lowest concentration.
   d. A clear well in a series of growth wells, e.g., growth at 1, 2 and 8 µg/ml, but not at 4 µg/ml is called a skipped well and should be ignored.
2. Breakpoint Results
   a. Following 16-20 hours incubation no growth in the antimicrobic is recorded as "S" (Susceptible).
   b. In most cases, growth in the lower concentration of the antimicrobic but not in the higher concentration is recorded as "I" (Intermediate).
   c. Growth in both concentrations of the antimicrobic or in a single well of a one diluton antimicrobic is recorded as "R" (Resistant).
   d. If there is growth in the higher concentration of the antimicrobic but not in the lower concentration, the well may be contaminated and the test should be repeated.
3. Spot growth in isolated wells indicates contamination. The test should be repeated.
4. For staphylococci with Oxacillin, any growth should be considered significant.

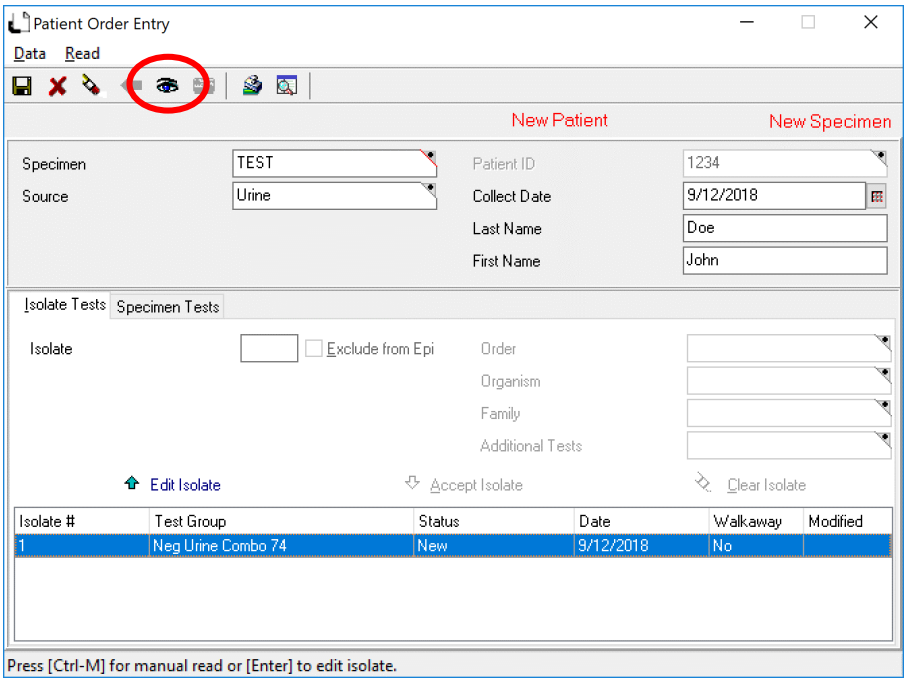
5. Accurate detection of resistance requires extended incubation times for the24 hour incubation is not required for *S. aureus* and *S. lugdunensis* for panels containing the Cefoxitin Screen Well.
6. The performance of vancomycin on MicroScan panels was compared to the microbroth dilution methods recommended by CLSI. Vancomycin results with staphylococci after 16-20 hours incubation (18-20 h for autoSCAN®-4 instrument reads) were comparable to those obtained at 24 hours with the reference method.
7. For panels containing Oxacillin only: Staphylococci should be reported as resistant to Ampicillin, Amoxicillin/K Clavulanate, Ampicillin/Sulbactam, Ertapenem, Imipenem, Meropenem, Penicillin, Piperacillin/Tazobactam, Ticarcillin/K Clavulanate and the Cephalosporin antimicrobics (regardless of the MIC) when Oxacillin MICs are > 2 µg/ml for *S. aureus* and *S. lugdunensis* and ≥ 0.5 µg/ml for coagulase negative staphylococci other than *S. lugdunensis*.
8. For panels containing both Oxacillin and the Cefoxitin Screen Well (CfxS): Staphylococci should be reported as resistant to Ampicillin, Amoxicillin/K Clavulanate, Ampicillin/Sulbactam, Ertapenem, Imipenem, Meropenem, Penicillin, Piperacillin/Tazobactam, Ticarcillin/K Clavulanate and the Cephalosporin antimicrobics (regardless of the MIC) when CfxS is > 4 µg/ml or Oxacillin MICs are >2 µg/ml for *S. aureus* and *S. lugdunensis* and Oxacillin is ≥ 0.5 µg/ml for other coagulase negative staphylococci.
9. For wells containing the Aminoglycosides (e.g. Gentamicin) and Macrolides (e.g. Erythromycin), growth may not be as heavy as that in the growth control well due to differences in basal media. Care should be taken in interpreting these
10. A “trailing effect” may be observed in some drug/organism combinations. Trailing with Trimethoprim/Sulfamethoxazole (T/S) with the use of the RENOK® Rehydrating/Inoculating System is due to the inoculum concentration. The end point should be read as the lowest concentration which when compared to the growth well shows:
    a. Approximately 80% reduction of growth (T/S)
    b. A white button which is less than 2 mm in diameteror
    c. A white button which is semi-translucent.1
11. A slight haze may be observed with the fluoroquinolone class of antimicrobics (e.g., Ciprofloxacin, Norfloxacin, Ofloxacin) when using the Prompt method of inoculation and staphylococci, including Quality Control organism *S. aureus* ATCC 29213. This should NOT be interpreted as growth.
12. If an organism does not grow in the Thymidine Free Growth (TFG) well, an MIC should not be reported for T/S.

Reading Panels Manually in LabPro

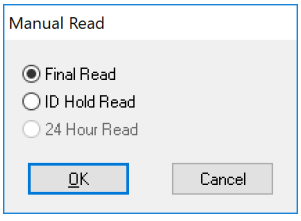
1. Receive all LIS data if necessary (Interface Monitor- Receive)
2. Click on Patient Order Entry 
3. Type in Specimen number (patient demographics should populate)
4. Click/highlight isolate panel order



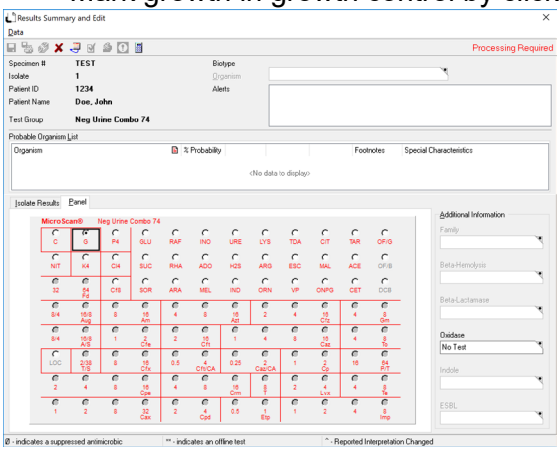
5. Click the eye icon to read panel manually



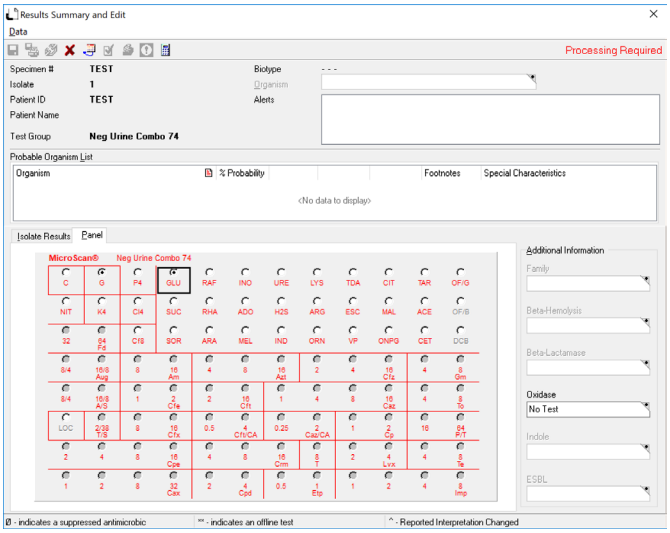
6. Click Ok to read as Final Read



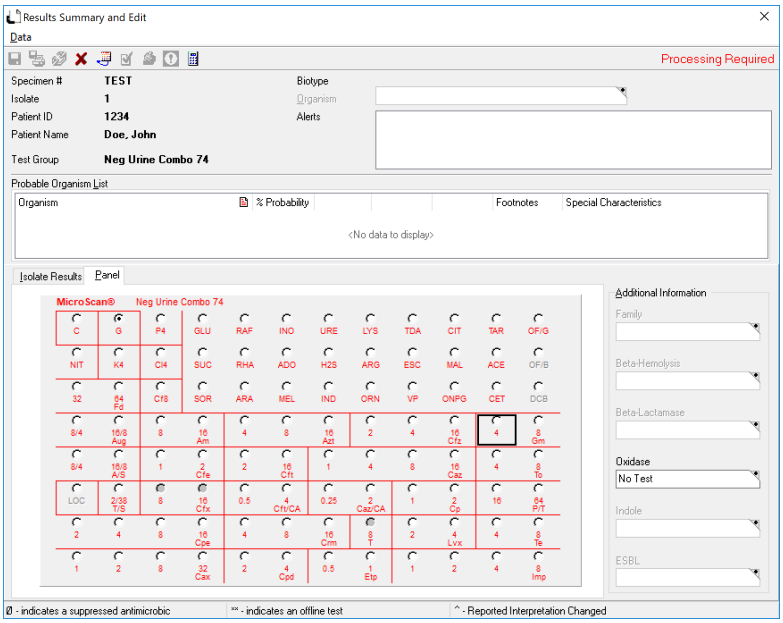
Mark growth in growth control by clicking into circle.



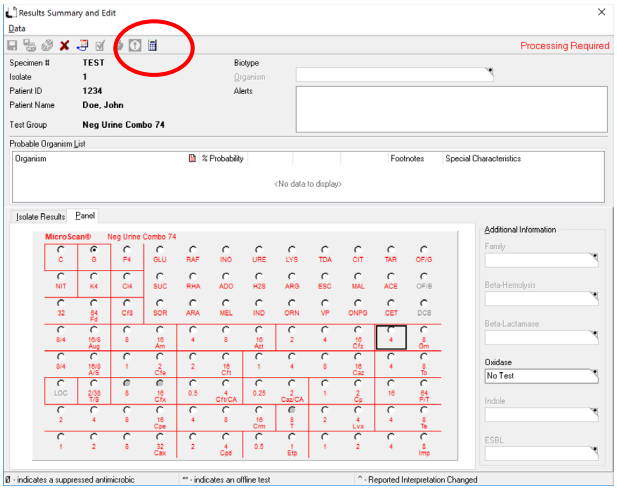
I. Read Identification reactions by using Color Chart, marking all positive wells



II. Click into the first well in each antibiotic (Note: gray wells will turn white. You MUST make ALL antibiotic wells white in order to finalize!)



III. Click the Calculator icon to process



IV. If you get a 'Panel data required' message, you missed an antibiotic. Click OK. The black square will show you where to click.

V. Save

VI. Transmit result to LIS as described in previous sections

Troubleshooting

Exception	Symptom	Corrective Action
Too many skipped wells	A no growth ("skip") well detected in a series of growth wells. >1 skipped well per panel will cause this exception	MIC results may be edited, if desired, through Read Micro Scan Panels. Save data.
Organism required	MIC only panel that was not assigned an organism not assigned an organism	Organism identification by number code MUST be entered through "select alternate organism" in Read Microscan

	number when it was selected for W/A processing.	Panels or Panel Status Inquiry. Store panel data.
Insufficient growth	Significant amount of growth not detected in growth control well.	Panel results not valid. Abandon the panel through Panel Status Inquiry.
Power failed	Power failure occurred for >2 hours during panel process.	Check power failure/ fluctuation log (2nd page of QC Instrument Diagnostics Report) to determine if panel incubation temperature has been affected. Operator discretion to store or abandon panel.
Aborted -missing	Time critical read has been missed due to panel	Abandon panel through Exceptions. If (a) Initial read -Reselect panel for W/A if it has been located & print new bar code label. -or if the panel has been incubated too long, inoculate new panel. Operator discretion. - or incubate off-line. (b) Final read-read panel manually and input results through Data Entry and Edit.
Aborted overtime	Time critical read has been missed due to power failure door open too long, or panel jam.	Abandon panel through Exceptions. Status Inquiry. If abandoned at (a) initial read- Reselect panel for AutoScan- W/A print new barcode label. - or if panel has incubated too long, inoculate new panel. operator discretion - or incubate off-line. (b) Final read - read panel manually and input results through Data Entry &. Edit.
Aborted - unrecognized	Locator well problem	Abandon panel through Exceptions. Check to be sure that panel type you selected for W/A is same as panel in process. If it is not, reselect for correct panel type. If it is, contact Technical services
Panel Missing	Bar code scan no longer "sees" panel that was previously seen in AutoScan-W/A.	Locate panel that was removed from W/A or check to be sure panel hasn't been turned around so that the bar code label is on - the wrong side. Load or correct panel placement through Load/Unload panel program only after verifying that the missing panel hasn't incubated too long by checking for biochemical reaction changes. If changes have occurred, inoculate new panel.
Invalid control	A Rapid Panel control well value is too low for valid interpretation	Abandon panel in Panel Status inquiry and repeat on new panel.

Limitations

Gram Pos Panels

I. MicroScan Dried Gram Positive Panels should not be used to determine the susceptibilities of *streptococci* other than *S. bovis* and *S. agalactiae*. Other streptococci should be tested using and accepted method such as MICroSTREP panels.

II. MicroScan Dried Gram Positive Panels should not be used to determine the susceptibilities of anaerobic isolates.

III. Certain antimicrobial agents commonly tested against Gram positives have limited use with both *Staphylococci* and *Streptococci.* While the correlation of the antimicrobial agent/organism combinations listed below was good when compared to an overnight reference method, MicroScan will not report MIC results for these combinations on the recommendation of our Antimicrobial Review Committee.

A. Gentamicin all *streptococci.*

B. Oxacillin all *streptococci* and *L. monocytogenes*.

C. Trimethoprim/Sulfamethoxazole - *Beta streptococci*, *Viridians streptococci*, and *S. Pneumoniae*.

IV. Read overnight positive panel for Enterococcus vs. Vancomycin at 24 hours.

V. Read overnight positive panels for Staphylococcus vs. oxacillin at 24 hours.

VI. The penicillin MIC's for coagulase negative Staphylococci may not be useful in predicting resistance due to β-lactamase.

Gram Neg panels

1. MicroScan Gram Negative Combo panels are intended for use with rapidly growing aerobic and facultative anaerobic gram negative bacilli isolated from human clinical specimens. They are not intended for use with anaerobic bacteria, fastidious bacteria such as Haemophilus species that require supplemented growth enrichments.

2. If panels are not covered by another panel or by a panel cover during incubation evaporation may occur, causing difficulties in interpretation of results.

3. The MIC and identification systems are designed to be incubated for a specific amount of time, as described in the procedural manual for each panel type. Attempts to read panels that have not been incubated for an appropriate amount of time may lead to erroneous results.

4. For some organisms where the ESBL is indeterminate, ESBL confirmation should be done by an alternate method (e.g. MicroScan Dried ESBL panel).

5. Interpretation of test results require trained clinical personnel who should use judgment, knowledge and applicable additional confirmatory tests prior to accepting the identification of any organism.

REFERENCES

I. LabPro Operator's Guide V4.42, Beckman Coulter , 2015

II. Operator's Manual, AutoScan-W/A, Baxter, Beckman Coulter, 2015.

III. Package Insert, Dried Gram Pos Procedure Manual, Beckman Coulter, 2019.

IV. Package Insert, Dried Gram Neg Procedure Manual, Beckman Coulter, 2019.

V. LabPro-MBT Implementation Guide. Beckman Coulter, 2015

DEFINITIONS: **N/A**

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES: 810.23 Microscan

EFFECTIVE: December 2019

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Bacteriology**

SUB SECTION: **Procedures**

SUBJECT: **MicroScan Rapid Pos. ID Type 2**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

MicroScan Rapid Pos. ID Type 2

TEST PRINCIPLE / PURPOSE

For use with MicroScan® Rapid Pos ID 2 Panels. Fluorogenic substrates or fluorometric indicators are used for the identification of gram-positive organisms. Identification is based on hydrolysis of fluorogenic substrates, pH changes following substrate utilization, production of specific metabolic byproducts and the rate of production of specific metabolic byproducts after 2 hours incubation at 35°C in the WalkAway® System.

Precautions

I. For *in vitro* diagnostic use only.
II. Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures, with special awareness that the inoculated panels contain potentially pathogenic organisms.
III. To date, no data have shown that fluorogenic substrates, at the manufactured panel concentrations used, are carcinogenic.
IV. Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

CLINICAL SIGNIFICANCE

Rapid and accurate identification of bacteria facilitates prompt and appropriate treatment of infections.

SPECIMEN TYPE(S)

Gram-positive organisms from a single isolate or pure culture must be isolated on blood agar for 18 – 24 hours in CO_2 at 35 °C prior to use.

REQUIRED REAGENT(S)

I. Materials Provided

A. Rapid Neg ID Type 2
B. Procedural and QC Manual

II. Materials required but not provided

A. 0.4% Saline with PLURONIC®*, 6.5 mL
B. Inoculum Water with PLURONIC-D 25 mL
C. RENOK Rehydrator/Inoculator (B1018-14) or equivalent
D. Inoculator-R
E. WalkAway *SI* System or upgrade
F. WalkAway System Tray Lids
G. WalkAway System Bar Code Label Paper
H. WalkAway System Bar Code Printer
I. Turbidity Meter
J. Quality Control Organisms (Refer to QC section of this manual)
K. TSA with 5% Sheep's Blood Agar Plate
L. 0.5 McFarland Barium Sulfate Standard
M. General Laboratory Equipment
N. Vortex

CALIBRATION

N/A

QUALITY CONTROL

The acceptability of the identification substrates should be checked by testing organisms with known reactions. The results for each reaction for the recommended American Type Culture Collection (ATCC[1]) control organisms are listed in the following tables. Following retest, should a quality control result be obtained which does not match the expected result, or if questions or concerns arise regarding MicroScan Rapid Panels or accessories, call MicroScan Technical Services.

Run identification quality control using the organisms listed in Table 2, Rapid Gram Positive Streamlined ID QC, and each week of use. (Refer to criteria for once-a-week QC susceptibility testing in Microscan panel's validation study binder).

Table 1. Extended Rapid Pos ID 2 Panels QC –Not for weekly/new lot QC (see below)

	A. baumannii **ATCC[1] 49139**	***E. casseliflavus*** **ATCC 700327**	***E. durans*** **ATCC 6056**	***K. oxytoca*** **ATCC 49131**	***S. bovis***[2] **ATCC 49133**	***S. epidermidis*** **ATCC 49134**	***S. putrefaciens*** **ATCC 49138**
AARG	-						+
AGAL	-				+		
AGL	-				+		
ARG	-						+

BDFU		+					-	
BGAL		+						-
BGL	-		+					
BGLR	-					+		
CEL	-			+				
CELB		+		-				
CHB	-	+						
CIT	-							+
FRU	-			+				
GLPR				-	+			
GLU				+				-
GLY							-	+
GLYC	-		+					
LAC	-				+			
MAL	-	+						
MAN	-			+				
MEL	-			+				
MNS	-	+						
MAG					-			+
PHO1			-	+				
PHO2			-	+				
PRO	-							+
PYET	+	-						
PYG	-							+
RAF	-			+				
SAL	-			+				
SOR	-			+				
SUC	-			+				
TRE	-			+				
TRYP				-				+
TYR				-				+
UREB					-	+		

1. ATCC = American Type Culture Collection, Manassas, VA USA
2. Incubation in CO_2 recommended.

Table 2. Rapid Gram Positive Streamlined ID QC* for weekly QC

QC Organisms	ATCC Number	Key Indicator Substrate	Expected Result	Out-of-Specification Result	Labile Substrate	Technique Indicator
E. durans	6056	PHO2	-	+	Yes	N/A
E. durans	6056	GLYC	+	-	N/A	YES
E. casseliflavus	700327	MNS	+	-	N/A	YES
A. baumannii	49139	SOR	-	+	N/A	YES

* streamlined QC based on CLSI document M50-A.

PROCEDURAL STEPS

I. Panel Preparation
   A. Remove the panels to be used from storage. Panels should not be used if the desiccant is not present or is broken or if the integrity of the packaging is compromised (unsealed, punctured, or torn).

II. Inoculum Preparation
   A. Using a sterile swab, or wooden applicator stick or bacteriological loop, touch the surface of 4-5 large or 5-10 small morphologically similar, well-isolated colonies from an 18-24 hour TSA with 5% Sheep's Blood agar plate. Note: If an isolate does not grow on TSA with 5% Sheep Blood Agar, growth can be taken from Chocolate Agar.
   B. Disperse in 6.5 mL of 0.4% saline with PLURONIC. To insure an even distribution of the bacterial cells in the saline PLURONIC, rub the stick or loop on the bottom or side of the tube. This will reduce “clumping” and will aid in dispersion of mucoid growth.
   C. Cap tightly and vortex the suspension for 2-3 seconds.
   D. The final turbidity should be equivalent to that of a 0.5 McFarland Barium Sulfate turbidity standard. Turbidity should be confirmed with a turbidity meter. A MicroScan Turbidity Meter range of 0.08 ± 0.02 (0.06 - 0.10) provides optimum performance.

III. Panel Rehydration/Inoculation
   A. Rehydration and inoculation is performed using the MicroScan RENOK Rehydrator/Inoculator system with Inoculator-R.
      1. Remove the transfer lid from the inoculator set. Pour the 6.5 mL standardized saline suspension into the bottom portion (3 rows) of the divided seed trough. Pour 25 mL of uninoculated Inoculum Water with PLURONIC-D into the top portion (5 rows) of the seed trough.
      2. Replace the transfer lid and attach the RENOK Rehydrator/Inoculator. Refer to the RENOK Rehydrator/Inoculator Operator’s Manual for use.
   B. If an alternative system is used, rehydrate each well with 115µL ± 10µL of the appropriate suspension.
      NOTE: The final organism concentration in the identification wells must be 1.5 X 10^8/mL. To ensure viability and purity of the organism tested, a purity plate may be prepared by streaking the inoculum to a blood agar plate and incubating for 16-20 hours. If two or more colony types are present on the purity plate, re-isolate the colonies and retest.

IV. Incubation
   A. Label each panel with a WalkAway System barcode label and place a WalkAway System tray lid on each panel.
   B. Insert panels into any available open slot in the WalkAway System. Refer to the WalkAway System Operator’s Manual for use.
   C. Panels are incubated for 2 hours prior to final reading.

V. Reading the Panels
   Readings of the identification substrates are complete after 2 hours incubation at 35 °C. Changes in intensity of fluorescence are used as an indication of positivity or negativity of identification tests.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS

I. Principles of Reactions

A. **Urea (URE)** - The enzyme urease splits urea forming ammonia and CO_2. The resultant increase in pH is detected by a fluorescent pH indicator.
B. **Fluorogenic tests (AGAL, AGL, BDFU, BGAL, BGL, BGLR, CELB, CHB, NAG, PHO1, PHO2, AARG, ARG, CIT, GLPR, GLY, PRO, PYG, TRYP, TYR)** - If the appropriate enzyme is present, the substrate conjugate complex is cleaved releasing either 4-methylumbelliferone or 7-amino-4-methylcoumarin resulting in an increase in fluorescence.
C. **Carbohydrate Fermentation (CEL, FRU, GLU, GLYC, LAC, MAL, MAN, MEL, MNS, RAF, SAL, SOR, SUC, TRE)** - The fermentation of a specific carbohydrate results in acid formation and pH drop which is detected by a fluorescent pH indicator.
D. **Protein/Yeast Extract (PYET) -** Protein utilization results in a breakdown of amino acids and the formation of basic amines which increases the pH and is detected by a fluorometric indicator**.**
E. **Fluorogenic rate tests (AAR2, ARG2, BGR2, CIT2, CIT3, MAL2, PH1A, PH2A, PYG2, TRP2) -** Multistate tests apply a second breakpoint to create a unique test.

II. Organism Identification

The MicroScan Data Management System and WalkAway System software include probability tables used in the identification of an unknown organism. The identification substrates are read by the WalkAway System. The results are converted to a 14 digit biotype number and compared to the database generated by MicroScan from clinical and stock isolates of gram-negative bacilli. Up to five possible identifications are listed in order of the highest probability up to a cumulative total of 99.9%. Should a biotype number occur that results in a "Very Rare Biotype", consult the LabPro Software (Utilities>System>Biotype Lookup), the Biotype Lookup Program on the Beckman Coulter website, or Beckman Coulter Representative or Distributor.

III. Limitations

A. Specimens from Additional tests may be necessary to determine the final identification when a low probability (<85%) identification is obtained.
B. Biotype numbers should not be used for phenotype identification of strains isolated from various the same patient.
C. The interpretation of test results require trained clinical personnel who should use judgment, knowledge and additional confirmatory tests where required prior to accepting the identification of an organism.

IV. Performance Characteristics

Performance claims for the MicroScan Rapid Pos ID Type 2 panels were established in a multicenter study. Clinical isolates and stock strains were tested on panels with the Rapid Pos. ID Type 2 database and conventional tube methodologies to represent the type of bacterial population expected in a routine clinical laboratory. The Rapid Pos. ID Type 2 results were in

agreement to species level with 97.4% of the strains tested. Refer to the MicroScan System Microbiologics Information Manual for a complete list of the organisms included in the database.

REFERENCES

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2. Bradbury, J.M. 1977. Rapid biochemical tests for characterization of the Mycoplasmatale. J. Clin. Microbiol. 5:531.
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4. Little, K.J., and P.A. Hartman. 1983. Fluorogenic selective and differential medium for isolation of fecal streptococci. Appl. Environ. Microbiol. 45:622.
5. Slifkin, M., and G.M. Gill. 1983. Rapid biochemical tests for the identification of groups A, B, C, F and G streptococci from throat cultures. J. Clin. Microbiol. 18:29.
6. Smith, R.E., E.R. Bissel, A.R. Mitchell, and K.W. Pearson. 1980. Direct photometric or fluorometric assay of proteinases using substrates containing 7- amino-4-trifluoromethylcoumarin. Thrombosis Research.17:393.
7. Watson, R.R. 1976. Substrate specificities of amino\peptidases: a specific method for microbial differentiation. Methods Microbiol. 9:1.
8. Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken, (eds), 2003. Manual of Clinical Microbiology, 8th ed. American Society for Microbiology, Washington D.C.
9. Quality Control for Commercial Microbial Identification Systems. 2008. CLSI Document M50-A. Clinical and Laboratory Standards Institute CLSI, Wayne, PA.

DEFINITIONS: **N/A**

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES: **N/A**

EFFECTIVE: December 2019

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Bacteriology**

SUB SECTION: **Procedures**

SUBJECT: **MicroScan Rapid Neg ID Type 4**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

MicroScan Rapid Neg ID Type 4

TEST PRINCIPLE / PURPOSE

For use with MicroScan Rapid Neg ID Type 4 Panels. MicroScan Rapid Panels (hereafter referred to as Rapid Panels) are designed for use in determining identification to the species level of rapidly growing aerobic and facultatively anaerobic gram-negative bacilli (*Enterobacteriaceae*, glucose non-fermenters, and non-*Enterobacteriaceae* glucose fermenters).

Fluorogenic substrates or fluorometric indicators are used for the identification of fermentative and non-fermentative gram-negative bacilli. Identification is based on hydrolysis of fluorogenic substrates, pH changes following substrate utilization, production of specific metabolic byproducts or the rate of production of specific metabolic byproducts after 2.5 hours incubation at 35°C in the WalkAway System.

Precautions

I. For in vitro diagnostic use only.

II. Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures, with special awareness that the inoculated panels contain potentially pathogenic organisms.

III. Product Deterioration: Exposure to storage conditions other than those recommended may result in loss of potency of the antimicrobial agents. Do not use beyond the expiration date. All wells on the panel, except the locator well, should appear clear prior to inoculation.

CLINICAL SIGNIFICANCE

Rapid and accurate identification of bacteria facilitate prompt and appropriate treatment of infections.

SPECIMEN TYPE(S)

Gram-negative organisms from a single isolate or pure culture must be isolated on McConkey agar and incubated 18 – 24 hours in Non-CO_2 at 35ºC. Organisms that don't grow on McConkey shoul be isolated to blood agar.

REQUIRED REAGENT(S)

I. Materials Provided

A. Rapid Neg ID Type 4
B. Procedural and QC Manual

II. Materials required but not provided

A. 0.4% Saline with PLURONIC®*, 6.5 mL
B. Inoculum Water with PLURONIC-D 25 mL
C. RENOK Rehydrator/Inoculator or equivalent
D. Inoculator-R
E. WalkAway *SI* System or upgrade
F. WalkAway System Tray Lids
G. WalkAway System Bar Code Label Paper
H. WalkAway System Bar Code Printer
I. Turbidity Meter
J. Quality Control Organisms (Refer to QC section of this manual)
K. TSA with 5% Sheep's Blood Agar Plate
L. 0.5 McFarland Barium Sulfate Standard
M. General Laboratory Equipment

CALIBRATION **N/A**

QUALITY CONTROL

The acceptability of the identification substrates should be checked by testing organisms with known reactions. The results for each reaction for the recommended American Type Culture Collection (ATCC[1]) control organisms are listed in the following tables.

Run identification quality control using the organisms listed in Table 2, Rapid Gram Negative Streamlined ID QC, and each week of use. (Refer to criteria for once-a-week QC susceptibility testing in Microscan panels validation study binder).

Table 1. Rapid Gram-Negative ID Type 4 Substrates Quality Control Table

	***E. coli* ATCC[1] 25922**	***P. aeruginosa* ATCC 27853**	***K. oxytoca* ATCC 49131**	***S. putrefaciens* ATCC 49138**	***E. faecalis* ATCC 29212**	***P. vulgaris* ATCC 49132**	***E. aerogenes* ATCC 51697**	***E. cloacae* ATCC 49141**
AARG	N/A	N/A	N/A	+	-[2]	N/A	N/A	N/A
AGAL	N/A	-	+	N/A	N/A	N/A	N/A	N/A
AGL	N/A	-	+	N/A	N/A	N/A	N/A	N/A
ALAR	N/A	-	N/A	N/A	N/A	N/A	N/A	+
ARG	N/A	N/A	-	+	N/A	N/A	N/A	N/A
BGAL	N/A	-	+	N/A	N/A	N/A	N/A	N/A
BGL	N/A	-	+	N/A	N/A	N/A	N/A	N/A
BGLR	+	-	N/A	N/A	N/A	N/A	N/A	N/A
CHB	N/A	-	N/A	N/A	+	N/A	N/A	N/A
DCB	N/A	+	N/A	N/A	-	N/A	N/A	N/A
DGA	N/A	-	+	N/A	N/A	N/A	N/A	N/A
FRU	N/A	N/A	+	N/A	N/A	-	N/A	N/A
GAL	N/A	-	N/A	N/A	N/A	N/A	+	N/A
GGA	-	N/A	N/A	+	N/A	N/A	N/A	N/A
GGLT	-	N/A	N/A	+	N/A	N/A	N/A	N/A
GLPR	N/A	N/A	-	+	N/A	N/A	N/A	N/A
GLU	N/A	N/A	+	-	N/A	N/A	N/A	N/A
GLY	N/A	N/A	N/A	+	-	N/A	N/A	N/A
GLYC	N/A	-	N/A	N/A	+	N/A	N/A	N/A
KETO	N/A	-	+	N/A	N/A	N/A	N/A	N/A
LAC	+	-	N/A	N/A	N/A	N/A	N/A	N/A
LYS	N/A	N/A	+	-	N/A	N/A	N/A	N/A
MAN	N/A	N/A	+	N/A	N/A	-	N/A	N/A
NAG	N/A	-	N/A	+	N/A	N/A	N/A	N/A
NGAL	N/A	-	N/A	+	N/A	N/A	N/A	N/A
ORN	+	N/A	N/A	N/A	N/A	-	N/A	N/A
PHO1	N/A	N/A	+	N/A	-	N/A	N/A	N/A
PHO2	N/A	N/A	N/A	+	-	N/A	N/A	N/A
PRO	N/A	N/A	N/A	+	-	N/A	N/A	N/A
PYG	N/A	N/A	N/A	+	N/A	-	N/A	N/A
SOR	N/A	-	+	N/A	N/A	N/A	N/A	N/A
SUC	-	N/A	+	N/A	N/A	N/A	N/A	N/A
TRE	N/A	-	+	N/A	N/A	N/A	N/A	N/A

	***E. coli* ATCC[1] 25922**	***P. aeruginosa* ATCC 27853**	***K. oxytoca* ATCC 49131**	***S. putrefaciens* ATCC 49138**	***E. faecalis* ATCC 29212**	***P. vulgaris* ATCC 49132**	***E. aerogenes* ATCC 51697**	***E. cloacae* ATCC 49141**
TRYP	N/A	N/A	-	+	N/A	N/A	N/A	N/A
TYR	N/A	-	N/A	+	N/A	N/A	N/A	N/A
URE	N/A	N/A	N/A	-	N/A	+	N/A	N/A

1.American Type Culture Collection, Manassas, VA USA

2. Retesting may require non-CO_2 incubation.

N/A = Not Applicable

NOTE: Quality Control organisms are selected to provide positive/negative reactions for all identification substrates. As a result, the organism identifications may vary from that stated in the QC chart. The acceptability of product performance should be determined by comparison of test results, not identification.

Table 2. Synergies plus Gram Neg Identification - Streamlined ID QC*

QC Organism	ATCC Number	Key Indicator Substrate	Expected Result	Out-of-Specification Result	Labile Substrate	Technique Indicator
E. faecalis	29212	PHO2	-	+	Yes	N/A
E. aerogenes	51697	GAL	+	-	N/A	Yes
P. aeruginosa	27853	DCB	+	-	N/A	Yes
P. aeruginosa	27853	GLYC	-	+	N/A	Yes

* Quality Control for Commercial Microbial Identification Systems. 2008. CLSI Document M50-A. Clinical and Laboratory Standards Institute CLSI, Wayne, PA

PROCEDURAL STEPS

I. Panel Preparation
   A. Remove the panels to be used from storage. Panels should not be used if the desiccant is not present or is broken or if the integrity of the packaging is compromised (unsealed, punctured, or torn).

II. Inoculum Preparation
   A. Using a sterile wooden applicator stick or bacteriological loop, touch the surface of 4-5 large or 5-10 small morphologically similar, well-isolated colonies from an 18-24 hour TSA with 5% Sheep's Blood agar plate.
   B. Disperse in 6.5 mL of 0.4% saline with PLURONIC. To insure an even distribution of the bacterial cells in the saline PLURONIC, rub the stick or loop on the bottom or side of the tube. This will reduce “clumping” and will aid in dispersion of mucoid growth.
   C. Cap tightly and vortex the suspension for 2-3 seconds.
   D. The final turbidity should be equivalent to that of a 0.5 McFarland Barium Sulfate turbidity standard. Turbidity should be confirmed with a turbidity meter. A MicroScan Turbidity Meter range of 0.08 ± 0.02 (0.06 - 0.10) provides optimal performance on Rapid Gram-Negative Panels.

III. Panel Rehydration/Inoculation

A. Rehydration and inoculation is performed using the MicroScan RENOK Rehydrator/Inoculator system with Inoculator-R.
   1. Remove the transfer lid from the inoculator set. Pour the 6.5 mL standardized saline suspension into the bottom portion (3 rows) of the divided seed trough. Pour 25 mL of uninoculated Inoculum Water with PLURONIC-D into the top portion (5 rows) of the seed trough.
   2. Replace the transfer lid and attach the RENOK Rehydrator/Inoculator. Refer to the RENOK Rehydrator/Inoculator Operator's Manual for use.

B. If an alternative system is used, rehydrate each well with 115µL ± 10µL of the appropriate suspension.
   NOTE: The final organism concentration in the identification wells must be 1.5 X 10^8/mL. To ensure viability and purity of the organism tested, a purity plate may be prepared by streaking the inoculum to a blood agar plate and incubating for 16-20 hours. If two or more colony types are present on the purity plate, re-isolate the colonies and retest.

IV. Incubation

A. Label each panel with a WalkAway System barcode label and place a WalkAway System tray lid on each panel.
B. Insert panels into any available open slot in the WalkAway System. Refer to the WalkAway System Operator's Manual for use.
C. Panels are incubated for 2.5 hours prior to final reading.

V. Reading the Panels

Readings of the identification substrates are complete after 2.5 hours. Refer to the WalkAway System Operator's Manual for use.
Changes in intensity of fluorescence are used as an indication of positivity or negativity of identification tests.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS

I. Principles of Reactions

A. **Urea (URE)** - The enzyme urease splits urea forming ammonia and CO_2. The resultant increase in pH is detected by a fluorescent pH indicator.
B. **Fluorogenic tests (AGAL, AGL, ALAR, BGAL, BGL, BGLR, CHB, NAG, NGAL, AARG, ARG, GGA, GGLT, GLPR, GLY, PRO, PYG, TRYP, TYR, PHO1, PHO2)** - If the appropriate enzyme is present, the substrate conjugate complex is cleaved releasing either 4-methylumbelliferone or 7-amino-4-methylcoumarin resulting in an increase in fluorescence.
C. **Carbohydrate Fermentation (DGA, FRU, GAL, GLU, GLYC, KETO, LAC, MAN, SOR, SUC, TRE)** - The fermentation of a specific carbohydrate results in acid formation and pH drop which is detected by a fluorescent pH indicator.

D. **Decarboxylases (DCB, LYS, ORN)** - Decarboxylation of these amino acids results in the formation of basic amines which increase the pH and are detected by a fluorescent pH indicator. The base medium (DCB) is used as a control and a unique test.
E. **Multistate tests (AAR2, ARG2, GLY2, ONB, ORN2, TYR2)** - Multistate tests apply a second breakpoint to create a unique test result.

II. Organism Identification

The MicroScan Data Management System and WalkAway System software include probability tables used in the identification of an unknown organism. The identification substrates are read by the WalkAway System. The results are converted to a 15 digit biotype number and compared to the database generated by MicroScan from clinical and stock isolates of gram-negative bacilli. Up to five possible identifications are listed in order of the highest probability up to a cumulative total of 99.9%.

III. Limitations

A. specimens from Additional tests may be necessary to determine the final identification when a low probability (<85%) identification is obtained.
B. Biotype numbers should not be used for phenotype identification of strains isolated from various the same patient.
C. Identification may be important for the application of some MIC determinations (e.g. interpretative categories or breakpoints, organism specific limitations, etc.) and additional testing may be required if identification results are questionable.
D. The interpretation of test results require trained clinical personnel who should use judgment, knowledge and additional confirmatory tests where required prior to accepting the identification of an organism.
E. A positive reaction may occur when testing *E. faecalis*, ATCC 29212, with AARG if incubated in a CO_2 environment.

IV. Expected Values

For expected values, please refer to the MicroScan Percent Charts for glucose fermenters and glucose non-fermenters located in the Microbiologics Information Manual.

V. Performance Characteristics

Performance claims for the MicroScan Rapid Neg ID Type 4 panels were established in a multicenter study. Clinical isolates and stock strains were tested on panels with the Rapid Neg ID Type 4 database and conventional tube methodologies to represent the type of bacterial population expected in a routine clinical laboratory. The Rapid Neg ID Type 4 results were in agreement to species level with 96.9% of the strains tested. Refer to the MicroScan System Microbiologics Information Manual for a complete list of the organisms included in the database.

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DEFINITIONS: **N/A**

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES: **N/A**

EFFECTIVE: December 2019

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Microbiology**

SUB SECTION: **Procedure**

SUBJECT: **LabPro-MBT**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

LabPro-MBT

TEST PRINCIPLE / PURPOSE

LabPro-MBT software is a software that connects LabPro software to Bruker MicroFlex™ MALDI TOF instruments and MALDI Biotyper software (Bruker MBT system). This enables you to assign test groups to wells on MALDI targets, and transmit the assignments to the Bruker MBT system.

The Bruker MBT system identifies microorganisms using Matrix Assisted Laser Desorption Ionization-Time of Flight mass spectrometry. MALDI Biotyper software compares results with organism protein patterns using an extensive organism database to identify microorganisms.

Organism identification results are transmitted from the MALDI Biotyper software to LabPro-MBT software. After you review and save results, LabPro software can transmit MBT results to an LIS, or send related MBT results and LabPro panel MIC results to an LIS.

CLINICAL SIGNIFICANCE

N/A

SPECIMEN TYPE(S)

N/A

REQUIRED REAGENT(S)

N/A

CALIBRATION

N/A

QUALITY CONTROL

N/A

PROCEDURAL STEPS

I. MBT Monitor Window

MBT Monitor is the primary interface for LabPro-MBT software. Use this window for all MBTID test group assignments, results processing functions, and data transmission interactions with the Bruker MBT system. The MBT Monitor window automatically displays each time you start up LabPro software. You can also access the MBT Monitor window from the LabPro Command Center.

Use the MBT Monitor window to assign MBTID test groups to positions on MALDI targets, transmit the assignments, and to process organism identification results received from the MALDI Biotyper software.

II. Targets Tab

Use the **Targets** tab in the **MBT Monitor** window to add, edit, or delete MALDI targets at any time depending on your laboratory workflow. After you add MALDI targets, you can select the appropriate MALDI target for well assignment. You can delete processed targets only after you resolve identification exceptions designated with red circles.

