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.

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. Two possible verification schemes, compatible with the BioFire RP2.1, have been designed. Each verification scheme provides 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 2.0 or BioFire 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:

Technical Note
BioFire Diagnostics, LLC
www.biofiredx.com
BFR0000-9430-02

QS-339C-01

Viruses	Bacteria
Adenovirus	
Coronavirus 229E	*Bordetella parapertussis*
Coronavirus HKU1	*Bordetella pertussis*
Coronavirus NL63	*Chlamydia pneumoniae*
Coronavirus OC43	*Mycoplasma pneumoniae*
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)	
Human Metapneumovirus	
Human Rhinovirus/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	

The complete intended use statement and additional information about the use of the BioFire System can be found in the *BioFire® Respiratory Panel 2.1 (RP2.1) Instructions for Use*.

Performance Verification Overview

Two different examples of performance verification procedures are described: (1) a Simple Protocol for the verification of the BioFire RP2.1and (2) a Transport Media Protocol that evaluates BioFire RP2.1 performance in a transport media sample 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 Systems.

The verification procedures described here may be used to evaluate the performance of each assay on the BioFire RP2.1. The performance verification protocols 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 BioFire RP2.1 assays. The procedures were developed using a Respiratory Verification Panel 2.1 available from ZeptoMetrix LLC, Buffalo, NY (part number NATRVP2.1-BIO).

A BioFire System is defined as all BioFire® FilmArray® Instruments or 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 instrument, it is advised that test replicates are evenly distributed among the instruments or 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.

Table 1. Overview of Verification Protocols

Verification Protocol	Organisms per Pool[a]	Number of Sample Pools	Replicates per Sample Pool	Pouches Required	Expected Positive Results[a]	Expected Negative Results	Approximate Days of Testing[b]
Example 1: Simple protocol	5 or 6	4	4	16	≥4 per organism	≤12 per organism	4
Example 2: Transport Media protocol	5 or 6	4	4	16	≥4 per organism	≤12 per organism	4

[a] 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 adenovirus positives would be 12 and the number of expected negatives would be 4.

[b] The approximate number of days for testing assumes a BioFire System configured with one instrument/module.

Performance Verification Materials

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

Table 2. Recommended materials for the verification protocols for RP2.1

Material	De Novo 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 Respiratory Verification Panel 2.1 (NATRVP2.1-BIO)
Transport Media (e.g. Remel M4 Viral Transport Media)	Various media are appropriate
2 mL or 5 mL Sample Tubes	Various manufacturers
Disposable Transfer pipets, graduated	VWR, 414004-024 (or equivalent)

[a]Any 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 verification materials (ZeptoMetrix NATRVP2.1-BIO) are 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 organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

Figures 1 and 2 illustrate 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 (or fewer) samples per day based on the number of modules in the BioFire System. The pooling scheme in Table 3 provides sufficient volume for testing more replicates if desired. Figure 2 provides an examples of user-to-user, day-to-day, and module-to-module testing for labs with multiple BioFire Modules.

Pooled samples can be stored overnight (or up to 3 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation.

Table 3. Proposed Organism Pooling Scheme for the Simple Protocol

Organism	Approximate Organism Volume	Approximate Pool Volume
Pool 1		
Adenovirus 3	0.3 mL	1.8 mL
Coronavirus OC43	0.3 mL	
SARS-CoV-2	0.3 mL	
Influenza A H1N1pdm	0.3 mL	
Influenza B	0.3 mL	
Parainfluenza 4	0.3 mL	
Pool 2		
Coronavirus 229E	0.3 mL	1.5 mL
Influenza AH3	0.3 mL	
Parainfluenza 1	0.3 mL	
Parainfluenza 2	0.3 mL	
Rhinovirus 1A	0.3 mL	
Pool 3		
Adenovirus 1	0.3 mL	1.8 mL
Coronavirus NL63	0.3 mL	
Influenza AH1	0.3 mL	
Parainfluenza 3	0.3 mL	
RSV A	0.3 mL	
Bordetella parapertussis	0.3 mL	
Pool 4		
Adenovirus 31	0.3 mL	1.8 mL
Coronavirus HKU-1	0.3 mL	
Metapneumovirus 8	0.3 mL	
Bordetella pertussis	03 mL	
Chlamydia pneumoniae	0.3 mL	
Mycoplasma pneumoniae	0.3 mL	

Simple Protocol Example

The estimated total time for completion for this Simple Protocol verification example is 4 days for a BioFire 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 3 days of preparation. The suggestion to prepare 3 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 System.

