PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC82200 – BioFire Respiratory Panel 2.1	Policy Number: 29-16-V1
Program Name: Laboratory Services	
Applicable Domain: Epidemic/Pandemic	
Additional Domain(s): Lab, DI and Pharmacy Services	
Effective Date:	Next Review Date:
Issuing Authority: Director of Health Services	Date Approved:
Accreditation Canada Applicable Standard: N/A	

GUIDING PRINCIPLE:
The BioFire Respiratory Panel 2.1 (RP2.1) is a real-time, nested multiplexed PCR test designed to simultaneously identify nucleic acids from 22 different viruses and bacteria associated with respiratory tract infection, including SARS-CoV-2.

PURPOSE/RATIONALE:
This standard operating procedure describes the BioFire Respiratory Panel 2.1 test using the BioFire FilmArray Torch at Stanton Territorial Hospital.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists processing specimens using the BioFire FilmArray Torch.

SAMPLE INFORMATION:

Type	Swab Saline gargle
Source	• Nasopharyngeal • Throat/oropharyngeal • Saline gargle rinse
Collection Container	• Viral transport media • Universal transport media • Saline gargle tube
Stability	VTM/UTM: • Room temperature up to 4 hours • Refrigerated up to 7 days • Frozen (≤-15 °C or ≤-70°C) up to 30 days Saline gargle: • Refrigerated up to 5 days

Storage Requirements	Room temperature or refrigerated
Criteria for rejection and follow up action	1. Unlabeled/mislabeled samples 2. Sample container label does not match patient identification on requisition 3. Sample not in UTM/VTM 4. Sample not stored correctly

REAGENTS and/or MEDIA:

- BioFire RP2.1 pouch
- Hydration Injection Vial (blue)
- Sample Injection Vial (red)
- Sample Buffer Ampoule
- Pouch loading station
- Accel TB 1 L bottle
- Accel TB wipes
- 70% isopropyl alcohol
- 10% bleach solution

SUPPLIES:

- Personal protective equipment
- Absorbent bench liner
- Sample racks
- Wet waste container
- Dry waste container
- Orange autoclave bag
- Spray bottles
- Transfer pipettes provided in kit
- Sharps container
- Blue pouch tray
- Cart

EQUIPMENT

- BioFire FilmArray Torch
- Printer
- Class II biosafety cabinet
- Refrigerator

ENVIRONMENTAL CONTROLS:

- Store the test kit, including reagent pouches and buffers, at room temperature (15°C to 25°C). **DO NOT REFRIGERATE**
- Avoid storage of any materials near heating or cooling vents or in direct sunlight
- All kit components should be stored and used together. Do not use components from one kit with those of another kit
- Once the pouch packaging has been opened, the pouch should be loaded with sample as soon as possible (within 30 minutes)
- Once a pouch has been loaded with sample, the test run should be started as soon as possible (within approximately 60 minutes)
- To prevent amplicon contamination: discard used pouches after the run has completed, avoid excessive handling of pouches after test runs and avoid exposing pouches to sharp edges or anything that might cause a puncture

SPECIAL SAFETY PRECAUTIONS:

- Patient samples should only be opened and prepared for testing in a contained environment (i.e. certified Class II BSC)
- Personal Protective Equipment (PPE) required when working with suspect SARS-CoV-2 samples includes:
  - Lab gown, double layer nitrile gloves and Class II BSC
- The test operator and all personnel in the immediate vicinity should be wearing appropriate PPE at all times when working with suspect respiratory pathogen samples, in the event of a spill outside of the BSC
- All items in the BSC must be sprayed or wiped with 70% isopropyl alcohol or Accel TB and allowed to disinfect for 5 minutes prior to removal
- All personnel handing potential respiratory pathogen samples should be knowledgeable in their laboratory's biological spill clean-up protocol for infectious respiratory samples
- Handle all samples and waste materials as if they were capable of transmitting infectious agents

QUALITY CONTROL:

- Maine Molecular BioFire RP2.1 Control Panel positive and negative controls need to be performed on every new lot number and/or shipment of BioFire Respiratory Panel 2.1 kits
- Refer to MIC82300-BioFire RP2.1 Quality Control for quality control procedure
- Record all results on MIC82310-BioFire RP2.1 Quality Control Results Record

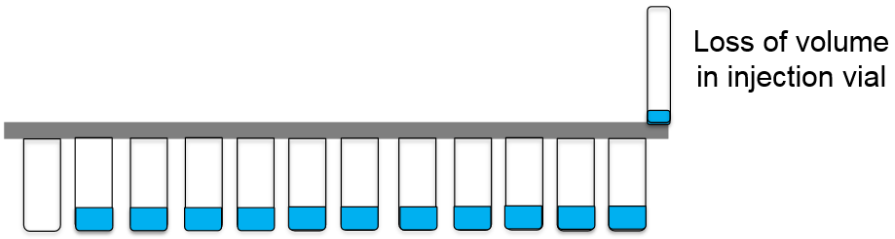
PROCEDURE INSTRUCTIONS:

Step	Action
Preparing the BSC	
1	Order BioFire RP2.1 testing in the LIS: • Medipatient the order if required • **If only SARS-CoV-2 testing is requested:** ➢ In SoftMic, accession the order using the test code **SCOV2** ➢ Collect, receive and plate the order ➢ Add any "copies to" if required • **If RPP is requested:** ➢ In SoftMic, accession the order using the test code **PCRES** ➢ Collect, receive and plate the order ➢ Add any "copies to" if required • **If extended RPP is requested:** ➢ In SoftMic, accession the order using the test code **PCRES** ➢ In SoftMic, add next order and order test code **VIRC** **NOTE:** Make sure the priority is STAT • Label the requisition with the requisition label and scan into SoftMedia • Place the sample barcode label and media barcode label in the pouch of the biohazard bag • Deliver samples to the blue bin labelled SCOV2/PCRES in the microbiology specimen fridge

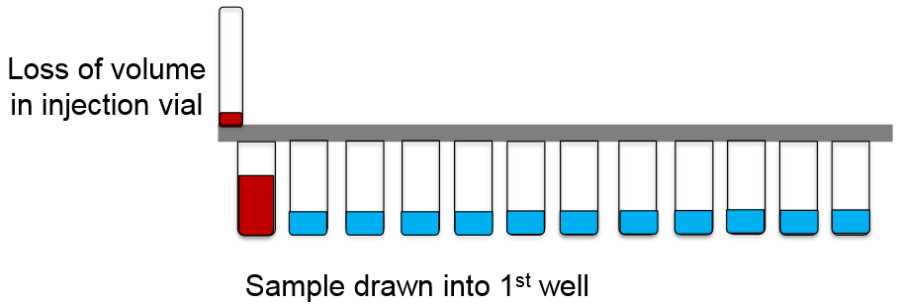
2	Collect accessioned samples from the microbiology specimen fridge and enter the TB workroom. Don one pair of gloves and turn on the blower to the BSC. Place the biohazard bag containing the sample in the BSC.
3	Ensure the daily maintenance for the BioFire has been completed and documented on MIC82110-Maintenance Record – BioFire FilmArray.
4	Set up the clean BSC with the following: • Absorbent pad on working surface moistened with Accel TB • Sample racks • Wet waste container half full with Accel TB • Dry waste container containing an autoclave bag • Sharps container • Spray bottles with 70% isopropyl alcohol and with Accel TB • Accel TB wipes with top opened • Clean blue tray • Pouch Loading Station • BioFire Respiratory Panel 2.1 pouch, Sample Buffer Ampoule, Hydration Injection Vial, Sample Injection Vial and transfer pipette **NOTE:** Only open pouches from packaging for each batch of 8 at a time

Step	Action
Preparing the Pouch	
1	Don an additional pair of gloves to ensure you are double gloved before working in the BSC.
2	Open biohazard bag and discard in the dry waste container. Wipe each sample with an Accel TB wipe and place in the sample rack. Once dry, label the sample with the sample label. Leave the media barcode label on the right hand side of the working area.
3	Move the first sample from the first rack to the second rack. Find the corresponding media barcode label and apply to the tab of the pouch ensuring the pouch barcode is not covered.
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 loading station. Place a blue-capped Hydration Injection Vial into the blue well of the Pouch Loading Station.