III. Assignment (Target view) tab

The Assignments (Target View) tab is the primary tool for assigning MBTID test groups to wells on a MALDI target. This tab provides informational displays, and tools you use to modify and manage assignments.

Use this tab to assign, unassign, or reassign MBTID test groups to wells on the MALDI target. You can also edit, reorder, clear, and view assignments, or create a pending assignment.

IV. Assignments (Table View) tab

After you assign test groups in the Assignments (Target View) tab, you can click the Assignments (Table View) tab to display a list of details about each assignment and the data associated with the wells on the MALDI target. Note that the Assignments (Target View) tab presents a pictorial display of the wells and assignments using status colors.

You can customize the columns of data and print the list as a Target report. You can use this printed report as a guide when you inoculate the wells.

V. Assignment modes

The **MBT Monitor** window supports single or double well assignment modes. The selected assignment mode is associated to all subsequent well assignments until you select a different mode. You can assign one MBTID test group to one or two wells using one of the following assignment modes:

A. **Single Down.** Assigns one MBTID test group to one well in a vertical (top-to-bottom) pattern.
B. **Single Across.** Assigns one MBTID test group to one well in a horizontal (left-to-right) pattern.

VI. Pending assignments

MBTID test group orders can be transmitted from the LIS dynamically (as the orders are entered) or accumulated and transmitted to the MBT Monitor in batches.
You can add pending assignments to wells as placeholders for a specific specimen number and isolate when:

A. You want to inoculate the MALDI target at the same time you are placing the order in the LIS.
B. The transmission of orders from the LIS is delayed or scheduled for a later time.
C. Your specific workflow requires you to assign all MALDI target before all MBTID test group orders are received from the LIS.

Use the **Well Details** box in the **Assignments (Target View)** tab to define and add new pending assignments for the current MALDI target.

VII. Results tab

Use this tab to view classification results. The Results list displays the following details:

A. State (well state)
B. Specimen
C. Isolate
D. Organism (identified by the Bruker MBT system)
E. Well status (text description of the well state)
F. Exception
G. Target Description (name of the target used in the classification run)

VIII. Communication Log tab

Click this tab to view the communication history between the Bruker MBT system and the MBT Monitor. This log also displays communication between the MBT Monitor and any external devices.

IX. Communication Log tab

Click this tab to view the details of any errors that the MBT Monitor records during target processing, or while attempting to process external test group order assignments.

X. Legend tab

Click this tab to view descriptions of the well states color indicators, results interpretation, device, and communication status indicators.

XI. Ordering MBTID test groups

The MBTID test group is included in the **LabPro Test Groups** table so you can order MBTID test groups the same way you order other MicroScan test groups. Typically, MicroScan test groups and MBTID test groups are entered in the Laboratory Information System (LIS) and then

transmitted to the LabPro database computer. However, you can use the **Patient Order Entry** window in LabPro software to create patient orders with modified accession numbers.

MBTID test groups are processed in the **MBT Monitor** window. Other MicroScan test groups are processed using LabPro and a MicroScan instrument.

A. You can order one ID and MIC only panel per isolate. You can have multiple isolates and each can have MBTID and MIC only panel.
B. You cannot order an MBTID test group and a MicroScan ID or Combo panel **for the same specimen and isolate**. However, you can order a MBTID test group for isolate 1 and an ID or Combo test group for isolate 1A from the same specimen.

CALCULATIONS

REFERENCE INTERVALS

INTERPRETATION OF RESULTS

REFERENCES:

DEFINITIONS:

ATTACHMENTS:

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES:

EFFECTIVE: *original date when p/p written and placed in use*

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Microbiology**

SUB SECTION: **Procedure**

SUBJECT: **RAPIDEC® CARBA NP**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

RAPIDEC® CARBA NP

TEST PRINCIPLE / PURPOSE

The RAPIDEC® CARBA NP is a phenotypic (colorimetric) *in vitro* diagnostic test for the qualitative detection of carbapenemase enzymes in *Enterobacteriaceae* and *Pseudomonas aeruginosa* that have elevated MICs to any carbapenem.

The RAPIDEC® CARBA NP test is based on the detection of imipenem hydrolysis by carbapenemase-producing bacteria. Hydrolysis acidifies the medium which results in a color change of the pH indicator. After bacterial lysis, which enables the extraction of the enzyme, the lysate is added to a detection solution containing a carbapenem (imipenem), phenol red (pH indicator), and zinc (required for the detection of metallo-dependent carbapenemase-producing strains). After incubating for a maximum of 2 hours, a visual reading is performed by comparing a control well without imipenem to a reaction well containing imipenem.
RAPIDEC® CARBA NP is not intended to guide or monitor the treatment for these bacterial infections. A negative result does not preclude the presence of carbapenemase enzymes.

CLINICAL SIGNIFICANCE

Carbapenemases are β-lactamases that are capable of inactivating most existing β-lactams. Their spread among Gram-negative bacilli is a major public health concern due to the extensive resistance they confer and their rapid global dissemination. The acquisition of a carbapenemase-producing strain by a patient represents a mortality risk factor. For these reasons, their rapid detection is of utmost importance for the determination of appropriate schemes for reducing the risk of primary and secondary infection, and the implementation of infection control measures in intensive care units.

SPECIMEN TYPE(S)

I. Routine subculture/incubation

A. The *Enterobacteriaceae* or *Pseudomonas aeruginosa* with elevated carbapenem MIC to be tested must first be isolated on a non-selective agar culture medium containing sheep blood.

II. Short subculture/incubation

A. In case of insufficient biomass to perform the test, or growth from incompatible media, the following short subculture procedure can be used:
1. Pick up one or several colonies from an 18-24 hour old culture
2. Inoculate and spread over sheep blood agar plate
3. Incubate at 35-37°C for at least 4 hours to obtain a sufficient biomass equivalent to one 10 µL calibrated loop

REQUIRED REAGENT(S)

I. Reagents and Materials Provided

A. Test strips
B. Ampules of API® Suspension Medium, 2 mL
C. Incubation lids
D. 1 sachet of stirring sticks
E. Two-colored (black and white) reading support card
F. Package insert provided in the kit or downloadable from www.biomerieux.com/techlib
G. Reading guide downloadable from www.biomerieux.com/techlib

II. Materials Required But Not Provided

A. Rack for holding the Ampule of API® Suspension Medium
B. Ampule protector
C. General microbiology laboratory equipment
D. Recommended culture media (sheep blood agar)
a. Columbia agar + 5% sheep blood
b. Trypticase soy agar + 5% sheep blood

III. Storage Requirements

A. The strips should be stored at 2-8°C in the dark.
B. After opening the kit, ensure that the ampules are intact. If any ampules are broken, the kit must be disposed of in an appropriate container.

CALIBRATION

N/A

QUALITY CONTROL

I. Quality control is performed on new lot kit in accordance with applicable local, state and/or federal regulations or accreditation requirements and user's laboratory's standard quality control procedures. See Rapid Carba NP Individualized Quality Control Plan.

II. The media, strip ingredients and reagents are systematically quality controlled at various stages of their manufacture. The following positive and negative QC strains will be used:

III. The following bacterial strains should be used for quality control:

A. Positive reaction: *Klebsiella pneumoniae* ATCC® BAA-1705™

B. Negative reaction: *Klebsiella pneumoniae* ATCC® BAA-1706™

PROCEDURES

I. Preparation of the strip

A. Remove the strip from its packaging.
B. Write the specimen reference number on the strip on the plastic extension on the bottom of the strip.

II. Preparation of the test

A. Open an ampule of API® Suspension medium
1. Place the ampule in the ampule protector
2. Hold the protected ampule in one hand in a vertical position (with the white plastic cap at the top)
3. Press the cap down as far as possible
4. Position the thumb on the striated portion of the cap and press forward to snap off the top of the ampule
5. Take the ampule out of the ampule protector
6. Save the protector for future use

B. Dispense 100 µL into each of the wells “a”, “b”, and “c”. NOTE: Do not use saline solution to rehydrate wells “a”, “b” or “c”
C. Place an incubation lid on the strip
D. Leave at room temperature (15-25°C) for 4 -10 minutes
E. Gently mix the contents of well “b” using a stirring stick provided in the kit

III. Preparation of the inoculum and bacterial lysis

A. Place the strip on the two-colored (black and white) reading support card
B. Position wells “b” and “c” on the black background to facilitate comparison of the turbidities
C. With the end of a new stirring stick, pick up several colonies of the same morphology, taking care not to pick up any fragments of the agar
D. Deposit the contents of the stick in well “c” and mix
1. Repeat this step several times until a turbidity equivalent to that of well “b” is obtained so that the bottom of the wells are not visible in the black background
2. The suspension in well must be perfectly homogeneous, with no aggregates

E. Place the lid on the test strip and leave at room temperature (15-25°C) for 30 minutes.

IV. Test Procedure

A. Use a micropipette to transfer 25 µL from well “c” to wells “d” and “e”
B. Use a new tip to transfer 25 µL from well “a” to wells “d” and “e”
C. Place the incubation lid on and incubate the strip for 30 minutes at 33- 38°C.

V. Reading and Interpretation

A. Place the strip on the two-colored (black and white) support. Position wells “d” and “e” on the white background to facilitate reading.
B. Remove the incubation lid
C. Reading is performed by comparing the colors in wells “d” and “e”, ensuring that the strip is firmly flattened against the two-colored (black and white) support
D. Perform the initial reading
1. Positive tests results are frequently obtained at 30 minutes of incubation
2. After 30 minutes, there is a risk that a positive result may change back to a negative. Therefore, it is imperative to perform the initial reading at 30 minutes of incubation

VI. In case of a negative or doubtful reaction, re-incubate the strip at 33- 38°C for an additional 1 hour and 30 minutes and perform a final reading

VII. The total test incubation time must not exceed 2 hours. A test is positive when a significant variation in color is observed between the two wells

VIII. Results

A. Refer to the chart below and the reading guide downloadable from www.biomerieux.com/techlib.

Control well “d”	Test well “e”	Interpretation
red	red	Negative (absence of carbapenemase)
orange	orange	
red	yellow, light orange, orange, dark orange	Positive (presence of carbapenemase)
orange	yellow	
any color other than red or orange	Not applicable	Uninterpretable
orange	red	

IX. Reporting Results

Result	Organism	Mnemonics
Positive	Carbapenemase Producing Entrobacteriaceae	CPCRE
Positive	Carbapenemase Producing *P. aeruginosa*	CPPSA
Negative	Non Carbapenemase Producing Carbapenem Resistant Entrobacteriaceae	NCPCRE
Negative	Non Carbapenemase Producing Carbapenem Resistant *P. aeruginosa*[1]	NCPPSA

[1] *P. aeruginosa* has intrinsic resistance to Ertapenem

X. Limitations of the assay

A. The performance of the RAPIDEC® CARBA NP assay for detection of carbapenemases enzymes encoded by genetic markers other than KPC, NDM, OXA-48, VIM and/or IMP has not been established. In addition, RAPIDEC® CARBA NP results may be influenced by the local epidemiology regarding genetic markers of resistance, i.e., depending on the local distribution/prevalence of different carbapenemase genetic markers, and more false negative results may occur. Conduct alternative testing if negative results are obtained and carbapenemase enzyme production is suspected based on local epidemiology.
B. RAPIDEC® CARBA NP testing should be used as an adjunct to other laboratory test(s) such as antimicrobial susceptibility testing.
C. The performance of the RAPIDEC® CARBA NP test with bacteria other than *Enterobacteriaceae* and *Pseudomonas aeruginosa* has not been evaluated. Organism identification and elevated carbapenem MICs should be determined prior to testing on the RAPIDEC® CARBA NP.
D. *Proteus species, Providencia species, Morganella species* may have elevated imipenem MICs due to intrinsic resistance mechanisms. *Pseudomonas aeruginosa* has been shown to exhibit resistance to ertapenem due to intrinsic resistance mechanisms.
E. The detection of OXA variants other than OXA-48 has not been evaluated sufficiently in the study.
F. Hypermucoid colonies may lead to false positive or false negative results and should not be tested by the RAPIDEC® CARBA NP test. A hypermucoid colony tends to stretch itself to form a continuous viscous filament > 5 mm in length when picked up from an agar plate using a bacteriology loop/needle (6).
G. Agar media containing pH indicator for colony color differentiation (e.g., Bromocresol Purple, MacConkey, Cysteine Lactose Electrolyte-Deficient, etc.) are not compatible with the RAPIDEC® CARBA NP and require subculturing growth/biomass on a sheep blood agar for testing.
H. The performance of RAPIDEC® CARBA NP has been evaluated for subculturing growth on 5% sheep blood agar incubated for 18- 24 hours (Routine procedure) and 4-5 hours (Short Incubation procedure) only. The performance with other culture media has not been evaluated and is therefore unknown.
I. The performance of the RAPIDEC® CARBA NP test when testing *Enterobacteriaceae* and *Pseudomonas aeruginosa* containing OXA-181, OXA- 232, SME, GIM, SPM, and IMI carbapenemase enzymes has not been established due to the low number of positive isolates available using the Composite Reference Method.

CALCULATIONS

N/A

REFERENCE INTERVALS

N/A

INTERPRETATION OF RESULTS

N/A

REFERENCES: RAPIDEC® CARBA NP [Package Insert]. Durham, NC: bioMérieux; 2017.

DEFINITIONS: N/A

ATTACHMENTS: N/A

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES: **N/A**

EFFECTIVE: _____

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Microbiology**

SUB SECTION: **Procedure**

SUBJECT: **BioFire® FilmArray® Respiratory Panel 2 (RP2) Testing**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

BioFire® FilmArray® Respiratory Panel 2 (RP2) Testing

TEST PRINCIPLE / PURPOSE

This procedure provides instructions for testing nasopharyngeal swabs (NPS) collected in transport media, using the FilmArray® Respiratory Panel 2 (RP2) Kit.

The FilmArray RP2 pouch is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple respiratory pathogens within a single NPS specimen. After sample collection, the user injects hydration solution and sample combined with sample buffer into the pouch, places the pouch into a FilmArray instrument, and starts a run. The entire run process takes about 45 minutes. Additional detail can be found in the appropriate FilmArray Operator's Manual.

During a run, the FilmArray® system:

I. Lyses the sample by agitation (bead beading).

II. Extracts and purifies all nucleic acids from the sample using magnetic bead technology.

III. Performs nested multiplex PCR by:

   A. First performing reverse transcription and a single, large volume, massively-multiplexed reaction (PCR1)

   B. Then performing multiple singleplex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products

IV. Uses endpoint melting curve data to detect and generate a result for each target on the FilmArray RP2 array.

CLINICAL SIGNIFICANCE

The FilmArray® Respiratory Panel 2 (RP2) is a multiplexed nucleic acid test intended for use with FilmArray® 2.0 or FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the FilmArray RP2:

1. Adenovirus
2. Coronavirus 229E
3. Coronavirus HKU1
4. Coronavirus NL63
5. Coronavirus OC43
6. Human Metapneumovirus
7. Human Rhinovirus/Enterovirus
8. Influenza A,
9. Influenza A/H1
10. Influenza A/H3
11. Influenza A/H1-2009
12. Influenza B
13. Parainfluenza Virus 1
14. Parainfluenza Virus 2
15. Parainfluenza Virus 3
16. Parainfluenza Virus 4
17. Respiratory Syncytial Virus
18. *Bordetella parapertussis* (IS*1001*)
19. *Bordetella pertussis* (*ptxP*)
20. *Chlamydia pneumoniae*
21. *Mycoplasma pneumoniae*

SPECIMEN TYPE(S)

The following table describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

Specimen Type	**Nasopharyngeal Swab** (NPS) collected according to standard technique and immediately placed in 1-3 mL of transport media
Minimum Sample Volume	0.3 mL (300 µL)
Transport and Storage	Specimens should be processed and tested with the FilmArray RP2 as soon as possible. If storage is required, specimens can be held: • At room temperature for up to 4 hours (15-25 °C) • Refrigerated for up to 3 days (2-8 °C) • Frozen (≤-15 °C or ≤-70°C) (for up to 30 days)

NOTE: NPS specimens should not be centrifuged before testing.

NOTE: Bleach can damage organisms/nucleic acids within the specimen, potentially causing false negative results. Contact between bleach and specimens during collection, disinfection, and testing procedures should be avoided.

REQUIRED REAGENT(S)

Materials Provided	Materials Required But Not Provided
Each kit contains sufficient reagents to test 30 or 6 specimens: 1. Individually-packaged FilmArray RP2 pouches 2. Single-use (1.0 mL) Sample Buffer ampoules 3. Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue) 4. Single-use Sample Injection Vials (red) 5. Individually-packaged Transfer Pipettes	FilmArray system including: 1. FilmArray®2.0 or FilmArray® Torch and accompanying software 2. FilmArray® Pouch Loading Station' 3. 10% bleach solution or a similar disinfectant

List of Acceptable Specimen Collection Swabs and Transport Media

Specimen Collection Materials		
Rayon Swabs (Copan 168C)	N/A	No Interference
Nylon Flocked Swabs (Copan 553C)	N/A	No Interference
Polyester Swabs (Copan 175KS01)	N/A	No Interference
Calcium Alginate Swabs (Puritan 25-801 A 50)	N/A	No Interference
M4® Transport Medium (Remel)	100%	No Interference
M4-RT® Transport Medium (Remel)	100%	No Interference
M5® Transport Medium (Remel)	100%	No Interference
M6™ Transport Medium (Remel)	100%	No Interference

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REF RFIT-ASY-0129
RFIT-ASY-0130

Substance Tested	Concentration Tested	Result
Universal Viral Transport vial (BD)	100%	No Interference
Sigma-Virocult™ Viral Collection and Transport System (Swab and Transport Medium)	100%	No Interference
Copan ESwab™ Sample Collection and Delivery System (Swab and Liquid Amies Medium)	100%	No Interference

[a] Nasal influenza vaccines (e.g. FluMist) were not evaluated, but are predicted to be reactive with the FilmArray RP2 Influenza A (subtype) and Influenza B assays.

[b] Not Detected results were reported for several analytes after incubation of the sample with 2% bleach for 10 minutes or overnight. It was concluded that interference resulted primarily from damage to the organisms/nucleic acids in the sample, rather than inhibition or interference with pouch function(s).

CALIBRATION N/A

QUALITY CONTROL

I. **Process Controls**
Two process controls are included in each pouch:

A. RNA Process Control

The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the FilmArray RP2 pouch were successful.

B. PCR2 Control

The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that PCR2 was successful. Both control assays must be positive for the test run to pass. If the controls fail, the sample should be retested using a new pouch.

II. **Monitoring Test System Performance**

The FilmArray software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable range (80.3-84.3°C for the RNA Process Control and 73.8-77.8°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices. Refer to the appropriate FilmArray operator's manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in several FilmArray pouch types (e.g., RP, BCID, GI, ME, and RP2) and can therefore be used to monitor the system when multiple pouch types are used on the same FilmArray system or instrument.

III. **External Controls**

External positive and negative controls are performed on New Lot # pouches and new shipments before they are placed for patient sample testing, or monthly, based on whichever comes first. ZeptoMetrix NATRPC2-BIO kit is used as an external quality control material for the RP2 pouches. Alternatively, transport media can be used as an external negative control. Previously characterized positive samples or negative samples spiked with well characterized organisms can be used as external positive controls. RP2 pouches will be used for patient testing only when positive and negative external controls provide expected results.

PROCEDURAL STEPS

A. Step 1: Prepare Pouch

1. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.

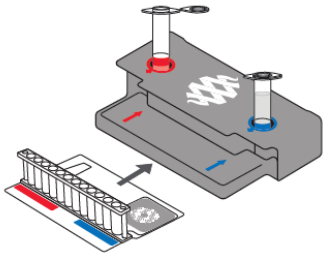
   Note: Mix 390 mL 8.25% bleach in 3450 mL water to make 10% working bleach solution

2. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

   NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

3. Check the expiration date on the pouch. Do not use expired pouches.

4. Insert the pouch into the FilmArray Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the FilmArray Pouch Loading Station.



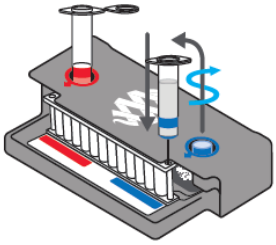
5. Place a red-capped Sample Injection Vial into the red well of the FilmArray Pouch Loading Station.

6. Place a blue-capped Hydration Injection Vial into the blue well of the FilmArray Pouch Loading Station.

B. Step 2: Hydrate Pouch

1. Unscrew the Hydration Injection Vial from the blue cap.

2. Remove the Hydration Injection Vial, leaving the blue cap in the FilmArray Pouch Loading Station.



3. Insert the Hydration Injection Vial's cannula tip into the pouch hydration port located directly below the blue arrow of the FilmArray Pouch Loading Station.

4. Forcefully push down in a firm and quick motion to puncture seal until a faint "pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.

   If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

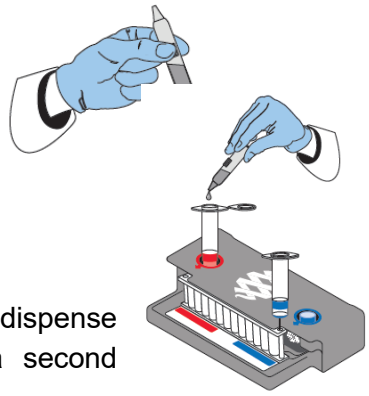
5. Verify that the pouch has been hydrated.

   a. Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.

   b. If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

C. Step 3: Prepare Sample Mix

1. Add Sample Buffer to the Sample Injection Vial.



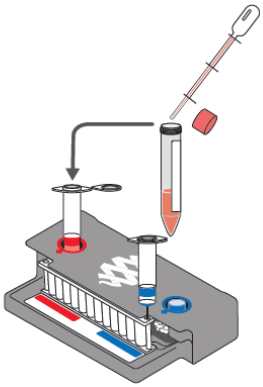
Hold the Sample Buffer ampoule with the tip facing up.

NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.

a. Firmly pinch at textured plastic tab on the side of the ampoule until the seal snaps.

b. Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

NOTE: Avoid squeezing the ampoule additional times. This will generate foaming, which should be avoided.

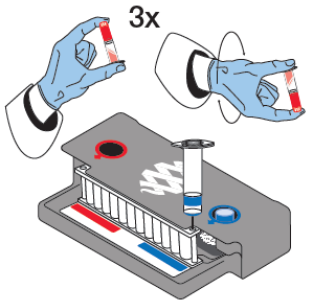


WARNING: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.

2. Thoroughly mix the NPS specimen by vortex or inversion.

3. Use the transfer pipette provided in the test kit to draw specimen to the third line (approximately 0.3 mL) of the transfer pipette.

4. Add the specimen to the Sample Buffer in the Sample Injection Vial.



5. Tightly close the lid of the Sample Injection Vial and discard the transfer pipette in a biohazard waste container.

NOTE: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.

6. Remove the Sample Injection Vial from the FilmArray Pouch Loading Station and invert the vial at least 3 times to mix.

7. Return the Sample Injection Vial to the red well of the FilmArray Pouch Loading Station.

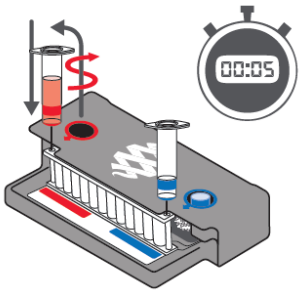
D. Step 4: Load Sample Mix

1. Slowly twist to unscrew the Sample Injection Vial from the red cap and wait for 5 seconds with the vial resting in the cap.

NOTE: Waiting 5 seconds decreases the risk of dripping and contamination from the sample.

2. Lift the Sample Injection Vial, leaving red cap in the well of the FilmArray Pouch Loading Station, and insert the Sample Injection Vial cannula tip into the pouch sample port located directly below the red arrow of the FilmArray Pouch Loading Station.

3. Forcefully push down in a firm and quick motion to puncture seal (a faint "pop" is heard) and sample is pulled into the pouch by vacuum.



4. Verify that the sample has been loaded.

    a. Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port.

    b. If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from *Step 1: Prepare Pouch*.

5. Discard the Sample Injection Vial and the Hydration Injection Vial in appropriate biohazard sharps container.

6. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the FilmArray Pouch Loading Station.

E. Step 5: Run Pouch

The FilmArray® Software includes step-by-step on-screen instructions that guide the operator through performing a run. Brief instructions for FilmArray 2.0 and FilmArray Torch systems are given below. Refer to the appropriate FilmArray Operator's Manual for more detailed instructions.

1. FilmArray 2.0

    a. Ensure that the FilmArray 2.0 system (instrument and computer) is powered on and the software is launched.

    b. Follow on-screen instructions and procedures described in the Operator's Manual to place the pouch in an instrument, enter pouch, sample, and operator information.

    c. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

        NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the FilmArray RP2 pouch.

    d. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

e. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The FilmArray RP2 has a single NPS2 protocol available in the drop down list.

f. Enter a user name and password in the Name and Password fields.

NOTE: The font color of the username is red until the user name is recognized by the software.

g. Review the entered run information on the screen. If correct, select Start Run.

Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.
NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

h. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.

i. The run file is automatically saved in the FilmArray database, and the test report can be viewed, printed, and/or saved as a PDF file.

2. FilmArray Torch

a. Ensure that the FilmArray Torch system is powered on.

b. Select an available Module (instrument) on the touch screen or scan the barcode on the FilmArray pouch using the barcode scanner.

c. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the FilmArray RP2 pouch.

d. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

e. Insert the pouch into the available Module (instrument).

Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module (instrument) will grab onto the pouch and pull it into the chamber.

f. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The FilmArray RP2 has a single NPS2 protocol available in the drop down list.

g. Enter operator user name and password, then select next.

NOTE: The font color of the username is red until the user name is recognized by the software.

h. Review the entered run information on the screen. If correct, select Start Run.

Once the run has started, the screen displays a list of the steps being performed by the Module (instrument) and the number of minutes remaining in the run.

NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

i. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.

The run file is automatically saved in the FilmArray database, and the test report can be viewed, printed, and/or saved as a PDF file.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS

When PCR2 is complete, the FilmArray instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see appropriate FilmArray Operator's Manual). The FilmArray Software then performs several analyses and assigns a final assay result. The steps in the analyses are described below.

Analysis of melt curves. The FilmArray Software evaluates the DNA melt curve for each well of the PCR2 array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve and compares it against the expected Tm range for the assay. If the software determines that the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is not in the appropriate Tm range, the melt curve is called negative.

Analysis of replicates. Once melt curves have been identified, the software evaluates the three re plicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive melt curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

For most organisms detected by the FilmArray RP2, the organism is reported as Detected if a single corresponding assay is positive. For example, Human Metapneumovirus will have a test report result of Human Metapneumovirus Detected if at least two of the three replicates of the one Human Metapneumovirus assay (hMPV) have similar positive melt peaks with Tm values that are within the assay-specific Tm range. The test results for Adenovirus and Influenza A depend on the interpretation of results from more than one assay. Interpretation and actions for these two multi-assay results are provided below.

A. Adenovirus

The FilmArray RP2 pouch contains five different assays (Adeno2, Adeno3, Adeno6, Adeno7.1, and Adeno8) for the detection of Adenovirus. The FilmArray Software interprets each of these assays independently (as described above) and the results are combined as a final test result for the virus. If one or any combination of assays is positive, the test report result will be Adenovirus Detected. If all assays are negative, the test report result will be Adenovirus Not Detected.

B. Influenza A

The assays in the FilmArray RP2 are designed to both detect Influenza A and to differentiate commonly occurring hemagglutinin subtypes. To accomplish this, the FilmArray RP2 uses two Influenza A assays, (FluA-pan-1 and FluA-pan-2) and three subtyping assays directed at the hemagglutinin gene (FluA-H1-2, FluA-H1-2009, and FluA-H3). Each of the individual assays is interpreted independently (as described above) and the test result reported for Influenza A is based on the combined results of the five assays as outlined in Table 1. Retest specimens having Equivocal results or multiple Influenza A subtypes detected.

Table 1. Possible Assay Results for Influenza A and the Corresponding Interpretation

Assay/Result	FluA-pan Assays (n=2)	FluA-H1 – 2	FluA-H1 – 2009	FluA-H3	Action
Influenza A Not Detected	Negative	Negative	Negative	Negative	None
Influenza A H1	**≥ 1 positive**	**Positive**	Negative	Negative	
Influenza A H3	**≥ 1 positive**	Negative	Negative	**Positive**	
Influenza A H1 - 2009	**≥ 1 positive**	Any Result	**Positive**	Negative	
Influenza A H1 Influenza A H3	**≥ 1 positive**	**Positive**	Negative	**Positive**	Multiple infections are possible but rare[a], retest to confirm result[b]
Influenza A H1 - 2009 Influenza A H3	**≥ 1 positive**	Any Result	**Positive**	**Positive**	
Influenza A (No subtype detected)	**2 positive**	Negative	Negative	Negative	Retest (see below)
Influenza A Equivocal	**1 positive**	Negative	Negative	Negative	Retest
Influenza A H1 Equivocal	Negative	**Positive**	Negative	Negative	
Influenza A H3 Equivocal	Negative	Negative	Negative	**Positive**	
Influenza A H1 - 2009 Equivocal	Negative	Any result	**Positive**	Negative	

[a]The FilmArray RP2 can simultaneously detect multiple influenza viruses contained in multivalent vaccines

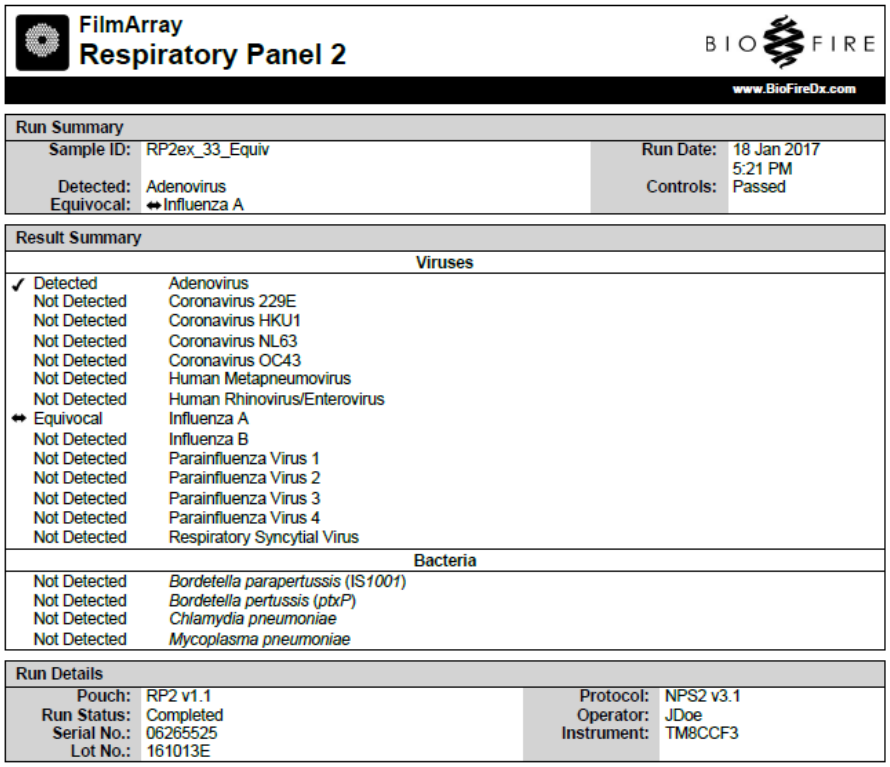
[b] Repeated multiple positives should be further confirmed by other FDA cleared Influenza subtyping tests

C. Influenza A (no subtype detected)

If both of the FluA-pan assays are positive, but none of the hemagglutinin subtyping assays are positive, then the interpretation is Influenza A (no subtype detected). This result could occur when the titer of the virus in the specimen is low and not detected by the subtyping assays. This result could also indicate the presence of a novel Influenza A strain. In both cases, the sample in question should be retested. If the retest provides a different result, test the sample a third time to ensure the accuracy of the result. If the retest provides the same result, then the function of the RP2 pouches should be verified by testing with appropriate external control materials (known positive samples for Influenza A H1, Influenza A H3 and Influenza A H1-2009), and a negative control should also be run to test for PCR-product contamination. If the FilmArray RP2 accurately identifies the external and negative controls, contact the appropriate public health authorities for confirmatory testing.

D. FilmArray RP2 Test Report

The FilmArray RP2 test report is automatically displayed upon completion of a run and can be printed or saved as a PDF file. Each report contains a Run Summary, a Result Summary, and a Run Details section.



FilmArray
Respiratory Panel 2

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Run Summary

Sample ID:	RP2ex_33_Equiv	Run Date:	18 Jan 2017 5:21 PM
Detected:	Adenovirus	Controls:	Passed
Equivocal:	⬌ Influenza A		

Result Summary

	Viruses
✓ Detected	Adenovirus
Not Detected	Coronavirus 229E
Not Detected	Coronavirus HKU1
Not Detected	Coronavirus NL63
Not Detected	Coronavirus OC43
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
⬌ Equivocal	Influenza A
Not Detected	Influenza B
Not Detected	Parainfluenza Virus 1
Not Detected	Parainfluenza Virus 2
Not Detected	Parainfluenza Virus 3
Not Detected	Parainfluenza Virus 4
Not Detected	Respiratory Syncytial Virus
	Bacteria
Not Detected	*Bordetella parapertussis* (IS*1001*)
Not Detected	*Bordetella pertussis* (*ptxP*)
Not Detected	*Chlamydia pneumoniae*
Not Detected	*Mycoplasma pneumoniae*

Run Details

Pouch:	RP2 v1.1	Protocol:	NPS2 v3.1
Run Status:	Completed	Operator:	JDoe
Serial No.:	06265525	Instrument:	TM8CCF3
Lot No.:	161013E		

1. Run Summary

The Run Summary section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the organism assays were negative then ‘None’ will be displayed in the Detected field. Controls are listed as Passed, Failed, or Invalid. Table 2 provides additional information for each of the possible control field results.

Table 2. Interpretation of Controls Field on the FilmArray RP2 Test Report

Control Result	Explanation	Action
Passed	The run was successfully completed AND Both pouch controls were successful	None Report the results provided on the test report
Failed	The run was successfully completed BUT At least one of the pouch controls (RNA Process Control and PCR2 Control) failed	Repeat the test using a new pouch. If the error persists, contact Technical Support for further instruction
Invalid	The controls are invalid because the run did not complete. (Typically this indicates a software or hardware error).	Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the appropriate FilmArray Operator's Manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument.

2. Result Summary

The Result Summary section of the test report lists the result for each target tested by the panel. Possible results for each organism are Detected, Not Detected, or Invalid. Table 3 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 3. Reporting of Results and Required Actions

Result	Explanation	Action
Detected[a]	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay (s) for the organism were POSITIVE (i.e., met the requirements for positive result described in the Assay Interpretation section above)	Report results
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay (s) for the organism were NEGATIVE (i.e., did met the requirements for positive result described in the Assay Interpretation section above)	Report results
Equivocal	The run was successfully completed AND The pouch controls were successful (Passed) AND The combination of positive and negative assay results for Influenza A were inconclusive (see Table 1)	Retest the original specimen using a new pouch and report the results of the retest.
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run status displayed: Aborted, Incomplete, Instrument error or software Error)	See Table 2, Interpretation of Control Field on the FilmArray Test Report for instruction.

[a] If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

3. Run Details

The **Run Details** section provides additional information about the run including: pouch information (type, lot number, and serial number), Run Status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test.

4. Change Summary

It is possible to edit the Sample ID once a run has completed. If this information has been changed, an additional section called **Change Summary** will be added to the test report. This Change Summary section lists the field that was changed, the original entry, the revised entry, the operator that made the change, and the date that the change was made. Sample ID is the only field of the report that can be changed.

Change Summary				
Field	**Changed To**	**Changed From**	**Operator**	**Date**
'Sample ID	RP2ex_36_ChangeID	RP2ex_33_Equiv	**JDoe**	18 Jan 2017

5. Reporting Results

a. Results are entered in MEDITECH under the Laboratory, not Microbiology.
b. Click on Laboratory → Result Entry → Enter results → Enter Bar Code # or MT # → On the result entry table, Enter **P** under Quality Control; under all organisms you will see **NOT DETECTED** as default result. If the test is Negative for all organisms, hit F12 (save) and release result. If the test is positive for any organism (s), carefully select the positive organism (s), and enter **DETECTED**, hit F12, and release result.
c. Call back Flu, RSV or *Bordetella pertussis* positive result with documentation in MEDITECH.
d. Label test report with a label from the lab. Slip, initial, date and file in appropriate results binder.
e. Dispose specimens in Biohazard trash can.

REFERENCES: FilmArray® Respiratory Panel (RP2) CE-IVD Instruction Booklet (RFIT-PRT-0522-01) BioFire Diagnostics, LLC.

DEFINITIONS: N/A

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES: **N/A**

EFFECTIVE: _____

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Microbiology- Molecular Test**

SUB SECTION: **Procedure**

SUBJECT: **Xpert® Xpress SARS-CoV-2 Test**

APPROVED BY: _____

Laboratory Medical Director or Designee

TEST NAME / POLICY

Xpert Xpress SARS-CoV-2 Test

TEST PRINCIPLE / PURPOSE

The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in either nasopharyngeal swab and/or nasal wash/ aspirate specimens collected from individuals suspected of COVID-19 by their healthcare provider.
Testing of nasopharyngeal swab and nasal wash/aspirate specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Dx system is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high and moderate complexity tests.

Testing of nasopharyngeal swab specimens using the Xpert Xpress SARS-CoV-2 test run on the GeneXpert Xpress System is authorized to be distributed and used in patient care settings outside of the clinical laboratory environment.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swab specimens and/or nasal wash/ aspirate specimens during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Xpert Xpress SARS-CoV-2 test is intended for use by trained operators who are proficient in performing tests using GeneXpert Xpress systems. The Xpert Xpress SARS-CoV-2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

CLINICAL SIGNIFICANCE

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.1 Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and exported cases in several Southeast Asian countries and more recently the United States. Cases of severe illness and some deaths have been reported. The International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.

The Xpert Xpress SARS-CoV-2 test is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2 test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in nasopharyngeal swab specimens and/or nasal wash/aspirate specimens.

SPECIMEN TYPE(S)

I. Specimen Types

A. Nasopharyngeal Swab in Viral Transport Medium (3 mL)

B. Nasal wash/aspirate specimens.

II. Specimen Collection, Transport, and Storage

Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section for swab collection procedure. Nasopharyngeal swab specimens can be stored at room temperature (15–30 °C) for up to 8 hours and refrigerated (2–8 °C) up to seven days until testing is performed on the GeneXpert Instrument Systems.

III. Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx. Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into a viral transport tube (3 mL). Break swab at the indicated break line and cap the specimen collection tube tightly.

IV. Nasal Wash/Aspirate Collection Procedure

Using a clean 300 µL transfer pipette (supplied), transfer 600 µL of the sample (two draws, using the same transfer pipette) into the 3 mL Xpert Viral Transport Medium tube and then cap the tube.

REQUIRED REAGENT(S)

The Xpert Xpress SARS-CoV-2 kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following:

A. Xpert Xpress SARS-CoV-2 Cartridges with Integrated Reaction Tubes 10

1. Bead 1, Bead 2, and Bead 3 (freeze-dried) 1 of each per cartridge
2. Lysis Reagent 1.5 mL per cartridge
3. Binding Reagent 1.5 mL per cartridge
4. Elution Reagent 3.0 mL per cartridge

B. Disposable Transfer Pipettes 12 per kit

C. CD 1 per kit

1. Assay Definition File (ADF)
2. Instructions to import ADF into GeneXpert software

D. Storage and Handling

1. Store the Xpert Xpress SARS-CoV-2 cartridges at 2-28°C.
2. Do not open a cartridge lid until you are ready to perform testing.
3. Do not use a cartridge that is wet or has leaked.

CALIBRATION N/A

QUALITY CONTROL

I. Internal Controls

a. Sample Processing Control (SPC)
Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC). Sample Processing Control (SPC) – Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

b. Probe Check Control (PCC)
Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

II. External Controls

a. AccuPlex SARS-CoV-2 reference material kit, Material Number, 0505-0126, from Seracare is used as positive and negative external controls.

b. External Controls are performed once a month or on a new lot number Cartridges before it is placed for patient sample testing.

PROCEDURES

I. Preparing the cartridge

a. Remove a cartridge from the package.

b. Check the specimen transport tube is closed.

c. Mix specimen by rapidly inverting the specimen transport tube 5 times. Open cap on the specimen transport tube.

d. Open the cartridge lid.

e. Remove the transfer pipette from the wrapper.

f. Squeeze the top bulb of the transfer pipette completely and then place the pipette tip in the specimen transport tube (see Figure 2).

Important: Start the test within 30 minutes of adding the sample to the cartridge.