Day 1

1. Organize materials needed (Table 2).

2. Prepare two sample pools (i.e. Pools #1 and 2) 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.
    a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 2 mL tube. Alternatively, a 5mL tube may be used.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The total volume for each pool will be approximately 1.5 or 1.8 mL.
    c. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. 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 (i.e. Figure 1: Pool # 1 replicates A and B). The replicate samples should be tested in a single day by different users.

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. Pool # 2 replicates A and B)

6. Refrigerate samples (2–8°C) for up to 3 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. Pool # 1 replicates C and D).

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. Pool # 3 replicates C and D).

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

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

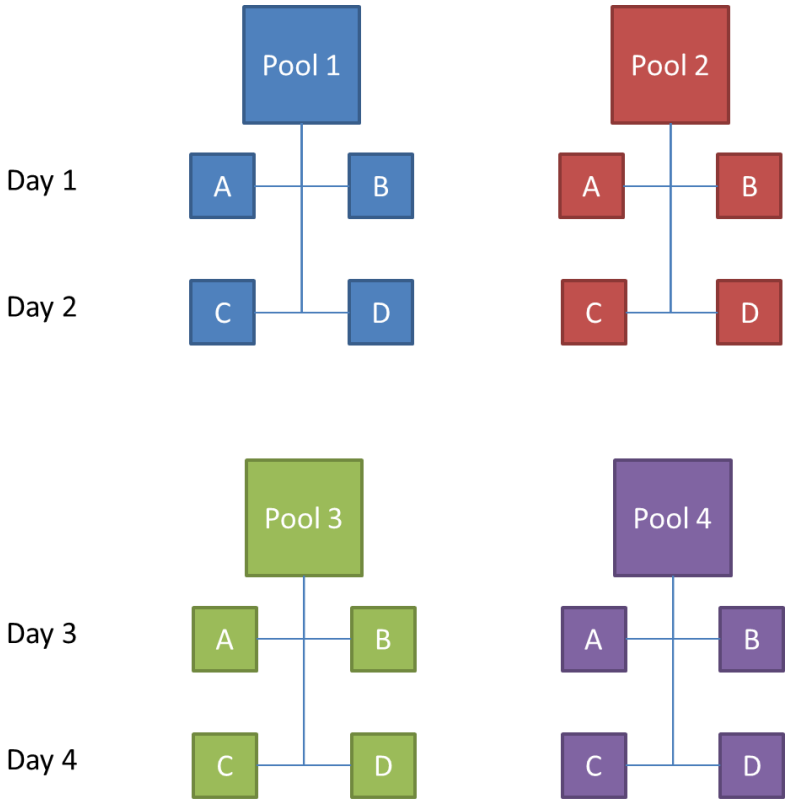

Figure 2. Example of a Verification workflow for use with multiple BioFire Modules

Verification with 2 modules	Module 1		Module 2	
Day 1	Pool 1/ User 1	Pool 2/ User 2	Pool 1/ User 2	Pool 2/ User 1
Day 2	Pool 1/ User 2	Pool 2/ User 1	Pool 1/ User 1	Pool 2/ User 2
Day 3	Pool 3 / User 1	Pool 4 / User 1	Pool 3/ User 2	Pool 4 / User 2
Day 4	Pool 3/ User 2	Pool 4 / User 2	Pool 3 / User1	Pool 4 / User 1

Verification with 4 modules	Module 1	Module 2	Module 3	Module 4
Day 1	Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2
Day 2	Pool 2/ User 2	Pool 2/ User 1	Pool 1/ User 2	Pool 1/ User 1
Day 3	Pool 3 / User 1	Pool 3/ User 2	Pool 4 / User 1	Pool 4 / User 2
Day 4	Pool 4 / User 2	Pool 4 / User 1	Pool 3/ User 2	Pool 3 / User1

Verification with 6 modules	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6
Day 1	Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2		
Day 2			Pool 1/ User 1	Pool 1/ User 2	Pool 2/ User 1	Pool 2/ User 2
Day 3	Pool 3 / User 1	Pool 3/ User 2			Pool 4 / User 1	Pool 4 / User 2
Day 4	Pool 4 / User 2	Pool 4 / User 1	Pool 3 / User1	Pool 3/ User 2		

Transport Media Protocol

The Transport Media Protocol evaluates the BioFire RP2.1 performance when verification 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.