Step	Action
Hydrating the Pouch	
1	Unscrew the Hydration Injection Vial from the blue cap.
2	Insert the Hydration Injection Vial's cannula tip into the Hydration Port located directly below the blue arrow of the Pouch Loading Station.
3	Forcefully push down in a firm and quick motion to puncture the 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.

4	Verify that the pouch has been hydrated: ➢ 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 bubbles may be seen.  Hydration fluid in last 11 wells

Step	Action
Preparing the Sample Mix	
1	Add Sample Buffer to the Sample Injection Vial. ➢ Hold the Sample Buffer Ampoule with the tip facing up. ➢ Firmly pinch at textured plastic tab on the side of the ampoule until the seal snaps. 
2	Invert the ampoule over the red-capped Sample Injection Vial and dispense the Sample Buffer using a slow forceful squeeze. Repeat this with a second squeeze. **NOTE:** Avoid generating foam
3	Thoroughly mix the patient sample by inverting rapidly 5 times.
4	Open the sample carefully to avoid touching droplets on the inner lid and place upright on the absorbent pad to avoid droplets falling on the work surface.
5	Use the transfer pipette provided to draw specimen to the third line (approximately 0.3 mL) on the transfer pipette.
6	Add the specimen to the Sample Injection Vial.
7	Rinse the pipette in the wet waste container with Accel TB and allow to soak.
8	Recap the patient sample tube and move to the back of the rack.
9	Tightly close the lid of the Sample Injection Vial and invert the vial 5 times to mix.
10	Return the Sample Injection Vial to the red well of the loading station.

Step	Action
Loading the Sample Mix	
1	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	Insert the Sample Injection Vial's cannula tip into the 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 until a faint "pop" is heard and sample is pulled into the pouch by vacuum.
4	Verify that the sample has been loaded: ➢ Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port. ➢ If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded and begin again with a new pouch.  Sample drawn into 1st well
5	Discard the Sample Injection Vial and the Hydration Injection Vial into the sharps container.
6	Remove the pouch from the Pouch Loading Station and place on the left hand side on the blue tray. **NOTE:** If any of the sample mix drips onto the Pouch Loading Station remove from use and soak in 10% bleach for 15 minutes before being put into use again
7	Wipe Pouch Loading Station with Accel TB before beginning next sample.
8	Spray outer gloves thoroughly with isopropyl alcohol, rub together and allow to air dry. **NOTE:** Always disinfect gloves between loading of each sample
9	Repeat pouch loading procedure for up to 15 additional samples.

Step	Action
Creating a Test Run	
1	Remove outer gloves and transfer the loaded pouches in the blue tray to the BioFire bench.
2	On the BioFire Torch dashboard, select an available module.
3	Using the built in scanner, scan the pouch barcode and the sample ID barcode. Insert the pouch into the module.

4	Enter the operator user name: **micro** and the password: **micro**. Select **Next**. Review the entered information and select **START RUN**.
5	After all the samples in the batch are loaded onto the BioFire, wipe the blue tray and return to the BSC. Remove and don a new pair of gloves.
6	Spray samples with Accel TB and label with the send out label if applicable. Place the sample rack on the cart.

Step	Action
Generating a Test Report	
1	A report is generated automatically upon completion of a run.
2	To view runs or reprint: Select **Browse Runs** on the main screen. Select the sample → View report → Print.