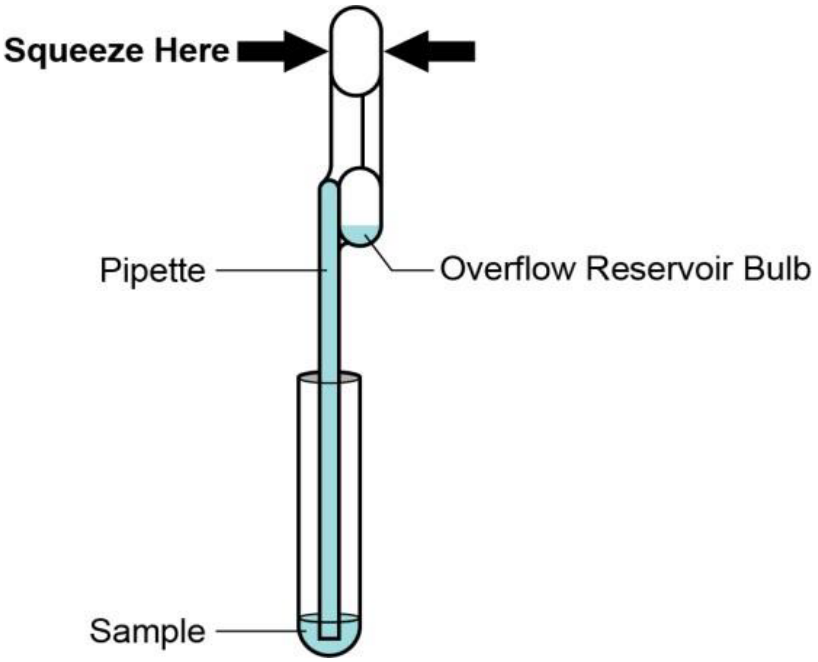


Figure 1. Nasopharyngeal Swab Collection

g. Release the top bulb of the pipette to fill the pipette before removing from the tube. After filling pipette, excess sample will be seen in the overflow reservoir bulb of the pipette (see Figure 2). Check that the pipette does not contain bubbles.

h. To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) in the cartridge shown in Figure 3. Dispose of the used pipette.

Figure 2. Xpert Xpress SARS-CoV-2 Cartridge (Top View)

i. Close the cartridge lid.

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

II. Starting the Test

A. Turn on the GeneXpert Instrument System:
Turn on the instrument and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double - clicking on the GeneXpert Dx shortcut icon on the Windows® desktop. Log on to the System software. The login screen appears. Type the user name and password.

B. Click **Create Test**

C. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test result.

D. Scan the barcode on the Xpert Xpress SARS-CoV-2 cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date and Selected Assay.
Note If the barcode on the Xpert Xpress SARS-CoV-2 cartridge does not scan, then repeat the test with a new cartridge.

E. Click **Start Test**

F. Locate the module with the blinking green light, open the instrument module door and load the cartridge.

G. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.

H. Dispose of used cartridges in red biohazard trash can.

III. Retests

A. Reasons to Repeat the Assay
If any of the test results mentioned below occur, repeat the test once according to instructions in Section 17.2, Retest Procedure.

1. A **PRESUMPTIVE POSITIVE** indicates the 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present.
2. An **INVALID** result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
3. An **ERROR** result could be due to, but not limited to, Probe Check Control failure, system component failure, or the maximum pressure limits were exceeded.

4. A **NO RESULT** indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.

   a. Put on a clean pair of gloves. Obtain a new Xpert Xpress SARS-CoV-2 cartridge and a new transfer pipette.
   b. Check the specimen transport tube or external control tube is closed.
   c. Mix the sample by rapidly invert the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
   d. Open the cartridge lid.
   e. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
   f. Close the cartridge lid.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

IV. Viewing and Printing Results

A. Results print automatically upon completion of the test. If not auto print,
B. Click the **View Results** icon to view results.
C. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file then print report.

V. Reporting Results

A. Results are entered in MEDITECH under the Laboratory, not Microbiology.
B. Click on Lab. → Result Entry → Enter results → Enter Bar Code # or MT # → enter N for Negative, P for Positive result or Presumptive Positive.
C. Call back positive result with documentation in MEDITECH.
D. Label test report with a label from lab. Slip, initial, date and file in appropriate folder.
E. Save both negative and positive Flu specimens in the rack in the freezer
F.

IV. Limitations

A. Performance of the Xpert Xpress SARS-CoV-2 has only been established in nasopharyngeal swab specimens. Specimen types other than nasopharyngeal swab may give inaccurate results.
B. A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.

C. As with any molecular test, mutations within the target regions of Xpert Xpress SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.

D. This test cannot rule out diseases caused by other bacterial or viral pathogens.

INTERPRETATION OF RESULTS

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress SARS-CoV-2 test provides test results based on the detection of two gene targets according to the algorithms shown in Table 1.

Table 1. Xpert Xpress SARS-CoV-2 Possible Results

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	**+**	**+**	**+/-**
SARS-CoV-2 POSITIVE	**+**	**-**	**+/-**
SARS-CoV-2 PRESUMPTIVE POSITIVE	**-**	**+**	**+/-**
SARS-CoV-2 NEGATIVE	**-**	**-**	**+**
INVALID	**-**	**-**	**-**

See Table 2 to interpret test result statements for the Xpert Xpress SARS-CoV-2 test.

Table 2. Xpert Xpress SARS-CoV-2 Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are detected. • The SARS-CoV-2 signal for the N2 nucleic acid target or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range and endpoint above the minimum setting SPC: NA; SPC is ignored because coronavirus target amplification occurred • Probe Check: PASS; all probe check results pass
SARS-CoV-2 PRESUMPTIVE POSITIVE	The 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present. Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. The SARS-CoV-2 signal for only the E nucleic acid target has a Ct within the valid range and endpoint above the minimum setting SPC: NA; SPC is ignored because a target amplification has occurred. Probe Check: PASS; all probe check results pass
SARS-CoV-2 NEGATIVE	The 2019 novel coronavirus (SARS-CoV-2) target nucleic acids are not detected. The SARS-CoV-2 signals for two nucleic acid targets (N2 and E) do not have a Ct within the valid range and endpoint above the minimum setting SPC: PASS; SPC has a Ct within the valid range and endpoint above the minimum setting Probe Check: PASS; all probe check results pass
INVALID	SPC does not meet acceptance criteria. Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in IFU (Section 17.2). SPC: FAIL; SPC and SARS-CoV-2 signals do not have a Ct within valid range and endpoint below minimum setting Probe Check – PASS; all probe check results pass
ERROR	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in IFU (Section 17.2). • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: FAIL1; all or one of the probe check results fail 1 If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range or by a system component failure.
NO RESULT	Presence or absence of the 2019 novel coronavirus (SARS-CoV-2) nucleic acids cannot be determined. Repeat test according to the Retest Procedure in IFU (Section 17.2). A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • SPC: NO RESULT • Probe Check: NA (not applicable)

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

REFERENCES:

1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed February 9, 2020.

2. bioRxiv. (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1). Accessed March 3, 2020.

3. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical laboratories* (refer to latest edition). http://www.cdc.gov/biosafety/publications/

4. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline*. Document M29 (refer to latest edition).

5. REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on the classification labeling and packaging of substances and mixtures amending and repealing, List of Precautionary Statements, Directives 67/548/EEC and 1999/45/EC (amending Regulation (EC) No 1907/2007).

6. Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March 26, 2012) (29 C.F.R., pt. 1910, subpt. Z).

DEFINITIONS: N/A

ATTACHMENTS: N/A

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES:

EFFECTIVE: April 10, 2020

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Microbiology**

SUB SECTION: **Procedure**

SUBJECT: **BioFire® FilmArray® COVID-19 TEST**

APPROVED BY: _____

Laboratory Medical Director or Designee

TEST NAME / POLICY

BioFire® FilmArray® COVID-19 TEST

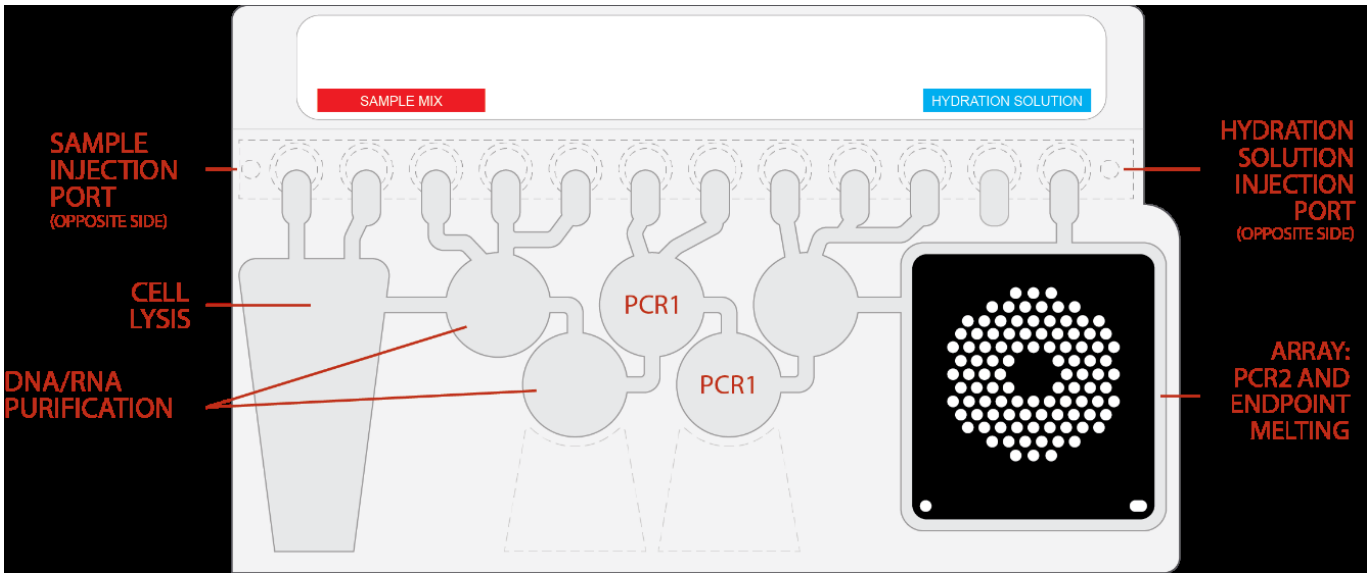
TEST PRINCIPLE / PURPOSE

The BioFire® COVID-19 Test is a nested multiplexed real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs in transport media from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate and high complexity tests

The BioFire COVID-19 Test is intended for use by laboratory personnel who have received specific training on the use of the FilmArray® 2.0 and/or the FilmArray® Torch Instrument Systems. The BioFire COVID-19 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

The BioFire COVID-19 Test is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from the SARS-CoV-2 virus within a single NPS specimen. After sample collection, the user injects hydration solution, and sample combined with sample buffer into the pouch, places the pouch into a FilmArray instrument, and starts a run. The entire run process takes about 50 minutes. Additional details can be found in the appropriate FilmArray operator's manual.

During a run, the FilmArray® system:Lyses the sample by agitation (bead beading); extracts and purifies all nucleic acids from the sample using magnetic bead technology; performs nested multiplex PCR by first performing reverse transcription and a single, large volume, multiplexed reaction (PCR1); then performing multiple single plex second-stage PCR reactions (PCR2) to amplify sequences within the PCR1 products; and uses endpoint melting curve data to detect and generate a result for each target assay on the BioFire COVID-19 Test.



CLINICAL SIGNIFICANCE

The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swabs in transport media during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The FilmArray® Respiratory Panel 2 (RP2) is a multiplexed nucleic acid test intended for use with FilmArray® 2.0 or FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the FilmArray RP2:

SPECIMEN TYPE(S)

See below for the recommended requirements for sample collection, preparation, and handling that will help ensure accurate test results.

Specimen Type	**Nasopharyngeal Swab** (NPS) collected according to standard technique and immediately placed in 1-3 mL of transport media.
Minimum Sample Volume	0.3 mL (300 µL)
Transport and Storage	Samples should be processed and tested with the BioFire COVID-19 Test as soon as possible. If storage is required, samples can be held: •At room temperature for up to 4 hours (15-25ºC) •Refrigerated for up to 3 days (2-8ºC) •Frozen (≤-15ºC or ≤-70ºC) for up to 30 days

NOTE: NPS specimens should not be centrifuged before testing.

NOTE: Bleach can damage organisms/nucleic acids within the specimen, potentially causing false negative results. Contact between bleach and specimens during collection, disinfection, and testing procedures should be avoided.

REQUIRED REAGENTS

I. Each BioFire COVID-19 Test Kit contains sufficient reagents to test or 30 samples (30-test kit; 423744):
   A. Individually-packaged BioFire COVID-19 Test pouches
   B. Single-use (1.0 mL) Sample Buffer tubes
   C. Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue)
   D. Single-use Sample Injection Vials (red)
   E. Individually-packaged Transfer Pipettes
   F. Instructions and Documentso*BioFire COVID-19 Test – Quick Guide*

II. Reagent Storage, Handling, and Stability
   A. Store the test and control kit, including reagent pouches and provided buffers, at room temperature (15-30°C). **DO NOT REFRIGERATE**.
   B. Avoid storage of any materials near heating or cooling vents, or in direct sunlight.
   C. All kit components should be stored and used together. Do not use components from one kit with those of another kit. Discard any extra components from the kit after all pouches have been consumed.
   D. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30minutes).
   E. Once a pouch has been loaded, the test run should be started as soon as possible (within approximately 60minutes). Do not expose a loaded pouch to temperatures above 40°C (104°F) prior to testing.

III. Warnings and Precautions
   A. General Precautions
      1. For *in vitro* diagnostic (IVD) use under Emergency Use Authorization only.
      2. Positive results are indicative of the presence of SARS-CoV-2 RNA.
      3. Laboratories within the Unites States and its territories are required to report all positive results to the appropriate public health authorities.
      4. BioFire COVID-19 Test pouches are only for use with FilmArray 2.0 and FilmArray Torch systems.
      5. BioFire COVID-19 External Control Kit (+) is only for use with FilmArray 2.0 and FilmArray Torch systems.
      6. Always check the expiration date on the pouch. Do not use a pouch after its expiration date.
      7. FilmArray pouches are stored under vacuum in individually-wrapped canisters. To preserve the integrity of the pouch vacuum for proper operation, be sure that a FilmArray instrument/module will be available and operational before unwrapping any pouches for loading.
      8. Bleach introduced in a sample may damage nucleic acids in the sample, which may lead to a false negative result.

9. If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

B. Safety Precautions

1. Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder-free gloves. Protect skin, eyes, and mucus membranes. Change gloves often when handling reagents or samples.
2. Process samples under Biological Safety Cabinet
3. Handle all samples and waste materials as if they were capable of transmitting infectious agents. Observe safety guidelines such as those outlined in:
4. Please refer to the BioFire COVID-19 Test Safety Data Sheet (SDS) for more information.
5. Sample Buffer will form hazardous compounds and fumes when mixed with bleach or other disinfectants.
   WARNING: Never add bleach to Sample Buffer or sample waste.
6. Bleach, a recommended disinfectant, is corrosive and may cause severe irritation or damage to eyes and skin. Vapor or mist may irritate the respiratory tract. Bleach is harmful if swallowed or inhaled.
   a. Eye contact: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses after the first 5 minutes and continue rinsing eye. Seek medical attention.
   b. Skin contact: Immediately flush skin with plenty of water for at least 15 minutes. If irritation develops, seek medical attention.
   c. Ingestion: Do not induce vomiting. Drink a glassful of water. If irritation develops, seek medical attention.
   d. Please refer to the appropriate Safety Data Sheet (SDS) for more information.

C. Laboratory Precautions

1. Preventing Organism Contamination

   a. Due to the sensitive nature of the BioFire COVID-19 Test, it is important to guard against contamination of the sample and work area by carefully following the testing process outlined in this instruction document, including these guidelines:
   b. Prior to processing samples, thoroughly clean both the work area and the FilmArray® Pouch Loading Station using a suitable cleaner such as freshly prepared 10% bleach or a similar disinfectant. To avoid residue buildup and potential damage to the sample or interference from disinfectants, wipe disinfected surfaces with 70% Ethanol.
   c. Samples and pouches should be handled and/or tested one-at-a-time. Always change gloves and clean the work area between each pouch and sample.
   d. Use clean gloves to remove materials from bulk packaging bags and reseal bulk-packaging bags when not in use.

2. Preventing Amplicon Contamination

   a. A common concern with PCR-based assays is false positive results caused by contamination of the work area with PCR amplicon. Because the BioFire COVID-19 Test pouch is a closed system, the risk of amplicon contamination is low if pouches remain intact after the test is completed. Adhere to the following guidelines, in addition to those above, to prevent amplicon contamination:
   b. Discard used pouches in a biohazard container immediately after the run has completed.

c. Avoid excessive handling of pouches after test runs.
d. Change gloves after handling a used pouch.
e. Avoid exposing pouches or sample injection vials to sharp edges or anything that might cause a puncture.
f. Change gloves after loading the External Control (+) material.
g. Clean thoroughly after loading the External Control (+) material to avoid contamination with the External Control (+).

WARNING: If liquid is observed on the exterior of a pouch, the liquid and pouch should be immediately contained and discarded in a biohazard container. The instrument and workspace must be decontaminated as described below and in the appropriate FilmArray operator's manual. DO NOT PERFORM ADDITIONAL TESTING UNTIL THE AREA HAS BEEN DECONTAMINATED.

CALIBRATION N/A

QUALITY CONTROL

I. Process Controls
Two process controls are included in each pouch:

A. RNA Process Control

The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the BioFire COVID-19 Test were successful.

B. PCR2 Control

The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that PCR2 was successful.
Both control assays must be positive for the test run to pass. If controls fail, the sample should be retested using a new pouch.

II. Monitoring Test System Performance

The FilmArray software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable range (80.3-84.4°C for the RNA Process Control and 73.8-78.2°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices. 5, 6 Refer to the appropriate FilmArray operator's manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in several FilmArray pouch types (e.g., RP2, BCID, GI, ME) and can therefore be used to monitor the system when multiple pouch types are used on the same FilmArray system or instrument.

III. Biofire® COVID-19 Test External Controls

For quality control and laboratory test verification, BioFire Defense provides an optional external positive assayed control kit to monitor the performance of *in vitro* laboratory nucleic acid testing procedures for the qualitative detection of the BioFire COVID-19 Test performed on FilmArray 2.0 and FilmArray Torch systems. The RNA in the external control includes RNA segments to monitor whether the PCR primers for each SARS-CoV-2 assay are present for both stages of the nested PCR. Test External Control (+) Kit contains no biological hazards and is 100% non-infectious. This control is stored at 15-30°C.

Evaluation of external controls is recommended prior to using a new shipment or new lot of BioFire COVID-19 Test Kits. Evaluation of external controls is also recommended when there is a new operator, and following replacement/repair of a FilmArray® 2.0 or FilmArray® Torch system.

IV. BioFire® COVID-19 Test External Control Procedure (+)

1. Follow Step 1 and Step 2 from the BioFire COVID-19 Test Procedure to prepare and hydrate the pouch.
2. Use the Transfer Pipette provided in the test kit to draw the transport media to the third line (approximately 0.3mL) of the Transfer Pipette. Add to the Sample Injection Vial.
3. Using clean gloves, obtain a Sample Buffer Tube from the BioFire COVID-19 Test Kit.
4. Uncap the External Control Vial (+) and place the cap on a clean surface (a paper towel may be used).
5. Add Sample Buffer to the External Control Vial (+).
    a. Hold the Sample Buffer Tube tip facing up and firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
    b. Invert the Sample Buffer tube over the uncapped External Control Vial (+) and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

   ***NOTE**: Avoid generating excessive foam.*
6. Recap the External Control Vial (+) and mix by gently inverting three (3) times.
7. Pour the rehydrated External Control (+) into the Sample Injection Vial and
8. Immediately dispose of the External Control Vial (+). **Change gloves**.
9. Tightly close lid of Sample Injection Vial and mix by gently inverting at least three (3) times.
10. Return Sample Injection Vial to red well of Pouch Loading Station.
11. Continue at Step 4 of the BioFire COVID-19 Test Panel Procedure to load the pouch and run the FilmArray.

V. BioFire® COVID-19 Test External Control Procedure (-)

1. Follow Step 1 and Step 2 from the BioFire COVID-19 Test Procedure to prepare and hydrate the pouch.
2. Use the Transfer Pipette provided in the test kit to draw the transport media to the third line (approximately 0.3mL) of the Transfer Pipette. Add to the Sample Injection Vial.
3. Using clean gloves, obtain a Sample Buffer Tube from the BioFire COVID-19 Test Kit.
4. Add Sample Buffer to the Sample Injection Vial.
    a. Hold the Sample Buffer Tube tip facing up and firmly pinch at textured plastic tab on the side of the tube until the seal snaps.
    b. Invert the Sample Buffer Tube over the Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.
5. ***NOTE:** Avoid generating excessive foam.*
6. Tightly close the lid of the Sample Injection Vial and mix by gently inverting at least three (3) times.

7. Remove the Sample Injection Vial from the Pouch Loading Station and mix by gently inverting the vial at least three (3) times to mix.

8. Return the Sample Injection Vial to the red well of the FilmArray Pouch Loading Station.
9. Continue at Step 4 of the BioFire COVID-19 Test Procedure to load the pouch and run the FilmArray.

PROCEDURAL STEPS

A. Step 1: Prepare Pouch

1. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.

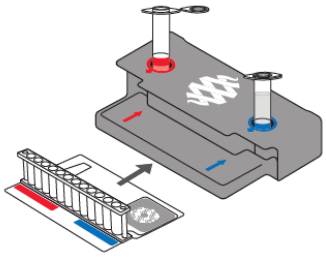
   Note: Mix 30 mL 8.25% bleach in 270 mL water to make 10% working bleach solution

2. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

   NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

3. Check the expiration date on the pouch. Do not use expired pouches.

4. Insert the pouch into the FilmArray Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the FilmArray Pouch Loading Station.



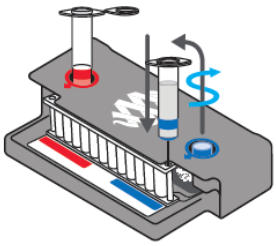
5. Place a red-capped Sample Injection Vial into the red well of the FilmArray Pouch Loading Station.

6. Place a blue-capped Hydration Injection Vial into the blue well of the FilmArray Pouch Loading Station.

B. Step 2: Hydrate Pouch

1. Unscrew the Hydration Injection Vial from the blue cap.

2. Remove the Hydration Injection Vial, leaving the blue cap in the FilmArray Pouch Loading Station.



3. Insert the Hydration Injection Vial's cannula tip into the pouch hydration port located directly below the blue arrow of the FilmArray Pouch Loading Station.

4. Forcefully push down in a firm and quick motion to puncture seal until a faint "pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.

   If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is

again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

5. Verify that the pouch has been hydrated.

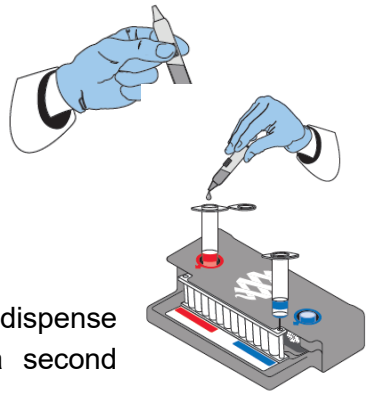
   a. Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.

   b. If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

C. Step 3: Prepare Sample Mix

1. Add Sample Buffer to the Sample Injection Vial.

   Hold the Sample Buffer ampoule with the tip facing up.



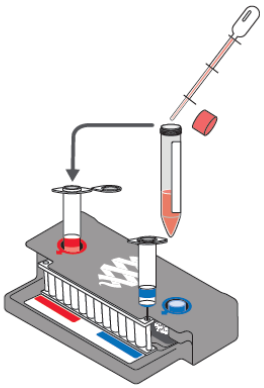
NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.

   a. Firmly pinch at textured plastic tab on the side of the ampoule until the seal snaps.

   b. Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

NOTE: Avoid squeezing the ampoule additional times. This will generate foaming, which should be avoided.

WARNING: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.

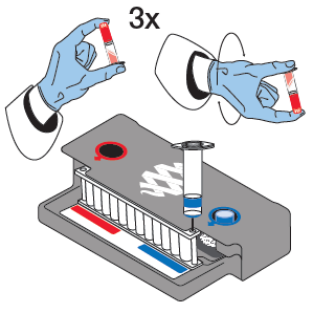


2. Thoroughly mix the NPS specimen by vortex or inversion.

3. Use the transfer pipette provided in the test kit to draw specimen to the third line (approximately 0.3 mL) of the transfer pipette.

4. Add the specimen to the Sample Buffer in the Sample Injection Vial.

5. Tightly close the lid of the Sample Injection Vial and discard the transfer pipette in a biohazard waste container.



NOTE: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.

6. Remove the Sample Injection Vial from the FilmArray Pouch Loading Station and invert the vial at least 3 times to mix.

7. Return the Sample Injection Vial to the red well of the FilmArray Pouch Loading Station.

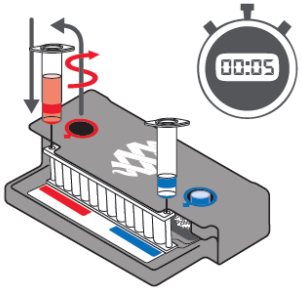
D. Step 4: Load Sample Mix

1. Slowly twist to unscrew the Sample Injection Vial from the red cap and wait for 5 seconds with the vial resting in the cap.

   NOTE: Waiting 5 seconds decreases the risk of dripping and contamination from the sample.

2. Lift the Sample Injection Vial, leaving red cap in the well of the FilmArray Pouch Loading Station, and insert the Sample Injection Vial cannula tip into the pouch sample port located directly below the red arrow of the FilmArray Pouch Loading Station.

3. Forcefully push down in a firm and quick motion to puncture seal (a faint "pop" is heard) and sample is pulled into the pouch by vacuum.



4. Verify that the sample has been loaded.

   a. Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port.

   b. If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from *Step 1: Prepare Pouch*.

5. Discard the Sample Injection Vial and the Hydration Injection Vial in appropriate biohazard sharps container.

6. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the FilmArray Pouch Loading Station.

E. Step 5: Run Pouch

The FilmArray® Software includes step-by-step on-screen instructions that guide the operator through performing a run. Brief instructions for FilmArray 2.0 and FilmArray Torch systems are given below. Refer to the appropriate FilmArray Operator's Manual for more detailed instructions.

1. FilmArray 2.0

   a. Ensure that the FilmArray 2.0 system (instrument and computer) is powered on and the software is launched.

   b. Follow on-screen instructions and procedures described in the Operator's Manual to place the pouch in an instrument, enter pouch, sample, and operator information.

   c. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually

entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the FilmArray RP2 pouch.

d. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

e. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The FilmArray RP2 has a single NPS2 protocol available in the drop down list.

f. Enter a user name and password in the Name and Password fields.

NOTE: The font color of the username is red until the user name is recognized by the software.

g. Review the entered run information on the screen. If correct, select Start Run.

Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.
NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

h. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.

i. The run file is automatically saved in the FilmArray database, and the test report can be viewed, printed, and/or saved as a PDF file.

2. FilmArray Torch

a. Ensure that the FilmArray Torch system is powered on.

b. Select an available Module (instrument) on the touch screen or scan the barcode on the FilmArray pouch using the barcode scanner.

c. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the FilmArray RP2 pouch.

d. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

e. Insert the pouch into the available Module (instrument).

Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module (instrument) will grab onto the pouch and pull it into the chamber.

f. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The FilmArray RP2 has a single NPS2 protocol available in the drop down list.

g. Enter operator user name and password, then select next.

NOTE: The font color of the username is red until the user name is recognized by the software.

h. Review the entered run information on the screen. If correct, select Start Run.

Once the run has started, the screen displays a list of the steps being performed by the Module (instrument) and the number of minutes remaining in the run.

NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

i. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.

The run file is automatically saved in the FilmArray database, and the test report can be viewed, printed, and/or saved as a PDF file.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS

The BioFire COVID-19 Test consists of three independent and non-overlapping assays targeting two SARS-CoV-2 open reading frame sequences: ORF1ab and ORF8. The target of each assay is shown in Table 1 below. The assays are designed to detect SARS-CoV-2 specifically. Detection of SARS-CoV-2 is based on the combined results of the three assays as described below.

Assay Name	SARS-COV-2 Genomic Region
SARS-CoV-2a	ORF1ab
SARS-CoV-2d	ORF1ab
SARS-CoV-2e	ORF8

I. Assay Interpretation

When PCR2 is complete, the FilmArray instrument performs a DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see appropriate FilmArray operator's manual). The FilmArray Software then performs several

analyses and assigns a final assay result for every well. The steps in the analyses are described below.

Analysis of Melt Curves. The FilmArray Software evaluates the DNA melt curve for each well of the PCR2 array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve and compares it against the expected Tm range for the assay. If the software determines that the Tm of the curve is within the assay specification Tm range, the melt curve is called positive. If the software determines that the Tm of the curve is not in the appropriate Tm range, the melt curve is called negative.

Analysis of Replicates. Once positive melt curves have been identified, the software evaluates the three replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive melt curves must be similar (within 1.0°C). Assays that do not meet these criteria are called negative.

II. Organism Interpretation

A. SARS-CoV-2

The BioFire COVID-19 Test contains three different assays (SARS-CoV-2a, SARS-CoV-2d, SARS-CoV-2e) for the detection of SARS-CoV-2. The FilmArray Software interprets each of these assays independently (as described above) and the results are combined as a final test result for the virus. For interpretation results, refer to Table 2.

If two or more assays are ‘Detected’, the result on the test report will be SARS-CoV-2 ‘Detected’. If all assays are ‘Not Detected’, the result on the test report will be SARS-CoV-2 ‘Not Detected’. If only one of the assays is ‘Detected’, the test report result will be SARS-CoV-2 ‘Equivocal’. If an ‘Equivocal’ result is obtained, retest the original sample using a new pouch. If the result of the retest is ‘Equivocal’ or ‘Detected’, the overall interpretation will be ‘Detected’. If the retest is ‘Not Detected’, seek confirmatory testing. In cases where either or both of the control assays have failed, all results are reported as ‘Invalid’ and retesting is required.

Table 2. Interpretation Rules for the BioFire COVID-19 Test SARS-CoV-2

Interpretation	Assay Results	Action
Detected	3/3 Assays ‘Detected’ 2/3 Assays ‘Detected’	Report the Results
Equivocal	1/3 Assays ‘Detected’	Retest the original sample and report the results of the retest. If the result of the retest is ‘Equivocal’ or ‘Detected’, the overall interpretation will be ‘Detected’. If the retest is ‘Not Detected’, seek confirmatory testing.
Not Detected	0/3 Assays ‘Not Detected’	Report the Results
		Retest the original sample. If repeated errors occur, contact the

Invalid	Invalid	BioFire Defense Customer Support Team. Refer to Table 3 for more

B. BioFire® COVID-19 Test Report

The BioFire COVID-19 test report is automatically displayed upon completion of a run and can be printed or saved as a PDF file. Each report contains a Run Summary, a Result Summary, and a Run Details section.

C. Run Summary

The **Run Summary** section of the test report provides the Sample ID, time and date of the run, internal control results, and an overall summary of the test results. If the SARS-CoV-2 interpretation is 'Detected', it will be listed in the 'Detected' field. If all of the assays are 'Not Detected' then 'None' will be displayed in the Detected field. Internal controls are listed as 'Passed', 'Failed', or 'Invalid'. Table 3 provides additional information for each of the possible internal control field results.

Table 3. Interpretation of Internal Controls Field on the BioFire COVID-19 Test Report

Internal Controls Result	Explanation	Action
Passed	The run was successfully completed AND Both pouch controls were successful.	None Report the results provided on the test report
Failed	The run was successfully completed BUT At least one of the pouch controls (RNA Process Control and/or PCR2 Control) failed.	Repeat the test using a new pouch. If the error persists, contact BioFire Defense Customer Support for further instruction.
Invalid	The controls are invalid because the run did not complete. (Typically this indicates a software or hardware error.)	Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the appropriate FilmArray operator's manual or contact BioFire Defense Customer Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument.

Table 4. Reporting of Results and Required Actions SARS-CoV-2

Results	Explanation	Action
Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND Two or three assays for the virus were 'Detected' (i.e., met the requirements for a positive result described in the Assay Interpretation section above)	Report results.
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The three assays for the virus were 'Not Detected' (i.e., did not meet the requirements for a positive result described in the Assay Interpretation section above)	Report results.
Equivocal	The run was successfully completed AND The pouch controls were successful (Passed) AND Only one of three assays was 'Detected' for the virus. The combination of 'Detected' and 'Not Detected' assay results were inconclusive	Retest the original sample using a new pouch and report the results of the retest. If the retest is 'Equivocal' or 'Detected', report the results as 'Detected'. If the result is 'Not Detected' seek confirmatory testing.
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run Status displayed as: Aborted, Incomplete, Instrument Error or Software Error)	See Table 3, Interpretation of Internal Controls Field on the BioFire Test Report for instruction.

D. Run Details

The **Run Details** section provide additional information about the run including: pouch information (type, lot number, and serial number), run status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test.

E. Change Summary

It is possible to edit the Sample ID once a run has completed. If this information has been changed, an additional section called **Change Summary** will be added to the test report. This Change Summary section lists the field that was changed, the original entry, the revised entry, the operator that made the change, and the date that the change was made. Sample ID is the only field of the report that can be changed.

F. Reporting Results

1. Results are entered manually in MEDITECH under the Laboratory, not Microbiology.
2. Click on Laboratory → Result Entry → Enter results → Enter Bar Code # or MT # → On the result entry table, Enter **P** under Quality Control; under all organisms you will see **NOT DETECTED** as default result. If the test is Negative for all organisms, hit F12 (save) and release result. If the test is positive for any organism (s), carefully select the positive organism (s), and enter **DETECTED**, hit F12, and release result.
3. Call back Flu, RSV or *Bordetella pertussis* positive result with documentation in MEDITECH.
4. Label test report with a label from the lab. Slip, initial, date and file in appropriate results binder.
5. Save specimens in a freezer.

LIMITATIONS

1. For *In Vitro* Diagnostic (IVD) Use under Emergency Use Authorization (EUA).
2. BioFire COVID-19 Test performance has only been established on the FilmArray 2.0 and FilmArray Torch systems.
3. The BioFire COVID-19 Test is a qualitative test and does not provide a quantitative value for the virus in the sample.
4. The BioFire COVID-19 Test has not been validated for testing of samples other than nasopharyngeal swab(NPS) specimens in transport media.
5. A false negative BioFire COVID-19 Test result may occur when the concentration of virus in the sample isbelow the device limit of detection.
6. The detection of viral nucleic acid is dependent upon proper sample collection, handling, transportation, storage and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
7. There is a risk of false positive and false negative results caused by improperly collected, transported, or handled samples. The RNA process control and the PCR2 control will not indicate whether or not nucleic acid has been lost due to inadequate collection, transport, or storage of samples.

8. As with any molecular test, mutations within the target regions of SARS-CoV-2 could affect primer binding, resulting in failure to detect the presence of virus.
9. All three assays show 80% or greater homology to Bat coronavirus RaTG13 (accession: MN996532). In addition, the SARS-CoV-2e assay shows greater than 80% homology to Pangolin coronavirus isolateMP789 (accession: MT084071). It is unlikely that these isolates would be found in our sample matrix of nasopharyngeal swabs; however, little is known about their potential to infect a human host, or their evolutionary relationship to SARS-CoV-2.

REFERENCES:

BioFire COVID-19 Test Instructions for Use, BioFire Defense, LLC

DEFINITIONS: N/A

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES: **N/A**

EFFECTIVE: April 27, 2020

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: Microbiology- Molecular Diagnostics

SUB SECTION: Procedure

SUBJECT: BioFire® Respiratory 2.1 (RP2.1) Panel (EUA) Testing
For Emergency Use Authorization (EUA) only

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

BioFire® Respiratory 2.1 (RP2.1) Panel (EUA) Testing - For Emergency Use Authorization (EUA) only

TEST PRINCIPLE / PURPOSE

The BioFire RP2.1 Panel (EUA) pouch is a closed system disposable that stores all the necessary reagents for sample preparation, reverse transcription, polymerase chain reaction (PCR), and detection in order to isolate, amplify, and detect nucleic acid from multiple respiratory pathogens within a single Nasopharyngeal swab (NPS) specimen. After sample collection, the user injects hydration solution and sample combined with sample buffer into the pouch, places the pouch into a BioFire® System instrument module, and starts a run. The entire run process takes about 45 minutes. Additional detail can be found in the appropriate BioFire® System Operator's Manual.

I. During a run, the BioFire System:

A. Lyses the sample by agitation (bead beading) in addition to chemical lysis mediated by the Sample Buffer.

B. Extracts and purifies all nucleic acids from the sample using magnetic bead technology.

C. Performs nested multiplex PCR by:

1. First performing reverse transcription, followed by a multiplexed first stage PCR reaction (PCR1).

2. Then performing multiple simultaneous second-stage PCR reactions (PCR2) in the array to amplify sequences within the PCR1 products

D. Uses endpoint melting curve data to detect target-specific amplicons and analyses the data to generate a result for each analyte on the BioFire RP2.1 Panel (EUA).

CLINICAL SIGNIFICANCE

The BioFire RP2.1 Panel (EUA) is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and differentiation of nucleic acids from multiple viral and bacterial respiratory organisms, including nucleic acid from Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), in NPS obtained from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high-complexity or moderate-complexity tests.

The BioFire RP2.1 Panel (EUA) is intended for the detection and differentiation of nucleic acid from SARS- CoV-2 and the following organism types and subtypes identified using the BioFire RP2.1 Panel (EUA).

Viruses	Bacteria
Adenovirus Coronavirus 29E Coronavirus KU1 Coronavirus L63 Coronavirus C43 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Human Metapneumovirus Human hinovirus/Enterovirus Influenza A, including subtypes H1, H3 and H1-2009 Influenza B Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 Respiratory Syncytial Virus	Bordetella parapertussis Bordetella pertussis Chlamydia pneumoniae Mycoplasma pneumoniae

SPECIMEN TYPE(S)

The following table describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

Specimen Type	Nasopharyngeal Swab (NPS) collected according to standard technique and immediately placed in 1-3 mL of transport media
Minimum Sample Volume	0.3 mL (300 µL)
Transport and Storage	• Specimens should be processed and tested with the RP2.1 Panel (EUA) as soon as possible. • If storage is required, specimens can be held: • At room temperature for up to 4 hours (15-25 °C) • Refrigerated for up to 3 days (2-8 °C) • Frozen (≤ -15 °C or ≤ -70°C) (for up to 30 days)

NOTE: NPS specimens should not be centrifuged before testing.

NOTE: Bleach can damage organisms/nucleic acids within the specimen, potentially causing false negative results. Contact between bleach and specimens during collection, disinfection, and testing procedures should be avoided.

REQUIRED REAGENT(S)

Materials Provided	Materials Required But Not Provided
Each kit contains sufficient reagents to test 30 or 6 specimens: 1. Individually-packaged RP2.1 PANEL (EUA) pouches 2. Single-use (1.0 mL) Sample Buffer ampoules 3. Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue) 4. Single-use Sample Injection Vials (red) 5. Individually-packaged Transfer Pipettes	system including: 1. BioFire ®2.0 or ® Torch and accompanying software 2. BioFire ® Pouch Loading Station' 3. 10% bleach solution or a similar disinfectant

List of Acceptable Specimen Collection Swabs and Transport Media

Specimen Collection Materials		
Rayon Swabs (Copan 168C)	N/A	No Interference
Nylon Flocked Swabs (Copan 553C)	N/A	No Interference
Polyester Swabs (Copan 175KS01)	N/A	No Interference
Calcium Alginate Swabs (Puritan 25-801 A 50)	N/A	No Interference
M4® Transport Medium (Remel)	100%	No Interference
M4-RT® Transport Medium (Remel)	100%	No Interference
M5® Transport Medium (Remel)	100%	No Interference
M6™ Transport Medium (Remel)	100%	No Interference

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REF RFIT-ASY-0129
RFIT-ASY-0130

Substance Tested	Concentration Tested	Result
Universal Viral Transport vial (BD)	100%	No Interference
Sigma-Virocult™ Viral Collection and Transport System (Swab and Transport Medium)	100%	No Interference
Copan ESwab™ Sample Collection and Delivery System (Swab and Liquid Amies Medium)	100%	No Interference

[a] Nasal influenza vaccines (e.g. FluMist) were not evaluated, but are predicted to be reactive with the FilmArray RP2 Influenza A (subtype) and Influenza B assays.

[b] Not Detected results were reported for several analytes after incubation of the sample with 2% bleach for 10 minutes or overnight. It was concluded that interference resulted primarily from damage to the organisms/nucleic acids in the sample, rather than inhibition or interference with pouch function(s).

CALIBRATION

N/A

QUALITY CONTROL

I. **Process Controls**

Two process controls are included in each pouch:

A. RNA Process Control

The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, PCR1, dilution, PCR2, and DNA melting. A positive control result indicates that all steps carried out in the BioFire ® RP2.1 PANEL (EUA) pouch were successful.

B. PCR2 Control

The PCR2 Control assay detects a DNA target that is dried into wells of the array along with the corresponding primers. A positive result indicates that PCR2 was successful. Both control assays must be positive for the test run to pass. If the controls fail, the sample should be retested using a new pouch.

II. **Monitoring Test System Performance**

The BioFire ®software will automatically fail the run if the melting temperature (Tm) for either the RNA Process Control or the PCR2 Control is outside of an acceptable range (80.3-84.3°C for the RNA Process Control and 73.8-77.8°C for the PCR2 Control). If required by local, state, or accrediting organization quality control requirements, users can monitor the system by trending Tm values for the control assays and maintaining records according to standard laboratory quality control practices. Refer to the appropriate BioFire ® operator's manual for instructions on obtaining control assay Tm values. The PCR2 Control is used in several BioFire ® pouch types (e.g., RP, BCID, GI, ME, and RP2.1 Panel (EUA)) and can therefore be used to monitor the system when multiple pouch types are used on the same BioFire ® system or instrument.

