



Protocols for Laboratory Verification of Performance of the BIOFIRE® Respiratory Panel 2.1 (RP2.1)

Laboratory Protocols for Use with ZeptoMetrix NATtrol™ Control Materials

Purpose

The Clinical Laboratory Improvement Amendments (CLIA), passed in 1988, establishes quality standards for all laboratory testing to ensure the accuracy and reliability of patient test results, regardless of where the test is performed. The CLIA regulations include a requirement for verifying the performance specifications of unmodified, moderate complexity tests cleared or approved by the FDA. The BIOFIRE® Respiratory Panel 2.1 (RP2.1) has been categorized by the FDA as a CLIA moderate complexity test.

This document provides examples of procedures to assist your laboratory in developing a protocol for the verification of BIOFIRE (RP2.1) performance on BIOFIRE® FILMARRAY® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems as required by CLIA. Verification schemes compatible with the BIOFIRE RP2.1, have been designed using non-clinical specimens. The methods described provide positive and negative tests for each organism detected by the BIOFIRE RP2.1 and may be easily modified or expanded to meet specific criteria. Day-to-day variation is evaluated by testing each sample on two separate days. To evaluate user-to-user variation, multiple laboratory technicians may test the same sample. In addition, testing patient samples for verification or to evaluate matrix effects on the performance of the BIOFIRE RP2.1 should be done under the guidance of the Laboratory Director, but is not described here.

As per the CLIA regulation, the Laboratory Director is ultimately responsible for ensuring that verification procedures meet the appropriate standards for CLIA and applicable laboratory accrediting agencies.

Intended Use

The BIOFIRE Respiratory Panel 2.1 (RP2.1) is a PCR-based multiplexed nucleic acid test intended for use with the BIOFIRE® FILMARRAY® 2.0 or BIOFIRE® 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, including COVID-19.

The following organism types and subtypes are identified using the BIOFIRE RP2.1:



Viruses	Bacteria
Adenovirus	<i>Bordetella parapertussis</i>
Coronavirus 229E	<i>Bordetella pertussis</i>
Coronavirus HKU1	<i>Chlamydia pneumoniae</i>
Coronavirus NL63	<i>Mycoplasma pneumoniae</i>
Coronavirus OC43	
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	
Human metapneumovirus	
Human rhinovirus/enterovirus	
Influenza A virus	
Influenza A virus A/H1	
Influenza A virus A/H3	
Influenza A virus A/H1-2009	
Influenza B virus	
Parainfluenza virus 1	
Parainfluenza virus 2	
Parainfluenza virus 3	
Parainfluenza virus 4	
Respiratory syncytial virus	

Always refer to the *BIOFIRE® Respiratory Panel 2.1 (RP2.1) Instructions for Use* for the complete intended use statement and additional information about the use of the BIOFIRE® FILMARRAY® 2.0 or BIOFIRE® FILMARRAY® TORCH Systems.

Performance Verification Overview

Two examples of performance verification procedures are described: (1) a Simple Protocol for the verification of the BIOFIRE RP2.1 and (2) a Transport Media Protocol that evaluates the performance of each assay on the Panel in a transport media clinical specimen matrix. These protocols are examples of procedures to assist your laboratory in developing a protocol for the verification of BIOFIRE RP2.1 performance on the BIOFIRE® FILMARRAY® Systems.



Note: Transport media or saline may contain non-viable organisms and/or nucleic acids at levels that can be detected by the BIOFIRE RP2.1 and may lead to false positive results. Transport media may be screened using the Panel prior to starting the verification procedure; the optimal transport media will be negative for all analytes tested on the BIOFIRE RP2.1.

The procedures have been designed to take advantage of the multiplex nature of the BIOFIRE RP2.1. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedures described below will generate multiple positive and negative detections for each of the Panel assays. The procedures were developed using the NATrol™ Respiratory Verification Panel 2.1 (NATRVP2.1-BIO) available from ZeptoMetrix LLC, Buffalo, NY.

A BIOFIRE® FILMARRAY® System is defined as all BIOFIRE® FILMARRAY® Modules that are connected to and controlled by a single computer system. If the laboratory director chooses not to perform the entire verification protocol on each individual module of the BIOFIRE® FILMARRAY® 2.0 or BIOFIRE® FILMARRAY® TORCH System,





it is advised that test replicates are evenly distributed among the modules. An example of a performance verification workflow using 2, 4, or 6 modules is provided in Figure 2.