CONTROL STATUS:

RESULT	INTERPRETATION
Passed	• The run was successfully completed • Both pouch controls were successful
Failed	• The run was successfully completed • At least one of the pouch controls (RNA Process Control and/or PCR2 Control) failed
Invalid	• The controls are invalid because the run did not complete • Typically this indicates a software or hardware error

INTERPRETATION OF RESULTS:

RESULT	INTERPRETATION
Not Detected	• The run was successfully completed • The pouch controls were successful (Passed) • The assay for the organism was negative • Results can be reported
Detected	• The run was successfully completed • The pouch controls were successful (Passed) • The assay for the organism was positive • Results can be reported
Equivocal	• The run was successfully completed • The pouch controls were successful (Passed) • The combination of positive and negative assay results for Influenza A were inconclusive
Invalid	• The pouch controls or run were not successful • Run status is displayed as: Aborted, Incomplete, Instrument Error or Software Error

REPORTING INSTRUCTIONS FOR SARS-CoV-2:

BF Results: SARS-CoV-2 **NOT DETECTED**	• Report: **NEGATIVE**

BF Results: SARS-CoV-2 **DETECTED**	• Repeat testing on the GeneXpert to determine Ct value ➢ Add comment in TCOMM that testing was repeated 1. <u>If GeneXpert results are negative</u>: • Mix sample well and repeat testing on both GeneXpert and BioFire • If repeat results are the same: ➢ Report: **INDETERMINANT** ➢ From the keypad add key R to add repeat sample collection comment ➢ Phone OCPHO (HPU1) at (867) 920 8646 ○ Inform OCPHO that discrepant results between the GeneXpert and BioFire were obtained ➢ Phone result to ordering location ➢ Add a copy to OCPHO (HPU1) in Order Entry ➢ Copy report to Stanton IPAC (SIPAC) if ER or inpatient 2. <u>If GeneXpert results are positive</u>: • Report: **POSITIVE** • Phone to OCPHO (HPU1) at (867)920 8646 ➢ If the SARS-CoV-2 Ct value from the GeneXpert is >38, inform OCPHO of the value • Phone result to ordering location • Report will automatically print to OCPHO (HPU1) • Copy report to Stanton IPAC (SIPAC) if ER or inpatient **NOTE:** If ordering location is not available, OCPHO will follow up with the patient. Document attempt in the call log

BF Results: SARS-CoV-2 **INVALID**	• Repeat testing on the BioFire ➢ Add comment in TCOMM that testing was repeated • If the repeat results are invalid: ➢ Repeat testing on the GeneXpert ➢ Add comment in TCOMM that testing was repeated • If the repeat results on the GeneXpert are invalid: ➢ Report: **INVALID** ➢ From the keypad add key R to add repeat sample collection comment ➢ Phone the ordering location and request a new sample be collected

REPORTING INSTRUCTIONS FOR INFLUENZA A, INFLUENZA B AND RSV:

BF Results: Influenza A Influenza B RSV Rhinovirus/ Enterovirus **NOT DETECTED**	• Report: **NEGATIVE** for each target

BF Results: RSV **DETECTED**	• Report: **POSITIVE** ➢ Phone result to the ordering location ➢ Report will automatically print to OCPHO (HPU1) ➢ Copy report to Stanton IPAC (SIPAC) if ER or inpatient
BF Results: Influenza B **DETECTED**	• Report: **POSITIVE** ➢ Report will automatically print to OCPHO (HPU1) ➢ Copy report to Stanton IPAC (SIPAC) if ER or inpatient
BF Results: Rhino/Entero **DETECTED**	• Report: **POSITIVE**
BF Results: Influenza A - H1 Influenza A - H1-2009 Influenza A - H3 **DETECTED**	• Report: **POSITIVE** for Influenza A • Report: **POSITIVE** for the FluA subtype detected ➢ In the results entry screen, select Add Test to add the test with the positive result ➢ The Influenza A test codes are: 1. FAH1T – Influenza A H1 2. FH109 – Influenza A H1-2009 3. FAH3T – Influenza A H3 ➢ Do not report the Influenza A targets that were not detected ➢ Report will automatically print to OCPHO (HPU1) ➢ Copy report to Stanton IPAC (SIPAC) if ER or inpatient
BF Results: Influenza A **DETECTED** No subtype detected	• Report: **POSITIVE** for Influenza A • Refer sample to APL for subtype confirmation: ➢ From the keypad add key V to add referred out comment ➢ Order test code VIRC for send out
BF Results: Influenza A - H1 Influenza A - H1-2009 Influenza A - H3 **EQUIVOCAL**	• If any of the Influenza A targets are equivocal: ➢ Repeat testing on the BioFire • If the repeat testing is equivocal: ➢ Report: **POSITIVE** for Influenza A ➢ Report: **POSITIVE** for the FluA subtype equivocal ➢ In the results entry screen, select Add Test to add the test with the equivocal result ➢ The Influenza A test codes are: 1. FAH1T – Influenza A H1 2. FH109 – Influenza A H1-2009 3. FAH3T – Influenza A H3 ➢ Do not report the Influenza A targets that were not detected ➢ Report will automatically print to OCPHO (HPU1) ➢ Copy report to Stanton IPAC (SIPAC) if ER or inpatient