III. **External Controls**

External positive and negative controls are performed on New Lot # pouches and new shipments before they are placed for patient sample testing, or monthly, based on whichever comes first. Main Molecular Quality Control, Inc., BioFire RP2.1 /RP2.1 Plus Control Panel, Part # 441 is used as an external quality control material for the RP2.1 PANEL (EUA).1 pouches. Alternatively, transport media can be used as an external negative control. Previously characterized positive samples or negative samples spiked with well characterized organisms can be used as external positive controls. RP2.1 Panel (EUA) pouches will be used for patient testing only when positive and negative external controls provide expected results.

PROCEDURAL STEPS

A. Step 1: Prepare Pouch

1. Thoroughly clean the work area and the Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse.

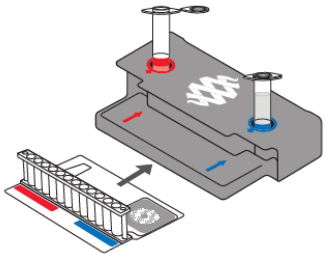
   Note: Mix 39 mL 8.25% bleach in 345 mL water to make 10% working bleach solution

2. Remove the pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

   NOTE: The pouch may still be used even if the vacuum seal of the pouch is not intact. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.

3. Check the expiration date on the pouch. Do not use expired pouches.

4. Insert the pouch into the Pouch Loading Station, aligning the red and blue labels on the pouch with the red and blue arrows on the Pouch Loading Station.



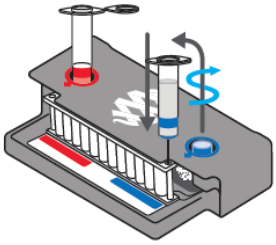
5. Place a red-capped Sample Injection Vial into the red well of the Pouch Loading Station.

6. Place a blue-capped Hydration Injection Vial into the blue well of the Pouch Loading Station.

B. Step 2: Hydrate Pouch

1. Unscrew the Hydration Injection Vial from the blue cap.

2. Remove the Hydration Injection Vial, leaving the blue cap in the Pouch Loading Station.



3. Insert the Hydration Injection Vial's cannula tip into the pouch hydration port located directly below the blue arrow of the Pouch Loading Station.

4. Forcefully push down in a firm and quick motion to puncture seal until a faint "pop" is heard and there is an ease in resistance. Wait as the correct volume of Hydration Solution is pulled into the pouch by vacuum.

   If the hydration solution is not automatically drawn into the pouch, repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is again not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

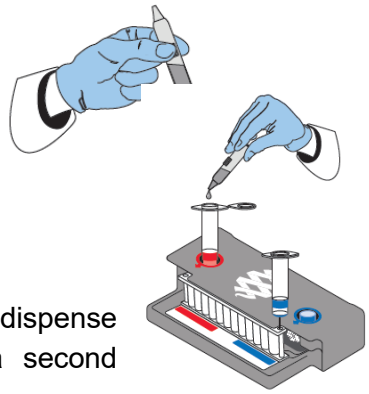
5. Verify that the pouch has been hydrated.

a. Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen.

b. If the pouch fails to hydrate (dry reagents appear as white pellets), repeat Step 2 to verify that the seal of the pouch hydration port was broken. If hydration solution is still not drawn into the pouch, discard the current pouch, retrieve a new pouch, and repeat from *Step 1: Prepare Pouch*.

C. Step 3: Prepare Sample Mix

1. Add Sample Buffer to the Sample Injection Vial.



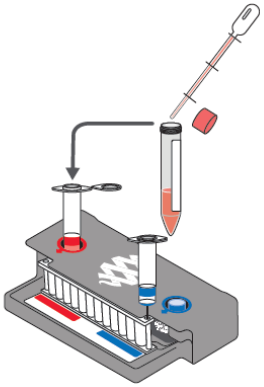
Hold the Sample Buffer ampoule with the tip facing up.

NOTE: Avoid touching the ampoule tip during handling, as this may introduce contamination.

a. Firmly pinch at textured plastic tab on the side of the ampoule until the seal snaps.

b. Invert the ampoule over the red-capped Sample Injection Vial and dispense Sample Buffer using a slow, forceful squeeze followed by a second squeeze.

NOTE: Avoid squeezing the ampoule additional times. This will generate foaming, which should be avoided.



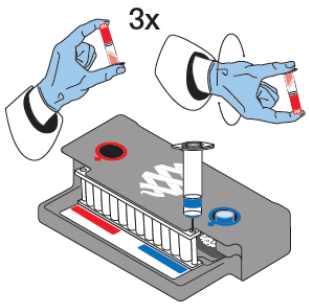
WARNING: The Sample Buffer is harmful if swallowed and can cause serious eye damage and skin irritation.

2. Thoroughly mix the NPS specimen by vortex or inversion.

3. Use the transfer pipette provided in the test kit to draw specimen to the third line (approximately 0.3 mL) of the transfer pipette.

4. Add the specimen to the Sample Buffer in the Sample Injection Vial.

5. Tightly close the lid of the Sample Injection Vial and discard the transfer pipette in a biohazard waste container.



NOTE: DO NOT use the Transfer Pipette to mix the sample once it is loaded into the Sample Injection Vial.

6. Remove the Sample Injection Vial from the Pouch Loading Station and invert the vial at least 3 times to mix.

7. Return the Sample Injection Vial to the red well of the Pouch Loading Station.

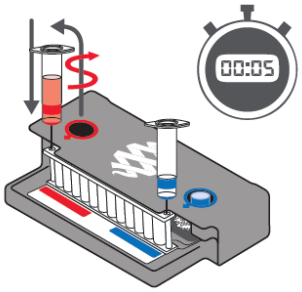
D. Step 4: Load Sample Mix

1. Slowly twist to unscrew the Sample Injection Vial from the red cap and wait for 5 seconds with the vial resting in the cap.

   NOTE: Waiting 5 seconds decreases the risk of dripping and contamination from the sample.

2. Lift the Sample Injection Vial, leaving red cap in the well of the Pouch Loading Station, and insert the Sample Injection Vial cannula tip into the pouch sample port located directly below the red arrow of the Pouch Loading Station.

3. Forcefully push down in a firm and quick motion to puncture seal (a faint "pop" is heard) and sample is pulled into the pouch by vacuum.



4. Verify that the sample has been loaded.

   a. Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port.

   b. If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from *Step 1: Prepare Pouch*.

5. Discard the Sample Injection Vial and the Hydration Injection Vial in appropriate biohazard sharps container.

6. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the BioFire® Pouch Loading Station.

E. Step 5: Run Pouch

The BioFire® Software includes step-by-step on-screen instructions that guide the operator through performing a run. Brief instructions for BioFire® 2.0 and Torch systems are given below. Refer to the appropriate BioFire® Operator's Manual for more detailed instructions.

1. BioFire 2.0

   a. Ensure that the BioFire 2.0 system (instrument and computer) is powered on and the software is launched.

   b. Follow on-screen instructions and procedures described in the Operator's Manual to place the pouch in an instrument, enter pouch, sample, and operator information.

   c. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BioFire® **RP2.1 Panel (EUA) pouch.**

d. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

e. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire RP2.1 Panel (EUA) has a single protocol available in the drop down list.

f. Enter a user name and password in the Name and Password fields.

NOTE: The font color of the username is red until the user name is recognized by the software.

g. Review the entered run information on the screen. If correct, select Start Run.

Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.
NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

h. When the run is finished, follow the on-screen instructions to remove the pouch, then immediately discard it in a biohazard waste container.

i. The run file is automatically saved in the BioFire® database, and the test report can be viewed, printed, and/or saved as a PDF file.

2. Torch

a. Ensure that the BioFire® Torch system is powered on.

b. Select an available Module (instrument) on the touch screen or scan the barcode on the BioFire®pouch using the barcode scanner.

c. Pouch identification (Lot Number and Serial Number), Pouch Type and Protocol information will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, Pouch Type and Protocol can be manually entered from the information provided on the pouch label into the appropriate fields. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

NOTE: When selecting a Pouch Type manually, ensure that the Pouch Type matches the label on the BioFire® **RP2.1 Panel (EUA) pouch.**

d. Enter the Sample ID. The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

e. Insert the pouch into the available Module (instrument).

Ensure that the pouch fitment label is lying flat on top of pouch and not folded over. As the pouch is inserted, the Module (instrument) will grab onto the pouch and pull it into the chamber.

f. If necessary, select and/or confirm the appropriate protocol for your sample type from the Protocol drop down list. The BioFire® RP2.1 PANEL (EUA) has a single NPS2 protocol available in the drop down list.

g. Enter operator user name and password, then select next.

NOTE: The font color of the username is red until the user name is recognized by the software.

h. Review the entered run information on the screen. If correct, select Start Run.

Once the run has started, the screen displays a list of the steps being performed by the Module (instrument) and the number of minutes remaining in the run.

NOTE: The bead-beater apparatus can be heard as a high-pitched noise during the first minute of operation.

i. At the end of the run, remove the partially ejected pouch, then immediately discard it in a biohazard waste container.

The run file is automatically saved in the BioFire® database, and the test report can be viewed, printed, and/or saved as a PDF file.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS

When PCR2 is complete, the BioFire instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see appropriate Operator's Manual). The BioFire Software then performs several analyses and assigns a final assay result. The steps in the analyses are described below.

Analysis of melt curves. The BioFire Software evaluates the DNA melt curve for each well of the PCR2 array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve and compares it against the expected Tm range for the assay. If the software determines that the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is not in the appropriate Tm range, the melt curve is called negative.

Analysis of replicates. Once melt curves have been identified, the software evaluates the three re plicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive melt curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

Organism Interpretation

For most organisms detected by the BioFire RP2.1 PANEL (EUA), the organism is reported as Detected if a single corresponding assay is positive. For example, Human Metapneumovirus will

have a test report result of Human Metapneumovirus Detected if at least two of the three replicates of the one Human Metapneumovirus assay (hMPV) have similar positive melt peaks with Tm values that are within the assay-specific Tm range. The test results for SARS-CoV-2, Adenovirus and Influenza A depend on the interpretation of results from more than one assay. Interpretation and actions for these two multi-assay results are provided below.

A. SARS-CoV-2

The BioFire RP2.1 Panel (EUA) pouch contains two different assays for the detection of the SARS-CoV-2. The target of each assay is shown in below. The BioFire Software interprets each assay independently and if either one or both of the assays is positive, the test report will show Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as Detected. If both assays are negative, the test report result will be Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Not Detected.

Gene Targets for SARS-CoV-2 Assays on the BioFire RP2.1 Panel (EUA)

Assay Name	Gene Target
SARSCoV2-1	Spike protein (S) gene
SARSCoV2-2	Membrane protein (M) gene

B. Adenovirus

The BioFire® RP2.1 Panel (EUA) pouch contains five different assays (Adeno2, Adeno3, Adeno6, Adeno7.1, and Adeno8) for the detection of Adenovirus. The BioFire® Software interprets each of these assays independently (as described above) and the results are combined as a final test result for the virus. If one or any combination of assays is positive, the test report result will be Adenovirus Detected. If all assays are negative, the test report result will be Adenovirus Not Detected.

C. Influenza A

The assays in the BioFire® RP2.1 PANEL (EUA) are designed to both detect Influenza A and to differentiate commonly occurring hemagglutinin subtypes. To accomplish this, the BioFire® RP2.1 PANEL (EUA) uses two Influenza A assays, (FluA-pan-1 and FluA-pan-2) and three subtyping assays directed at the hemagglutinin gene (FluA-H1-2, FluA-H1-2009, and FluA-H3). Each of the individual assays is interpreted independently (as described above) and the test result reported for Influenza A is based on the combined results of the five assays as outlined in Table 1. Retest specimens having Equivocal results or multiple Influenza A subtypes detected.

Table 1. Possible Assay Results for Influenza A and the Corresponding Interpretation

Assay/Result	FluA-pan Assays (n=2)	FluA-H1 – 2	FluA-H1 – 2009	FluA-H3	Action
Influenza A Not Detected	Negative	Negative	Negative	Negative	None
Influenza A H1	**≥ 1 positive**	**Positive**	Negative	Negative	
Influenza A H3	**≥ 1 positive**	Negative	Negative	**Positive**	
Influenza A H1 - 2009	**≥ 1 positive**	Any Result	**Positive**	Negative	
Influenza A H1 Influenza A H3	**≥ 1 positive**	**Positive**	Negative	**Positive**	Multiple infections are possible but rare[a], retest to confirm result[b]
Influenza A H1 - 2009 Influenza A H3	**≥ 1 positive**	Any Result	**Positive**	**Positive**	
Influenza A (No subtype detected)	**2 positive**	Negative	Negative	Negative	Retest (see below)
Influenza A Equivocal	**1 positive**	Negative	Negative	Negative	Retest
Influenza A H1 Equivocal	Negative	**Positive**	Negative	Negative	
Influenza A H3 Equivocal	Negative	Negative	Negative	**Positive**	
Influenza A H1 - 2009 Equivocal	Negative	Any result	**Positive**	Negative	

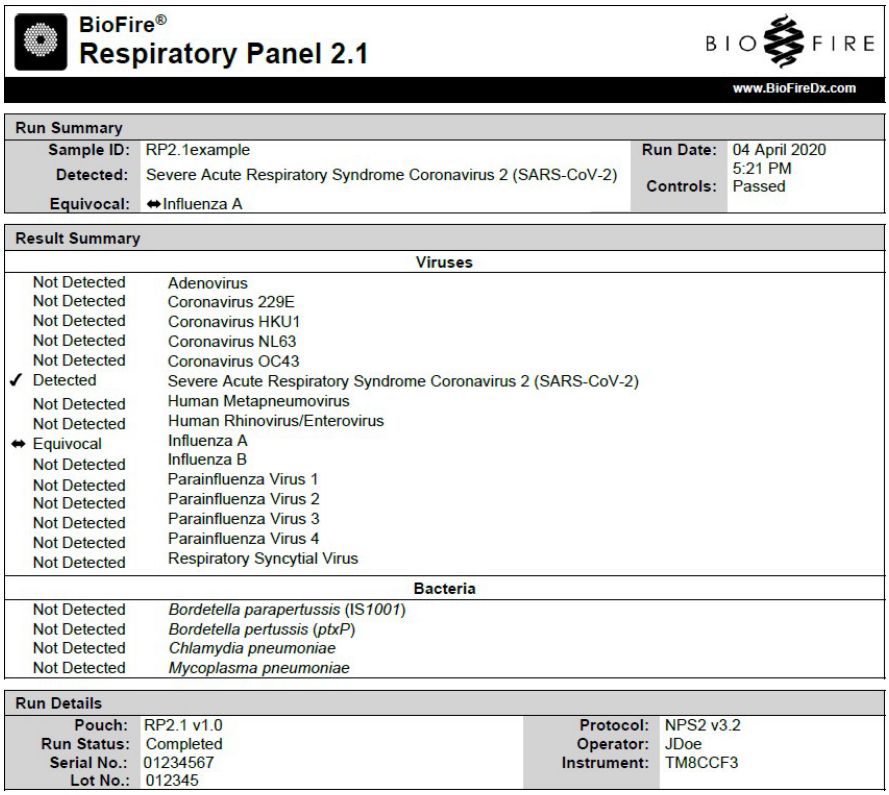
[a]The BioFire RP2.1 PANEL (EUA) can simultaneously detect multiple influenza viruses contained in multivalent vaccines

[b] Repeated multiple positives should be further confirmed by other FDA cleared Influenza subtyping tests

D. Influenza A (no subtype detected)

If both of the FluA-pan assays are positive, but none of the hemagglutinin subtyping assays are positive, then the interpretation is Influenza A (no subtype detected). This result could occur when the titer of the virus in the specimen is low and not detected by the subtyping assays. This result could also indicate the presence of a novel Influenza A strain. In both cases, the sample in question should be retested. If the retest provides a different result, test the sample a third time to ensure the accuracy of the result. If the retest provides the same result, then the function of the RP2.1 Panel(EUA) pouches should be verified by testing with appropriate external control materials (known positive samples for Influenza A H1, Influenza A H3 and Influenza A H1-2009), and a negative control should also be run to test for PCR-product contamination. If the BioFire® RP2.1 Panel (EUA) accurately identifies the external and negative controls, contact the appropriate public health authorities for confirmatory testing.

E. RP2.1 Panel (EUA) Test Report



BioFire®
Respiratory Panel 2.1

BIOFIRE
www.BioFireDx.com

Run Summary

Sample ID:	RP2.1example	**Run Date:**	04 April 2020 5:21 PM
Detected:	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	**Controls:**	Passed
Equivocal:	⬌Influenza A		

Result Summary

	Viruses
Not Detected	Adenovirus
Not Detected	Coronavirus 229E
Not Detected	Coronavirus HKU1
Not Detected	Coronavirus NL63
Not Detected	Coronavirus OC43
✓ Detected	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
Not Detected	Human Metapneumovirus
Not Detected	Human Rhinovirus/Enterovirus
⬌ Equivocal	Influenza A
Not Detected	Influenza B
Not Detected	Parainfluenza Virus 1
Not Detected	Parainfluenza Virus 2
Not Detected	Parainfluenza Virus 3
Not Detected	Parainfluenza Virus 4
Not Detected	Respiratory Syncytial Virus
	Bacteria
Not Detected	*Bordetella parapertussis* (IS*1001*)
Not Detected	*Bordetella pertussis* (*ptxP*)
Not Detected	*Chlamydia pneumoniae*
Not Detected	*Mycoplasma pneumoniae*

Run Details

Pouch:	RP2.1 v1.0	**Protocol:**	NPS2 v3.2
Run Status:	Completed	**Operator:**	JDoe
Serial No.:	01234567	**Instrument:**	TM8CCF3
Lot No.:	012345		

The BioFire® RP2.1 PANEL (EUA) test report is automatically displayed upon completion of a run and can be printed or saved as a PDF file. Each report contains a Run Summary, a Result Summary, and a Run Details section.

1. Run Summary

The Run Summary section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. Any organism with a Detected result will be listed in the corresponding field of the summary. If all of the organism assays were negative then 'None' will be displayed in the Detected field. Controls are listed as Passed, Failed, or Invalid. Table 2 provides additional information for each of the possible control field results.

Table 2. Interpretation of Controls Field on the BioFire RP2.1 PANEL (EUA) Test Report

Control Result	Explanation	Action
Passed	The run was successfully completed AND Both pouch controls were successful	None Report the results provided on the test report
Failed	The run was successfully completed BUT At least one of the pouch controls (RNA Process Control and PCR2 Control) failed	Repeat the test using a new pouch. If the error persists, contact Technical Support for further instruction
Invalid	The controls are invalid because	Note any error codes displayed during the run and the Run Status field in the

	the run did not complete. (Typically this indicates a software or hardware error).	Run Details section of the report. Refer to the appropriate BioFire Operator's Manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument.

2. Result Summary

The Result Summary section of the test report lists the result for each target tested by the panel. Possible results for each organism are Detected, Not Detected, or Invalid. Table 3 provides an explanation for each interpretation and any follow-up necessary to obtain a final result.

Table 3. Reporting of Results and Required Actions

Result	Explanation	Action
Detected[a]	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay (s) for the organism were POSITIVE (i.e., met the requirements for positive result described in the Assay Interpretation section above)	Report results
Not Detected	The run was successfully completed AND The pouch controls were successful (Passed) AND The assay (s) for the organism were NEGATIVE (i.e., did met the requirements for positive result described in the Assay Interpretation section above)	Report results
Equivocal	The run was successfully completed AND The pouch controls were successful (Passed) AND The combination of positive and negative assay results for Influenza A were inconclusive (see Table 1)	Retest the original specimen using a new pouch and report the results of the retest.
Invalid	The pouch controls were not successful (Failed) OR The run was not successful (Run status displayed: Aborted, Incomplete, Instrument error or software Error)	See Table 2, Interpretation of Control Field on the BioFire® Test Report for instruction.

[a] If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

3. Run Details

The **Run Details** section provides additional information about the run including: pouch information (type, lot number, and serial number), Run Status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test.

4. Change Summary

It is possible to edit the Sample ID once a run has completed. If this information has been changed, an additional section called **Change Summary** will be added to the test report. This Change Summary section lists the field that was changed, the original entry, the revised entry,

the operator that made the change, and the date that the change was made. Sample ID is the only field of the report that can be changed.

Change Summary				
Field	**Changed To**	**Changed From**	**Operator**	**Date**
[1]Sample ID	New Example Id	Old Example Id	Anonymous	06 Apr 2020

5. Reporting Results

a. Results are entered in MEDITECH under Laboratory, not Microbiology.
b. Click on Laboratory → Result Entry → Enter results → Enter Bar Code # or MT # → On the result entry table, Enter P under Quality Control; under all organisms you will see NOT DETECTED as default result. If the test is Negative for all organisms, hit F12 (save) and release result. If the test is positive for any organism (s), carefully select the positive organism (s), and enter DETECTED, hit F12, and release result.
c. Call back SARS-CoV-2, Flu, RSV or Bordetella pertussis positive result with documentation in MEDITECH.
d. Label test report with a label from the lab. Slip, initial, date and file in appropriate results binder.
e. Dispose specimens in Biohazard trash can.

REFERENCES:

BioFire® Respiratory Panel 2.1 (RP2.1) Instructions for Use, for Emergency Use Only (BFR0000-8303-01) BioFire Diagnostics, LLC.

DEFINITIONS: N/A

ATTACHMENTS: **N/A**

APPROVAL DATE:

N/A	**Policy, Procedure and Standards Committee**
7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
	Board of Supervisors **Approved by the Governing Body**

REPLACES: **N/A**

EFFECTIVE: _____

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Microbiology**

SUB SECTION: **Policies**

SUBJECT: **Antibiotics Reporting Guide**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

ANTIBIOTICS REPORTING GUIDE

TEST PRINCIPLE / PURPOSE

N/A

CLINICAL SIGNIFICANCE

N/A

SPECIMEN TYPE(S)

N/A

REQUIRED REAGENT(S)

N/A

CALIBRATION

N/A

QUALITY CONTROL

N/A

PROCEDURAL STEPS

I. Microscan Antibiotics Reporting Guide: Gram Negative Panel, Neg. MIC 53

Interpretive Breakpoints*

Antimicrobial Agents	Abbr.	Susceptible	Intermediate	Resistant
Amikacin	Ak	≤16	32	≥64
Amoxicillin/K Clavulanate - Enterobacteriaceae	Aug	≤8/4	16/8	≥32/16
Ampicillin[2] - Enterobacteriaceae and *V. cholerae*	Am	≤8	16	≥32
Ampicillin/Sulbactam - Enterobacteriaceae and *Acinetobacter* spp.	A/S	≤8/4	16/8	≥32/16
Aztreonam[3] - Enterobacteriaceae (CLSI M100-S19) and Non-Enterobacteriaceae	Azt	≤8	16	≥32
Cefazolin - Enterobacteriaceae (CLSI M100-S19)	Cfz	≤8	16	≥32
Cefepime[11]	Cpe	≤8	16	≥32
Cefotaxime[3] - Enterobacteriaceae (CLSI M100-S19) and Non-Enterobacteriaceae other than *P. aeruginosa*	Cft	≤8	16-32	≥64
Cefotaxime/K Clavulanate[6,7]	Cft/CA	--	--	--
Cefoxitin - Enterobacteriaceae	Cfx	≤8	16	≥32
Ceftazidime[2,3] - Enterobacteriaceae (CLSI M100-S19), Non-Enterobacteriaceae and *B. pseudomallei*	Caz	≤8	16	≥32
Ceftazidime/Avibactam	CZA			
Enterobacteriaceae: *C. freundii* complex, *C. koseri, E. aerogenes, E. cloacae, E. coli, K. oxytoca, K. pneumoniae, M. morganii, P. mirabilis, P. rettgeri, P. stuartii, S. marcescens*		≤8/4	--	≥16/4
P. aeruginosa		≤8/4	--	≥16/4
Ceftazidime/K Clavulanate[6,7]	Caz/CA	--	--	--
Ceftolozane/Tazobactam	C/T			
Enterobacteriaceae: *E. cloacae, E. coli, K. oxytoca, K. pneumoniae, P. mirabilis, C. koseri, M. morganii, P. vulgaris, P. rettgeri, P. stuartii, S. liquefaciens, S. marcescens*		≤2/4	4/4	≥8/4
P. aeruginosa		≤4/4	8/4	≥16/4
Ceftriaxone	Cax			
Enterobacteriaceae[9]		≤1	2	≥4
Non-Enterobacteriaceae other than *P. aeruginosa*		≤8	16-32	≥64
Ceftriaxone[3] - Enterobacteriaceae (CLSI M100-S19) and Non-Enterobacteriaceae other than *P. aeruginosa*	Cax	≤8	16-32	≥64
Cefuroxime axetil (oral) - Enterobacteriaceae	Crm	≤4	8-16	≥32
Cefuroxime sodium (parenteral) - Enterobacteriaceae	Crm	≤8	16	≥32
Ciprofloxacin[2] – Enterobacteriaceae[12] *Y. pestis* (CLSI M100-S17) and Non-Enterobacteriaceae	Cp	≤1	2	≥4
Ciprofloxacin-S *Salmonella* ser. Typhi	Cp-S	≤0.06	0.12-0.5	≥1
Doripenem[1]	Dor			
Enterobacteriaceae		≤0.5	--	--
A. baumannii		≤1	--	--
P. aeruginosa		≤2	--	--
Ertapenem – Enterobacteriaceae[10]	Etp	≤0.5	1	≥2

Antimicrobial Agents	Abbr.	Susceptible	Intermediate	Resistant
Ertapenem - Enterobacteriaceae (CLSI M100-S20)	Etp	≤2	4	≥8
Gentamicin[2] - Enterobacteriaceae, *Y. pestis* and Non-Enterobacteriaceae	Gm	≤4	8	≥16
Imipenem	Imp			
Enterobacteriaceae: *Citrobacter* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *M. morganii*, *P. vulgaris*, and *P. rettgeri*		≤1	2	≥4
P. aeruginosa		≤2	4	≥8
Acinetobacter spp.		≤2	4	≥8
Levofloxacin[8]	Lvx	≤2	4	≥8
Meropenem	Mer			
Enterobacteriaceae (CLSI M100-S20)		≤4	8	≥16
P. aeruginosa (CLSI M100-S21)		≤4	8	≥16
Non-Enterobacteriaceae		≤4	8	≥16
Minocycline	Min			
Enterobacteriaceae		≤4	8	≤16
Acinetobacter spp.		≤4	8	≤16
Moxifloxacin[1,4] - Enterobacteriaceae	Mxf	≤2	4	≥8
Nitrofurantoin[5] - Enterobacteriaceae	Fd	≤32	64	≥128
Piperacillin/Tazobactam	PT			
Enterobacteriaceae and Non-Enterobacteriaceae		≤16/4	32/4-64/4	≥128/4
P. aeruginosa (CLSI M100-S21)		≤64/4	--	≥128/4
Tetracycline[2] - Enterobacteriaceae, *V. cholerae*, *Y. pestis, B. pseudomallei,* and Non-Enterobacteriaceae other than *P. aeruginosa*	Te	≤4	8	≥16
Tigecycline[1] - Enterobacteriaceae	Tgc	≤2	4	≥8
Tobramycin	To	≤4	8	≥16
Trimethoprim/Sulfamethoxazole[2] - Enterobacteriaceae, *V. cholerae*, *Y. pestis, B. pseudomallei,* and Non-Enterobacteriaceae other than *P. aeruginosa*	T/S	≤2/38	--	≥4/76

*Based on Interpretive Breakpoints as indicated in CLSI Document M100-27th ed. or M45-A2. The antimicrobials included in this panel are not proven to be safe and effective in treating clinical infections for all organisms tested. For reporting of antimicrobial results which have shown to be active against organism groups *in vitro* or in clinical infections refer to CLSI M100, Tables 1 and 2 or the pharmaceutical package insert.

1. Based on manufacturer's breakpoints.
2. Interpretations for *V. cholerae*, *Y. pestis* or *B. pseudomallei* exist only for the antimicrobics designated.
3. Clinical isolates of *Klebsiella oxytoca*, *K. pneumoniae*, and *Escherichia coli* with increased MICs (≥2 µg/mL) of ceftazidime, aztreonam, cefotaxime, ceftriaxone, or MICs of ≥2 or ≥8 µg/mL (depending on panel type) of cefpodoxime should be suspected of harboring an extended-spectrum beta-lactamase. For *Proteus mirabilis* strains, only ceftazidime, cefotaxime, and cefpodoxime can be used for ESBL screening purposes.[17]
4. Only systemic therapy will be reported.
5. Only urine therapy will be reported (based on CLSI Document M100-S20 for cephalothin).
6. No breakpoints exist for this test.
7. Clinical isolates of *Klebsiella oxytoca*, *K. pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* with a ≥3 two-fold dilution drop (i.e. a 3 well decrease) in an MIC value for the antibiotic tested with clavulanic acid as compared to the MIC value of that antibiotic tested alone, are considered ESBL phenotypic-confirmation positive (CLSI).[23] For further details see the LabPro Operator's Guide.
8. Interpretations for *Salmonella* species are based on CLSI M100-S21.
9. Updated Enterobacteriaceae breakpoints are only implemented on panel types which minimally contain dilutions 1 and 2 µg/mL.

10. Updated Enterobacteriaceae breakpoints are only implemented on panel types which minimally contain dilutions 0.5 and 1 µg/mL.
11. Interpretations for Enterobacteriaceae are based on CLSI M100-S23.
12. Interpretations for *Salmonella* species (other than *Salmonella* ser. Typhi) are based on CLSI M100-S21. Refer to Cp-S for reporting of *Salmonella* ser. Typhi.

II. Microscan Antibiotics Reporting Guide: Gram Positive Panel, Pos. MIC 38

Interpretive Breakpoints*

Antimicrobial Agents	Abbr.	Susceptible	Intermediate	Resistant
Amoxicillin/K Clavulanate[5,6] – Staphylococci (CLSI M100-S22)	Aug	≤4/2	-	≥8/4
Ampicillin	Am			
Staphylococci (CLSI M100-S22)		≤0.25	-	≥0.5
L. monocytogenes[2] (CLSI M45-A2)		≤2	-	-
Enterococci		≤8	-	≥16
β-hemolytic Streptococci (*S. agalactiae*, Group B)[2]		≤0.25	-	-
Viridans Group Streptococci (*S. bovis* group)		≤0.25	0.5-4	≥8
Ampicillin/Sulbactam[5,6] – Staphylococci (CLSI M100-S22)	A/S	≤8/4	16/8	≥32/16
Azithromycin[3] - Staphylococci	Azi	≤2	4	≥8
Cefazolin[5] – Staphylococci (CLSI M100-S22)	Cfz	≤8	16	≥32
Cefepime[5] – Staphylococci (CLSI M100-S22)	Cpe	≤8	16	≥32
Cefotaxime[5] – Staphylococci (CLSI M100-S22)	Cft	≤8	16-32	≥64
Ceftaroline[1] – *S. aureus*[7]	Cpt	≤1	2	≥4
Ceftriaxone[5] – Staphylococci (CLSI M100-S22)	Cax	≤8	16-32	≥64
Cefuroxime axetil (oral) – Staphylococci (CLSI M100-S22)	Crm	≤4	8-16	≥32
Cefuroxime sodium (parenteral) – Staphylococci (CLSI M100-S22)	Crm	≤8	16	≥32
Cephalothin[5] – Staphylococci (CLSI M100-S22)	Cf	≤8	16	≥32
Ciprofloxacin - Staphylococci and Enterococci	Cp	≤1	2	≥4
Clindamycin[3]	Cd			
Staphylococci		≤0.5	1-2	≥4
β-hemolytic Streptococci (*S. agalactiae*, Group B)		≤0.25	0.5	≥1
Viridans Group Streptococci (*S. bovis* group)		≤0.25	0.5	≥1
Daptomycin[2]	Dap			
Staphylococci		≤1	-	-
Enterococci		≤4	-	-
β-hemolytic Streptococci (*S. agalactiae*, Group B)		≤1	-	-
Viridans Group Streptococci (*S. bovis* group)		≤1	-	-
Erythromycin[3]				
Staphylococci and Enterococci	E	≤0.5	1-4	≥8
β-hemolytic Streptococci (*S. agalactiae*, Group B)		≤0.25	0.5	≥1
Gentamicin - Staphylococci	Gm	≤4	8	≥16
Imipenem[5] – Staphylococci (CLSI M100-S22)	Imp	≤4	8	≥16
	Lvx			
Levofloxacin		≤2	4	≥8
Staphylococci (CLSI M100-S14) and Enterococci		≤2	4	≥8

β-hemolytic Streptococci (*S. agalactiae*, Group B) Viridans Group Streptococci (*S. bovis* group)		≤2	4	≥8
Linezolid	Lzd			
Staphylococci[2] (CLSI M100-S19)		≤4	-	-
Enterococci		≤2	4	≥8
β-hemolytic Streptococci (*S. agalactiae*, Group B)[2]		≤2	-	-
Viridans Group Streptococci (*S. bovis* group)[2]		≤2	-	-
Meropenem[3,5] – Staphylococci (CLSI M100-S22)	Mer	≤4	8	≥16
Nitrofurantoin[4] - Staphylococci and Enterococci	Fd	≤32	64	≥128
Oxacilline	Ox			
Coagulase - négative Staphylococcie		≤0.25	-	≥0.5
S. aureus/S. lugdunensis		≤2	-	≥4
Penicillin G	P			
Staphylococci		≤0.12	-	≥0.25
L. monocytogenes[2] (CLSI M45-A2)		≤2	-	-
Enterococci		≤8	-	≥16
β-hemolytic Streptococci (*S. agalactiae*, Group B)[2]		≤0.12	-	-
Viridans Group Streptococci (*S. bovis* group)		≤0.12	0.25-2	≥4
Piperacillin/Tazobactam[6] – Staphylococci (CLSI M100-S22)	P/T	≤8/4	-	≥16/4
Rifampin - Staphylococci and Enterococci	Rif	≤1	2	≥4
Synercid - Staphylococci	Syn	≤1	2	≥4
Tetracycline	Te			
Staphylococci and Enterococci		≤4	8	≥16
β-hemolytic Streptococci (*S. agalactiae*, Group B)		≤2	4	≥8
Viridans Group Streptococci (*S. bovis* group)		≤2	4	≥8
Tigecycline[1,8] – *S. aureus* and *S. epidermidis*[9]	Tgc	≤0.5	-	-
E. faecalis (Vancomycin-susceptible isolates)		≤0.25	-	-
Trimethoprim/Sulfamethoxazole - Staphylococci	T/S	≤2/38	-	≥4/76
Vancomycin	Va			
Staphylococci and Enterococci		≤4	8-16	≥32
S. aureus		≤2	4-8	≥16

* Based on Interpretative Breakpoints as indicated in CLSI Document M100, 27th ed. There are antimicrobials included in this panel that are not proven to be safe and effective in treating clinical infections for all organisms tested. For reporting of antimicrobial results which have shown to be active against organism groups *in vitro* or in clinical infections refer to CLSI M100, Tables 1 and 2 or the pharmaceutical package insert.

1. Based on manufacturer's breakpoints.
2. The absence of resistant strains precludes the CLSI from defining any result categories other than "Susceptible" at this time. Strains yielding results suggestive of a "non-susceptible" category should be submitted to a reference laboratory for further testing.
3. Only systemic therapy will be reported.
4. Only urine therapy will be reported.
5. For streptococci, refer to the penicillin result.
6. For beta-lactamase negative enterococci, refer to the penicillin result.
7. Ceftaroline should be used for methicillin-susceptible and methicillin-resistant *S. aureus* isolates from skin and skin structure infections and methicillin-susceptible *S. aureus* isolates from community acquired bacterial pneumonia.
8. The current absence of resistant isolates precludes defining any category other than Susceptible. Isolates yielding MIC results suggestive of a Nonsusceptible category should be subjected to additional testing.
9. There are no CLSI/FDA Interpretive Breakpoints for *S. epidermidis*. Due to the lack of CLSI/FDA interpretive breakpoints for *S. epidermidis*, overall performance of tigecycline was determined using *S. aureus* breakpoints for *S. epidermidis*.

Cefoxitin Screen Interpretive Criteria

Test	Negative	Positive
Cefoxitin Screen Well	≤4	>4

The MicroScan Cefoxitin Screen Well is intended to determine the susceptibility of *S. aureus* and *S. lugdunensis* to the penicillinase-stable beta-lactams (e.g. oxacillin), using the Cefoxitin Screen Well (CfxS) and the oxacillin MIC result at 16-20 hours. The CfxS result and oxacillin MIC are read independently at 16-20 hours and then processed through the LabPro software or interpreted manually to determine the final interpretation to oxacillin. The interpretation rules are shown in the following table:

		Final Oxacillin Interpretation
CfxS Result	Oxacillin MIC	*S. aureus* or *S. lugdunensis*
≤ 4 µg/mL Negative	≤0.25	S
	0.5	S
	1 or 2	S
	>2	R
> 4 µg/mL Positive	≤0.25	R*
	0.5	R*
	1 or 2	R*
	>2	R

Interpretations of R* are used by the LabPro software when the Cefoxitin Screen Well result changes the interpretation of the oxacillin MIC result. These criteria should also be followed when interpreting the results manually; however, the asterisk is not required.

Inducible Clindamycin

The MicroScan Inducible Clindamycin test is intended to detect inducible clindamycin resistance in staphylococci resistant or intermediate to erythromycin and susceptible or intermediate to clindamycin. Expression of resistance due to the erm gene may require induction by erythromycin. Results of ICd are equivalent to the D-zone disk approximation test. The interpretive criteria are shown in the following table:

Antimicrobial Test	**Negative**	**Positive**
Inducible Clindamycin Test - Staphylococci	≤4/0.5 µg/mL	> 4/0.5 µg/mL

When erythromycin is I or R and clindamycin is S or I, and the ICd test is positive, clindamycin should be reported as resistant.

Streptomycin and Gentamicin Synergy Screen

In enterococcal endocarditis the use of penicillin or ampicillin alone results in frequent treatment failures. When enterococci are susceptible in vitro to high levels of streptomycin or gentamicin, the addition of this antimicrobic to penicillin or ampicillin is synergistic and correlates clinically with an improved cure rate.[23,26] According to CLSI Document M07-A9, the recommended method for detection of high level aminoglycoside resistance (HLAR) for broth microdilution is as follows:

Antimicrobic Concentration	**Medium**	**Incubation**
Gentamicin 500 µg/mL	BHI*	24 hours
Streptomycin 1000 µg/mL	BHI*	24 - 48 hours

* Comparable results have been shown in limited testing with dextrose phosphate broth.

The performance of Gentamicin and Streptomycin Synergy Screens on the MicroScan Panels was compared to the microbroth reference methods recommended by the CLSI. Any evidence of turbidity should be considered growth or reincubated to confirm results. The results obtained with Gentamicin Synergy after 18 hours incubation were comparable to those obtained at 24 hours with the reference method. For best detection of resistance with Streptomycin Synergy Screen, MicroScan Panels should be incubated for 24 to 48 hours.

Thymidine Free Growth Well

Some bacteria require thymidine for growth. These bacteria may exhibit false susceptibility to the sulfonamides due to lack of thymidine in the Mueller-Hinton broth. If an organism does not grow in the TFG well, an MIC should not be reported for T/S.

Daptomycin

Daptomycin includes the CLSI recommended calcium supplementation. No additional supplementation is required.

III. Microscan Antibiotics Reporting Guide: MICroSTREP *plus* Type 1 Panel

Streptococci Interpretive Criteria

Antimicrobial Agents	Abbr.	Susceptible	Intermediate	Resistant
Amoxicillin/K Clavulanate				
S. pneumoniae	Aug	≤2/1	4/2	≥8/4
Ampicillin				
β-hemolytic streptococci[1]	Am	≤0.25	-	-
viridans streptococci		≤0.25	0.5 – 4	≥8
Azithromycin[3,5]	Azi	≤0.5	1	≥2
Cefaclor				
S. pneumoniae	Cfr	≤1	2	≥4
Cefepime				
S. pneumoniae	Cpe	≤1	2	≥4
β-hemolytic streptococci[1]		≤0.5	-	-
viridans streptococci		≤1	2	≥4
Cefotaxime				
S. pneumoniae (meningitis)[2,4]	Cft	≤0.5	1	≥2
S. pneumoniae (non-meningitis)[2,4]		≤1	2	≥4
β-hemolytic streptococci[1]		≤0.5	-	-
viridans streptococci		≤1	2	≥4
Ceftriaxone				
S. pneumoniae (meningitis)[2,4]	Cax	≤0.5	1	≥2
S. pneumoniae (non-meningitis)[2,4]		≤1	2	≥4
β-hemolytic streptococci[1]		≤0.5	-	-
viridans streptococci		≤1	2	≥4
Cefuroxime axetil (oral)				
S. pneumoniae	Crm	≤1	2	≥4
Cefuroxime sodium (parenteral)				
S. pneumoniae	Crm	≤0.5	1	≥2
Chloramphenicol[3]				
S. pneumoniae	C	≤4	-	≥8
Streptococcus spp. other than *S. pneumoniae*		≤4	8	≥16
Clindamycin[3]	Cd	≤0.25	0.5	≥1
Erythromycin[3,5]	E	≤0.25	0.5	≥1
Levofloxacin	Lvx	≤2	4	≥8
Meropenem				
S. pneumoniae[4]	Mer	≤0.25	0.5	≥1
viridans streptococci[1]		≤0.5	-	-
Penicillin				
S. pneumoniae (meningitis) [2,4]	P	≤0.06	-	≥0.12
S. pneumoniae (non-meningitis) [2,4]		≤2	4	≥8
S. pneumoniae (oral penicillin V)		≤0.06	0.12 – 1	≥2
β-hemolytic streptococci[1]		≤0.12	-	-
viridans streptococci		≤0.12	0.25 – 2	≥4
Tetracycline[6]	Te	≤2	4	≥8
Trimethoprim/Sulfamethoxazole				
S. pneumoniae	T/S	≤0.5/9.5	1/19 – 2/38	≥4/76
Vancomycin[1,4]	Va	≤1	-	-

1. For some organism/antimicrobial combinations the absence or rare occurrence of resistant strains precludes defining any results categories other than “susceptible.” For strains yielding results suggestive of a “nonsusceptible” category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory that will confirm results using a CLSI reference dilution method.