Clinical/patient samples may be used in place of, or in addition to the verification schemes described here in order to assess clinical sensitivity/specificity and sample matrix effects as part of the performance verification of the BIOFIRE RP2.1.



Note: The laboratory should only perform the verification study with analytes that will be reported using the BIOFIRE RP2.1 in their laboratory setting.

Table 1. Overview of Verification Protocols

Verification Protocol	Organisms per Pool	Number of Sample Pools	Replicates per Sample Pool	Pouches Required ^a	Expected Positive Results ^b	Expected Negative Results	Approximate Days of Testing ^c
Simple protocol	5 or 6	4	4	16	≥4 per organism	≤12 per organism	4
Transport Media protocol	5 or 6	4	4	16	≥4 per organism	≤12 per organism	4

^a Pouches required does not include pouches that may be needed for screening transport media.

^b The expected number of positives and negatives per organism is dependent upon the number strains of a particular organism used to complete the verification. The proposed verification procedure recommends multiple adenovirus strains; therefore the number of expected positive and negative detections for adenovirus will vary.

^c The approximate number of days for testing assumes a BIOFIRE FILMARRAY System configured with one module.

Performance Verification Materials

The following materials may be used to perform the verification procedure:

Table 2. Recommended materials for the verification protocols

Material	Part Number
BIOFIRE® Respiratory Panel 2.1 (RP2.1) Kit (30 tests)	BIOFIRE Diagnostics, LLC 423742
BIOFIRE® Respiratory Panel 2.1 (RP2.1) Instructions for Use	BIOFIRE Diagnostics, LLC BFR0000-8579
BIOFIRE® Respiratory Panel 2.1 (RP2.1) Quick Guide	BIOFIRE Diagnostics, LLC BFR0000-8793
Control Organism ^a	ZeptoMetrix, NATtrol™ Respiratory Verification Panel 2.1, NATRVP2.1-BIO
Transport Media	Various media are appropriate
2 mL or 5 mL Sample Tubes	Various manufacturers
Disposable Transfer pipets, graduated	Avantor, 414004-024 (or equivalent)

^aAny appropriate source of organism may be used for verification of any or all of the assays in the BIOFIRE RP2.1 panel. However, when alternate organism sources are used (i.e. not the ZeptoMetrix control material), the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.



Performance Verification Protocols

Simple Protocol

The Simple Protocol evaluates the BIOFIRE RP2.1 performance when control material (ZeptoMetrix NATRVP2.1-BIO) is pooled in the absence of clinical matrix. The proposed organism pooling scheme (Table 3) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.



Note: Dilution of ZeptoMetrix control organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

Figures 1 and 2 illustrate protocol and workflow schemes for testing 4 replicates per pool for 4 different pools over multiple days. This produces a total of 16 verification sample test runs and provides at least 4 positive results and as many as 12 negative results per assay. Some organisms, such as adenovirus, are represented multiple times. This is done to ensure all adenovirus assays are represented in the verification protocol.

The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run more samples per day based on the number of modules in the BIOFIRE® FILMARRAY® System. The pooling scheme in Table 3 provides sufficient volume for testing more replicates if desired.

To evaluate day-to-day variation: pooled organisms may be stored overnight or up to 14 days at refrigeration temperature (2–8°C). To evaluate operator-to-operator variation, multiple laboratory technicians/ staff may perform testing. Figure 2 provides an example of user-to-user, day-to-day, and module-to-module testing for labs with multiple BIOFIRE Modules.

