Protocols for Laboratory Verification of Performance of the BioFire® FilmArray® Respiratory Panel 2 (RP2)

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 RP2 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 performance on BioFire® FilmArray® 2.0 and BioFire® FilmArray® Torch Systems as required by CLIA. Two possible verification schemes, compatible with the BioFire RP2, have been designed. Each verification scheme provides positive and negative tests for each organism detected by the BioFire RP2 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 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.

BioFire Intended Use

The BioFire RP2 is a multiplexed nucleic acid test intended for use with BioFire 2.0 and 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. The following organisms and subtypes are identified using the BioFire RP2: adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, enterovirus, human metapneumovirus, influenza A, influenza A subtype H1, influenza A subtype H3, influenza A subtype 2009 H1, influenza B, parainfluenza

Technical Note
BioFire Diagnostics, LLC
www.biofiredx.com
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virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, rhinovirus, respiratory syncytial virus, *Bordetella parapertussis*, *B. pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*.

The complete intended use statement and additional information about the use of the BioFire System can be found in the *BioFire® FilmArray® Respiratory Panel 2 (RP2) Instructions for Use*.

Performance Verification: Overview

Each procedure described below will generate multiple positive and negative results for each of the BioFire RP2 assays. The procedures were developed using a Respiratory Verification Panel available from ZeptoMetrix Corporation, Buffalo, NY (part number NATRVP2-BIO or NATRVP2.1-BIO).

Two different examples of performance verification procedures are described: (1) a Simple Protocol for the verification of BioFire RP2 performance and (2) a Transport Media Protocol that evaluates BioFire RP2 performance when organisms are 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 Panel performance on BioFire 2.0 and BioFire Torch Systems.

A BioFire® FilmArray® 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 verification protocol on each individual instrument, it is advised that test replicates are evenly distributed among the instruments.

Performance verification protocols should be designed to take advantage of the multiplex nature of the BioFire RP2. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run.

In addition to, or in place of, the verification protocol examples described here, a laboratory may choose to test clinical/patient samples to assess clinical sensitivity and sample matrix effects in its performance verification of the BioFire RP2.

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 needed to perform verification procedures:

Table 2. Recommended materials for the verification protocols for RP2

Material	Part Number
BioFire® FilmArray® Respiratory Panel 2 (RP2) Kit (30 tests)	BioFire Diagnostics, LLC (RFIT-ASY-0129)
BioFire® FilmArray® Respiratory Panel 2 (RP2) Instruction for Use	BioFire Diagnostics, LLC (RFIT-PRT-0522)
BioFire® FilmArray® Respiratory Panel 2 (RP2) Quick Guide	BioFire Diagnostics, LLC (RFIT-PRT-0541)
Control organism	ZeptoMetrix NATRVP2-BIO or NATRPV2.1-BIO
Transport medium (e.g. Remel M4 Viral Transport Media)	Various media are appropriate
5mL sample tubes	VWR Part # 89497-740 (or similar)
Transfer pipettes	VWR Part # 13-711-43 (or similar)

[a] Any appropriate source of organism may be used for verification of any or all of the assays in the BioFire RP2. However, when alternate organism sources are used (i.e. not the ZeptoMetrix NATRVP2-BIO or NATRVP2.1-BIO 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 utilizes samples prepared by pooling together either 5 or 6 different organisms (ZeptoMetrix NATRVP2-BIO or NATRVP2.1-BIO). 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 Respiratory Verification Panel organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

The Simple Protocol can be followed to test a total of 16 pooled samples, providing at least 4 positive results and as many as 12 negative results per assay. The number of samples tested per day should be determined by the individual laboratory. Some organisms, such as adenovirus, are represented multiple times. This is done to ensure all adenovirus assays are represented in the verification protocol.

The testing scheme can be modified to run more samples per day based on the number of instruments configured on the BioFire System.

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 Type 3	0.3 mL	1.5 mL
Coronavirus OC43	0.3 mL	
Influenza A 2009 H1N1	0.3 mL	
Influenza B	0.3 mL	
Parainfluenza virus Type 4	0.3 mL	
Pool 2		
Coronavirus 229E	0.3 mL	1.5 mL
Influenza A 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 A H1	0.3 mL	
Parainfluenza virus Type 3	0.3 mL	
Respiratory Syncytial Virus A	0.3 mL	
Bordetella parapertussis	0.3 mL	
Pool 4		
Adenovirus Type 31	0.3 mL	1.8 mL
Coronavirus HKU1	0.3 mL	
Human 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 2.0 or BioFire Torch System configured with 1 module. A proposed organism pooling scheme is presented above in Table 3.

Day 1

1. Organize materials needed (Table 2).

2. Prepare two sample pools (i.e. Pools #1 and # 2, in Table 3 above) from ZeptoMetrix NATRVP2-BIO or NATRVP2.1-BIO control material. Organism vials should be well mixed prior to preparing each pool. Refer to Table 3 for example organism pooling schemes and specific volumes for each pool.

   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 4 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of instruments connected within a BioFire System.

   a. Transfer 0.3 mL 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 (approximately 1.5 mL total volume for five organisms or 1.8 mL for six organisms).
   c. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. Repeat Step 2 for the remaining sample pools (i.e. Pool # 2) to be tested that day.

4. Test two replicates from a single sample pool (i.e. Figure 1: Pool # 1 replicates A and B). The duplicate samples should be tested in a single day by different users.

   Note: For each sample, follow instructions in the *BioFire® FilmArray® Respiratory Panel 2 (RP2) Instructions for Use* and *BioFire® FilmArray® Respiratory Panel 2 (RP2) Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for the remaining sample pools (i.e. Pool #2) to be tested that day.