2. For *S. pneumoniae* isolated from cerebral spinal fluid (CSF), report only meningitis interpretations. For *S. pneumoniae* isolated from all specimens other than CSF, report interpretations for both meningitis and non-meningitis.
3. Not routinely reported on isolates from the urinary tract.
4. Only results of testing with penicillin, vancomycin, and cefotaxime or ceftriaxone or meropenem should be reported routinely for CSF isolates of *S. pneumoniae*.

5. Susceptibility and resistance to azithromycin, clarithromycin, and dirithromycin can be predicted by testing erythromycin.
6. Interpretations for *S. pneumoniae* are based on CLSI M100-S22.

NOTE: Results may be inaccurate if specified criteria are applied to organisms other than those listed.

NOTE: Streptococci susceptible to penicillin can be considered susceptible to other beta-lactam antimicrobics (e.g. cephalosporins, ampicillin, etc.).

NOTE: Interpretative criteria listed in the labeling may differ from the criteria in the LabPro Information Manager due to differences in software and panel releases.

IV. Gram Negative Kirby Bauer disc diffusion Test Reporting Guide: Interpretive Categories and Zone Diameter Breakpoints

Available antimicrobial Agents		*Entrobacteriaceae spp.*			*P. aeruginosa*			*Acinetobacter Spp.*			*Burkholderia cepaciae*			*Stentrop. Multophilia*			*Aeromonas Spp.*		
	code	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Amikacin	AN-30	≥17	15-16	≤ 14	≥17	15-16	≤ 14	≥17	15-16	≤ 14							≥17	15-16	≤14
Ampicillin	AM-10	≥17	14-16	≤13															
Ampicillin/Sulbactam	SAM-20	≥15	12-14	≤11				≥15	12-14	≤11									
Aztreonam	ATM-30	≥21	18-20	≤17	≥22	16-21	≤15										≥21	18-20	≤17
Cefepime	FEP-30	≥25	19-24	≤18	≥18	15-17	≤14	≥18	15-17	≤14							≥18	15-17	≤14
Cefotaxime	CTX-30	≥26	23-25	≤22				≥23	15-22	≤14							≥26	23-25	≤22
Ceftazidime	CAZ-30	≥21	18-20	≤17	≥18	15-17	≤14	≥18	15-17	≤14	≥21	18-20	≤17				≥21	18-20	≤17
Ceftazidime/Avibactam	CZA-50	≥21	-	≤20	≥21	-	≤20												
Ceftolozane/Tazobactam	C/T-40	≥21	18-20	≤17	≥21	17-20	≤16												
Ceftriaxone	CRO-30	≥23	20-22	≤19				≥21	14-20	≤13							≥23	20-22	≤19
Cefuroxime	CXM-30	≥18	15-17	≤14															
Ciprofloxacin	CIP-5	≥26	22-25	≤21	≥25	19-24	≤18	≥21	16-20	≤15							≥21	16-20	≤15
Ertapenem	ETP-10	≥22	19-21	≤18															
Gentamicin	GM-10	≥15	13-14	≤12	≥15	13-14	≤12	≥15	13-14	≤12							≥15	13-14	≤12
Imipenem	IPM-10	≥23	20-22	≤19	≥19	16-18	≤15	≥22	19-21	≤18							≥16	14-15	≤13
Levofloxacin	LVX-5	≥21	17-20	≤16	≥22	15-21	≤14	≥17	14-16	≤13				≥17	14-16	≤13	≥17	14-16	≤13
Meropenem	MEM-10	≥23	20-22	≤19	≥19	16-18	≤15	≥18	15-17	≤14	≥20	16-19	≤15				≥16	14-15	≤13
Nitrofurantoin	F/M-300	≥17	15-16	≤14															
Piperacillin/Tazobactam	TZP-110	≥21	18-20	≤17	≥21	15-20	≤14	≥21	18-20	≤17							≥21	18-20	≤17
Tetracycline	Te-30	≥15	12-14	≤11				≥15	12-14	≤11							≥15	12-14	≤11
Tobramycin	NN-10	≥15	13-14	≤12	≥15	13-14	≤12	≥15	13-14	≤12									
Trimethoprim/Sulfamethoxazole	SXT	≥16	11-15	≤10				≥16	11-15	≤10	≥16	11-15	≤10	≥16	11-15	≤10	≥16	11-15	≤10

For All Other Non-fermenters- Disc diffusion testing is not recommended, INTERPRETATION AVAILABLE IN MIC ONLY PER FDA/CLSI

V. Gram positive Kirby Bauer disc diffusion Test Reporting Guide

Interpretive Categories and Zone Diameter Breakpoints

Available antimicrobial Agents		Staphylococcus spp.			Enterococcus spp.			Streptococcus Viridans spp.[1,2]			Streptococcus B-Hemolytic Group[3,4]			Moraxella catarrhalis			Streptococcus pneumoniae		
	code	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin	AM-10	≥29	-	≤28	≥17	-	≤16	MIC ONLY			≥24	-	-						
Cefotaxime	CTX-30							≥28	26-27	≤25	≥24	-	-						
Chloramphenicol	C-30	≥18	13-17	≤12	≥18	13-17	≤12	≥21	18-20	≤17	≥21	18-20	≤17						
Clindamycin	CC-2	≥21	15-20	≤14				≥19	16-18	≤15	≥19	16-18	≤15						
Ciprofloxacin	CIP-5	≥21	16-20	≤15	≥21	16-20	≤15												
Erythromycin	EE-15	≥23	14-22	≤13	≥23	14-22	≤13	≥21	16-20	≤15	≥21	16-20	≤15	≥21	-	-			
Oxacillin	OX-1	≥18	-	≤17													≥20*	≤19[5]	
Penicillin	P-10	≥29	-	≤28	≥15	-	≤14	MIC ONLY			≥24	-	-						
Tetracycline	Te-30	≥19	15-18	≤14	≥19	15-18	≤14	≥23	19-22	≤18	≥23	19-22	≤18	≥29	25-28	≤24			
Trimethoprim/ Sulfamethoxazole	SXT	≥16	11-15	≤10										≥13	11-12	≤10			
Vancomycin	Va-30	MIC ONLY			≥17	15-16	≤14	≥17	-	-	≥17	-	-						
Nitrofurantoin	F/M300	≥17	15-16	≤14	≥17	15-16	≤14												

[1] PERFORM MICroSTREP *plus*, Type 1 Panel ON ISOLATES FROM NORMALLY STERILE SITES (CSF, BLOOD, BONE, BODY FLUIDES)

[2] INCLUDES mutans group, salvarious group, bovis group, anginosus group (previously S. milleri group) and mitis group

[3] PERFORM GRAM POS. MIC 38 PANEL ON GROUP B STEREP SPP.

[4] INCLUDES GROUP A (S. pyogenes)*, C, OR G.* SMALL COLONY FORMING B-HEMOLYTIC GROUPA, C, F, OR G ANTIGENS ARE CONSIDERED PART OF THE VIRIDANS GROUP

[5] DO NOT REPORT AS RESISTANT. PERFORM MIC TO CONFIRM

*Can predict susceptibility to ampicillin, ampicillin-sulbactam, cephalosporin, carbapenems.

VI. Gram Negative Fastidious Organisms Kirby Bauer disc diffusion Test Reporting Guide

Interpretive Categories and Zone Diameter Breakpoints

Available antimicrobial Agents		Pasteurella spp.			Hemophilus and Parainfluenzae spp.			Neisseria meningitidis			Neisseria gonorrhoeae		
	code	S	I	R	S	I	R	S	I	R			
Ampicillin	AM-10	≥27	-	-	≥22	19-21	≤18	MIC ONLY					
Ampicillin/ Sulbactam	SAM-20				≥20	-	≤19						
Azithromycin								≥20	-	-			
Cefotaxime	CTX-30				≥26	-	-	≥34	-	-			
Ceftriaxone	CRO-30	≥34	-	-	≥26	-	-	≥34					
Cefuroxime	CXM-30				≥20	17-19	≤16						
Chloramphenicol	C-30	≥28	-	-	≥29	26-28	≤25	≥26	20-25	≤19			
Ciprofloxacin	CIP-5				≥21	-	-	≥35	33-34	≤32			
Erythromycin	EE-15	≥27	25-26	≤24									
Meropenem	MEM-10				≥20	-	-	≥30	-	-			
Penicillin	P-10	≥25	-	-	X	X	x	MIC ONLY					
Tetracycline	Te-30	≥23	-	-	≥29	26-28	≤25						
Trimethoprim/ Sulfamethoxazole	SXT	≥24	-	-	≥16	11-15	≤10	≥30	26-29	≤25			
Oxacillin	OX1												
Cefinase test					Hemophilus –rapid test for AM resistance						POS/NEG		

VII. Gram-Negative and Gram-Positive Anaerobic Organisms E-Test MIC Reporting Guide

Antimicrobial Agents	Interpretive Categories and MIC Breakpoints, ug/mL		
	S	I	R
Penicillin	≤0.5	1	≥2
Clindamycin	≤2	4	8
Imipenem	≤4	8	≥16
Metronidazole	≤8	16	≥32
Ampicillin/Sulbactam	≤8/4	16/8	≥32/16
Piperacillin/Tazobactam	≤16/4	32/4 - 64/4	≥128/4

XI. *Campylobacter spp*. E-Test MIC Reporting Guide

Interpretive Categories and Zone Diameter and E-Test Breakpoints

Antimicrobial Agents	Zone Diameter Interpretive Criteria			Interpretive Categories and MIC Breakpoints, ug/Ml RUO		
	S	I	R	S	I	R
Erythromycin	–	–	6	≤8	16	≥32

XII. Testing Conditions and Quality Control for Kirby Bauer Disk Diffusion or E-Test Susceptibility tests on Frequently Isolated or Fastidious Bacteria

Organism/Organism Group	Media	Inoculum McFarland	Incubation Condition	Quality Control
Entrobacteriaceae *PSA*	MHA	0.5	35ºC ± 2ºC, Ambient Air for 16-18 hours	*E.coli ATCC 25922* *PSA ATCC 27853*
Acinitobacter *Burkholderia* *Stenotrophomonas*	MHA	0.5	35ºC ± 2ºC, Ambient Air for **20-24** hours	*E.coli ATCC 25922* *PSA ATCC 27853*
Non- Entrobacteriaceae	MHA	0.5	35ºC ± 2ºC, Ambient Air for 16-20 hours	*E.coli ATCC 25922* *PSA ATCC 27853*
Staphylococcus spp. *Entrococcus spp*	MHA	0.5	35ºC ± 2ºC, Ambient Air for 24 hours	*S.aureus ATCC 25923*
Haemophilus flu and H. paraflu	HTM	0.5	35ºC ± 2ºC, 5% CO_2 16-18 hours	*H.flu ATCC 49247* *H.flu ATCC 49766*
Streptococcus pneumoniae *Strep Beta Hemolytic Group* *Streptococcus spp. Viridans Group* *Nisseria meningitidis*	MHA with 5% Blood	0.5	35ºC ± 2ºC, 5% CO_2 20-24 hours	*S. pnemoniae ATCC49619*
Anaerobes	Brucella Agar +5% blood+ Vitamin K and Hemin	1 MH Broth	35ºC ± 2ºC, anaerobically, 24 – 72 hours	*B. fragilis ATCC 25285* *B.thetaiotaomicron ATCC 29741*
Campylobacter Spp.	MHA + 5% blood	1 BHI Broth	42ºC, Microaerophilic, 24 hours or 35ºC ± 2ºC 48 – 72 hours	*S.aureus ATCC 25923*
Moraxella catarrhalis	MHA	0.5	35ºC ± 2ºC, 5% CO_2 20-24 hours	*S.aureus ATCC 29213*
Pasturella spp.	MHA with 5% Blood	0.5	35ºC ± 2ºC, Ambient Air for 16-18 hours	*S.aureus ATCC 25923*

MHA, Muller-Hinton Agar; BHI, Brain Heart Infusion; HTM, Haemophilus Test Media

CALCULATIONS

N/A

REFERENCE INTERVALS

N/A

INTERPRETATION OF RESULTS

N/A

REFERENCES:

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2. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guidelines-Second Edition. Clinical and Laboratory Standards Institute (CLSI), M45-A2, Vol.30, No.18, 09/06/2012.

3. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. Clinical and Laboratory Standards Institute (CLSI), M100 S24, Vol. 34 No.1, 2014.

4. Performance Standards for Antimicrobial Susceptibility Testing, Clinical and Laboratory Standards Institute (CLSI), M100, 29th Edition, 02/05/2019.

5. Package Insert, Dried Gram Pos Procedure Manual, Beckman Coulter, 2019.

6. Package Insert, Dried Gram Neg Procedure Manual, Beckman Coulter, 2019

7. Package Insert, MICroSTREP *plus* Type 1 Panel, Beckman Coulter, 2019

DEFINITIONS: **N/A**

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES:

EFFECTIVE: _____

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off P**

SECTION: **Microbiology- Molecular Diagnostics**

SUB SECTION: **Procedure**

SUBJECT: **Xpert® Xpress SARS-CoV-2/Flu/RSV**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

Xpert® Xpress SARS-CoV-2/Flu/RSV

TEST PRINCIPLE / PURPOSE

The Xpert Xpress SARS-CoV-2/Flu/RSV test is a rapid, multiplexed real-time RT-PCR test intended for the simultaneous qualitative detection and differentiation of SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA in either nasopharyngeal swab, nasal swab or nasal wash/aspirate specimens collected from individuals suspected of respiratory viral infection consistent with COVID-19 by their healthcare provider. [1] Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar due to SARS-CoV-2, influenza, and RSV can be similar.

Testing of nasopharyngeal swab, nasal swab, or nasal wash/aspirate specimens using the Xpert Xpress SARS-CoV-2/Flu/RSV test run on the GeneXpert Dx and GeneXpert Infinity systems is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high and moderate complexity tests.

Testing of nasopharyngeal or nasal swab specimens using the Xpert Xpress SARS-CoV-2/Flu/RSV test run on the GeneXpert Xpress System (Tablet and Hub Configurations) is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

Results are for the simultaneous detection and differentiation of SARS-CoV-2, influenza A virus, influenza B virus and RSV nucleic acids in clinical specimens and is not intended to detect influenza C virus. SARS-CoV-2, influenza A, influenza B and RSV RNA identified by this test are generally detectable in upper respiratory specimens during the acute phase of infection.

Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test.

Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive SARS-CoV-2 results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus and/or RSV infection and should not be used as the sole basis for treatment or other patient management decisions. Negative

results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Xpert Xpress SARS-CoV-2/Flu/RSV test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx, GeneXpert Infinity and/or GeneXpert Xpress systems. The Xpert Xpress SARS-CoV-2/Flu/RSV test is only for use under the Food and Drug Administration's Emergency Use Authorization.

CLINICAL SIGNIFICANCE

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019.1 Chinese authorities identified a novel coronavirus (2019-nCoV), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and exported cases in several Southeast Asian countries and more recently the United States. Cases of severe illness and some deaths have been reported. The International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily airborne (i.e., coughing or sneezing) and the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, malaise, cough, and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting or diarrhea) may also occur, primarily in children, but are less common. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication due to influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations.

Influenza viruses are classified into types A, B, and C, the former two of which cause the most human infections. Influenza A (Flu A) is the most common type of influenza virus in humans and is generally responsible for seasonal flu epidemics and potentially pandemics. Flu A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B (Flu B) virus are generally restricted to humans and less frequently cause epidemics. Flu A viruses are further divided into subtypes based on two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by subtypes H1, H2, H3, N1 and N2.

Respiratory Syncytial Virus (RSV), a member of the *Pneumoviridae* family (formerly *Paramyxoviridae*), consisting of two strains (subgroups A and B) is also the cause of a contagious disease that affects primarily infants, and the elderly who are immunocompromised (e.g., patients with chronic lung disease or undergoing treatment for conditions that reduce the strength of their immune system). The virus can remain infectious for hours on countertops and toys and can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia. By the age of two years, most children have already been infected by RSV and because only weak immunity develops, both children and adults can be re-infected. Symptoms appear four to six days after infection and are usually self-limiting, lasting approximately one to two weeks in infants. In adults, infection lasts about 5 days and presents as symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season mirrors influenza somewhat as infections begin to rise during the fall through early spring.

Active surveillance programs in conjunction with infection prevention precautions are important components for preventing transmission of SARS-CoV-2, influenza, and RSV. The use of assays providing rapid results to identify patients infected with these viruses can be an important factor for effective control, proper choice of treatment, and prevention of widespread outbreaks.

The Xpert Xpress SARS-CoV-2/Flu/RSV test is a molecular *in vitro* diagnostic test that aids in the detection and differentiation of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus and is based on widely used nucleic acid amplification technology. The Xpert Xpress SARS-CoV-2/Flu/RSV test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection and

differentiation of RNA from Flu A, Flu B, RSV and SARS-CoV-2 virus in upper respiratory specimens.

SPECIMEN TYPE(S)

I. Specimen Types

A. Nasopharyngeal Swab in Viral Transport Medium (3 mL)

B. Nasal Swab

C. Nasal wash/aspirate specimens.

1. Specimen Collection, Transport, and Storage

Nasopharyngeal swab, nasal swab, and nasal wash/aspirate specimens can be stored at room temperature (15-30 °C) for up to 24 hours in viral transport medium until testing is performed on the GeneXpert Instrument Systems. Alternatively, nasopharyngeal swab, nasal swab, and nasal wash/aspirate specimens can be stored refrigerated (2–8 °C) up to seven days in viral transport medium until testing is performed on the GeneXpert Instrument Systems. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.

2. Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx. Rotate swab by firmly brushing against the nasopharynx several times. Remove and place the swab into a viral transport tube (3 mL). Break swab at the indicated break line and cap the specimen collection tube tightly.

3. Nasal Swab Collection Procedure

Insert a nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril to avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril. Remove and place the swab into the tube containing 3 mL of viral transport medium. Break swab at the indicated break line and cap the specimen collection tube tightly.

4. Nasal Wash/Aspirate Collection Procedure

Using a clean 300 µL transfer pipette (supplied), transfer 600 µL of the sample (two draws, using the same transfer pipette) into the 3 mL Xpert Viral Transport Medium tube and then cap the tube.

REQUIRED REAGENT(S)

The Xpert Xpress SARS-CoV-2/Flu/RSV kit contains sufficient reagents to process 10 specimens

or quality control samples. The kit contains the following:

I. Bead 1, Bead 2, and Bead 3 (freeze-dried) 1 of each per cartridge

II. Lysis Reagent 1.5 mL per cartridge

III. Binding Reagent 1.5 mL per cartridge

IV. Elution Reagent 3.0 mL per cartridge

V. Disposable Transfer Pipettes 12 per kit

VI. CD 1 per kit

A. Assay Definition File (ADF)
B. Instructions to import ADF into GeneXpert software

VII. Storage and Handling

A. Store the Xpert Xpress SARS-CoV-2/Flu/RSV cartridges at 2-28°C.
B. Do not open a cartridge lid until you are ready to perform testing.
C. Do not use a cartridge that is wet or has leaked.

CALIBRATION N/A

QUALITY CONTROL

I. Internal Controls

A. Sample Processing Control (SPC)
Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC). Sample Processing Control (SPC) – Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

B. Probe Check Control (PCC)
Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

II. External Controls

External controls in the form of inactivated virus(es) are available from ZeptoMetrix (Buffalo, NY).

A. External Positive Control: Catalog #NATFRC-6C (NATtrol Flu/RSV/SARS-CoV-2)

B. External Negative Control: Catalog #NATCV9-6C (Coxsackievirus CVA9)
C. Report all out of range results to the Microbiolgy Supervisor or Tech III for corrective action. Control results are reviewed for acceptability before reporting patient results.

PROCEDURES

I. Preparing the cartridge

A. Remove a cartridge from the package.

B. Check the specimen transport tube is closed.

C. Mix specimen by rapidly inverting the specimen transport tube 5 times or vortex. Open cap on the specimen transport tube.

D. Open the cartridge lid.

E. Remove the transfer pipette from the wrapper.

F. Squeeze the top bulb of the transfer pipette completely and then place the pipette tip in the specimen transport tube (see Figure 2).

Important: Start the test within 30 minutes of adding the sample to the cartridge.

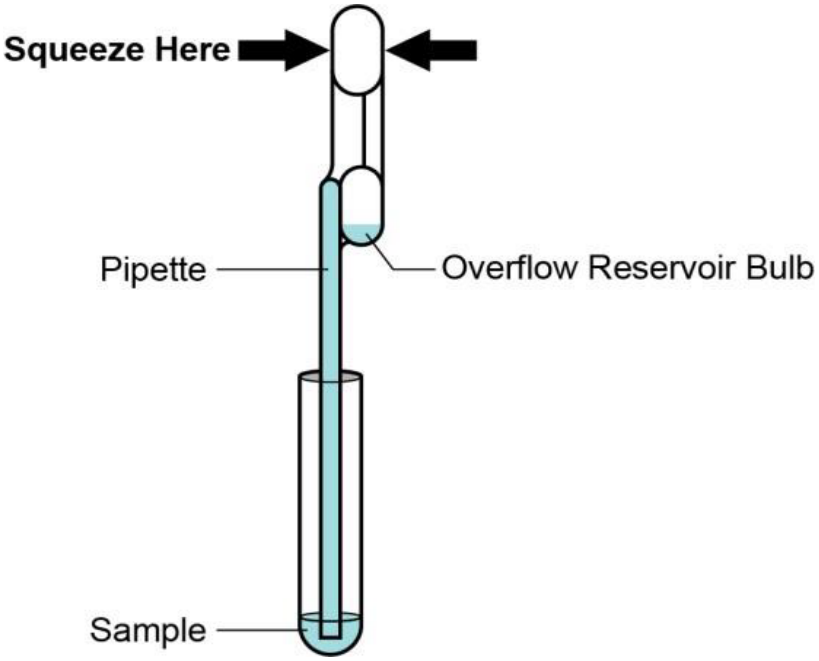

Figure 1. Nasopharyngeal Swab Collection

G. Release the top bulb of the pipette to fill the pipette before removing from the tube. After filling pipette, excess sample will be seen in the overflow reservoir bulb of the pipette (see Figure 2). Check that the pipette does not contain bubbles.

H. To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette into the large opening (Sample Chamber) in the cartridge shown in Figure 3. Dispose of the used pipette.

Figure 2. Xpert Xpress SARS-CoV-2 /Flu/RSV Cartridge (Top View)

I. Close the cartridge lid.

Note Take care to dispense the entire volume of liquid into the Sample Chamber. False negative results may occur if insufficient sample is added to the cartridge.

II. Starting the Test

A. Turn on the GeneXpert Instrument System:
Turn on the instrument and then turn on the computer. Log into the Windows operating system. The GeneXpert software may launch automatically or may require double - clicking on the GeneXpert Dx shortcut icon on the Windows® desktop. Log on to the System software. The login screen appears. Type the username and password.
B. Click Create Test
C. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is shown on the left side of the View Results window and is associated with the test result.
D. Scan the barcode on the Xpert Xpress SARS-CoV-2 /Flu/RSV cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, Expiration Date and Selected Assay.

Note If the barcode on the Xpert Xpress SARS-CoV-2 /Flu/RSV cartridge does not scan, then repeat the test with a new cartridge.

E. Click Start Test
F. Locate the module with the blinking green light, open the instrument module door and load the cartridge.
G. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off and the door will unlock. Remove the cartridge.
H. Dispose of used cartridges in red biohazard trash can.

III. Retests

A. Reasons to Repeat the Assay
If any of the test results mentioned below occur, repeat the test once according to instructions in for Retest Procedure.

1. A PRESUMPTIVE POSITIVE indicates the 2019 novel coronavirus (SARS-CoV-2) nucleic acids may be present.

2. An INVALID result indicates that the control SPC failed. The sample was not properly processed, PCR is inhibited, or the sample was not properly collected.
3. An ERROR result could be due to, but not limited to, Probe Check Control failure, system component failure, or the maximum pressure limits were exceeded.
4. A NO RESULT indicates that insufficient data were collected. For example, cartridge failed integrity test, the operator stopped a test that was in progress, or a power failure occurred.
    a. Put on a clean pair of gloves. Obtain a new Xpert Xpress SARS-CoV-2 cartridge and a new transfer pipette.
    b. Check the specimen transport tube or external control tube is closed.
    c. Mix the sample by rapidly invert the specimen transport medium tube or external control tube 5 times. Open the cap on the specimen transport tube or external control tube.
    d. Open the cartridge lid.
    e. Using a clean transfer pipette (supplied), transfer sample (one draw) to the sample chamber with the large opening in the cartridge.
    f. Close the cartridge lid.

If an External Control fails to perform as expected, repeat external control test and/or contact Cepheid for assistance.

IV. Viewing and Printing Results

A. Results print automatically upon completion of the test. If not auto print.
B. Click the View Results icon to view results.
C. Upon completion of the test, click the Report button of the View Results window to view and/or generate a PDF report file then print report.

V. Reporting Results

A. Results are entered in computer.
B. Call back positive result with documentation in computer.
C. Label test report with a label from lab slip, initial, date and file in appropriate folder.
D. Save both negative and positive Flu specimens in the rack in the freezer

VI. Limitations

A. Performance of the Xpert Xpress SARS-CoV-2/Flu/RSV test has only been established in nasopharyngeal swab specimens. Use of the Xpert Xpress SARS-CoV-2/Flu/RSV test with other specimen types has not been assessed and performance characteristics are unknown.
B. Nasal swabs (self-collected under supervision of, or collected by, a healthcare provider) and nasal wash/aspirate specimens are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2/Flu/RSV test but performance with these specimen types has not been established.
C. As with any molecular test, mutations within the target regions of the Xpert Xpress SARS-CoV-2/Flu/RSV test could affect primer and/or probe binding resulting in failure to detect the presence of virus, or the virus being detected less predictably.

D. This test cannot rule out diseases caused by other bacterial or viral pathogens.
E. The performance of this test was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
F. Erroneous test results might occur from improper specimen collection; failure to follow the recommended sample collection, handling, and storage procedures; technical error; or sample mix-up. Careful compliance with the instructions in this insert is necessary to avoid erroneous results.
G. False negative results may occur if virus is present at levels below the analytical limit of detection.
H. Negative results do not preclude SARS-CoV-2, influenza or RSV infection and should not be used as the sole basis for treatment or other patient management decisions.
I. Results from the Xpert Xpress SARS-CoV-2/Flu/RSV test should be correlated with the clinical history, epidemiological
data, and other data available to the clinician evaluating the patient.
J. Viral nucleic acid may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious or are the causative agents for clinical symptoms.
K. This test has been evaluated for use with human specimen material only.
L. This test is a qualitative test and does not provide the quantitative value of detected organism present.
M. This test has not been evaluated for monitoring treatment of infection.
N. This test has not been evaluated for screening of blood or blood products for the presence of SARS-CoV-2, influenza or RSV.
O. The effect of interfering substances has only been evaluated for those listed within the labeling. Interference by substances other than those described can lead to erroneous results.
P. Results from analytical studies with contrived co-infected samples showed potential for competitive interference when SARS-CoV-2, Flu or RSV was present at LoD levels.
Q. Cross-reactivity with respiratory tract organisms other than those described herein can lead to erroneous results.
R. Recent patient exposure to FluMist® or other live attenuated influenza vaccines may cause inaccurate positive results.
S. As the Xpert Xpress SARS-CoV-2/Flu/RSV test does not differentiate between the N2 and E gene targets, the presence of other coronaviruses in the B lineage, *Betacoronavirus* genus, including SARS-CoV-1 may cause a false positive result. None of these other coronaviruses is known to currently circulate in the human population.
T. This test is not intended to differentiate RSV subgroups, influenza A subtypes or influenza B lineages. If differentiation of specific RSV or influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
U. This test has not been FDA cleared or approved.
V. This test has been authorized by FDA under an EUA for use by authorized laboratories.
W. This test has been authorized only for the simultaneous qualitative detection and differentiation of nucleic acids from SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV), and not for any other viruses or pathogens.
X. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or

diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

INTERPRETATION OF RESULTS

The results are interpreted automatically by the GeneXpert System and are clearly shown in the View Results window. The Xpert Xpress SARS-CoV-2/Flu/RSV test provides test results based on the detection of respective gene targets according to the algorithms.

The format of the test results presented will vary depending on the user's choice to run either an Xpert Xpress_SARS-CoV-2_Flu_RSV, Xpert Xpress_SARS-CoV-2_Flu or Xpert Xpress_SARS-CoV-2 test. See Table 1 to interpret test result statements for the Xpert Xpress SARS-CoV-2/Flu/RSV test.

Table 1. Xpert Xpress_SARS-CoV-2_Flu_RSV Possible Results and Interpretation

Result	Interpretation
SARS-CoV-2 POSITIVE	The SARS-CoV-2 target nucleic acids are detected. • The SARS-CoV-2 signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA (not applicable); SPC is ignored because SARS-CoV-2 target amplification occurred. • Probe Check: PASS; all probe check results pass.
Flu A POSITIVE	• The Flu A signal for either the Flu A1 nucleic acid target or the Flu A2 nucleic acid target or signals for both nucleic acid targets have a Ct within the valid range and endpoint above the threshold setting. • SPC - NA; SPC is ignored because the Flu A target amplification occurred. • Probe Check - PASS; all probe check results pass.
Flu B POSITIVE	• The Flu B signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA; SPC is ignored because Flu B target amplification occurred. • Probe Check: PASS; all probe check results pass
RSV POSITIVE	• The RSV signal has a Ct within the valid range and endpoint above the minimum setting. • SPC: NA; SPC is ignored because RSV target amplification occurred. • Probe Check: PASS; all probe check results pass.

SARS-CoV-2 NEGATIVE; Flu A NEGATIVE; Flu B NEGATIVE; RSV NEGATIVE	SARS-CoV-2 target RNA is not detected; Flu A target RNA is not detected; Flu B target RNA is not detected; RSV target RNA is not detected. • SARS-CoV-2, Flu A, Flu B and RSV target RNAs are not detected. • SPC - PASS; SPC has a Ct within the valid range and endpoint above the minimum setting. • Probe Check - PASS; all probe check results pass.
INVALID	SPC does not meet acceptance criteria and all targets not detected. Repeat test according to the Retest Procedure. • SPC: FAIL; SPC and SARS-CoV-2, Flu A, Flu B and RSV signals do not have a Ct within valid range and endpoint is below minimum setting. • Probe Check - PASS; all probe check results pass
ERROR	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV nucleic acids cannot be determined. Repeat test according to the Retest Procedure • SARS-CoV-2: NO RESULT • Flu A: NO RESULT • Flu B: NO RESULT • RSV: NO RESULT • SPC: NO RESULT • Probe Check: FAIL[1]; all or one of the probe check results fail [1] If the probe check passes, the error is caused by the maximum pressure limit exceeding the acceptable range, no sample added, or by a system component failure.
NO RESULT	Presence or absence of SARS-CoV-2, Flu A, Flu B and RSV nucleic acids cannot be determined. Repeat test according to the Retest Procedure in A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress. • SARS-CoV-2: NO RESULT • Flu A: NO RESULT • Flu B: NO RESULT • RSV: NO RESULT • SPC: NO RESULT • Probe Check: NA

If the SPC is negative and the results for any of the targets are positive, the results for all targets are considered valid.

If only one viral target is positive but coinfection with multiple targets is suspected, the sample should be re-tested with another FDA cleared, approved, or authorized test, if coinfection would change clinical management.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

REFERENCES:

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DEFINITIONS **N/A**

ATTACHMENTS **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	11/5/2020	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES **N/A**

EFFECTIVE November 5, 2020

REVISED **See Review/Revise Sign off Page**

REVIEWED **See Review/Revise Sign off Page**

SECTION: **Microbiology- Molecular Diagnostics**

SUB SECTION: **Procedure**

SUBJECT: **PerkinElmer® New Coronavirus Nucleic Acid Detection**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

PerkinElmer® New Coronavirus Nucleic Acid Detection Kit

TEST PRINCIPLE / PURPOSE

The PerkinElmer® New Coronavirus Nucleic Acid Detection kit uses TaqMan based real-time PCR technique to conduct *in vitro* reverse transcription of SARS-CoV-2 RNA, DNA amplification and fluorescence detection. The assay targets specific genomic regions of SARS-CoV-2: nucleocapsid (N) gene and ORF1ab.The TaqMan probes for the two amplicons are labeled with FAM and ROX fluorescent dyes respectively to generate target-specific signal. The assay includes an RNA internal control (IC, bacteriophage MS2) to monitor the processes from nucleic acid extraction to fluorescence detection. The IC probe is labeled with VIC fluorescent dye to differentiate its fluorescent signal from SARS-CoV-2 targets.

The assay also uses a dUTP/UNG carryover prevention system to avoid contamination of PCR products and subsequent false positive results.

CLINICAL SIGNIFICANCE

The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit is a real-time RTPCR *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 virus in human oropharyngeal swab, nasopharyngeal swab, and anterior nasal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263 a, that meet requirements to perform high complexity tests. Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in human respiratory specimens during the acute phase of infection. Positive results are indicative of presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for

patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

SPECIMEN TYPE(S)

I. Collection, Storage & Shipment of Specimens

A. Specimen Collection

Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 3 ml of viral transport media. For initial testing, nasopharyngeal swab specimens are recommended. Collection of oropharyngeal swabs is a lower priority and is acceptable if other swabs are not available.

1. Nasopharyngeal swab (NP): Insert a swab into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.
2. Oropharyngeal swab (e.g., throat swab, OP): Swab the posterior pharynx, avoiding the tongue.
3. Anterior Nasal Swab (NS): Using a flocked or spun polyester swab, insert the swab at least 1 cm (0.5 inch) inside the nostril (naris) and firmly sample the nasal membrane by rotating the swab and leaving in place for 10 to 15 seconds. Sample both nostrils with same swab.

B. Storage

Store specimens at 2- 8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.

C. Shipping

Specimens PUI's must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Store specimens at 2-8°C and ship overnight to the lab on ice pack. If a specimen is frozen at -70°C ship overnight to the lab on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

D. For more information, refer to:

Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)
https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinicalspecimens.html

Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)
https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html

REQUIRED REAGENT(S)

A. Kit Components and Packaging Specifications

Catalog Number: 2019-nCoV-PCR-AUS (48 tests/kit). Enough for 96 tests or one full run.

Component Name	Specifications & Loading	Main Ingredients	Storage Conditions	
nCoV Reagent A	950 µL	×1 tube	Buffers, dNTPs, Mg2+	-25 to -15°C
nCoV Reagent B	230 µL	×1 tube	TE buffer, primers, probes	-25 to -15°C
nCoV Enzyme Mix	170 µL	×1 tube	Taq DNA polymerase, MMLV, RNasin, UNG	-25 to -15°C
nCoV Internal Control	1.4 mL	×1 tube	TE buffer, bacteriophage MS2	-25 to -15°C
nCoV Positive Control	1.4 mL	×2 tubes	SARS-CoV-2 RNA fragments capsulated in bacteriophage	-25 to -15°C
nCoV Negative Control	1.4 mL	×2 tubes	TE buffer	-25 to -15°C

Notes: 1) The reference materials and other components in the kit should be treated as potential sources of infection. 2) The use of this kit should be strictly in accordance with the nucleic acid amplification guidelines to operate in compliance with the requirements of the appropriate laboratories. 3) The components in different batches of the kit cannot be used interchangeably.

B. Materials Required

1. RNA extraction reagents and instrument
   a. The PerkinElmer® Nucleic Acid Extraction Kits (KN0212) and PreNAT II (SY61) (software version 1.00.06).
   b. chemagic™ Viral DNA/RNA 300 Kit special H96 (CMG-1033, CMG-1033-S) and chemagic™ 360 (2024-0020) with chemagic™ Rod Head Set 96 (CMG 371) (chemagic MSM I software version 6.1.0.5).

2. PCR amplification instrument and software
   Analytik Jena qTower3 / qTower3 G Real-Time PCR System: 844-00553-x, 844-00554-x, 844-00555-x, 844-00556-x, 844-00563-x, 844-00564-x, 844 00503-2, 844-00503-4, 844-00504-2 software version qPCRsoft 4.1.

3. 96-well PCR plate:
   a. VWR® Heat-Resistant Films for Real-Time qPCR, Ultra-Clear Polyester: 60941-078
   b. VWR® 96-WELL Real Time PCR plates

4. Additional tools and consumables required for automatic nucleic acid extraction and PCR setup using Pre-NAT II and chemagic™ 360.

Items	Cat. No.	Pre-NAT II	chemagic™360
Centrifuge	TDL-80-2B	x	x
Vortex mixer	XW-80A	x	x
900 µL conductive tip Sterilized	AF01MP-9-XS	x	
175 µL conductive tip Sterilized	AF200P-9-XS	x	
50 µL conductive tip Sterilized	ATO5OP-9- XS-LB	x	
150 mL Reagent Trough	C3040016	x	
33 mL Reagent Trough	CJ222161115	x	
2 mL U type 96 deep-well plate	DP20UR-9-N	x	
2 mL deep-well-plate (replate SW)	CMG-555		x
Low-well-plate	CMG-555-1		x
Magnetic rods disposable tips	CMG-550	x	x
1.5 mL transparent centrifugal tube	MCT-150-C	x	
0.2 mL PCR 8-trip tubes	PCR-0208-C	x	
Caps for 0.2 mL PCR 8-trip tubes	PCR-2CP-RT C	x	
Deep-well plate sealing film	HY3020011	x	

C. Storage & Handing Requirements
1. Store all reagents at -25 to -15°C.
2. Use the reagents within 30 days once opened.
3. Completely thaw the reagents before use.
4. Avoid excessive freeze/thaw cycles for reagents.

D. Warnings and Precautions
1. For *in vitro* diagnostic use under Emergency Use Authorization only.
2. Positive results are indicative of the presence of SARS-CoV-2 RNA.
3. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
4. Keep the kit upright during storage and transportation.
5. Before using the kit, check tubes for leakage or damage. Each component in the kit should be thawed at room temperature, thoroughly mixed, and centrifuged before use.
6. Cross-contamination may occur when inappropriate handling of reference materials and specimens, which will cause inaccurate results. It is recommended to use sterile disposable filter-tips to aspirate reagents and specimens.
7. All specimen to be tested and the reference materials of the kits should be considered as infectious substances and processed strictly in accordance with Laboratory biosafety requirements. Sterile centrifuge tubes and filter-tips should be used. After use, the tips should be disposed into a waste bin containing a 10% sodium hypochlorite solution. After the operation, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution, and then cleaned with 75% ethanol or pure water. Finally, turn on UV light to disinfect working surfaces for 30 minutes. The PCR instrument used for this assay should be calibrated regularly according to instrument's instructions to eliminate cross-talks between channels.
8. This kit uses PCR-based technology and experiments should be conducted in three separate areas: reagent preparation area, specimen preparation area, amplification area. Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation and protective equipment accessories should be changed when entering and leaving different work areas. Protective equipment accessories in each work area are not interchangeable.

E. Instruments

1. PerkinElmer® PreNAT II Automated Workstation
2. PerkinElmer® PreNAT II Automated Workstation, PCR plate re-formatter
3. chemagic™ 360 for RNA extraction
4. Analytik Jena qTower3 / qTower3 G Real-Time PCR System

CALIBRATION

N/A

QUALITY CONTROL

The product provides negative control, positive control, and internal control to monitor the reliability of the results for the entire batch of specimens from sample 25 extraction to PCR amplification. All test controls should be examined prior to interpretation of patient results. Positive control, negative control and IC in positive and negative control should meet the requirements listed in the below table to ensure valid results. If the controls are not valid, the patient results cannot be interpreted.

Result Interpretation of Test Controls for 60uL reaction:

Control type	**Ct**		
	N (FAM)	**ORF1ab (ROX)**	**IC (HEX/VIC)**
Negative	Undet or > 42	Undet or > 42	Ct ≤ 40
Positive	≤ 35	≤ 35	/

/: No requirements on the Ct value.
Undet: Undetermined

PROCEDURAL STEPS

I. Nucleic Acid Extraction and PCR Setup

A. Extraction and PCR setup on Pre-NAT II

Pre-NAT II Automated Workstation is designed to process 1-96 samples for downstream molecular assays. It contains a liquid handling system which automatically pipettes and mixes reagents and samples, a purification module that extracts and purifies nucleic acids, and an automatic PCR setup function which is also conducted by the liquid handling system. The entire workflow is automatic without manual intervention. Detailed operation instructions of Pre-NAT II can be found in the Pre-NAT II Automated Workstation User Manual. A quick-start instruction for the SARS-CoV-2 assay is described as below.

1. Take the nCoV Internal Control, nCoV Positive Control and nCoV Negative Control out from freezer, place them in a biological safety cabinet and completely thaw them at room temperature. Vortex the tubes to mix the contents, then centrifuge the tubes briefly at 1000 rpm to collect the liquid to the bottom of the tubes.