Table 3. Proposed Organism Pooling Scheme for the Simple Protocol

Control Organism: NATRVP2.1-BIO	Approximate Organism Volume	Approximate Final Pool Volume
Pool 1		
Adenovirus Type 3	0.3 mL	1.8 mL
Coronavirus OC43	0.3 mL	
SARS-CoV-2 (USA-WA1/2020)	0.3 mL	
Influenza A H1N1pdm (A/NY/02/092) subtype H1-2009	0.3 mL	
Influenza B (B/Florida/02/06)	0.3 mL	
Parainfluenza virus Type 4	0.3 mL	
Pool 2		
Coronavirus 229E	0.3 mL	1.5 mL
Influenza AH3 (A/Brisbane/10/07)-subtype H3	0.3 mL	
Parainfluenza virus Type 1	0.3 mL	
Parainfluenza virus Type 2	0.3 mL	
Rhinovirus 1A	0.3 mL	





Pool 3		
Adenovirus Type 1	0.3 mL	1.8 mL
Coronavirus NL63	0.3 mL	
Influenza AH1 (A/New Caledonia/20/99)- subtype H1	0.3 mL	
Parainfluenza virus Type 3	0.3 mL	
Respiratory Syncytial Virus A	0.3 mL	
<i>Bordetella parapertussis</i> (A747)	0.3 mL	
Pool 4		
Adenovirus Type 31	0.3 mL	1.8 mL
Coronavirus HKU1 (recombinant)	0.3 mL	
Metapneumovirus 8 (Peru6-2003)	0.3 mL	
<i>Bordetella pertussis</i> (A639)	0.3 mL	
<i>Chlamydia pneumoniae</i> (IOL-207)	0.3 mL	
<i>Mycoplasma pneumoniae</i> (M129)	0.3 mL	

Simple Protocol Example

The estimated total time for completion for the Simple Protocol verification example is 4 days for a BIOFIRE® FILMARRAY® System configured with 1 module. A proposed organism pooling scheme is presented above in Table 3.



Note: It is important to prepare only the number of sample pools that will be tested within 14 days. The number of samples prepared may be modified based on the laboratory's work schedule and number of modules connected within a BIOFIRE FILMARRAY System.

Day 1

- Organize materials needed (Table 2).
- Prepare one sample pool (i.e. Pool 1) from ZeptoMetrix NATRVP2.1-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 3 for example organism pooling schemes and specific volumes for each pool.
 - Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 2 mL tube. Alternatively, a 5mL tube may be used.
 - Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The combined volume of organisms for each pool will be approximately 1.5 or 1.8 mL.
- Repeat Step 2 for the remaining sample pool (i.e. Pool 2) to be prepared on Day 1.



4. Test 2 replicates from a single sample pool (Figure 1: Pool 1 replicates A and B). Ensure the pooled sample is well mixed prior to removing a sample for testing. Replicate samples A and B should be tested in a single day by different operators. Refer to Figure 2 for suggested workflows depending upon the module configuration in the verification study.



Note: For each sample, follow instructions in the *BIOFIRE® Respiratory Panel 2.1 (RP2.1) Instructions for Use* and the *BIOFIRE® Respiratory Panel 2.1 (RP2.1) Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for the remaining sample pool replicates to be tested that day (i.e. replicates A and B for Pool 2)
6. Refrigerate samples (2–8°C) for up to 14 days for the evaluation of day-to-day variation.



Note: The proposed organism pooling scheme (Table 3) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more samples if desired.

Day 2

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 1 by repeating Step 4 and 5 above (i.e. replicates C and D from Pools 1 and 2).

Day 3

Prepare 2 new sample pools (i.e. Pools 3 and 4) as described in Steps 2 and 3. Test replicates as described in Steps 4 and 5 above.

Day 4

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 3 by repeating Step 4 and 5 above (i.e. replicates C and D from Pools 3 and 4).



Note: A BIOFIRE RP2.1 Verification Record is provided and may serve as a template for recording your results.



Figure 1. Verification Protocol Workflow for the Simple Protocol and the Transport Media Protocol