BF Results: Influenza A Influenza B RSV Rhinovirus/ Enterovirus **INVALID**	• Repeat testing on the BioFire ➢ Add comment in TCOMM that testing was repeated • If the repeat results are invalid: ➢ Report: **INVALID** ➢ From the keypad add key R to add repeat sample collection comment ➢ Phone the ordering location and request a new sample be collected

NOTE: All calls are to be documented in the Call Box in SoftMic

Step	Action
Completing the Run	
1	Check the Resulting Worklist – Torch to ensure all ordered samples are complete.
2	In the BSC, with gloved hands, remove the used pipettes from the wet waste container and place into the dry waste container.
3	Remove the autoclave bag from the dry waste container. Tie and wipe outside with an Accel TB wipe. Place in the biohazard garbage.
4	Ensure supplies in BSC are stocked up and sharps container is not full.
5	Wipe the BSC with an Accel TB wipe. Turn off the blower and lower the sash. Remove gloves and don a fresh pair.
6	Ensure all used pouches from the BioFire are discarded in the biohazard waste.
7	When all testing of patient samples and disinfection of surfaces is complete, remove PPE and place in the biohazard waste. Retrieve samples from the refrigerator and place in correct storage location.

LIMITATIONS:

1. The BioFire RP2.1 is a qualitative test and does not provide a quantitative value for the organism(s) in the specimen
2. Results from this test must be correlated with clinical history
3. The performance of BioFire RP2.1 has not been validated for specimens collected from individuals without signs or symptoms of respiratory infection
4. The effect of antibiotic treatment on test performance has not been evaluated
5. The detection of viral and bacterial nucleic acid is dependent upon proper specimen collection, handling, transportation, storage and preparation.
6. A negative BioFire RP2.1 result does not exclude the possibility of viral or bacterial infection
7. If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result
8. Recent administration of nasal influenza vaccines prior to NPS specimen collection could lead to accurate virus detection by the BioFire RP2.1 of the viruses contained in the vaccine, but would not represent infection by those agents

CROSS-REFERENCES:

- MIC82110-Maintenance Record – BioFire FilmArray
- MIC82300-BioFire RP2.1 Quality Control
- MIC82310-BioFire RP2.1 Quality Control Results Record

REFERENCES:

- *BioFire Respiratory Panel 2.1 (RP2.1)* Instructions for Use (EUA). BFR0000-8303-01, May 2020
- BioFire FilmArray Torch Operator's Manual, HTFA-PRT-0001-05, March 2020
- Canadian Laboratory Response Network Training: COVID-19 Point of Care Diagnostics. *BioFire FilmArray Torch and the FilmArray Respiratory Panel 2.1 (RP2.1)*.
- Biosafety Advisory Committee. *STHA Biosafety Program Manual*. January 2016

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	10 Aug 20	Initial Release	L. Steven
2.0	02 Oct 20	Updated to remove "presumptive" from reporting	L. Steven
3.0	23 Dec 20	Updated to reflect positive results report to OCPHO, inform OCPHO when CT ≥38 and record repeat testing in TCOMM	L. Steven
4.0	05 Feb 21	Updated to include the reporting of Influenza A, Influenza B, RSV and Enterovirus/Rhinovirus	L. Steven
5.0	28 Apr 21	Updated to reflect new Xpert Xpress SARS-CoV-2/Flu/RSV cartridge	L. Steven