2. Prepare specimens and place them in a biological safety cabinet. If the specimens are frozen, completely thaw them at room temperatures and follow the operations described in 1) for the controls.
3. Take the Magnetic Beads from the PerkinElmer® Nucleic Acid Extraction Kits (KN0212) kit, vortex the tube for one minute to completely suspend the beads in the solution.
4. Turn on the PreNAT II instrument, double click the “Pre-NAT II” software icon, follow software guidance to initialize the instrument.
5. After initialization, click “Program Input” to choose an extraction protocol. For the SARS-CoV-2 assay, choose “2019-nCoV” from the protocol list.
6. In the same window, input the number of specimens that are going to be processed at the indicated box, positive control and negative control should not be counted, as they are pre-set in the 2019-nCoV protocol. After the sample number is entered, click “Set Complete” to proceed to the loading guidance for reagents and consumables.
7. Remove the lids from reagents, controls, and specimens, load the consumables, reagents, specimens, and controls according to software guidance, then double check to confirm that all items are at the positions indicated by software. Close instrument door after finish loading. Click “Run” to start the protocol, the procedures automatically performed by Pre-NAT II are described below.

    a. Add 300 µL of each specimen, Negative Control and Positive Control to the wells of a 96 deep-well plate, and add 5 µL nCoV Internal Control, 800 µL Lysis/Binding Buffer and 15 µL Magnetic Beads to each well.
    b. Magnetic rods take rod tips and rotate in 96 deep-well plate to mix (magnetic force off status), during which stage DNA/RNA is released through lysis and binds to magnetic beads.
    c. During lysis and binding, automatic liquid handler pipettes Wash Buffer A to a 96 deep-well plate.
    d. Magnetic force is turned on for magnetic rods and beads are collected from Lysis/Binding reaction to Wash Buffer A.
    e. Magnetic rods (magnetic force off) rotate to wash beads in Wash Buffer A and proceed in a same manner to wash beads in Wash Buffer B.
    f. Finally, the beads are collected and placed into 60 µL elution buffer to elute DNA/RNA.
    g. During elution, liquid handler pipettes/mixes PCR reagents to prepare a PCR mix and aliquot 20 µL to PCR tubes.
    h. For each sample, 40 µL of eluted DNA/RNA is added to PCR mix in each tube, which is ready for amplification.

B. Extraction and PCR Setup on chemagicTM 360

Please follow chemagicTM 360 User Manual for extraction setup. A quick start instruction is described as below.

1. Place specimens in a biological safety cabinet. If the specimen is frozen, completely thaw it at room temperature before use.
2. Take a 2 mL deep-well-plate (riplate SW), add 300 µL Lysis buffer, 300 µL specimen, 5 µL Internal Control, 4 µL Poly (A) RNA and 10 µL Proteinase K to each well in a sequential order.
   Please note:
    a. Dissolve lyophilized Poly(A) RNA by adding 440 µL of the Poly(A) RNA Buffer to the Poly(A) RNA tube and mix thoroughly before use;
    b. Dissolve lyophilized Proteinase K in H2O before use (volume is given on the label).
3. Take a low-well-plate, add 150 µL magnetic beads into each well.

4. Take a new deep-well-plate (riplate SW), add 60 µL Elution Buffer 5 into each well.
5. Turn on the chemagicTM 360, double click the software icon “chemagic_360”, select username and enter password to start. Follow the chemagicTM 360 User Manual to select the appropriate protocol.
6. Load the magnetic rods disposable tips onto the tip rack according to the number of specimens, positive control and negative control being tested.
7. Load the plates manually onto the tracking system (table) according to the instructions given by the chemagic software. The plates should be at the positions indicated in the below table.
8. Please note:
   a. Specimens and Magnetic Beads should be thoroughly vortex mixed before use;
   b. Never move the tracking system (table) manually. All movements must be performed with the [Turn Table] function.

chemagic 360 layout：	
Position 1	Magnetic rods disposable tips
Position 2	Low-well-plate (MICROTITER SYSTEM) prefilled with 150 µL Magnetic Beads
Position 3	Deep-well-plate (riplate SW) containing: 300 µL Lysis Buffer 1 300 µL specimen 5 µL Internal Control 4 µL Poly(A) RNA 10 µL Proteinase K Binding Buffer 2 (added automatically)
Position 4	Empty deep-well-plate (riplate SW) [Wash Buffer 3 added automatically]
Position 5	Empty deep-well-plate (riplate SW) [Wash Buffer 4 added automatically]
Position6	Empty deep-well-plate (riplate SW) [purified water added automatically]
Position 7	Deep-well-plate (riplate SW) prefilled with 60 µL Elution Buffer 5

9. Double check the positions and directions of all consumables according to the tracking system.
10. Click “Start” to start the extraction process.
11. Proceed to downstream assay with the extracted nucleic acids or store the nucleic acids at -25°C to -15°C.

C. Setup PCR Manually

Setup PCR manually according to the procedures described below after nucleic acid extraction using chemagicTM 360.

1. Prepare PCR mix in Reagent Preparation Area according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

Component	Volume/ test	Volume for N Samples and 2 Controls	110% of volume
nCoV Reagent A	15 µL	15 x (n + 2) µL	16.5 x (n + 2) µL
nCoV Reagent B	3 µL	3 x (n + 2) µL	3.3 x (n + 2) µL
nCoV Enzyme mix	2 µL	2 x (n + 2) µL	2.2 x (n + 2) µL

2. Completely vortex the prepared PCR mix, aliquot 20 µL into each PCR tube or each well of a 96-well PCR plate.
3. Add 40 µL of extracted nucleic acid into each tube or well containing PCR mix, close lids for the PCR tubes or seal PCR plates with an appropriate film, slightly vortex the tubes and briefly centrifuge them to get rid of bubbles.

D. Amplification

1. Analytik Jena qTOWER3 / 3G Real-Time PCR system: Set up and run the Analytik Jena qTOWER3 / 3G Real-Time PCR instrument. Refer to Analytik Jena qTOWER3 / 3G Real-Time PCR Operating Manual for detailed instructions. In general, double-click qPCRsoft software 4.1 › File | New › Settings › Thermal Cycler | Scan | Samples, then click Start.

2. Settings | General:
   a. Title: as appropriate for this run
   b. Operator: appropriate operator designation
   c. Start and End: populated automatically as part of the run
   d. Comment: any additional information regarding the run

3. Settings | Thermal Cycler:
   a. Set Run method as following for PCR amplification and fluorescence detection, using default ramping rate.
   b. Lid Temp: 100 ºC (or 105 ºC), ☑ Preheat lid.
   c. Device: qTOWER3 or qTower3 G, depending on instrument type

Step	Scan	Temperature	Time(m:s)	GoTo	Loops
1		37ºC	02:00	-	-
2		50 ºC	05:00	-	-
3		42 ºC	35:00	-	-
4		94 ºC	10:00	-	-
5		94 ºC	00:10	-	-
6		55 ºC	00:15	-	-
7	X	65 ºC	00:45	5	44

4. Settings | Scan:
   Activate the following measurement detectors. The passive reference (Pass. Ref.) cells/column must be left empty.

Pos.	Channel	Dye	Gain	Measurement
1	Blue	FAM	5	X
2	Green	JOE	5	
3	Yellow	HEX_3 or TAMRA	5	X
4	Orange	ROX	5	X
5	Red	Cy5	5	
6	NIR1	Cy5.5	5	

• Color compensation: Standard 1 for 4.1

NOTE – all six Pos. and Channel options must be activated in Edit color modules before opening any new project files (on software main page, click Extras>Edit color modules). Otherwise, corresponding Pos. and Channel options may not show up in Scan setting.

5. Settings | Samples:

    a. Set up the plate layout by assigning a unique sample name to each well.
    b. Assign a sample type to each well:
    c. Positive control
    d. Negative control
    e. Unknown (patient sample)

6. Double check all settings, save the project, and then click Start qPCR run to initialize amplification.

Note – Device selection must match the specific device in thermal cycler setting, otherwise qPCR run will not start, and error message may pop up.

II. BENCH-SIDE SHORT PROCEDURAL STEPS

A. Pre-analytical steps

1. Receive specimen in the computer
2. Place Lab. slip in the bag
3. Label specimen with barcode under Biological Safety Cabinet
4. Place specimen in a metal test tube rack
5. Create work batch.
    - Laboratory------Worksheet------Create-----Type COVID----Enter all fields, and save------print
    - Match specimen with the Lab. worksheet
    - Remove from the batch specimens not found/missing.

III. JANUS (RE-FORMATTER)/LIQUID HANDELER

A. Daily maintenance

1. Wet Kimwipes with 70% alcohol and gently clean the bottom of the versa tips
2. Use 70% alcohol to clean the deck
3. Check if valves are finger tight on all syringes
4. Dispose wastewater into the sink.
5. Re-fill the system liquid with ddH2O. Use 2 L Glass Flask located in Micro.
6. Run Flush and Wash Tips as part of morning maintenance as follows:

    a. On Janus computer go to Utilities---Diagnostics----Flush and wash Tip---Click Deck Layout Ok Run Protocol now--------Click on Next steps------ ----Start
    b. Look for air bubbles in the syringes and the tubing. If bubbles persist, repeat the Flush and Wash.

B. Load consumables and reagents

1. Use the attached picture as a guide to load consumables and reagents. For one 96 sample run, load only supplies needed for a run.
2. Label empty plates with A1 at the corner
3. See also on the Janus computer monitor for one 96 tests lay out positions
4. Load 900 ul/1000ul tips and 200 ul tips @ the right position
5. Label and place magnetic beads plate, elution buffer plate, lysates plate @ correct position
6. Mix magnetic bid thoroughly and pour into a trough and place it @position 1
7. Pour Elution buffer into trough and place it @ position 2
8. Wait for lysis buffer until prompted by the instrument after dispensing patient samples

C. Poly A, Internal Control (IC) and Proteinase K Mix

1. Reconstitute Lyophilize Poly A: Add 440 uL **Poly (A) RNA Buffer** to **Poly (A) RNA** tube and vortex to mix
2. Reconstitute Proteinase K: add 11 mL ddH_2O and mix well. Store at 4°C for 30 days
3. Click on Excel icon “Calculation Old for PKI” on the monitor to obtain volume of the reagents calculated based on the number of tests:
4. For one full run, add 444 uL Poly A; 1110 uL Protinase K and 555 uL of IC, total 2109 uL into sterile tube, vortex and divided into 2 Eppendorf tubes (1054.5uL/tube).
5. Place tubes @ position A1 and B1 in chill box

D. Positive and Negative Controls

1. Open the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit located in -20°C freezer and place it under Biological Safety Cabinet (BSC) to thaw at room temp
2. Vortex to mix, place Negative control @ position A6 in chill box
3. Vortex to mix, place Positive control @ position B6 in chill box

E. Load patient samples

1. Neutralize sample in oven at 65°C for 15 minutes
2. Remove cap under the flame hood. If swab pulls out with the cap, remove it, and dispose it in the biohazard bag
3. Make sure swab is not bent in the tube. Remove bent swabs
4. place the caps upside down in order of removal. It will be used to recap the tubes
5. Load samples into PE specimen rack. Push the tubes all the way to the bottom. Do not leave open space in between
6. Place the samples on the instrument rail, the metal end facing inward

F. Start Protocol

1. Press on Run (green button)
2. **Select** ---Type the plate ID for plate 1: Today’s date, batch X. E.g., **112320batch1,** enter next step
3. **Gather** ---- lists the consumables and reagents---hit next step
4. **Place** ------shows reagent positions—next step
5. **START**

6. The instrument starts with reading the barcodes and proceeds with dispensing reagents. Make sure all barcodes are read. The instrument flags the incorrect barcode reads/missing specimens. If missed reading, abort the run and restart.
7. Pause and resume pipetting by the instrument to check if the right volume is dispensed
8. Before proceeding further, check if the correct volume of Poly A mix is dispensed into a lysate well. If not, dispense 19 ul manually
9. Watch the versa tips when patient samples are pipetted. If swab is pulled up with the pipette, press pause, with kimwipes push dawn the swab into the tube, and resume pipetting.
10. When finished pipetting patient samples, check if correct volume of sample (300 uL) is pipetted into each well. If sample is short, or missing, identify the tube make sure it is the correct patient, identify the position and pipette manually. Use the Sample tracking icon on the computer and the chart on the shelf to locate the position of the sample.
11. After pipetting patient samples are complete, the instrument asks to load the lysis buffer.
12. Fil the lysis trough
13. Resume pipetting
14. The protocol is complete after lysis buffer is dispensed.

G. Save the Run-on USB flash drive

1. Insert USB in the drive, close the file
2. Click on sample tracking file on the monitor
3. Select the run name in **.csv** format file from the list, click on **date modify** if not on top of the list
4. Open
5. File, ---- Save As ----Browse----Select PKBACD----Save
6. 2nd save
7. File, ---- Save As ----Browse----Select PKBACD. Go to second line ---Pull down menu ---- select Text (Tab Delimited)**txt-----Save---Eject USB, safely.
8. Use the USB on Chemagic 360

Thaw PerkinElmer® New Coronavirus Nucleic Acid Detection Kit located in the -20ºC Freezer by placing under BSC at room Temp.

I. CHEMAGIC 360 PRPTOCOL

A. Insert the USB from Janus into USB drive
B. Select the protocol "**check manifolds 1 – 6**" and press the [**Insert IDs**] to prime manifolds.
C. Open the side door, place kimwipes on the pressure sensor
D. Enter ok and then observe for a steady flow of liquid through all the nozzles. The flow starts from the first manifold. If failed, repeat one more time. If failed again, use the corresponding priming protocol for this manifold.
E. Select the protocol "**chemagic Viral300 360 H96 drying prefilling VD141211.che**" and press the [**Insert IDs**] button
F. Check buffer, for 96 samples, make sure 125 mL buffer in each bottle is available and hit OK
G. Number of samples for prefilling: if 96 samples, press Ok, if less, de select the full empty columns
H. Kit Lot #: If new kit, Scan in with a scanner. If old kit, press OK
I. Click Finished

J. Elution ID same as Sample ID's? Press Yes
K. Enter plate ID on Tracking System: press Yes
L. Rack ID: place the plates on the tracking system according to the instructions given by the chemagic QA software. MAKE SURE THE CORRECT PLATE IS PLACED AT THE CORRECT POSITION
M. Now, enter Read ID's from file
N. Browse for today's text file
O. Make sure that all Plate Columns where Samples are used, are filled with empty wells.
P. Make sure the A1 position is at the correct position for all plates.
Q. Close the door. press Start. The protocol takes 1 hour to complete. After finished, use the turn table function to unload the plates. ***Never move the tracking system (table) manually. All movements must*** press ***be performed with the [Turn Table] function***.
R. Save the Elution Buffer plate
S. Before disposing off all plates, make sure of the following:
    1. Make sure most beads have been picked up from Magnetic bead plate
    2. Make sure there are no missed wells or columns in Lysate Plate
    3. Make sure there are no missed wells or columns in wash plates
    4. Make sure all sleeves or tips have been dropped in Wash 5 plate
    5. Dispose all other plates in biohazard trash

II. JANUS qPCR WORKSTATION

A. Perform daily maintenance like listed above
B. See the Janus q PCR workstation computer monitor for one 96 tests lay out
C. Load 200 ul tips @ the right position
D. Load the elution buffer from Chemagic 360 @ the correct position
E. Load the solid white PCR plate @ the correct position
F. Prepare a PCR master mix as follows: add 825 ul of **reagent A**, 165 uL of **reagent B**, and 110 uL of **reagent C**, total 1100 uL into an Eppendorf tube, and vortex to mix -then centrifuge.
G. Place @ position A6 on chill box
H. Insert USB flash card in USB Drive; Click Import Path Short Cut Icon; Select the Run ID as .csv file from the USB----Copy----Paste to Import Path,---- Select Run ID as .csv ---rename---copy;
I. Click on Run the instrument; two windows appear
J. Bottom window (Elution plate ID): paste
K. Upper window (qPCR plate) paste and then type **AJ** at the end
L. When run is complete: Click Import Path Short Cut Icon -----Lims Path-----Copy .trf file and save it to USB **Packard E** drive

III. ANALYTIC JENA (AJ) q TOWER[3]

A. Insert USB Flash Drive into the Lab top USB drive, close files
B. Click on File
    1. Select Import LIMS
    2. Type in the RUN ID, select your RUN ID in **.trf** file
    3. Click Open
    4. PCR window opens,
    5. Under General: make sure the correct file name appears under title
    6. under operator, put your initial

C. Press Thermocycler: default set up uploads. DO NOT CHANGE ANY SETTING
D. Press Scan: select standard 1 from the pull-down menu
E. Press Sample: Make sure Negative control and positive control at A1 and A2 positions. Make sure patient barcode numbers appear for each sample and under patient sample type unknown listed under each patient sample
F. Start run by clicking on the play button
G. PCR takes 2 hour and 30 minutes
H. When the run is complete
I. Save
    1. Name: type Run ID, keep the title. Just remove .trf
J. Click on Calculate Ct
K. Set threshold for each channel; ROX. FAM, HEX_3, change it to 5
L. All colors
M. Move cursor to sample result area, Right click
N. Save as xls
O. File name: use the same name, save

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS

I. Baseline and threshold setting for qTower^3

After the run completion, save and analyze the data according to PCR instrument instructions.

Under Settings tab, for color compensation configuration, select “Standard1” for qTower3 / 3 G (version 4.1)

Under monitoring tab, click “Calculate Ct”, the following view shows up.

A. Set baseline for each target

In most of the cases, the default baseline can be used. In order to adjust baseline, click icon The default setting is “Sample specific crop first cycles”, which is good for most of the cases.

The default is 5, which can be adjusted, for example 10, 15 to minimize background noises in some cases.

To set up different baseline, click “For all samples”, from cycle X (default 3) to Y (default 15) as the following window.

To switch back to the default setting, click “Sample specific Crop first cycles”.

B. Set threshold for each target

Under Monitoring tab, view the threshold values under “linear” scaling (showed in above figure) for each target. Thresholds should be adjusted to fall within exponential phase of the

fluorescence curves and above any background signal. The threshold value for different instruments varies due to different signal intensities. It is recommended to setup threshold manually instead of default settings. For manual threshold setup, it can either remove threshold bar up and down, or manually input threshold number to the threshold window, shown in the following figure. It is recommended to setup the threshold in the range 5-15 as general.

II. Result Interpretation of Test Controls for 60uL reaction:

A. Negative Control: both ORF1ab and N of SARS-CoV-2 must be not detected, and the Ct value of internal control should be ≤40;

B. Positive Control: both ORF1ab and N of SARS-CoV-2 must be detected, and their Ct values should fall within the ranges described in the above tables, the Ct value of internal control does not have to be ≤40 for positive control.

III. Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and confirmed to be valid 26 and acceptable. If the controls are not valid, the patient results cannot be interpreted.

The table below lists the expected results for the kit with valid positive control and negative control:

Ct		**Result Interpretation**
IC (VIC/HEX)	N(FAM), ORF1ab (ROX)	
≤40	Both targets Undetermined or > 42	SARS-CoV-2 Not Detected
/	Both targets ≤ 42	SARS-CoV-2 Detected
/	One of the targets ≤ 42	SARS-CoV-2 Detected
>40 or Undetermined.	Both targets Undetermined or > 42	Invalid result. Specimen needs to be re-tested from re-extraction or re-collected from patient for test

A. */*: No requirements on the Ct value. If the result for a specimen is SARS-CoV- 2 RNA not detected, the Ct value of the internal control must be ≤40, otherwise the result of that specimen is invalid.

B. If the result for a specimen is SARS-CoV-2 RNA detected, the Ct value of the internal control is not required to be considered valid.

IV. Examination and Interpretation of Pooled Patient Specimen Results

Negative—Negative results from pooled sample testing should not be treated as definitive. If the patient's clinical signs and symptoms are inconsistent with a negative result and if results are necessary for patient management, then the patient should be considered for individual testing. The utilization of sample pooling should be indicated for any specimens with reported negative results.

Positive—Specimens with a positive sample pool result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Invalid—Specimens with an invalid pool result must be tested individually prior to reporting a result. However, in instances of an invalid run, repeat testing of pooled specimens may be appropriate depending on laboratory workflow and required result reporting time.

V. Kit Limitations

A. The use of this assay as an in vitro diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

B. This kit is used for qualitative detection of SARS-CoV-2 RNA from human oropharyngeal swab, nasopharyngeal swab, and anterior nasal swab. The results cannot directly reflect the viral load in the original specimens.

C. Nasal swab specimens self-collected under the supervision of or collected by a health care provider can be tested with the PerkinElmer New Coronavirus Nucleic Acid Detection Kit; however, performance with this specimen type has not been determined.

D. Sample pooling has only been validated using nasopharyngeal and oropharyngeal swab specimens.

E. Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.

F. The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit performance has only been established with the specimen types described in the Intended Use section. Testing other types of specimen may cause inaccurate results.

G. The specimens to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.

H. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

I. Amplification and detection of SARS-CoV-2 with the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit has only been validated with the Applied Biosystems® 7500 Real-Time PCR instrument. Use of other instrument systems may cause inaccurate results.

J. The limit of detection (LoD) is determined based on a 95% confidence of detection. When SARS-CoV-2 presents at or above the LoD concentration in the test specimen, there will be a low probability that SARS-CoV-2 is not detected. When SARS-CoV-2 presents below the LoD concentration in the test specimen, there will also be certain probability that SARS- CoV-2 can be detected.

K. Primers and probes for this kit target highly conserved regions within the 28 genome of SARS-CoV-2. Mutations occurred in these highly conserved regions (although rare) may result in RNA being undetectable.

L. This kit uses an UNG/dUTP PCR products carryover prevention system which can prevent contamination caused by PCR products. However, in the actual operation process, the amplicon contamination can be avoided only by strictly following the instructions of PCR laboratories.

M. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.

N. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs have not been evaluated.

O. Laboratories are required to report all positive results to the appropriate public health authorities.

VI. Conditions of Authorization for the Laboratory

The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit (“your product” in the conditions below), the relevant

VII. Conditions of Authorization are listed below:

A. Authorized laboratories1 using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRHEUA-Reporting@fda.hhs.gov) and You (via email: COVID- 19.TechnicalSupport@PerkinElmer.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

The letter of authorization refers to, “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests” as “authorized laboratories.

VIII. REPORTING RESULTS

Identify invalid results. Remove invalid results from the batch.

A. Positive Results
Enter results for each sample : Lab. ---Result Entry -----Enter result (standard) ----Type D (Detected) ------Physician notification window pops-up ---call back result or for drive-through-clinics enter DTC ---release result as final and verified.

B. Negative results

Lab----Results Entry------Enter spreadsheet results---------COVID---Pull up the worksheet ------- Select worksheet options----Select Propagate -------Enter ND for Not Detected -----Enter ----- Save---- release result as final, verified

C. Print worksheet report and file in appropriate binder. Close worksheet.

REFERENCES

1. Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: World Health Organization; 2020.

2. Innis, MA, et Al, and the PCR Protocols Applications: A Laboratory Manual Academic, New York, 1989.

3. Mahony JB. Detection of Respiratory Viruses by Molecular Methods. Clinical Microbiology Reviews. 2008; 21 (4): 716-747.

4. China CDC Virus Disease Control and Prevention. Novel coronavirus nucleic acid detection primer and probe sequences (Specific Primers and Probes for Detection Novel coronavirus 2019) [EB / OL]., 2020-01-21.

DEFINITIONS **N/A**

ATTACHMENTS **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/21/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES **N/A**

EFFECTIVE **November 25, 2020**

REVISED **See Review/Revise Sign off Page**

REVIEWED **See Review/Revise Sign off Page**

SECTION: **Bacteriology**

SUB SECTION: **Procedures**

SUBJECT: **PREVI® Color Gram V2**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

PREVI® Color Gram V2

TEST PRINCIPLE / PURPOSE

The PREVI® Color Gram is designed to Gram stain specimen smears on microscope slides.
A Gram stain aids in the presumptive diagnosis of the illness, and it gives preliminary classification of the causative agent.

The chemical mechanism of Gram differentiation is based on the differential cell wall permeability to the Crystal Violet-Iodine complex. The Gram stain yields a specimen in which the Gram-positive organisms are blue-black, and the Gram-negative organisms are pink to red.

CLINICAL SIGNIFICANCE

SPECIMEN TYPE(S)

The PREVI® Color Gram has been validated with the following cell suspensions:

I. Blood
II. Bronchoalveolar liquid (BAL)
III. Cerebrospinal fluid (CSF)
IV. Feces
V. Sputum
VI. Vaginal swab
VII. Urine
VIII. Wounds
IX. Pure strain isolate smears (gram positive, gram negative, yeast)
Any other specimen type must be validated by the user.

REQUIRED REAGENT(S)

PREVI® Color Gram V2 Stain instrument

I. 12 (P/N 29557) slide carousel
II. D & E Empty 5-liter Level-sensing (P/N 415437) or 500 mL (P/N 29559) bottles for ethanol or methanol and distilled water
III. Acetone Safranin-A solution, P/N 29582
IV. Safranin-A solution, P/N 29583
V. Acetone Fuchsin-A solution, P/N 29584
VI. Fuchsin-A solution, P/N 29585
VII. Iodine-B solution, P/N 29586
VIII. Crystal Violet-C solution, P/N 29587
IX. Distilled water
X. Ethanol or methanol (see specifications in the PREVI® Color Gram User's Manual)
Clean and grease free glass slides
XI. Immersion oil
XII. Microscope with oil-immersion objective Nozzle Cleaning Solution 5-liter (P/N 29588) or 500 mL (P/N 414291)
XIII. Waste tubing, P/N 29694
XIV. Waste container V2, P/N 415436
XV. Maintenance kit V2, P/N 415593
XVI. Barcode reader, P/N 415592

CALIBRATION **N/A**

QUALITY CONTROL

Daily perform a Gram stain on a smear containing Staphylococcus and another smear containing *E. coli*. *Staphylococcus* should stain Gram-positive (blue-black) and *E. coli* should stain Gram-negative (pink to red). If the stain fails to work properly, report findings to the supervisor or a technologist III for corrective action.

PROCEDURAL STEPS

I. **Smear Preparation Tips**

A. Use clean, grease-free glass slides that are not cracked, chipped, or etched.
B. All specimens should be prepared as thinly as possible.
C. For the best results, the center area of the slide should be used for specimen loading.
D. If using the blood smear method, the entire slide is likely to be covered, but only the center of the slide should be examined. Samples at the Samples at the extreme edges of the slide may under- or over decolorized, depending on the state of the nozzle spray pattern.
E. Do not overheat slides if heat fixing.

II. **PREVI® Color Gram V2 Daily Workflow**

A. Check reagent levels and expiration dates and liquid level of waste container.

NOTE: Never allow a reagent to run dry. When the reagent level is near the bottom of the bottle, perform the Replacing a Reagent Bottle procedure in the User Manual. Never allow

the waste level to go above the maximum safety level. When the waste container is full, perform the Emptying the Waste Container procedure in the User Manual.

B. Perform a Hub Pattern Test (Pattern Test)

NOTE: The Pattern test is used to ensure the nozzles are clear of debris and spraying properly.

1. Select **Maintenance**.
2. Select **Pattern Test** to start the pattern test.
3. Hold sheet of white paper towel in front of nozzle A.
4. Select the **A** prime button.
5. Check the quality of the pattern. If the pattern is not correct, refer to the **Maintaining Nozzles** section in the User Manual.
6. Repeat the previous steps for each nozzle, pressing the corresponding prime button nozzle (A, B, C, D, and E).

C. Loading Slides

1. Remove the carousel from the bowl and place it on a solid, level surface.
2. Remove the carousel lid by pressing the button and lifting the lid.
3. Insert the slides into the carousel with the first slide in position 1.

Note: The slides must be diametrically opposite one another to balance the carousel.

a. Be sure that the slides are loaded in balanced pairs. If an odd number of slides needs to be stained, use a blank slide to balance the carousel.
b. If there are empty slots in the carousel, use blocking slides to prevent overspray. Refer to **Using Blocking Slides** section in the User Manual.
c. If using a 12-slide carousel, make sure the slides are loaded with all the slide labels toward the outer rim of the carousel.
d. If using a 30-slide carousel, make sure the slides are loaded with all the slide labels toward the center of the carousel.
e. Always load slides with the specimen facing clockwise.
f. Always begin loading the slides in position 1, then in position 2, position 3, position 4 and so on.

4. Replace the carousel lid by pressing the button and lowering the lid over the indexing posts.
5. Release the button and press the lid handle until it clicks

NOTE: Carousels must be loaded with similar specimens for a similar level of staining. Otherwise bioMérieux does not guarantee the staining performance.

D. Starting a Staining Cycle

1. Insert a carousel and close the instrument lid.
2. Select **Load Slides**.
3. Enter your passcode if required.
4. Select the number of slides loaded into the carousel to be stained.

NOTE: Do not count blocking slides as part of the total.

5. Select **Start**. The instrument will indicate the progress of the program, and a signal tone will indicate the end of the cycle.
6. Select the **Checkmark** button and open the lid, or just open the lid.

NOTE: Wait until the carousel rotation ceases prior to opening the lid.

E. Unloading the Carousel

NOTE: Treat slides in accordance with good laboratory practice guidelines and local regulations.

1. Remove the carousel from the bowl and place it on a solid, level surface.
2. Remove the carousel lid by pressing the button and lifting the lid.
3. Carefully remove each slide already dried and read the gram results with a microscope.

F. CLEAN alcohol purge stage

NOTE: The Clean Alcohol Purge stage requires the use of 10ml of alcohol divided equally among nozzles A, B, and C. This used to clean the carousel after staining. The same function is performed when **Standby/Ready** is pressed.

1. Place the empty carousel in the instrument and close the lid.
2. Press CLEAN button.
3. Open the lid and remove the carousel when the Clean Cycle has finished.
4. Spray and wipe the interior of the bowl and nozzles with ethanol 70% solution.

NOTE: The Clean Alcohol Purge stage automatically occurs after each staining cycle.

NOTE: At the end of the day, the customer will press the shutdown button and another CLEAN alcohol purge will begin to run. This step assists in the prevention of clogging of the nozzles and tubing.

G. INSTRUMENT MAINTENANCE:
Daily, before first cycle: Check reagent levels and expiration dates, check the waste container empty if necessary), and perform the Hub Pattern test procedure.

Daily, after the last cycle: Clean the bowl and nozzles, clean the instrument lid

Weekly: Perform the volume test, wipe carousel tray and lid, flush waste tubing, and ensure the monthly maintenance procedures listed on the Maintenance Log have been performed and initial the chart or enable the Preventive Maintenance Log to track the QC/PM within the instrument's software.

Monthly: Disassemble and clean all the nozzles, disinfect the Reagent D Bottle and ensure the monthly maintenance -procedures listed on the Maintenance Log have been performed, and initial the chart or enable the Preventative Maintenance Log to track the QC/PM within the instrument's software.

Yearly: Preventative Maintenance every two years by a bioMérieux Field Service Engineer.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS

I. Observe under the microscope (oil-immersion objective, magnification x1000)
II. Gram-positive micro-organisms appear blue-black. Gram-negative micro-organisms appear red-pink. Note: Yeasts appear blue-black.
III. Additional techniques (culture, identification, etc.) must be used to establish a diagnosis.

REFERENCES:

PREVI® Color Gram V2. Package Insert. 2020 bioMerieux. 800-682-2666 or bioMérieux, Inc., 100 Rodolphe Street, Durham, NC 27712.

DEFINITIONS: **N/A**

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	1/1/2021	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES: **N/A**

EFFECTIVE: January 2021

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Bacteriology**

SUB SECTION: **Procedures**

SUBJECT: **The PREVI COLOR Cytocentrifuge Rotor**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

The PREVI® COLOR Cytocentrifuge Rotor

TEST PRINCIPLE / PURPOSE

The PREVI® COLOR Cytocentrifuge Rotor is an *in vitro* diagnostic medical device for professional use only. It is an accessory for fixing biological cell suspensions on a glass microscope slide for cytological examination.

It is recommended that you use the Default program for the PREVI® COLOR Cytocentrifuge Rotor. The PREVI® COLOR Cytocentrifuge Rotor can be used with the following cell suspensions: Bronchoalveolar liquid (BAL), Cerebrospinal fluid (CSF), Urine, and Synovial fluid.

The PREVI® COLOR Cytocentrifuge Rotor allows rapid sedimentation of specimen cells onto microscope slides for staining or other purposes.

Up to eight disposable sample chamber assemblies with absorbent pads and glass microscope slides can be loaded into the PREVI® COLOR Cytocentrifuge Rotor. Cytocentrifuge and staining functions are independent of one another.

The PREVI® COLOR Cytocentrifuge Rotor has patented features to reduce cell loss during collection and prevent accidental damage to the collected specimen. The rotor is sealed to control aerosol release during cytocentrifugation.

CLINICAL SIGNIFICANCE

Specimens from Bronchoalveolar liquid (BAL), Cerebrospinal fluid (CSF), Urine, and Synovial fluid can be used for PREVI® COLOR Cytocentrifuge Rotor preparations. The primary requirements are that the specimen be a cell suspension, preferably of single cells, and that the cells are fresh and intact enough to yield diagnostic information. With proper application of the general principles of PREVI® COLOR Cytocentrifuge Rotor operation, consistent preparations of well-preserved cell monolayers should result.

SPECIMEN TYPE(S) **N/A**

REQUIRED REAGENT(S)

I. Single sample chamber - Fast (White)
II. Single sample chamber - Slow (Tan)
III. Poly-L-Lysine slides for single sample chamber
IV. Cyto slide protection

CALIBRATION **N/A**

QUALITY CONTROL **N/A**

PROCEDURAL STEPS

The PREVI® COLOR Cytocentrifuge Rotor is intended for use with the PREVI® COLOR GRAM instrument. For more information, refer to the Intended Use and Users section of the package insert.

Table 9: General Information (Single Chamber)

Sample Preparation	Cyto. pad Type *	Sample Volume µL	Prewet µL**	Speed rpm	Time min	Acceleration
Bronchoalveolar Liquid (BAL)	Tan/ White	200 minimum	0 to 100	1000	5	High
Cerebrospinal Fluid (CSF)	Tan	200 minimum	0 to 100	1000	5	High
Synovial Fluid	White	200 minimum	0 to 100	1000	5	High
Urine	Tan	200 minimum	0 to 100	1000	5	High

It is possible to dilute fluids that are too concentrated.
Thin sample: slow (tan); thick sample: fast (white)
** Load up to 100 µL balanced saline in tunnel

I. The PREVI® COLOR Cytocentrifuge Rotor should always be opened and closed in a biological safety hood.
II. Always use appropriate individual protection equipment when handling biological specimens.
III. CAUTION: Never load chipped or cracked slides into the instrument. Slides in poor condition may break during the staining cycle. If a slide breaks in the bowl, refer to the Cleaning Broken Slides section.
IV. IMPORTANT: The rotor must be balanced by placing the chambers and slides opposite each other. Use an empty chamber and slide if necessary.
V. It is strongly recommended that you use slides pre-coated with Poly-L-Lysine to reduce cell loss during wet fixation and staining.

VI. Place each slide into a slide bracket with the labeled side facing the rotor.
VII. Depress the release lever and insert a chamber assembly
VIII. Release the lever while gently pressing down on the top of the chamber frame.
IX. Load the specimen and prewetting fluids through the cap vents.

X. Place the lid on the rotor by lifting the locking pin while placing the center pin into the rotor lid
XI. IMPORTANT: Ensure that the caps are tightly closed
XII. Push down on the locking pin until it locks.
XIII. Changing from the Staining Carousel to the Cytocentrifuge Rotor

A. Select **Cyto** to start the Cytocentrifuge mode.
B. Place the rotor into the instrument by gently lowering it into place on the drive hub. Make sure the rotor is firmly seated on the hub.
C. Close the instrument lid.

XIV. Starting a Cytocentrifuge Cycle
A. Select **Cyto**.
B. Select Cytocentrifuge program to be started.
C. Select **Start**. A tone will sound at the end of the cycle.
D. Open the lid.
E. Remove the rotor and place it in a biological safety hood
Note: *Selecting* ***Cyto*** *brings up the Cytocentrifuge mode. You can return to the Stain mode by selecting the Back Arrow.*

XV. Unloading Specimens
CAUTION: Never attempt to release the lid by holding the lid knob and shaking the rotor with the locking pin released. This will cause the rotor to drop, resulting in damage to the slides and the rotor.

IMPORTANT: Cells dry rapidly when slides are removed from the rotor. Transporting exposed slides exposes them to air flow and greatly accelerates drying.
XVI. To unload specimens from the rotor:

A. Remove the rotor lid.
B. Check the chambers for residual fluid in the tunnel. If fluid remains, make the specimen return to the well by carefully pressing the release lever.
C. Remove the chamber and discard it in a biohazard container.
D. Wet fix or air dry the slides as quickly as possible.
E. CAUTION: Removing fluid by wet fixing or air drying causes some cell loss. The remaining cells may not be completely flattened against the slide**.**

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS **N/A**

REFERENCES

I. PREVI® Color Gram V2. Package Insert. 2020 bioMerieux. 800-682-2666 or bioMérieux, Inc., 100 Rodolphe Street, Durham, NC 27712.

DEFINITIONS: **N/A**

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	1/1/2021	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES: Policy 810.11 Cytospin 3

EFFECTIVE: January 2021

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Microbiology**

SUB SECTION: **Procedures**

SUBJECT: **chromID® Strepto B agar for the screening of group B streptococci (Streptococcus agalactiae) in Pregnant women**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY **N/A**

chromID® Strepto B agar for the screening of group B streptococci (*S. agalactiae*)

TEST PRINCIPLE / PURPOSE

chromID® Strepto B agar is a selective chromogenic medium that is intended to aid in the qualitative determination of Group B Streptococcus (GBS) colonization in pregnant women. This medium supports the growth of, but does not differentiate between, hemolytic and non-hemolytic GBS strains. The test is performed on 18–24-hour LIM broth enrichments of vaginal/rectal swabs obtained from pregnant women. chromID® Strepto B agar results can be interpreted after 24 hours incubation with confirmation of characteristic GBS colonies from the media.

chromID® Strepto B agar is not intended to diagnose infection nor to guide or monitor treatment for infections. chromID® Strepto B agar does not provide susceptibility results. Subculture to non-selective media should be performed as needed for susceptibility testing. chromID® Strepto B agar is intended for use by laboratory health practitioners in a clinical laboratory.

CLINICAL SIGNIFICANCE

Among babies, there are 2 main types of GBS disease: Early-onset — occurs during the first week of life, and Late-onset — occurs from the first week through three months of life.
According to the CDC, in the United States, GBS bacteria are a leading cause of meningitis and bloodstream infections in a newborn's first three months of life. Newborns are at increased risk for GBS disease if their mother tests positive for the bacteria during pregnancy. Two to 3 in every 50 babies (4–6%) who develop GBS disease die.

SPECIMEN TYPE(S)

Specimens consist of vaginal/rectal swabs from pregnant women enriched in LIM broth (Todd Hewitt with Colistin 10 mg/L and Nalidixic Acid 15 mg/L).

Good laboratory practices for collection and transport should be respected and adapted to the type of specimen.

REQUIRED REAGENT(S)

I. Cary-Blair swab transport medium

II. Ready-to-use medium: REF: 419751 Pack of 2x10 plates (90 mm) STRB, printed on each plate

III. LIM broth

IV. Ancillary culture media, BAP

V. Other laboratory equipment as required

VI. Warnings and Precautions

A. For in vitro diagnostic use only.
B. For professional use only.
C. This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
D. All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure.
E. Do not use reagents after the expiry date.
F. Do not use reagents if the packaging is damaged.
G. Do not use contaminated plates or plates that exude moisture.
H. Interpretation of the test results should be made taking into consideration the patient's history, the source of the specimen, colonial, and microscopic morphology and, if necessary, the results of any other tests performed.
I. Use of the medium may be difficult for people who have problems recognizing colors.
J. Do not use plates which are a pink color.
K. The performance data were obtained using the procedure indicated in the package insert. Any change or modification in the procedure may affect the results.
L. Storage Conditions

1. Store the plates in their box at +2°C - +8°C until the expiry date.
2. If not in the box, plates can be stored in the cellophane wrapper for 2 weeks at +2°C - +8°C in the dark.

CALIBRATION N/A

QUALITY CONTROL

Check performance of each new lot media by inoculating plates with pure cultures of control organisms that produce known desired reactions. The nutrient capacity of the medium can be tested using the following quality control strains:

I. Streptococcus agalactiae ATCC® 13813™

II. Staphylococcus aureus ATCC® 29213™

III. Expected results after incubation for 24 hours:

Strain	Results at +35°C - +37°C
Streptococcus agalactiae ATCC 13813	Pale pink to red colonies
Staphylococcus aureus ATCC 29213	No growth after 24 hours

PROCEDURAL STEPS

I. Culture Setup

chromID® Strepto B agar can be used after enrichment of the vaginal/rectal swab specimen in LIM broth.

The medium should not be exposed to light apart from during the inoculation and reading steps.

A. Allow the plates to come to room temperature in the dark.
B. Inoculate chromID® Strepto B agar with 100 µL of the enriched LIM broth specimen.
C. Incubate at +35°C - +37°C for 24 hours in aerobic (non – CO_2) incubator in the dark.