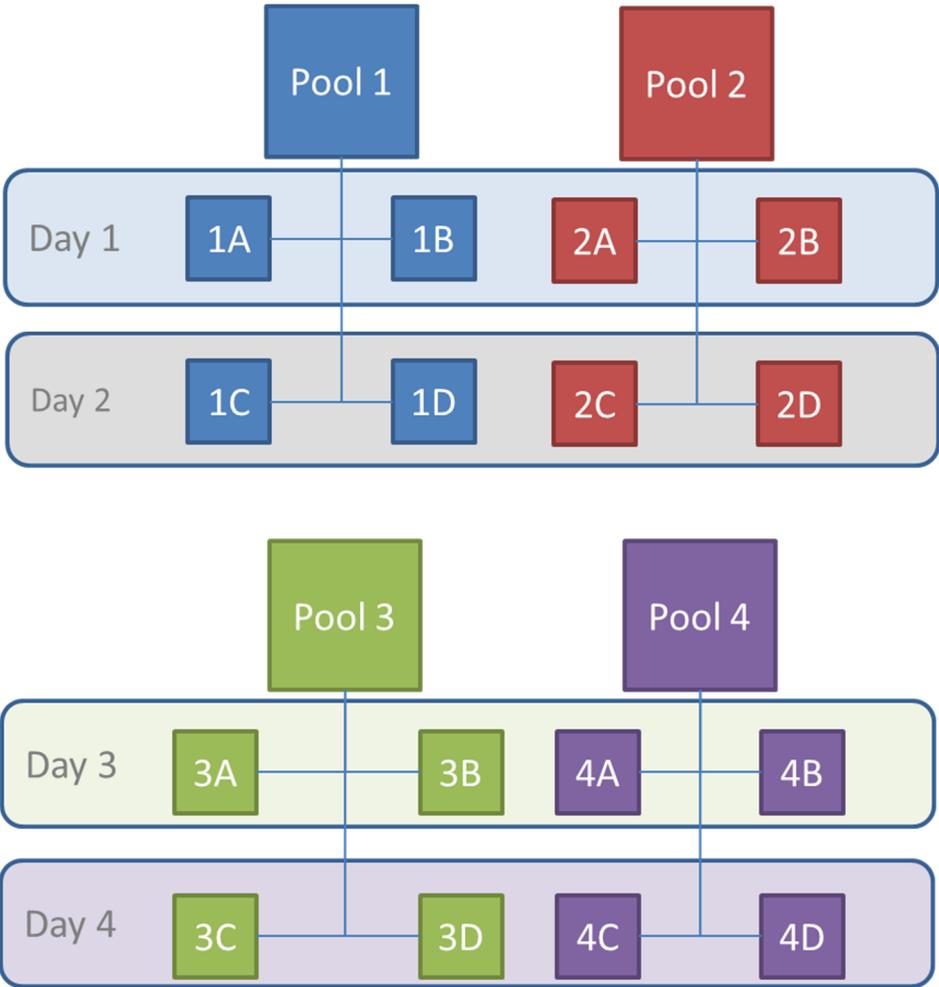




Figure 2. Example of a Verification Workflows for Use with Multiple BIOFIRE Modules

Verification with 2 modules				
Testing Day	Module 1		Module 2	
Day 1	Pool 1A/ Operator 1	Pool 2B/ Operator 2	Pool 1B/ Operator 2	Pool 2A/ Operator 1
Day 2	Pool 1D/ Operator 2	Pool 2C/ Operator 1	Pool 1C/ Operator 1	Pool 2D/ Operator 2
Day 3	Pool 3A / Operator 1	Pool 4B / Operator 1	Pool 3B/ Operator 2	Pool 4A / Operator 2
Day 4	Pool 3D/ Operator 2	Pool 4C / Operator 2	Pool 3C / Operator 1	Pool 4D / Operator 1

Verification with 4 modules				
Testing Day	Module 1	Module 2	Module 3	Module 4
Day 1	Pool 1A/ Operator 1	Pool 1B/ Operator 2	Pool 2A/ Operator 1	Pool 2B/ Operator 2
Day 2	Pool 2D/ Operator 2	Pool 2C/ Operator 1	Pool 1D/ Operator 2	Pool 1C/ Operator 1
Day 3	Pool 3A / Operator 1	Pool 3B/ Operator 2	Pool 4B / Operator 1	Pool 4A / Operator 2
Day 4	Pool 4C / Operator 2	Pool 4D / Operator 1	Pool 3D/ Operator 2	Pool 3C / Operator 1

Verification with 6 modules						
Testing Day	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6
Day 1	Pool 1A/ Operator 1	Pool 1B/ Operator 2	Pool 2A/ Operator 1	Pool 2B/ Operator 2		
Day 2			Pool 1C/ Operator 1	Pool 1D/ Operator 2	Pool 2C/ Operator 1	Pool 2D/ Operator 2
Day 3	Pool 3A / Operator 1	Pool 3B/ Operator 2			Pool 4B / Operator 1	Pool 4A / Operator 2
Day 4	Pool 4C / Operator 2	Pool 4D / Operator 1	Pool 3C / Operator 1	Pool 3D/ Operator 2		



Transport Media Protocol

The Transport Media Protocol evaluates the BIOFIRE RP2.1 performance when verification control materials (ZeptoMetrix NATRVP2.1-BIO) are tested in the presence of a transport media sample matrix. The proposed organism pooling scheme (Table 4) should be followed to obtain the expected number of positive and negative results for each assay in a time and resource-efficient manner.