The protocol and workflow schemes (Figures 1 and 2) illustrate 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 (or fewer) samples per day based on the number of modules in the BioFire System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples can be stored overnight (or up to 3 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

Organism	Approximate Organism Volume	Volume Transport Media	Approximate Pool Volume
Pool 1			
Adenovirus 3	0.3 mL	1.8 mL	3.6 mL
Coronavirus OC43	0.3 mL		
SARS-CoV-2	0.3 mL		
Influenza A H1N1pdm	0.3 mL		
Influenza B	0.3 mL		
Parainfluenza 4	0.3 mL		
Pool 2			
Coronavirus 229E	0.3 mL	1.5 mL	3.0 mL
Influenza AH3	0.3 mL		
Parainfluenza 1	0.3 mL		
Parainfluenza 2	0.3 mL		
Rhinovirus 1A	0.3 mL		
Pool 3			
Adenovirus 1	0.3 mL	1.8 mL	3.6 mL
Coronavirus NL63	0.3 mL		
Influenza AH1	0.3 mL		
Parainfluenza 3	0.3 mL		
RSV A	0.3 mL		
Bordetella parapertussis	0.3 mL		
Pool 4			
Adenovirus 31	0.3 mL	1.8 mL	3.6 mL
Coronavirus HKU-1	0.3 mL		
Metapneumovirus 8	0.3 mL		
Bordetella pertussis	03 mL		
Chlamydia pneumoniae	0.3 mL		
Mycoplasma pneumoniae	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 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 3 days of preparation. The suggestion to prepare 2 sample pools is based on testing up to 41.5 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 System.

Day 1

1. Organize materials needed (Table 2).

2. Prepare two sample pools (i.e. Pools #1 and 2) from ZeptoMetrix NARVP2.1-BIO control material. 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.

    a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 5mL tube.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The total volume for each pool will be approximately 1.5 to 1.8 mL.
    c. 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.
    d. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. 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 (i.e. Figure 1: Pool # 1 replicates A and B). The replicate samples should be tested in a single day by different users.

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. Pool # 2 replicates A and B)

6. Refrigerate samples (2–8°C) for up to 3 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. Pool # 1 replicates C and D).

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. Pool # 3 replicates C and D).

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

Implementing the BioFire® Respiratory Panel 2.1 (RP2.1) De Novo

The BioFire RP2.1 EUA and the BioFire RP2.1 De Novo are identical; assays for all analytes and the reaction conditions of the test are unchanged. Part number labeling of EUA and De Novo components differ to distinguish each product.

Laboratories that have performed verification studies for the BioFire RP2.1 EUA should consult with their Laboratory Director to determine the need for and the extent of the verification for the BioFire RP2.1 De Novo. If limited verification studies were performed initially, the laboratory may need to supplement with additional data. Retrospective data can be used for this (e.g. patient specimens/proficiency samples). Reference CAP accreditation checklist requirements: COM. 30980, COM.40300, COM.40475, and COM.40500.

Verification studies should include an adequate number and a representative distribution of samples for each type of specimen collected as determined by the laboratory director. The Transport Media protocol provided offers guidance on testing control materials in a transport media sample matrix. The verification study may use multiple types of transport media in the pools, as needed. Reference CAP accreditation checklist requirements: MIC.64960.

If your laboratory previously implemented an individualized quality control plan (IQCP) for a non-waived panel, the laboratory needs to determine if there are any additional risks that were not considered and update the existing risk assessment and quality control plan if appropriate Reference CAP accreditation checklist requirement: MIC.65220.

The protocol provided can be expanded or condensed, as necessary, in order to meet guidelines set by the regulatory body governing your laboratory.

Expanding 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.

Verification of Loaner, Repaired, and Permanent Replacement Instruments or Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement instrument or 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 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 instrument or module and document the results.

Technical Support Contact Information

BioFire is dedicated to providing the best customer support available. If you have any questions or concerns about this process, please contact the BioFire Technical Support team for assistance.

BioFire Technical Support
Email: support@biofiredx.com
Phone: +1-801-736-6354, select Option 5

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 #** _____

	Organism and Representative Strain		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	# Users	# Days	# Modules	Patient Samples?
Pool 1	Adenovirus	Type 3																						
	Coronavirus OC43																							
	Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-																							
	Influenza A H1-2009																							
	Influenza B																							
	Parainfluenza Virus 4																							
Pool 2	Coronavirus 229E																							
	Influenza A H3																							
	Parainfluenza Virus 1																							
	Parainfluenza Virus 2																							
	Human Rhinovirus/Enterovirus	Rhinovirus 1A																						
Pool 3	Adenovirus	Type 1																						
	Coronavirus NL63																							
	Influenza A H1																							
	Parainfluenza Virus 3																							
	Respiratory Syncytial Virus																							
	Bordetella parapertussis (IS1001)																							
Pool 4	Adenovirus	Type 31																						
	Coronavirus HKU1																							
	Human Metapneumovirus																							
	Bordetella pertussis (ptxP)																							
	Chlamydia pneumoniae																							
	Mycoplasma pneumoniae																							

Reviewed by: _____

Signature **Date**

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