II. Reading and Interpretation

A. After incubation, observe the bacterial growth and the appearance of the colonies: a typical *Streptococcus agalactiae* colonies are pale pink to red.
B. The results must be confirmed by a latex agglutination tests performed directly from the chromID® Strepto B agar. If no pale pink to red colonies is observed after 24 hours incubation, the specimen is considered negative.
C. The growth of micro-organisms belonging to other species is either inhibited or the colonies produced are of a different color (e.g.: violet, blue, colorless etc.)
D. Subculture to a non-selective media, tryptic soy agar with 5% sheep blood, if susceptibility testing is indicated.
E. When susceptibility is requested, setup Microstrep Plus1 Manual Read GBS. Refer to Procedure 810.59 page 16 – 22.

III. Result reporting

A. Negative results

1. Enter pneumonic, NGBS, for NO GROUP B STREP ISOLATED under results or in culture results page, hit F5 for canned message and enter NGBS

B. Positive results

1. Enter GBS under Organism ID page. No quantitation is required

C. Positive result with sensitivity

1. From Microstrep plus panel, test Vancomycin, Erythromycin, Clindamycin, and perform inducible clindamycin by a D-test, when indicated, and report only Cd and hide vancomycin. Do not report Penicillin on penicillin-allergic pregnant women.

IV. Waste Disposal

Unused reagents may be considered as non-hazardous waste and disposed of accordingly. Dispose of used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

V. Limitations of the method

A. Growth depends on the requirements of each individual micro-organism. It is therefore possible that certain strains of *S. agalactiae* which have specific requirements (substrate, temperature, incubation conditions, etc.) may not develop.

B. Susceptibility and Identification testing should not be performed directly from chromID® Strepto B media. If either a susceptibility test or an identification test is performed using colonies from chromID® Strepto B media, the result obtained will not be interpretable. Certain strains of *S. porcinus* may exhibit pigmentation characteristic of *S. agalactiae*.

C. The following organisms (not S. agalactiae) may grow on chromID® Strepto B but do not produce characteristic colonies after 24 hours: *Aerococcus urinae, Arthrobacter cumminsii, Bifidobacterium spp, Candida albicans, Candida glabrata, Candida krusei, Candida tropicalis Corynebacterium amycolatum/xerosis, Dermabacter hominis, Enterococcus casseliflavus, Enterococcus durans, Enterococcus faecalis (VRE strain), Enterococcus feacium (VRE strain), Enterococcus gallinarum, Enterococcus hirae, Enterococcus raffinosus, Facklamia hominis, Geobacillus thermoglucosidasius, Globicatella sanguinis, Lactobacillus acidophilus, Lactobacillus acidophilus/gasseri, Lactobacillus casei/paracasei, Lactobacillus crispatus, Lactobacillus jensenii, Lactobacillus plantarum, Lactococcus garvieae, Lactobacillus salivarius, Micrococcus luteus/lylae, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus pasteuri, Staphylococcus warneri, Streptococcus australis, Streptococcus bovis, Streptococcus group D, Streptococcus equi, Streptococcus equinus, Streptococcus gallolyticus, Streptococcus gordonii, Streptococcus infantarius, Streptococcus pyogenes, Streptococcus uberis, Streptococcus vestibularis, Vagococcus fluvialis, Weissella confuse*

D. The following organisms (not S. agalactiae) may produce characteristic colonies after 24 hours on chromID® Strepto B: *Escherichia coli, Lactococcus lactis, Streptococcus canis*. The following organisms (not S. agalactiae) may produce characteristic or non-characteristic colonies after 24 hours on chromID® Strepto B: *Klebsiella pneumoniae (KPC strain), Lactobacillus sakei, Streptococcus group C., Streptococcus anginosus, Streptococcus pyogenes. Enterococcus avium, Enterococcus faecalis, Enterococcus faecium, Streptococcus constellatus, Streptococcus dysgalactiae, Streptococcus mitis/oralis, Streptococcus parasanguinis, Streptococcus porcinus, Streptococcus salivarius, Staphylococcus aureus (MRSA).*

E. Analytical studies demonstrated that the recovery of group B Streptococci is dependent on incubation time and organism load. Use of medicines that contain naproxen sodium 27.5 mg/mL or topical product (body powder) 0.125g/mL may demonstrate partial inhibition of the growth of group B Streptococci. Use of compounds containing the active ingredients listed below may have an inhibitory effect on GBS growth that is unrelated to chromID® Strepto B medium performance: nystatin (104 UI/mL), hydrocortisone (0.625 mg/mL), aluminum hydroxide (2.125 mg/mL)/magnesium hydroxide (2.250 mg/mL), mesalazine (5 mg/mL), barium

sulfate (5 mg/mL), esomeprazole (1 mg/mL), loperamide (1 mg/mL), sennosides (40 mg/mL), metronidazole (25 mg/mL), lidocaine (2.5 mg/mL), econazole (7.2 mg/mL), naproxen sodium (27.5 mg/mL), nonoxynol-9 (one condom/50 mL sterile water; used at 1:1 dilution), benzalkonium chloride (1 wipe/100 mL sterile water; used at 1:1 dilution). In the presence of high concentrations of Streptococcus group C, GBS may grow as no characteristic colored (purple) colonies on chromID® Strepto B medium at 24 hrs.

CALCULATIONS **N/A**

REFERENCE INTERVALS **N/A**

INTERPRETATION OF RESULTS **N/A**

REFERENCES:

I. Package Insert; chromID® Strepto B agar for the screening of group B streptococci (S. agalactiae). Biomerieux, Inc. 100 Rodolphe Street, Durham, North Carolina, 2017.
II. Centers for Disease Control and Prevention. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, Group B *Streptococcus*, 2018

DEFINITIONS: **N/A**

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	8/1/2021	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES: **Policy 800.48 Group B Beta Strep Culture Station**

EFFECTIVE: **August 2021**

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Point-of-Care**

SUB SECTION: **Procedures**

SUBJECT: **Abbott ID NOW COVID-19 Testing**

APPROVED BY: _____

Laboratory Medical Director or Designee

TEST NAME / POLICY

Abbott ID NOW Covid-19 test is a Point-of-Care (POC) test. It is a waived (emergency use authorization only) laboratory test that is performed according to the manufacturer's instructions under the regulations set forth by the Clinical Laboratory Improvement Act (CLIA), College of American Pathologists (CAP), and the State of California Business and Professions Code. Testing is performed by licensed healthcare providers who have successfully completed appropriate training and competency.

TEST PRINCIPLE / PURPOSE

ID NOW™ COVID-19 assay performed on the ID NOW™ Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology intended for the qualitative detection of nucleic acid from the SARS-CoV-2 viral RNA in direct nasal, nasopharyngeal or throat swabs from individuals who are suspected of COVID-19 by their healthcare provider. Testing is authorized for laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity/high complexity tests. The ID NOW™ COVID-19 assay is also authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

ID NOW™ COVID-19 is an automated assay that utilizes isothermal nucleic acid amplification technology for the qualitative detection of SARS-CoV-2 viral nucleic acids. It is comprised of a Sample Receiver, containing elution/lysis buffer, a Test Base, comprising two sealed reaction tubes, each containing a lyophilized pellet, a Transfer Cartridge for transfer of the eluted sample to the Test Base, and the ID NOW™ Instrument.

The reaction tubes in the Test Base contain the reagents required for amplification of SARS-CoV-2, as well as an internal control. The templates (similar to primers) designed to target SARS-CoV-2 RNA amplify a unique region of the RdRp segment. Fluorescently labeled molecular beacons are used to specifically identify each of the amplified RNA targets.

To perform the assay, the Sample Receiver and Test Base are inserted into the ID NOW™ Instrument. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base, initiating target amplification. Heating, mixing and detection are provided by the instrument.

CONDITIONS OF AUTHORIZATION FOR LABORATORY AND PATIENT CARE SETTINGS

The ID NOW™ COVID-19 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm.
However, to assist clinical laboratories and patient care settings using the ID NOW™ COVID-19 ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

I. Authorized laboratories and patient care settings using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

II. Authorized laboratories and patient care settings using your product will use your product as outlined in the package insert. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

III. Authorized laboratories and patient care settings that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

IV. Authorized laboratories and patient care settings using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

V. Authorized laboratories and patient care settings will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Abbott Diagnostics Scarborough, Inc. technical support (via email: ts.scr@abbott.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

VI. All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

CLINICAL SIGNIFICANCE

The ID NOW system is used as an aid in screening patients for COVID-19. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory samples during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Testing facilities within the United States and its territories are required to report all positive results to the appropriate public health authorities.
Negative results should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, should be tested with different authorized or cleared molecular tests. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results should be

considered in the context of a patient's recent exposures history and the presence of clinical signs and symptoms consistent with COVID-19.
The ID NOW™ COVID-19 test is intended for use by medical professionals or trained operators who are proficient in performing tests using the ID NOW™ Instrument. The ID NOW™ COVID-19 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

SPECIMEN TYPE(S)

I. Collection and Handling:
   A. Use freshly collected specimens for optimal test performance. Inadequate specimen collection or improper sample handling/storage/transport may yield erroneous results. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

   B. ID NOW™ COVID-19 is intended for testing a swab directly without elution in viral transport media as dilution will result in decreased detection of low positive samples that are near the limit of detection of the test.

   C. To minimize the risk of contamination of PPE and swab package during sample collection, it is recommended to widely open the package by pulling from the top down. Carefully remove the swab and perform sample collection.

II. Acceptable specimen
   A. Throat Swab:
   For optimal test performance, use the swabs provided in the test kit. Alternatively, foam, polyester, HydraFlock® and nylon flocked throat swabs can be used to collect throat swab samples. Collect patient specimen by swabbing the posterior pharynx, tonsils and other inflamed areas. Avoid touching the tongue, cheeks and teeth with the swab.
   B. Nasal Swab:
   For optimal test performance, use the swabs provided in the test kit. Alternatively, rayon, foam, HydraFlock® Flocked swab (standard tip), HydraFlock® Flocked swab (mini tip), Copan Mini Tip Flocked Swab, or Copan Standard Flocked swabs can be used to collect nasal swab samples. To collect a nasal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Using gentle rotation, push the swab until resistance is met at the level of the turbinates (less than one inch into the nostril). Rotate the swab several times against the nasal wall then slowly remove from the nostril. Using the same swab, repeat sample collection in the other nostril.
   C. Nasopharyngeal Swab**:**
   Use sterile rayon, foam, polyester or flocked flexible-shaft NP swabs to collect a nasopharyngeal sample.
   To collect a nasopharyngeal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Pass the swab directly backwards without tipping the swab head up or down. The nasal passage runs parallel to the floor, not parallel to the bridge of the nose. Using gentle rotation, insert the swab into the anterior nare parallel to the palate advancing the swab into the nasopharynx, leave in place for a few seconds, and then slowly rotate the swab as it is being withdrawn.
   To ensure proper collection, the swab should be passed a distance that is halfway of that from the nose to the tip of the ear. This is about half the length of the swab. **DO NOT USE**

FORCE while inserting the swab. The swab should travel smoothly with minimal resistance; if resistance is encountered, withdraw the swab a little bit without taking it out of the nostril. Then elevate the back of the swab and move it forward into the nasopharynx.

III. Specimen Transport
   A. Direct nasal, throat or nasopharyngeal swabs should be tested as soon as possible after collection. If immediate testing is not possible, the nasal, throat or nasopharyngeal swab can be held in a conical tube and capped tightly at room temperature (15-30°C) for up to one (1) hour prior to testing. If greater than one (1) hour delay occurs, dispose of sample. A new sample must be collected for testing.

IV. Unacceptable specimen
   A. Rayon swabs are not suitable for use in this assay
   B. Puritan PurFlock Standard Tip Ultra Flocked Swabs, Puritan PurFlock Mini Tip Ultra Flocked Swabs and Copan Standard Rayon Tip Swabs are not suitable for use in this assay.
   C. Swab received in viral transport media.

LIMITATIONS / PRECAUTIONS

I. For *in vitro* diagnostic use.
II. This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. . §263a, to perform moderate complexity/high complexity tests and at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.
III. Federal Law restricts this device to sale by or on the order of a licensed practitioner (US only).
IV. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
V. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
VI. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health laboratories.
VII. To be used in conjunction with the ID NOW™ Instrument.
VIII. Treat all specimens as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
IX. Proper sample collection, storage and transport are essential for correct results.
X. Leave test pieces sealed in their foil pouches until just before use.
XI. Do not tamper with test pieces prior to or after use.
XII. Do not use kit past its expiration date.
XIII. Do not mix components from different kit lots or from other ID NOW™ assays.
XIV. Solutions used to make the positive control swab are inactivated using standard methods. However, patient samples, controls, and test pieces should be handled as though they could transmit disease. Observe established precautions against microbial hazards during use and disposal.
XV. Wear clean personal protection equipment and gloves when running each test. Change gloves between the handling of specimens suspected of COVID-19.
XVI. **If any assay components are dropped, cracked, found to be damaged or opened when received, DO NOT USE and discard. Do not use scissors or sharp objects to open foil pouches as damage to test pieces can occur.**
XVII. Do not open the Sample Receiver before placing in the instrument. It will prohibit the Elution Buffer

from reaching temperature and may impact test performance.

XVIII. If the Sample Receiver is spilled while opening, clean the instrument per instructions provided in the instrument User Manual and cancel test. Repeat test with a new Sample Receiver.

XIX. All test pieces must be removed from the instrument according to removal instructions displayed on the instrument and disposed of according to country and local requirements. **Pieces must not be separated once they are assembled**.

XX. All test pieces are single use items. Do not use with multiple specimens.

XXI. Once reacted, the Test Base contains large amounts of amplified target (Amplicon). **Do not disassemble the Test Base and Transfer Cartridge**. In the case of a positive sample, this could lead to amplicon leakage and potential ID NOW™ COVID-19 false positive test results.

XXII. At a low frequency, clinical samples can contain inhibitors that may generate invalid results. Site to site invalid rates may vary.

XXIII. Due to the high sensitivity of the assays run on the instrument, contamination of the work area with previous positive samples may cause false positive results. Handle samples according to standard laboratory practices. Clean instruments and surrounding surfaces according to instructions provided in the cleaning section of the instrument User Manual. Refer to Section 1.6, Maintenance & Cleaning, for further information.

XXIV. The performance of the ID NOW™ COVID-19 test was evaluated using the procedures provided in this product insert only. Modifications to these procedures may alter the performance of the test.

XXV. Negative results should be treated as presumptive and tested with an alternative FDA authorized molecular assay, if necessary for clinical management, including infection control.

XXVI. False negative results may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate levels of viruses are present in the specimen. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

XXVII. As with any molecular test, mutations within the target regions of the Abbott ID NOW™ COVID-19 test could affect primer and/or probe binding resulting in failure to detect the presence of the virus.

XXVIII. The test cannot rule out diseases caused by other bacterial or viral pathogens.

XXIX. ID NOW™ COVID-19 is intended for testing a swab directly without elution in viral transport media as dilution will result in decreased detection of low positive samples that are near the limit of detection of the test.

XXX. Swab samples eluted in VTM are not appropriate for use in this test.

REQUIRED REAGENT(S)

I. Abbott ID NOW COVID-19 Test Kit 190-000
   A. Abbott ID NOW test base
   B. Abbott ID NOW sample receivers
   C. Abbott ID NOW transfer cartridges
   D. Sterile foam swab
   E. Abbott ID NOW positive control swab
   F. Abbott ID NOW sterile swab used as negative control swab

II. Abbott ID NOW COVID-19 External Control Kit 190-080

III. Reagent Storage requirements:

   A. Each test base, sample receiver, and transfer cartridge are individually packaged in a foil pouch for single-use only.
   B. Testing pieces must be brought to room temperature before use.

C. Unopened test pieces are stored at 2-30°C until the printed expiration date

CALIBRATION

I. The ID NOW instrument is factory calibrated and does not require any further calibration and verification

II. If the instrument is transported or moved, a performance check using ID NOW positive and negative controls is recommended to ensure proper functionality

QUALITY CONTROL

I. ID NOW™ COVID-19 has built-in procedural controls. The result of the Procedural Control is displayed on the screen and is automatically stored in the instrument with each test result. This can be reviewed later by selecting Review Memory on the instrument.

A. Procedural Control Valid displayed on the instrument screen indicates that the assay reagents maintained their functional integrity and the sample did not significantly inhibit assay performance.

B. Procedural control **must be valid** for test results to be interpreted.

II. Positive and Negative Controls should be tested following the Run QC Test instructions on the ID NOW™ Instrument. A Positive Control Swab is included in the kit. Use a sterile swab provided in the kit as the Negative Control Swab. Refer to Quality Control Swab Test Procedure or Instrument User Manual for further details. If the correct control results are not obtained, do not perform patient tests or report patient results. Contact Technical Support during normal business hours before testing patient specimens

Note: *The ID NOW™ Instrument reports QC results as Pass or Fail.*

III. External Positive and Negative Controls:
positive and negative controls are run to ensure that test reagents are working and that the test is correctly performed. ID NOW™ COVID-19 kits contain a Positive Control Swab and Sterile Swabs that can be used as a Negative Control Swab. These swabs will monitor the entire assay. Test these swabs once with each new shipment received and once for each untrained operator. Further controls may be tested in order to conform with local, state and/or federal regulations, accrediting groups, or your lab's standard Quality Control procedures.

PROCEDURAL STEPS

I. Please refer to the ID NOW™ Instrument User Manual for full instructions.
II. Before testing with ID NOW™ COVID-19:
A. **Put on a clean pair of gloves.**
B. Allow all samples to reach room temperature.
C. Allow all test pieces to reach room temperature.
D. Check that a reagent pellet is visible at the bottom of each of the reaction tubes prior to inserting the Test Base in the ID NOW™ Instrument. Do not use the Test Base if a pellet is not visible at the bottom of each reaction tube.

To Perform a Test:

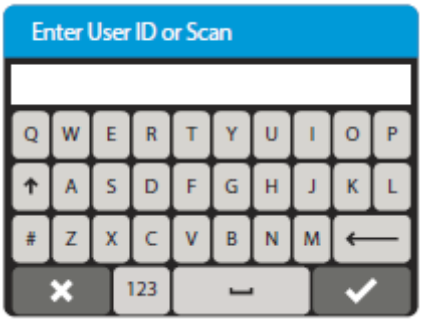
Step 1

Turn on the ID NOW™ Instrument - press the power button ⏻ on the side of the instrument.

Note: *If the unit is unattended for one hour, the instrument will go to a black screen power save mode. Touch the screen to return the unit to active display operation.*

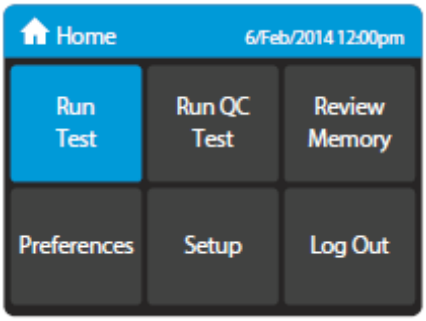
Enter User ID

Press '✓' after entry.



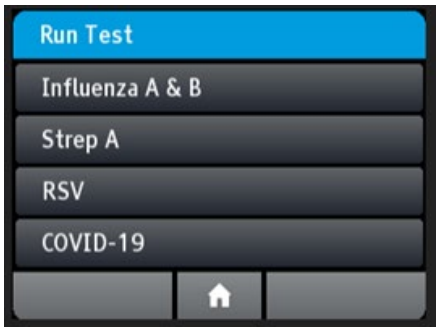
Touch 'Run Test'

This will begin the test process.



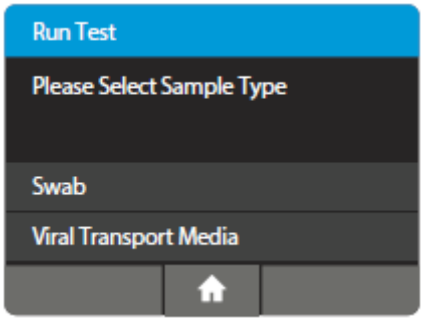
Touch 'COVID-19 Test'

This starts a COVID-19 test.



Select Swab Sample Type (if prompted)

If the sample type has already been specified by the Admin, the instrument will automatically advance to the next step.

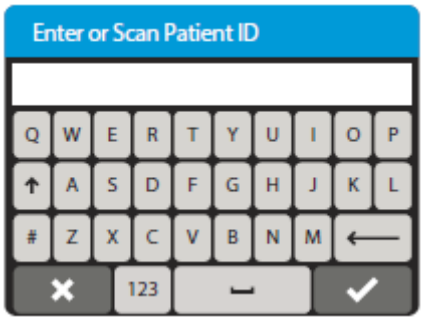


⚠ **Caution: VTM Samples are not an appropriate sample type for the ID NOW™ COVID-19 test.**

Enter Patient ID using on screen keyboard or barcode scanner.

Touch '✓'.

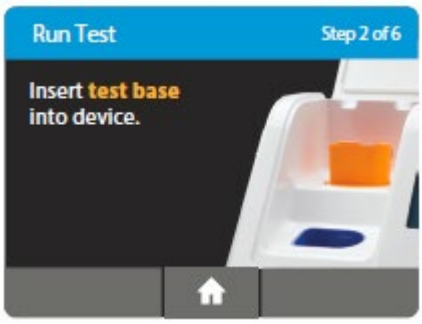
Verify that the ID was entered correctly, then touch '✓' to confirm entry.



Step 2

Open the Lid and Insert Orange Test Base into Orange Test Base holder

⚠ **Caution: Do not apply excessive force. Excessive force could damage the instrument.**



Confirm that the correct test is displayed on the screen.

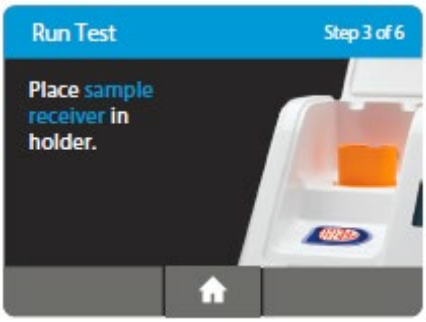
Touch 'OK' to proceed.

⚠ **Caution: Once the Test Base has been placed in the holder, the user will have 10 minutes to confirm the test. If the test is not confirmed within 10 minutes, the instrument will time out and the Test Base must be removed and discarded.**

If the incorrect Test Base has been inserted, remove and dispose of the incorrect Test Base. Close the lid. The instrument will then run a self-test before proceeding to the Home screen. Press Run Test and restart the test using the correct Test Base.

Step 3

Insert Blue Sample Receiver into the Blue Sample Receiver holder



⚠ **Caution: Do not apply excessive force. Excessive force could damage the instrument.**

⚠ **Caution: Once the Sample Receiver has been placed in the holder, the user will have 10 minutes to start the test (Steps 3 through 5). If the test is not started within 10 minutes, the instrument will time out and all test pieces (Test Base and Sample Receiver) must be removed and discarded. The instrument will proceed to the Home screen. Press Run Test and restart the test using a new Test Base and Sample Receiver.**

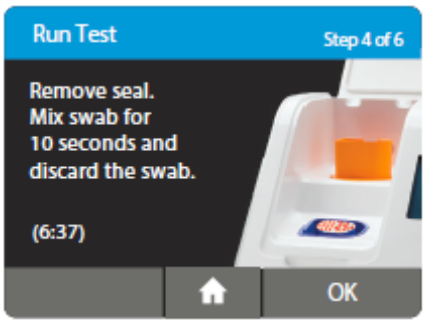
Wait for the Sample Receiver to Warm Up. Do not remove the Sample Receiver from the instrument once the Warm Up begins.

⚠ **Caution: DO NOT REMOVE THE FOIL SEAL UNTIL PROMPTED BY THE INSTRUMENT. DO NOT** close the lid or insert the sample until prompted by the instrument.

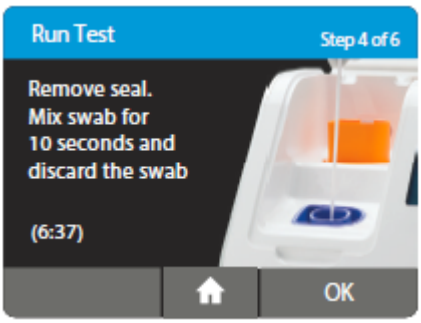
Step 4

Direct Nasal, Throat or Nasopharyngeal Swab Test Procedure

When prompted, remove the foil seal and place the patient swab to be tested into the Sample Receiver.



Mix the swab in the liquid for 10 seconds. This helps remove the sample from the swab. Lift the swab out of the liquid and press the swab head against the side of the Sample Receiver to remove excess liquid. Once the swab is removed, touch 'OK' to proceed.



Discard the swab into a biohazard waste container.

Caution: To ensure that the Sample Receiver remains in the instrument while removing the foil seal, place two fingers along the outer edge of the Sample Receiver to hold it in place. If the Sample Receiver spills after warm up, cancel the test by pressing the Home button. Remove and discard the test pieces (Sample Receiver and Test Base) and clean the instrument. Press Run Test to start a new test using a new Test Base and Sample Receiver.

Step 5a

Press the White Transfer Cartridge into the Blue Sample Receiver

Listen for a click.

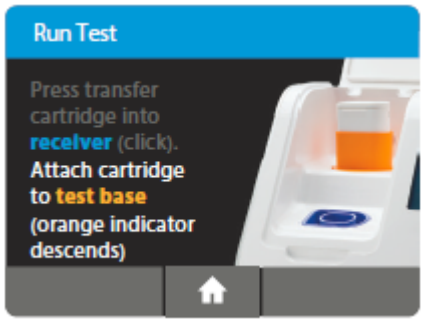
When the Transfer Cartridge is properly attached to the Sample Receiver, the orange indicator on the Transfer Cartridge will rise. If the orange indicator does not rise, continue pushing onto the Sample Receiver until it does.

⚠ **Caution: The orange indicator should be observed closely. If the orange indicator does not fully rise, the Transfer Cartridge may not collect enough sample.**

Step 5b

Lift and then connect the Transfer Cartridge to the Test Base

When the Transfer Cartridge is properly attached to the Test Base, the orange indicator on the Transfer Cartridge will descend. If the orange indicator does not descend, continue pushing onto the Test Base until it does.



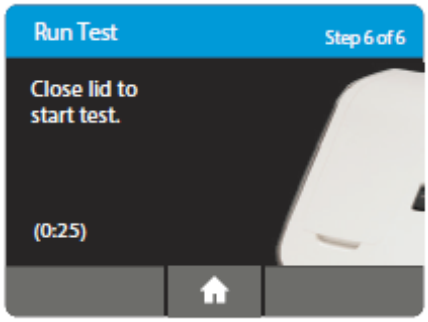
⚠ **Caution: If the orange indicator does not fully descend, not enough sample will be dispensed. This may potentially result in invalid or false test results.**

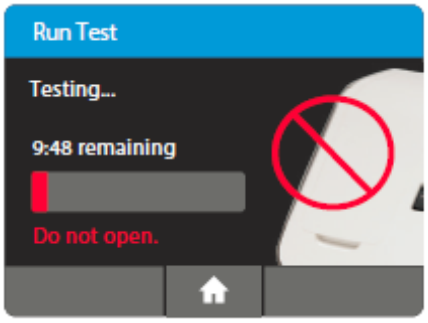
Step 6

Close the Lid.

DO NOT OPEN THE LID until the **Test Complete** message appears on the screen.

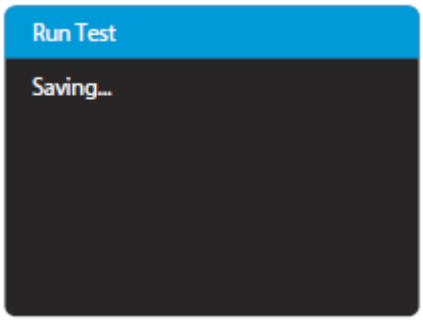
Note: *The test will be cancelled if the lid is opened.*





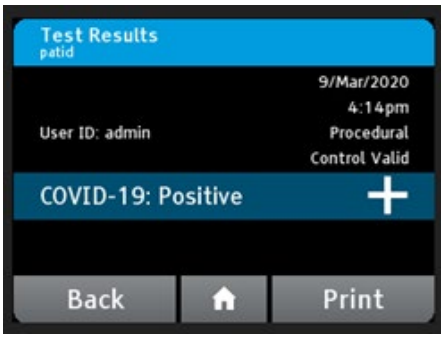
⚠ **Caution: This screen will be displayed for up to 30 seconds once the Transfer Cartridge is detected. If the instrument does not detect that the lid has been closed by then, it will time out and all test pieces (Sample Receiver, Test Base, and Transfer Cartridge) must be removed and discarded. The instrument will proceed to the Home screen. Collect a new sample from the patient. Press Run Test and restart the test using a new Test Base and Sample Receiver.**

⚠ **Caution: DO NOT OPEN THE LID. The test will be cancelled and all test pieces (Sample Receiver, Test Base, and Transfer Cartridge) must be removed and discarded. A test result will not be reported or saved in the instrument memory.**



When amplification and detection is complete, the instrument will automatically save the data before advancing to the results screen.

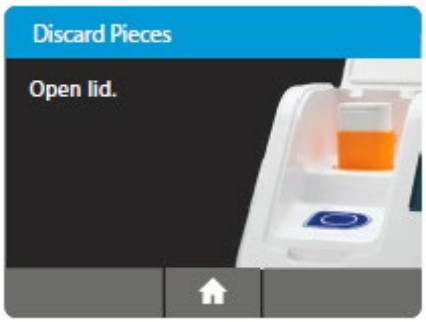
⚠ **Caution: The test is not saved until the completed result is displayed. Do not open the lid until the results are displayed.**



The **Test Results** screen displays either a Negative or Positive result for a successfully completed test. If a test error occurs, the display will read 'Invalid'. Refer to the Result Interpretation Section for Interpretation of Results.

Press Print to print test results, press New Test to run another test, Press Home to return to the Home screen

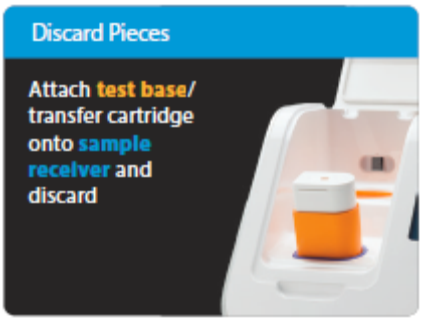
After printing, or if New Test or Home are selected, the instrument will prompt to open the lid and discard the used test pieces.



Remove test pieces by lifting the Transfer Cartridge attached to the Test Base, and clicking it into the Sample Receiver, by pressing into the Sample Receiver.

⚠ **Caution: Do not try to remove the Sample Receiver by any other method as there is a risk of spilling the patient sample.**

All test pieces will be connected and can now be removed from the instrument and disposed of according to federal, state and local regulations.



⚠ **Caution: DO NOT disassemble the Transfer Cartridge and the Test Base before disposal.**

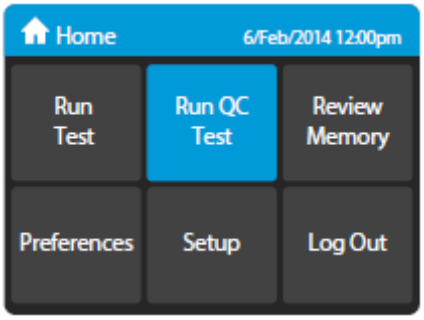
Close the lid. The instrument will then run a Self-Test before showing the Home screen or Enter Patient ID screen, depending on the previous selection.

Remove and dispose of gloves.

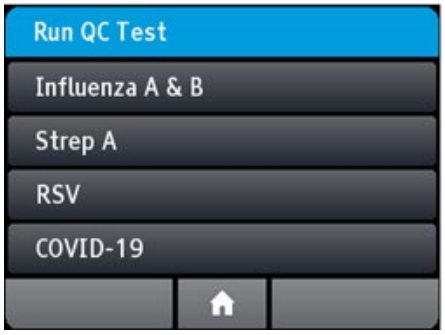
1. Quality Control Swab Test Procedure

For QC testing, select Run QC Test on the Home screen, and follow the displayed instructions. Refer to Running a QC Test in the ID NOW™ Instrument User Manual for further details.

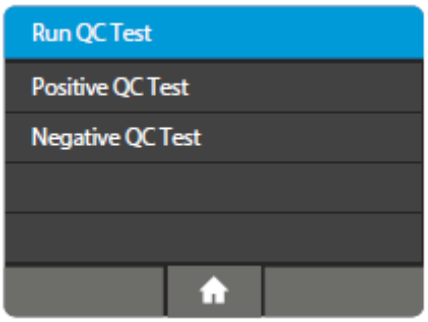
1 Touch 'Run QC Test'



2 Touch 'COVID-19'



3 Select the QC Test to be Run

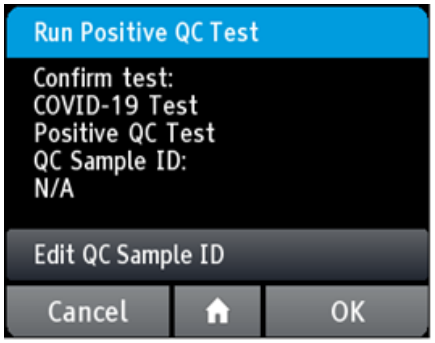


4 Confirm Test

Confirm the test type to match the QC sample intended for testing by touching 'OK' and following the on screen prompts to complete testing.

The user has the option to enter an ID for the QC Sample being run.

Note: *The QC test is run in the same manner as a Direct Nasal/Throat/Nasopharyngeal Swab Patient Test. See the* ***To Perform a Test*** *section above for step by step instructions for direct nasal/throat/nasopharyngeal swab samples.*



MAINTENANCE:

I. Clean instrument and surrounding bench area DAILY with acceptable cleaning agents listed below:

A. 70% ethanol (commercial wipes or on damp lint free cloth)

B. 70% isopropanol (commercial wipes or on damp lint free cloth)

C. 10% bleach (on damp lint free cloth)

SAFETY:

I. All operators must use appropriate personal protective equipment (PPE) in accordance with CDC guidelines and hospital infection control.

A. Follow Standard Precautions when handling clinical specimens, including hand hygiene and the use of proper PPE, such as laboratory coats, gloves, and eye protection. If needed, additional precautions can be used, such as a surgical mask or face shield, or other physical barriers, such as a splash shield to work behind. (CDC, 2020)

II. Clean gloves should be used for all patient sample handling.

CALCULATIONS: N/A

REFERENCE INTERVALS:

I. Expected COVID-19 test result is negative.

INTERPRETATION OF RESULTS:

I. Procedural control **must be valid** for test results to be interpreted.

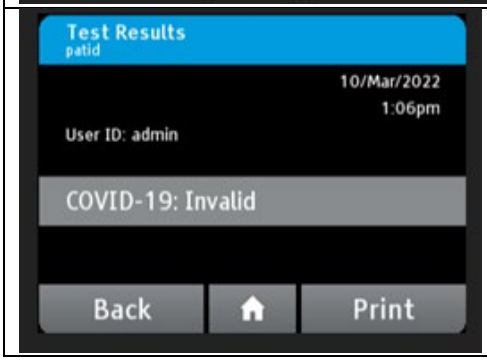
II. If an Invalid result is received, one additional test may be run using the same Sample Receiver. See instructions below to repeat testing.

III. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory samples during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Testing facilities within the United States and its territories are required to report all positive results to the appropriate public health authorities.

IV. Negative results should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, should be tested with different authorized or cleared molecular tests. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results should be considered in the context of a patient's recent exposures history and the presence of clinical signs and symptoms consistent with COVID-19.

V. The ID NOW™ COVID-19 test is intended for use by medical professionals or trained operators who are proficient in performing tests using the ID NOW™ Instrument. The ID NOW™ COVID-19 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

VI. When the test is complete, the results are clearly displayed on the instrument screen.

Instrument Display	Interpretation of Results and Follow-up Actions
	**COVID-19 Positive** Positive results do not rule out bacterial infection or co-infection with other viruses.
	**COVID-19 Negative** Negative results should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, should be tested with an alternative molecular assay. A negative result does not rule out co-infections with other pathogens.
	**The presence or absence of COVID-19 Viral RNAs cannot be determined.** Repeat testing of the sample using new test components (test base and transfer cartridge only). If repeated Invalid results are obtained, results should be confirmed by another method prior to reporting the results.

VII. Repeat Testing

A. The used, connected, orange Test Base and white Transfer Cartridge **MUST** be attached to a blue Sample Receiver prior to disposal

1. Open a new Specimen Receiver/ Transfer Cartridge (#2)

2. Remove the blue sample Receiver from the package and open by removing the foil seal

3. Remove the used connect, orange Test Base and white Transfer Cartridge from the instrument

4. Connect the used pieces to the new, **UNUSED**, blue Sample Receiver and dispose

B. Retain the used, blue Sample Receiver for repeat testing

1. Remove the used, blue Sample Receiver from the instrument

2. Keep upright to avoid spilling the liquid contents

C. Repeat test

1. Close the lid (of analyzer) to initiate the Self-Test
From the Home Screen Begin a new test

2. Use a new orange Test Base and white Transfer Cartridge

3. Follow the screen prompts; however, when asked to insert the blue Sample Receiver, **reuse the existing blue Sample Receiver from the initial test.**

4. **DO NOT** re-elute the swab or add additional sample

5. If an inaccurate result is obtained using the repeat procedure, do not retest the sample again. The result should be documented. Additional testing should only be attempted with an alternate method

TROUBLESHOOTING:

I. Sample dispense errors

A. Visually inspect the orange indicator of the white Transfer Cartridge to verify that it fully descended. If the orange indicator is still visible at the top of the white Transfer Cartridge, the specimen was not transferred into the reaction tubes of the orange Test Base.

B. Visually inspect the orange Test Base reaction tubes to confirm the liquid levels in both tubes are equal and that all dry (lyophilized) reagents dissolved properly. If the orange indicator was fully descended and the reaction tubes are dry, the sample was never pipetted from the blue Sample Receiver

II. Procedural errors

A. Confirm test kits are stored at proper temperatures per package insert

B. Do not remove the foil seal on blue Sample Receiver until prompted by instrument

C. Timing is important; follow procedural steps as displayed on the screen

D. Press OK when prompted

E. If any test pieces are accidently dropped, do not use any of the pieces for testing

III. Interfering substances – Listed in the package insert

REFERENCES:

I. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed February 9, 2020.

II. Clinical Laboratory Policy 107.01, Safe Work Practices and Infection Control Policy 107.01 Issue 1

III. Center of Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html Accessed August 21, 2020.

IV. bioRxiv. (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1). Accessed March 3, 2020.

V. Manual of Clinical Microbiology, 11th Edition, Vol. 1, ASM. (2015) pg. 279.

VI. https://www.iata.org/en/programs/cargo/dgr

DEFINITIONS: **N/A**

ATTACHMENTS: **N/A**

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	8/25/2020	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES: **N/A**

EFFECTIVE: **08/25/2020**

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

SECTION: **Point-of-Care**

SUB SECTION: **Procedures**

SUBJECT: **Hemochron Signature Elite Activated Clotting Time Plus (ACT+)**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

The Activated Clotting Time Plus (ACT+) is a non-waived moderately complex Point-of-Care test performed under the regulations set forth by the Clinical Laboratory Improvement Act (CLIA), College of American Pathologists (CAP), and the State of California Business and Professions Code. Testing is performed by licensed personnel who have completed appropriate training and competency, including Registered Nurses (RN), Licensed Vocational Nurses (LVN) or Respiratory Therapists (RT), to monitor heparin therapy. Testing is performed following manufacturer's instructions.

TEST PRINCIPLE / PURPOSE

The Hemochron microcoagulation system utilizes a mechanical endpoint clotting mechanism in which testing occurs within the disposable ACT+ cuvette. The instrument measures 15 microliters (µL) of whole blood**.** Sample/reagent mixing, and test initiation are performed automatically, requiring no operator interaction. The clot detection mechanism consists of several LED (light emitting diode) optical detectors aligned with the test channel of the cuvette. The speed at which the sample moves between the two detectors is measured. As clot formation begins, blood flow is impeded, and the movement slows. The instrument recognizes that a clot endpoint has been achieved when the movement decreases below a predetermined rate. Electronic optical detection of a fibrin clot in the blood sample automatically terminates the test. The instrument's digital timer displays the Celite-equivalent ACT result in seconds in order to provide a familiar clinical format

CLINICAL SIGNIFICANCE

ACT+ is a quantitative assay intended for monitoring moderate to high heparin doses up to 1-6 units/ml blood frequently associated with Cardiopulmonary bypass surgery and Cardiac angioplasty.

SPECIMEN TYPE(S)

I. Acceptable specimen:

   A. Fresh whole blood collected in a syringe using a 23 or larger gauge needle. Test immediately.

II. Unacceptable specimen:

A. Sample that is clotted or hemolyzed.
B. Sample collected with any anticoagulant (including Blue top Sodium Citrate tube.)
C. Sample collected in a pre-heparinized syringe.
D. Sample contaminated with tissue thromboplastin, indwelling intravenous (I.V.) fluids, or alcohol cleansing solutions are unacceptable.