 Note: Dilution of ZeptoMetrix organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

Figures 1 and 2 (above) illustrate protocol and workflow schemes for testing 4 replicates per pool for 4 different pools over multiple days. This produces a total of 16 verification sample test runs and provides at least 4 positive results and as many as 12 negative results per assay. Some organisms, such as adenovirus, are represented multiple times. This is done to ensure all adenovirus assays are represented in the verification protocol.

The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run more replicates per day based on the number of modules in the BIOFIRE® FILMARRAY® System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples can be stored overnight (or up to 14 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation.

Table 4. Proposed Organism Pooling Scheme for the Transport Media Protocol

Control Organism: NATRVP2.1-BIO	Approximate Organism Volume	Volume Transport Media	Approximate Final Pool Volume
Pool 1			
Adenovirus Type 3	0.3 mL	1.8 mL	3.6 mL
Coronavirus OC43	0.3 mL		
SARS-CoV-2 (USA-WA1/2020)	0.3 mL		
Influenza A H1N1pdm (A/NY/02/092) subtype H1-2009	0.3 mL		
Influenza B (B/Florida/02/06)	0.3 mL		
Parainfluenza virus Type 4	0.3 mL		
Pool 2			
Coronavirus 229E	0.3 mL	1.5 mL	3.0 mL
Influenza AH3 (A/Brisbane/10/07)-subtype H3	0.3 mL		
Parainfluenza virus Type 1	0.3 mL		
Parainfluenza virus Type 2	0.3 mL		
Rhinovirus 1A	0.3 mL		



Pool 3			
Adenovirus Type 1	0.3 mL	1.8 mL	3.6 mL
Coronavirus NL63	0.3 mL		
Influenza AH1 (A/New Caledonia/20/99)- subtype H1	0.3 mL		
Parainfluenza virus Type 3	0.3 mL		
Respiratory Syncytial Virus A	0.3 mL		
<i>Bordetella parapertussis</i> (A747)	0.3 mL		
Pool 4			
Adenovirus Type 31	0.3 mL	1.8 mL	3.6 mL
Coronavirus HKU1 (recombinant)	0.3 mL		
Metapneumovirus 8 (Peru6-2003)	0.3 mL		
<i>Bordetella pertussis</i> (A639)	0.3 mL		
<i>Chlamydia pneumoniae</i> (IOL-207)	0.3 mL		
<i>Mycoplasma pneumoniae</i> (M129)	0.3 mL		

Transport Media Protocol Example

The estimated total time for completion for this Transport Media Protocol verification example is 4 days for a BIOFIRE FILMARRAY System configured with 1 module. A proposed organism pooling scheme is presented above in Table 4.

Note: It is important to prepare only the number of sample pools that will be tested within 14 days of preparation. The suggestion to prepare 2 sample pools is based on testing up to 4 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of modules connected within a BIOFIRE FILMARRAY System.

Day 1

- Organize materials needed (Table 2). Screen Transport media on the BIOFIRE RP2.1 Panel in order to characterize the sample prior to preparing pools.
- Prepare one sample pool (i.e. Pool 1) from ZeptoMetrix NARVP2.1-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 4 for example organism pooling schemes and specific volumes for each pool.
 - Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 5mL tube.
 - Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The combined volume of organisms for each pool will be approximately 1.5 to 1.8 mL.
 - Add 1.5 or 1.8 mL of transport media (as described in Table 4) to the tube containing the organism pool (step b). The total volume will be approximately 3.0 to 3.6 mL.
- Repeat Step 2 for the remaining sample pool (i.e. Pool 2) to be prepared on Day 1.