III. Limitations / Interfering Substances:

A. ACT+ is unaffected by aprotinin
B. Samples with hematocrit of <20 or >55% are not recommended.

REQUIRED REAGENT(S)

I. Hemochron Elite ACT+ Test Cuvettes Catalog number, Cat. No. JACT+
II. Normal ACT Direct Check control, Cat. No. DCJACT-N
III. Abnormal ACT Direct Check control, Cat. No. DCJACT-A
IV. Reagent Storage requirements:

A. Each cuvette is individually packaged in a foil pouch with a desiccant and for single use only.
B. Cuvettes must be brought to room temperature prior to use (may take 30 to 60 minutes).
C. Unopened cuvettes can be stored refrigerated (2 to 8°C) until the printed expiration date.
D. Unopened cuvettes can be stored at room temperature (15 - 30°C) for up to twelve weeks, not to exceed the marked expiration date (cuvette packages must be re-dated.)
E. An opened (folded) pouch is good for seven days refrigerated 2 - 8°C (cuvette package must be re-dated.)
F. Liquid controls stored refrigerated (2 – 8 °C) are stable until the vial expiration date.
G. Liquid controls stored at room temperature (not to exceed 37°C) are stable 4 weeks not to exceed the manufacturer's expiration date.

CALIBRATION

Calibration is completed by the manufacturer**.** No additional calibration is necessary.
Instrument will perform a "self-check" every time a test is performed**.** This includes verification of adequate battery power, verification of test type, verification that the cuvette temperature is warmed to 37°C ± 1°C, verification that sample size is adequate and verification of internal timer function**.**

QUALITY CONTROL

I. Electronic Quality Control (EQC): Used to provide a two-level electronic verification of instrument performance.

A. Manufacturer recommends performing EQC every 8 hours of patient testing.
B. Instrument is programmed to perform:
1. Normal level target: 30 seconds. (Range = 29-31 seconds)
2. Abnormal level target: 500 seconds. (Range = 499-501)
3. Internal temperature check target: 37°C. (Range = 36-38°C)

C. View EQC results by selecting the “database” key, then 5 – Display QC. Use the “enter” and “0” keys to view results.
D. EQC can also be requested manually at any time.
E. If one test fails to meet specifications, the EQC test will stop and record all results as failed.
F. If the user aborts the EQC, the test is not saved to the database or printed.
G. If a cuvette is inserted during testing, the EQC will be aborted and recorded as a failed test.
H. Results are stored in the database and downloaded monthly.

II. Liquid Quality Control (LQC):

A. Per manufacturer: Run 2 levels of liquid control for each lot of cuvettes, when a new shipment is received or at least once per 30 calendar days.
B. Each new lot number of control is paralleled to confirm acceptability at least once prior to use.
C. This is a nonwaived test system which uses an internal (electronic/procedural/built-in) quality control system and is eligible for the Individualized Quality Control Plan (IQCP) option per CAP eligibility worksheet.
D. An IQCP approved by the laboratory director may be used to reduce the external control analysis to a frequency less than the limits defined in the CLIA regulations and CAP checklists. However, the frequency of external quality control analysis cannot be less than that required by the manufacturer instructions.
E. An IQCP has been approved by the laboratory director.

III. Any instrument/cuvette combination must not be used until control values obtained are within manufacturer’s range.

IV. All controls, both electronic and liquid, must be determined to be acceptable before any patient results are reported.

V. The lead nurse in the Operating Room will monitor the EQC on a weekly basis and the Point-of-Care Coordinator, or designee, will review both the EQC and LQC on a monthly basis.

PROCEDURAL STEPS

I. Always allow refrigerated cuvettes to come to room temperature for at least 30 - 60 minutes prior to testing (patient or control.)

II. A new lot number of cuvette, control and range, or operator identification can be entered using the “Configuration Manager” software located on the point-of-care coordinator’s computer in the laboratory or through the supervisor function on the instrument. See Operator’s Manual for specific instructions.

III. Quality Control Testing, Electronic and Liquid

A. EQC (Normal, Abnormal and Temperature Verification)
1. Instrument programmed to perform a normal (30 ± 1 ACT seconds), abnormal (500 ± 1 ACT seconds) and a temperature check every 8 hours of operation if instrument is “ON” and connected to a DC (direct current) source by the transformer.
2. If instrument is not in use, the EQC will automatically initiate when the instrument is activated.
3. To perform EQC manually:

a. Press “QC” key before a cuvette is inserted.

b. Then, press “1” key to run EQC.

4. Note: to view last EQC result, select the “database” key, then 5 – Display QC. Use the “enter” and “cancel” keys to view results.
5. If an ERROR message is displayed:

   a. Repeat EQC.
   b. If ERROR message still displayed, Call ITC (International Technidyne Corporation) Technical Support at 1-800-631-5945.
   c. Notify the Supervisor or Charge RN.
   d. Notify the Laboratory Point-of-Care Coordinator.

6. Record results on Hemochron Elite Electronic Quality Control and Maintenance Log.

B. Liquid Controls Procedure

1. Perform 2 levels of Liquid QC on each lot of Hemochron cuvettes, with each new shipment and once per 30 days.
2. Remove the ACT+ test cuvettes and the DirectCheck control vials from the refrigerator and allow all test materials to come to room temperature for a minimum of 30 minutes but recommend 60 minutes.
3. Visually inspect each vial to ensure that the glass ampule inside the plastic vial is intact.
4. Insert the ACT+ cuvette into instrument.
5. Scan barcodes by holding print/scan key.
6. Scan cuvette barcode (lot number) or enter manually.

   a. Note: new lot numbers must be added to instrument through supervisor menu or “Configuration Manager” software.
   b. Can enter manually (select “7” key)
   c. Enter lot number using keypad, press and hold enter key.
   d. Enter expiration <u>code</u> on cuvette package (bottom right) and hold enter key.

7. Scan Operator Identification (OID) barcode or enter manually (must be a valid OID.)
8. Press “QC” key, select QC level (1- Normal or 2- Abnormal). Follow prompts.
9. Scan control barcode or enter manually.

   a. Note: new lot numbers must be added to instrument through supervisor menu or“Configuration Manager” software.

10. The instrument will signal when ready with a beep and display alternating messages, “add sample” and “press start.”

    a. Instrument will remain in ready mode for 5 minutes.
    b. If testing is not started within 5 minutes, a “Start timeout” error will occur, and test sequence must be started again.

11. Remove the plastic seal from the control vial.
12. Insert the control vial into the white protective sleeve.
13. Holding the vial upright, tap the vial on the tabletop to settle the inner glass ampule to the bottom of the plastic vial.
14. Note: the following steps from initially crushing the glass ampule to pressing the “START” key should not take longer than 15-18 seconds.
15. Crush the inner glass ampule at least 3 times by bending the vial over the edge of a tabletop.

a. Crush the middle of vial.
b. Twist vial quarter of turn and crush top.
c. Twist vial quarter of turn and crush bottom.

16. Quickly invert the control with dropper tip down end-to-end 10 times (use a downward snapping motion of the wrist to ensure the control material flows to the dropper tip)
17. Remove cap and discard first drop.
18. Immediately dispense into cuvette sample well until full. If a large dome extends over the top, push it over into the outer sample well.
19. Press "START" key.
20. Note: Dispose of control vial and test cuvette into biohazard sharps container.
21. Record results on Hemochron Elite Liquid Quality Control Log maintained by the clinical laboratory.
22. Evaluate control results by comparing with the manufacturer's acceptable range for a given lot number. Repeat any unacceptable results.
23. Troubleshooting QC failure:

a. The most likely causes of error:
   1) Control or test cuvettes not warmed to room temperature long enough.
   2) Glass ampule not crushed adequately.
   3) Control not mixed properly.
   4) Entire process completed too slowly (>15 – 18 seconds).
b. Contact technical services (**1-800-678-0710**), consult the control package insert or the Hemochron Elite Operator's Manual if any results are still unacceptable.
c. **The instrument/cuvette combination must not be used until all control values are within acceptable limits.**

IV. Patient Testing

A. Insert the ACT+ cuvette into the cuvette opening on the side of the instrument.
B. The instrument will automatically identify the test cuvette inserted and display the test choice. This initiates the pre-warm/self-check mode.
C. If any fault messages appear, test should be terminated.
D. Reinsert test cuvette to repeat pre-warm/self-check. If fault message appears again, ACT+ testing cannot be completed. Notify Supervisor or Charge Nurse Immediately. Refer to attached troubleshooting guide for a list of error messages and corrective actions. If unable to resolve issue, contact ITC Technical Services Department at **1-800-678-0710.**
E. Follow prompts to enter cuvette lot number, patient identification and operator identification.
F. Scan barcodes by holding print/scan key.
G. Scan cuvette barcode (lot number) or enter manually.

1. Can enter manually (select "7" key)
2. Enter lot number using keypad, press and hold enter key.
3. Enter expiration code on cuvette package (bottom right) and hold enter key.

H. Scan or manually enter valid OID.
I. Scan patient identification barcode or enter manually.
J. Instrument will signal when "Ready" with an audible tone.
K. Screen will display "Add Sample" and "press Start".

1. Instrument will remain in ready mode for 5 minutes.
2. If testing not started within 5 minutes, a "Start timeout" error will occur.

3. Discard cuvette and obtain new cuvette.
4. Recollect a fresh whole blood sample.

L. Obtain a fresh whole blood (0.2cc) sample. Do not use a sample that has been contaminated with tissue thromboplastin, IV solutions or alcohol cleansing solution. In addition, do not use a sample with any signs of clotting. Refer to Specimen section for a complete list of limitations and interferences.

M. Collection Procedure:

1. Identify patient using at least two patient identifiers (name, visit number (V#), Medical Record number (MR#), birthdate).
2. Collect a blood sample from the patient by venipuncture or an indwelling catheter line.
3. If syringe is used: Use 1 or 3 cc syringe and a 23- or 21-gauge needle.
4. Syringe collection from an indwelling line:

   a. Adequately flush the line until it is free of contaminants (this depends on the amount of solution contained within the line).
   b. Then, collect enough sample to fill ACT+ cuvette with 0.2 cc.

5. Syringe collection from venipuncture:

   a. Cleanse site with alcohol and allow to air-dry completely.
   b. Using a two-syringe technique, fill the first syringe with 2.0 cc of blood and discard.
   c. Obtain enough sample to be able to fill the ACT+ cuvette with 0.2 cc.

N. Immediately dispense one drop of blood into ACT+ test cuvette sample well. Fill from the bottom up until sample is flush with the top.

1. If excess sample is added creating a “dome-like” appearance, push the excess sample off into the outer ring with the tip of the syringe.

O. Depress the START key.
P. Discard syringe and transfer needle in appropriate biohazard sharps or trash container.
Q. Test completion will be indicated by a single beep.
R. ACT+ results are automatically converted to a reference Celite ACT result and displayed as the Celite equivalent results in seconds.
S. Record ACT result: Place ACT sticker or stamp (which includes normal range) on the Operative/Invasive Procedure Report. Document result, time, date and initials. This Report is scanned into the electronic medical record (EMR).
T. Immediately report all test results, in seconds, to ordering physician.
U. Discard used cuvette and blood collection devices into appropriate biohazard container.
V. Note: Should a fault message appear on the display at any time during the procedure, refer to “TROUBLESHOOTING’ in the Hemochron Elite Operator’s Manual.

V. Maintenance (cleaning):

A. Inspect and clean the cuvette opening as needed. Use water-moistened cotton swab.
B. After use of disinfectant solution, wipe instrument with water dampened cloth to remove bleach from the plastic surfaces.
C. Clean and disinfect the meter between each patient use as per the ARMC Infection Control Policy.

1. For patients with known or suspected Clostridium difficile (C. diff) infections use Clorox Germicidal Wipes or for all other patients use Super Sani-Cloth Germicidal Disposable Wipes to gently wipe the outside of the meter and carefully wipe around the test strip area.
2. The surface of the meter must remain damp with solution for manufacturer recommended contact times. Clorox Germicidal Wipes have a 3-minute contact time and Super Sani-Cloth Wipes have a 2-minute contact time.

CALCULATIONS **N/A**

REFERENCE INTERVALS

I. Manufacturer's Normal range for the ACT+ (non-heparinized patients) = 96 -152 seconds.
II. *Target ACT range

A. Cardiopulmonary bypass target range = > 500 seconds
B. Cardiac angioplasty target range

1. No Reopro = > 300 – 350 seconds
2. With Reopro = 200-300 seconds

C. *May vary according to medication used during procedure.

III. Validation studies were completed for the ACT+ test on the Hemochron Elite. The validation data was reviewed and the ACT+ test was approved for patient testing by the Laboratory Director. Validation data is saved in the Hemochron Signature Elite validation file.

INTERPRETATION OF RESULTS

I. Legibly, record the results on the patient's chart using the ACT chart sticker applied to the Conscious Sedation Flow Sheet.
II. The Analytic Measurement Range (AMR)or reportable range for this method is 68-1005 seconds
III. "Out of range - Hi" results are reported as "greater than 1005" (>1005).
IV. "Out of range – Lo" results are reported as "less than 68" (<68).
V. If result is inconsistent with patient therapy or questionable for any reason, the test should be repeated immediately. At the discretion of the Attending Physician, a STAT PTT may be ordered and sent to the Laboratory if results and/or operation of the instrument are questionable.

REFERENCES

I. International Technidyne Corporation (ITC) Hemochron Elite Operator's Manual.
II. International Technidyne Corporation (ITC) Hemochron Elite Procedure for Activated Clotting Time Plus Testing (ACT+).
III. DirectCHECK Control Package Insert
IV. Hemochron Jr. Whole Blood Microcoagulation Systems ACT+ cuvette package inser
V. Clinical Laboratory Improvement Act (CLIA)
VI. College of the American Pathologists – POC Checklist rev. 7.28.201
VII. State of California Business and Professions Code Title 22
VIII. ARMC Infection Control Manual, Policy No. 308, Cleaning and Disinfection of Patient Care Items and Equipment, and Policy No. 402, Standard and Isolation Precautions

IX. Hemochron Elite ACT + Individualized Quality Control Plan
X. Hemochron Signature Elite Technical Bulletin 1215 16833
XI. Heparin therapy during extracorporeal circulation Bull, B. et al., 1974, Loma Linda University School of Medicine.

DEFINITIONS **N/A**

ATTACHMENTS

Attachment A: Hemochron Elite Operator's Manual, Troubleshooting and Maintenance sections

Attachment B: ACT management on CPB during Cardiothoracic Surgery using The Hemochron Signature Elite

Attachment C: Bivalirudin Protocol Heparin Induced Thrombocytopenia

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	7/26/2021	**Carolyn Leach, MD, Laboratory Medical Director** Applicable Administrator, Hospital or Medical Committee
	9/1/2022	**Quality Management Committee** Applicable Administrator, Hospital or Medical Committee
	9/22/2022	**Medical Executive Committee** Applicable Administrator, Hospital or Medical Committee
		Board of Supervisors Approved by the Governing Body

REPLACES: **N/A**

EFFECTIVE: 07/26/2021

REVISED: **See Review/Revise Sign off Page**

REVIEWED: **See Review/Revise Sign off Page**

TROUBLESHOOTING

Configuration Manager Error Messages

Problem	Cause	Corrective Action
CHKSUM error during data transfer	Other software applications are open during data transfer.	Close all applications including anti-virus programs during communications.
Communication error/ Initialization error	Incorrect cable is used or cable is not completely connected.	Ensure proper cable is being used. Check cable connections.
No COM Port	No COM Port available.	Use HCM to detect available COM Port
Text crowded / Overlapping on screen	Older computer graphics card	Does not affect program. Printouts will be correct.

Instrument Error Messages

The error messages that may be displayed while operating the HEMOCHRON *Signature Elite* instrument are listed below. The probable cause and corrective action are shown for each message. Some messages designate a test or sample fault. This message is included in the printed results for the affected sample. A list of these messages can be found in *Acronyms for Error Messages* on page 46.

If needed, contact ITC Technical Support by phone at +1-732-548-5700, by FAX at +1-732-548-9824, or by e-mail at techsupport@itcmed.com.

Error Message	Cause	Corrective Action
RTC....FAULT	Can not communicate with external Real Time Clock.	Contact ITC Technical Support.
CHARGE BATTERY	Battery power depleted below predetermined level.	Connect to AC/DC Power Module and charge battery for 8 hours.
BATTERY FAULT	Battery is discharged.	Charge battery for 8 hours. If message persists, contact ITC Technical Support.
EXTERNAL TOO HIGH	AC/DC Power Module voltage exceeds 12.7 volts.	Disconnect the AC/DC Power Module. Contact ITC Technical Support.
BATTERY TOO HIGH	Battery voltage exceeds 8.8 volts.	Disconnect the AC/DC Power Module. Contact ITC Technical Support.
Heater Too Cool	Incubator remained below 36 °C after 90 seconds of warming on external power or for up to 150 seconds on battery.	Repeat test, then charge battery. If message persists, contact ITC Technical Support.
Heater Too Hot	Incubator exceeded 38 °C for 2.5 seconds.	Repeat test with new cuvette. If message persists, contact ITC Technical Support.

Error Message	Cause	Corrective Action
Detector Fault	Light path between LED and detectors is blocked.	Repeat test with new cuvette. If message persists, contact ITC Technical Support.
Detector Blocked	EQC is being run while a cuvette is inserted.	Remove the cuvette and repeat the EQC.
Sample Pos Fault	Sample has moved outside of testing area in cuvette.	Contact ITC Technical Support.
ASSAY LOCKED CALL SUPERVISOR	The QC failure limit has been exceeded.	Remove the cuvette. Contact the supervisor.
MACHINE LOCKED CALL SUPERVISOR	The EQC failure limit has been exceeded.	Contact the Supervisor.
Sample Not Seen	Sample has not reached front detector in specified time period.	Repeat test with new cuvette. If message persists, contact ITC Technical Support.
Cuvette Removed	Cuvette was prematurely removed from instrument while testing in progress.	Repeat test with new cuvette.
Sample Too Large/ Sample Too Small	Excess or insufficient sample.	Repeat test with new cuvette.
Premature Sample	Sample was detected at front detector before specified time period. May occur if sample is added before pump-priming sequence is complete.	Repeat test with new cuvette. If message persists, contact ITC Technical Support.
MEMORY FAULT	Malfunction in the computer's memory.	Contact ITC Technical Support.
START Timed Out	START key was not pressed within 5 minutes after entering ready mode.	Remove cuvette and repeat test with new cuvette.
Unsupported Assay	Cuvette cannot be identified by instrument.	Remove cuvette and repeat test with new cuvette. Use ITC cuvettes only.
No Data Stored	Operator attempted to print data when none is stored in database.	N/A
User Abort	The test was aborted by the user.	Repeat test.
Invalid Lot #	The barcoded cuvette or QC lot number label that was scanned was not recognized.	Check the label for damage, then repeat the scan.
	The incorrect format was entered for the lot number.	Repeat the entry using the correct format.
	The lot that was scanned does not match the cuvette test type.	Verify the test type, then rescan the lot.

Error Message	Cause	Corrective Action
Lot Expired	The cuvette and/or QC material that is being used has reached its expiration date.	Remove the cuvette and repeat the test using supplies that are within their expiration date.
Action Denied	Date/Time cannot be changed if QC Lockout is required, or Date/Time function is denied through Configuration Manager.	Supervisor needs to reconfigure instrument using HCM.
Disallowed Assay	Assay performance has been prohibited via *Configuration Manager*.	Supervisor needs to reconfigure instrument using HCM.
	The table of cuvette or QC lots is empty or contains only expired lots.	Supervisor needs to reconfigure instrument using HCM.
	The QC interval is set to zero in *Configuration Manager*.	Supervisor needs to reconfigure instrument using HCM.
Cannot erase DB	Erasing patient and/or QC database is not allowed.	Supervisor needs to reconfigure instrument using HCM.
INR< 0.8 INR > 10.0 APTT < 20	Test result is outside clinical range.	Repeat test with new cuvette.
Out of Range-Lo	Test result is outside clinical range. Sample has clotted prematurely, or did not mix correctly in cuvette. Bubbles may be present.	Repeat test with new cuvette.
Out of Range-Hi	Test result is outside clinical range.	Repeat test with new cuvette.
Check Time/Date	Low Battery or RTC has lost its Time/Date Tracking.	Enter or verify Time & Date. Charge Battery.
Dark Photo Fault	Hardware malfunction.	Contact ITC Technical Support.
Unsupported POCT >> COM	The POCT-1A communication protocol was attempted to be set while the COM Port is selected, or vice versa.	Set POCT-1A to run over NET, or set ITC to run over COM or NET.

SERVICE AND MAINTENANCE

Routine Maintenance

Inspect and clean the cuvette opening as required. Remove residual dried blood or other foreign matter using water-moistened cotton swabs.

Remove any residual water with dry cotton swabs. If a disinfectant is needed, use a 0.5% solution of sodium hypochlorite or a 10% dilution of household bleach in water. Wipe instrument with a water-dampened cloth to remove residual bleach from the plastic surfaces.

Apply solution to clean and disinfect areas contaminated with blood. DO NOT use solvents or strong cleaning solutions as they may damage the instrument's plastic components. Routine maintenance other than cleaning normally is not required.

It is recommended to clean and decontaminate prior to service or transfer of equipment.

Service

The HEMOCHRON *Signature Elite* instrument is almost completely self-monitoring and has no user serviceable parts.

It monitors internal circuitry and reports problems to the display screen automatically. Malfunctions are indicated by error messages detailed in the section "Troubleshooting."

Battery Care

To optimize battery life, it is recommended that the HEMOCHRON *Signature Elite* instrument be run on its battery during the day. It can be plugged in overnight to allow the batteries to recharge. The life of the lithium battery is optimized when the battery is exercised in this manner.

A fully charged battery will operate as defined in the *Operation* specifications section on page 13.

When the batteries are drained to the point that valid testing cannot be performed, the instrument will display "CHARGE BATTERY" (see "Troubleshooting"). At this point, the instrument must be plugged in for operation and recharging. Once plugged into a Hospital Grade AC outlet, the instrument can be used immediately.

Caution

The battery used in this device may present a risk of fire or chemical burn if mistreated. Do not disassemble, heat above (60°C), or incinerate. Use of another battery may present a risk of fire or explosion.

Dispose of used battery promptly. Keep away from children. Do not disassemble and do not dispose of in fire.

Instrument Disposal

If disposal is necessary, follow local regulations for disposal of Li-Ion batteries and electronic devices.

ACT management on CPB during Cardiothoracic Surgery using
The HEMOCHRON Signature Elite

PRINCIPLES OF OPERATION:

The HEMOCHRON Signature Elite Whole Blood Microcoagulation System provides many features for ease of use and reliability, including a patented clot detection system, a data storage module, interfaces for a laboratory computer and/or printer, a streamlined user-interface panel, and an integrated barcode scanner. The system measures whole blood clotting times using HEMOCHRON Jr. disposable single-use cuvettes. Each cuvette contains all of the reagents necessary for a specified test. The operator inserts a cuvette for the test into the instrument and then (if desired) enters information about the sample or scans the information from a barcode using the integral barcode scanner. After the cuvette has warmed to 37 °C ±1.0 °C, the instrument beeps, signaling the operator that a blood sample can be added to the cuvette and the test started. The operator then places a drop of blood in the sample well of the cuvette and presses the START key. The instrument measures the required volume of blood and automatically moves it into the cuvette test channel, where it is mixed with reagents. The remainder of the blood sample, not needed for testing, is automatically drawn out of the sample well and into an enclosed waste channel on the cuvette. After mixing with the reagent, the sample is moved back and forth at a predetermined rate within the test channel and monitored for clot formation. The test channel is maintained at 37 °C ±1.0 °C during the test. The rate of movement of the sample is monitored by a series of LED optical detectors that are aligned with the test channel. When the blood clots, the flow of the blood sample within the test channel is impeded, reducing its rate of flow between the optical detectors. This reduction in flow below a predetermined value signals to the instrument that a clot has formed. The instrument also emits an audible beep when clot formation occurs, indicating the end of the test. An internal timer measures the elapsed time between the start of the test and the clot formation. During the test, the whole blood clotting time (in seconds) is displayed.

The HEMOCHRON® Jr. ACT**+** test uses a mixture of silica, kaolin, and phospholipids as an activator to create a rapid and highly sensitive alternative to existing ACT tests. This test demonstrates linearity at heparin concentrations ranging from 1.0 to 6.0 units of heparin per mL of blood.

ACT management on CPB:

Target ACT required on bypass is >480 seconds.

- Obtaining a base line ACT for each patient before heparin is given.
- Give the anesthesiologist 400 units/kg of heparin for each adult patient.
- If patient weigh 100kg or above 100kg, give no more than 300units/kg
- After 5 minutes of the initial dose of heparin is given to the patient, a whole blood sample from the patient is tested using **ACT+ cartridge**
- If ACT above 480 seconds, no extra heparin is needed.
- If ACT result is below 480 seconds, administer 100 units/kg of heparin
- Test ACT in 5minutes after the additional dose given to the patient to achieve a target ACT value of 480 seconds.
- ACT should be tested every 20-30 minutes after the initial dose is given and target ACT result is obtained.
- ACT should be tested within 5-10 minutes once bypass is initiated, then tested again every 20-30 minutes during the length of bypass.
- Based on ACT result, additional heparin (5,000-10,000 units) will be given during CPB to achieve ACT of 480 seconds.
- If ACT target value of 480 seconds is not achieved after the initial dosage and additional dose up to 600 units/kg, ask the anesthesiologist to order one bottle of Anti-Thrombin III Concentrate (AT III) (500-600 units), to be given to patient once bypass is initiated. (*If the patient was planned to receive blood product during surgery, then FFP should be used instead of ATIII*).

Bivalirudin Protocol
Heparin Induced Thrombocytopenia

Objective: To describe alternative anticoagulation for patients unable to accept either fractionated or unfractionated heparin.

Bivalirudin Dosing Schedule
On - pump procedure
Angiomax to support on pump anticoagulation for bypass contained in this protocol is a bolus dose of 1.0 mg/kg IV and an infusion of 2.5 mg/kg/hr for the duration of anticoagulation, along with separate priming and separation dosing for cardiopulmonary bypass pump. This should achieve a mean steady state plasma Angiomax concentration of 12.3+_ 1.7mcg/mL.

Dosing Recommendations

	Prior to CPB	**During CPB**	**After CPB**
Patient	1.0mg/kg IV bolus 2.0 mg/kg/hr IV infusion	2.5 mg/kg/hr IV infusion until 20 mins prior to CPB discontinuation	
Flush solution	0.1 mg/ml Angiomax	0.1 mg/ml Angiomax	0.1 mg/ml Angiomax
Graft storage	(blood-based) 1:12 CPD[1] OR 0.1 mg/ml Angiomax in crystalloid solution	blood-based) 1:12 CPD[1] OR 0.1 mg/ml Angiomax in crystalloid solution	
Cell saver	1:11 or 1:12 sodium citrate/CPD	1:11 or 1:12 sodium citrate/CPD	1:11 or 1:12 sodium citrate/CPD
CPB pump	50 mg priming dose[2]		50 mg priming dose followed by 50mg/hr[3]

[1] Sodium Citrate/ Citrate Phosphate Dextrose.
[2] Suitable for all priming volumes
[3] Due to a low remaining blood volume in the circuit resulting in high concentrations of bivalirudin, it should be processed in the cell saver prior to reinfusion to the patient.

Dosing of Patient
Angiomax is given as a 1.0 mg/kg intravenous bolus immediately followed by a 2.5 mg/kg/hr IV infusion beginning at the time anticoagulation is requested by the surgeon and will be discontinued when it is estimated that 15-20 minutes remain prior to the discontinuation of CPB. The infusion dose will not be titrated for the period of anticoagulation unless medically indicated. If CPB is not terminated within 20 minutes following discontinuation of the infusion, then an additional bolus of 0.5 mg/kg of Angiomax is to be given, and the infusion at 2.5 mg/kg/hr restarted, until CPB is terminated.

If a higher level of anticoagulation is desired, administer additional boluses of 0.1-0.5 mg/kg. These can be repeated as clinically indicated. Adjustments in plasma concentration of Angiomax are best achieved with bolus dosing.

Off- pump procedures
Angiomx to support off0-pump anticoagulation for CABG is given as a bolus dose of 0.75 mg/kg IV and an infusion of 1.75 mg/kg/hr for the duration of anticoagulation.

Dosing Recommendations

Prior to Graft Occlusion

Patient	0.75 mg/kg IV bolus 1.75 mg/kg/hr IV infusion
Flush solutions	0.1 mg/ml Angiomax
Graft storage	1:12 CPD OR 0.1 mg/ml Angiomax in crystalloid solution
Cell Saver	1:11 vor 1:12 sodium citrate/CPD

If a higher level of anticoagulation is desired, administer additional boluses of 0.1-.0.5 mg/kg. These can be repeated as clinically indicated. Adjustments in plasma concentration of Angiomax are best achieved with bolus dosing.

Permitted concomitant Medications
The us of antifibrinolytics such as transexamic acid or Amicar are allowed at the discretion of the investigator.

SECTION: **Point-of-Care**

SUB SECTION: **Procedures**

SUBJECT: **Abbott BinaxNOW COVID-19 AG Card Testing**

APPROVED BY: _____
Laboratory Medical Director or Designee

TEST NAME / POLICY

Abbott BinaxNOW Covid-19 Ag Card test is a Point-of-Care (POC) test. It is a waived (emergency use authorization only) laboratory test that is performed according to the manufacturer's instructions under the regulations set forth by the Clinical Laboratory Improvement Act (CLIA), College of American Pathologists (CAP), and the State of California Business and Professions Code. Testing is performed by licensed healthcare providers who have successfully completed appropriate training and competency.

TEST PRINCIPLE / PURPOSE

The BinaxNOW™ COVID-19 Ag Card is an immunochromatographic membrane assay that uses highly sensitive antibodies to detect SARS-CoV-2 nucleocapsid protein from nasal swab specimens. SARS-CoV-2 specific antibodies and a control antibody are immobilized onto a membrane support as two distinct lines and combined with other reagents/pads to construct a test strip. This test strip and a well to hold the swab specimen are mounted on opposite sides of a cardboard, book-shaped hinged test card.
The BinaxNOW™ COVID-19 Ag Card does not differentiate between SARS- CoV and SARS-CoV-2.
Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. Antigen is generally detectable in nasal swabs during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
Negative results from patients with symptom onset beyond seven days, should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.
The BinaxNOW™ COVID-19 Ag Card is intended for use by medical professionals or trained operators who are proficient in performing rapid lateral flow tests. BinaxNOW™ COVID-19 Ag Card is only for use under the Food and Drug Administration's Emergency Use Authorization.

CONDITIONS OF AUTHORIZATION FOR LABORATORY AND PATIENT CARE SETTINGS

The BinaxNOW™ COVID-19 Ag Card Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www. fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.
However, to assist clinical laboratories using the BinaxNOW™ COVID-19 Ag Card, the relevant Conditions of Authorization are listed below:

I. Authorized laboratories[1] using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

II. Authorized laboratories using your product will use your product as outlined in the "BinaxNOW™ COVID-19 Ag Card" Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

III. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

IV. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

V. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Abbott Diagnostics Scarborough, Inc. (via email: ts.scr@abbott.com, or via phone by contacting Abbott Diagnostics Scarborough, Inc. Technical Service at 1-800-257- 9525 any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

VI. All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

VII. Abbott Diagnostics Scarborough, Inc., authorized distributors, and authorized laboratories and patient care settings using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high, moderate, or waived complexity tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation." as "authorized laboratories."

CLINICAL SIGNIFICANCE

The BinaxNOW™ COVID-19 Ag Card is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid protein antigen from SARS- CoV-2 in direct nasal swabs from individuals suspected of COVID-19 by their healthcare provider within the first seven days of symptom onset. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.

§263a, that meet the requirements to perform moderate, high, or waived complexity tests. This test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

SPECIMEN TYPE(S)

I. Collection and Handling:
   A. Test specimens immediately after collection for optimal test performance. Inadequate specimen collection or improper sample handling/storage/ transport may yield erroneous results. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019- nCoV/lab/guidelines-clinical-specimens.html
   B. Only the swab provided in the kit is to be used for nasal swab collection.

      1. To collect a nasal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Using gentle rotation, push the swab until resistance is met at the level of the turbinates (less than one inch into the nostril). Rotate the swab 5 times or more against the nasal wall then slowly remove from the nostril. Using the same swab, repeat sample collection in the other nostril.

   C. To minimize the risk of contamination of PPE and swab package during sample collection, it is recommended to widely open the package by pulling from the top down. Carefully remove the swab and perform sample collection.

II. Acceptable specimen

III. Nasal Swab:

   A. For optimal test performance, use the swabs provided in the test kit. Alternatively, rayon, foam, HydraFlock® Flocked swab (standard tip), HydraFlock® Flocked swab (mini tip), Copan Mini Tip Flocked Swab, or Copan Standard Flocked swabs can be used to collect nasal swab samples. To collect a nasal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Using gentle rotation, push the swab until resistance is met at the level of the turbinates (less than one inch into the nostril). Rotate the swab several times against the nasal wall then slowly remove from the nostril. Using the same swab, repeat sample collection in the other nostril.

IV. Specimen Transport
   A. Do not return the nasal swab to the original paper packaging.
      1. For best performance, direct nasal swabs should be tested as soon as possible after collection. If immediate testing is not possible, and to maintain best performance and avoid possible contamination, it is highly recommended the nasal swab is placed in a clean, unused plastic tube labeled with patient information, preserving sample integrity, and capped tightly at room temperature (15-30°C) for up to (1) hour prior to testing. Ensure the swab fits securely within the tube and the cap is tightly closed. If greater than 1-hour delay occurs, dispose of sample. A new sample must be collected for testing.

V. Unacceptable specimen

   A. Rayon swabs are not suitable for use in this assay

B. Puritan PurFlock Standard Tip Ultra Flocked Swabs, Puritan PurFlock Mini Tip Ultra Flocked Swabs and Copan Standard Rayon Tip Swabs are not suitable for use in this assay.
C. Swab received in viral transport media.

1. Throat and nasopharyngeal swabs

LIMITATIONS / PRECAUTIONS

I. This test detects both viable (live) and non-viable, SARS-CoV and SARS- CoV-2. Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture results performed on the same sample.

II. A negative test result may occur if the level of antigen in a sample is below the detection limit of the test.

III. The performance of the BinaxNOW™ COVID-19 Ag Card was evaluated using the procedures provided in this product insert only. Modifications to these procedures may alter the performance of the test.

IV. False negative results may occur if a specimen is improperly collected, transported, or handled.

V. False results may occur if specimens are tested past 1 hour of collection. Specimens should be tested as quickly as possible after specimen collection.

VI. False negative results may occur if inadequate extraction buffer is used (e.g., <6 drops).

VII. False negative results may occur if specimen swabs are not twirled within the test card.

VIII. False negative results may occur if swabs are stored in their paper sheath after specimen collection.

IX. Positive test results do not rule out co-infections with other pathogens.

X. Positive test results do not differentiate between SARS-CoV and SARS- CoV-2.

XI. Negative test results are not intended to rule in other non-SARS viral or bacterial infections.

XII. The presence of mupirocin may interfere with the BinaxNOW™ COVID-19 Ag test and may cause false negative results.

XIII. Negative results, from patients with symptom onset beyond seven days, should be treated as presumptive and confirmation with a molecular assay, if necessary, for patient management, may be performed.

XIV. If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.

REQUIRED REAGENT(S)

I. Abbott BinaxNOW COVID-19 Test Card

II. Abbott BinaxNOW COVID-19 Extraction reagent

CALIBRATION N/A

QUALITY CONTROL

I. BinaxNOW™ COVID-19 Ag Card has built-in procedural controls. For daily quality control, Abbott suggests that you record these controls for each test run.
   A. Procedural Controls:

      1. The pink-to-purple line at the “Control” position is an internal procedural control. If the test flows and the reagents work, this line will always appear.
      2. The clearing of background color from the result window is a negative background control. The background color in the window should be light pink to white within 15 minutes. Background color should not hinder reading of the test.

II. Positive and Negative Controls should be tested following the Run QC Test instructions on the BinaxNOW™ procedure. A Positive Control Swab is included in the kit. Use a sterile swab provided in the kit as the Negative Control Swab. Refer to Quality Control Swab Test Procedure. If the correct control results are not obtained, do not perform patient tests or report patient results. Contact Technical Support during normal business hours before testing patient specimens.

III. External Positive and Negative Controls:

Good laboratory practice suggests the use of positive and negative controls to ensure that test reagents are working and that the test is correctly performed. BinaxNOW™ COVID-19 Ag Card kits contain a Positive Control Swab and Sterile Swabs that can be used as a Negative Control Swab. These swabs will monitor the entire assay. Test these swabs once with each new shipment received and once for each untrained operator. Further controls may be tested in order to conform with local, state and/or federal regulations, accrediting groups, or your lab’s standard Quality Control procedures.
If the correct control results are not obtained, do not perform patient tests or report patient results. Contact Technical Support during normal business hours before testing patient specimens.

PROCEDURAL STEPS

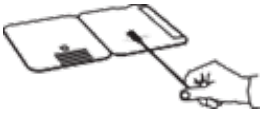
I. Please refer to the BinaxNOW™ User Manual for full instructions.
II. Before testing with BinaxNOW™ COVID-19,

   A. Put on a clean pair of gloves.

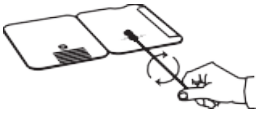
III. Procedure for Patient Specimens
Open the test card just prior to use, lay it flat, and perform assay as follows. The test card must be flat when performing testing, do not perform testing with the test card in any other position.

1.Hold Extraction Reagent bottle vertically. Hovering 1/2 inch above the TOP HOLE, slowly add 6 DROPS to the TOP HOLE of the swab well. DO NOT touch the card with the dropper tip while dispensing.

2. Insert sample into BOTTOM HOLE and firmly push upwards so that the swab tip is visible in the TOP HOLE.



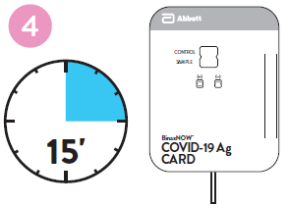
3. Rotate (twirl) swab shaft 3 times CLOCKWISE (to the right). Do not remove swab.



Correct

Note: False negative results can occur if the sample swab is not rotated (twirled) prior to closing the card.

4.Peel off adhesive liner from the right edge of the test card. Close and securely seal the card. Read result in the window 15 minutes after closing the card. In order to ensure proper test performance, it is important to read the result promptly at 15 minutes, and not before. Results should not be read after 30 minutes.



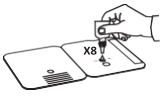
Note: When reading test results, tilt the card to reduce glare on the result window if necessary. Individuals with color-impaired vision may not be able to adequately interpret test results.

Procedure for BinaxNOW™ Swab Controls

I. Open the test card just prior to use, lay it flat, and perform assay as follows.

1. Hold Extraction Reagent bottle vertically Hovering 1/2 inch above the TOP HOLE, slowly add 8 DROPS to the TOP HOLE of the swab well. DO NOT touch the card with the dropper tip while dispensing.

CORRECT WRONG



2. Follow Steps 2 – 4 of the Test Procedure for Patient specimens.

SAFETY:

I. All operators must use appropriate personal protective equipment (PPE) in accordance with CDC guidelines and hospital infection control.

A. Follow Standard Precautions when handling clinical specimens, including hand hygiene and the use of proper PPE, such as laboratory coats, gloves, and eye protection. If needed, additional precautions can be used, such as a surgical mask or face shield, or other physical barriers, such as a splash shield to work behind. (CDC, 2020).

II. Clean gloves should be used for all patient sample handling.

CALCULATIONS: N/A

REFERENCE INTERVALS:

I. Expected COVID-19 test result is negative.

INTERPRETATION OF RESULTS:

I. Procedural control must be valid for test results to be interpreted.

II. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory samples during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with

other viruses. Testing facilities within the United States and its territories are required to report all positive results to the appropriate public health authorities.

III. Negative

A. A negative specimen will give a single pink/purple colored Control Line in the top half of the window, indicating a negative result. This Control Line means that the detection part of the test was done correctly, but no COVID-19 antigen was detected.

IV. Positive

A. A positive specimen will give two pink/purple colored lines. This means that COVID-19 antigen was detected. Specimens with low levels of antigen may give a faint Sample Line. Any visible pink/ purple colored line is positive.

V. Invalid

A. If no lines are seen, if just the Sample Line is seen, or the Blue Control Line remains blue, the assay is invalid. Invalid tests should be repeated.

VI. Repeat Testing

A. No repeat test may be performed

TROUBLESHOOTING:

I. Procedural errors

A. Confirm test kits are stored at proper temperatures per package insert

B. Timing is important, result must be read with in 15-30 minutes of testing

II. Interfering substances – Listed in the package insert

MAINTENANCE: N/A

REFERENCES:

I. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-ncov/index.html. Accessed February 9, 2020.

II. Clinical Laboratory Policy 107.01, Safe Work Practices and Infection Control Policy 107.01 Issue 1

III. Center of Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html Accessed August 21, 2020.

IV. bioRxiv. (https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1). Accessed March 3, 2020.

V. Manual of Clinical Microbiology, 11th Edition, Vol. 1, ASM. (2015) pg. 279.

VI. https://www.iata.org/en/programs/cargo/dgr

DEFINITIONS: **N/A**

ATTACHMENTS: N/A

APPROVAL DATE:	**N/A**	**Policy, Procedure and Standards Committee**
	11/20/2020	**Carolyn Leach, MD, Laboratory Medical Director** **Applicable Administrator, Hospital or Medical Committee**
	9/1/2022	**Quality Management Committee** **Applicable Administrator, Hospital or Medical Committee**
	9/22/2022	**Medical Executive Committee** **Applicable Administrator, Hospital or Medical Committee**
		Board of Supervisors **Approved by the Governing Body**

REPLACES:

EFFECTIVE: **11/20/2020**

REVISED: **See Review/Revise Sign off Page**

REVIEWED: See Review/Revise Sign off Page