4. Test 2 replicates from a single sample pool (Figure 1: Pool 1 replicates A and B). Ensure the pooled sample is well mixed prior to removing a sample for testing. Replicate samples A and B should be tested in a single day by different operators. Refer to Figure 2 for suggested workflows depending upon the module configuration in the verification study.



Note: For each sample, follow instructions in the *BIOFIRE® Respiratory Panel 2.1 (RP2.1) Instructions for Use* and the *BIOFIRE® Respiratory Panel 2.1 (RP2.1) Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for the remaining sample pool replicates to be tested that day (i.e. replicates A and B for Pool 2)
6. Refrigerate samples (2–8°C) for up to 14 days for the evaluation of day-to-day variation.



Note: The proposed organism pooling scheme (Table 4) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more samples if desired.

Day 2

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 1 by repeating Step 4 and 5 above (i.e. replicates C and D from Pools 1 and 2).

Day 3

Prepare 2 new sample pools (i.e. Pools 3 and 4) as described in Steps 2 and 3. Test replicates as described in Steps 4 and 5 above.

Day 4

To evaluate day-to-day variation, test replicates from the same sample pools prepared on Day 3 by repeating Step 4 and 5 above (i.e. replicates C and D from Pools 3 and 4).



Note: A BIOFIRE RP2.1 Verification Record is provided and may serve as a template for recording your results.

Expanding or Modifying the Protocols

The protocols described above can be expanded by increasing the number of tests from each of the organism pools. Each organism pool contains sufficient volume for testing additional replicates. The verification study may use multiple types of transport media in the pools, as needed. Reference CAP accreditation checklist requirements: MIC.64960 Validation or Verification Studies-Specimen Selection.



Note: The laboratory should perform the verification study with specimen types and media that will be used with the BIOFIRE RP2.1 Panel in their laboratory setting.

Verification of Loaner, Repaired, and Permanent Replacement Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement module, the following protocol may serve as a guideline but should be verified by the Laboratory Director.

1. Select a few specimens and/or proficiency samples (any combination of positives and negatives) previously tested on the BIOFIRE® Respiratory Panel 2.1 (RP2.1). The Laboratory Director should determine the appropriate number of samples to test. Proficiency samples should not be pooled or diluted.
2. Select a set of controls that verify detection of all targets on the BIOFIRE RP2.1.
3. Test the selected samples on the loaner, repaired, or permanent replacement module and document the results.

Technical Support Contact Information

bioMérieux is dedicated to providing the best customer support available. If you have questions or concerns about this process, please contact your local bioMérieux representative or your authorized distributor.

*All product names, trademarks and registered trademarks are property of their respective owners.



BIOFIRE® Respiratory Panel 2.1 (RP2.1) Verification Record

BIOFIRE® Respiratory Panel 2.1 (RP2.1) Verification Record

 Kit Part # _____
 Lot # _____

Module Serial # _____
 Module Serial # _____
 Module Serial # _____
 Module Serial # _____

BIOFIRE RP2.1 Panel Detection		Replicate Testing - Record Organism Detections																Summary					
		1-A	1-B	1-C	1-D	2-A	2-B	2-C	2-D	3-A	3-B	3-C	3-D	4-A	4-B	4-C	4-D	# Positives	# Negatives	# Operators	# Days	# Modules	Patient Samples?
Pool 1	Adenovirus Type 3																						
	Coronavirus OC43																						
	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)																						
	Influenza A H1-2009																						
	Influenza B																						
Pool 2	Parainfluenza Virus 4																						
	Coronavirus 229E																						
	Influenza A H3																						
	Parainfluenza Virus 1																						
	Parainfluenza Virus 2																						
Pool 3	Human Rhinovirus/Enterovirus 1A																						
	Adenovirus Type 1																						
	Coronavirus NL63																						
	Influenza A H1																						
	Parainfluenza Virus 3																						
Pool 4	Respiratory Syncytial Virus																						
	Bordetella parapertussis (IS 1001)																						
	Adenovirus Type 31																						
	Coronavirus HKU1																						
	Human Metapneumovirus																						
Bordetella pertussis (ptxP)																							
Chlamydia pneumoniae																							
Mycoplasma pneumoniae																							

Reviewed by: _____
 Signature Date