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 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 samples according to Steps 4 and 5 above.

Day 4

To evaluate day-to-day variation, test the samples prepared on Day 3 by repeating Steps 4 and 5 above.

Note: The remaining material from ZeptoMetrix NATRVP2-BIO and NATRVP2.1-BIO may be stored according to manufacturer's instructions for use at a later date. NATRVP2.1-BIO contains an additional organism that is not used for RP2 verification; this material may be disregarded for RP2 verification purposes.

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

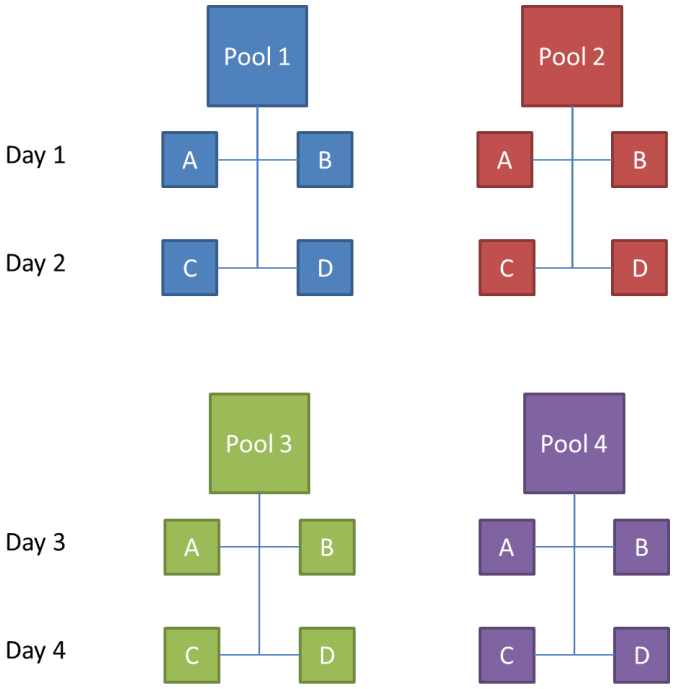

Figure 2. Example of a Verification workflow for use with multiple BioFire Modules

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 BioFire RP2 performance in a Transport media sample matrix. Sample material is pooled and added to an equal volume of Transport Media matrix.

The Transport Media Protocol can be followed to test a total of 16 pooled samples, providing at least 4 positive results and as many as 12 negative results per assay. The number of samples tested per day should be determined by the individual laboratory. The testing scheme can be modified to run more samples per day based on the number of instruments or modules configured on the BioFire System.

Transport Media 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. To evaluate user-to-user variation, multiple laboratory technicians may perform testing.

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

Organism	Approximate Organism Volume	Volume Transport Media	Approximate Pool Volume
Pool 1			
Adenovirus Type 3	0.3 mL	1.5 mL	3.0 mL
Coronavirus OC43	0.3 mL		
Influenza A 2009 H1N1	0.3 mL		
Influenza B	0.3 mL		
Parainfluenza virus Type 4	0.3 mL		
Pool 2			
Coronavirus 229E	0.3 mL	1.5 mL	3.0 mL
Influenza A 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 A H1	0.3 mL		
Parainfluenza virus Type 3	0.3 mL		
Respiratory Syncytial Virus A	0.3 mL		
Bordetella parapertussis	0.3 mL		
Pool 4			
Adenovirus Type 31	0.3 mL	1.8 mL	3.6 mL
Coronavirus HKU1	0.3 mL		
Human 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 to completion for this Transport Media Protocol verification example is 4 days for a BioFire 2.0 or BioFire Torch System configured with 1 module. Refer to Figure 1 for the suggested workflow.

Day 1

1. Organize materials needed (Table 2).

2. Prepare two sample pools (i.e. Pools #1 and #2, in Table 4 above) from ZeptoMetrix NATRVP2-BIO or NATRVP2.1 control material. Organism vials should be well mixed prior to preparing each pool. Refer to Table 3 for example organism pooling schemes and specific volumes for each pool.

   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 4 pouches per day. The number of samples prepared may be increased or decreased based on the laboratory's work schedule and number of instruments connected within a BioFire System.

   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 pools (i.e. Pool # 2) to be tested that day.

4. Test two replicates from a single sample pool (i.e. Figure 1: Pool # 1 replicates A and B). The duplicate samples should be tested in a single day by different users.

   Note: For each sample, follow instructions in the *BioFire® FilmArray® Respiratory Panel 2 (RP2) Instructions for Use* and *BioFire® FilmArray® Respiratory Panel 2 (RP2) Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for the remaining sample pools (i.e. pool 2) to be tested that day.

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 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 samples according to Steps 4 and 5 above.

Day 4

To evaluate day-to-day variation, test the samples prepared on Day 3 by repeating Steps 4 and 5 above.

Note: The remaining material from ZeptoMetrix NATRVP2-BIO and NATRVP2.1-BIO may be stored according to manufacturer's instructions for use at a later date. NATRVP2.1-BIO contains an additional organism that is not used for RP2 verification; this material may be disregarded for RP2 verification purposes.

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.

Verification of Loaner, Repaired, and Permanent Replacement Instruments

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement instrument, the following protocol may serve as a guideline.

1. Select a few specimens and/or proficiency samples (any combination of positives and negatives) previously tested on the BioFire RP2. The Laboratory Director should determine the appropriate number of samples to test.

2. Select a set of controls that verify detection of all targets on the BioFire RP2.

3. Test the selected specimens/samples on the loaner, repaired, or replacement instrument 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® FilmArray® Respiratory Panel 2 (RP2) Verification Record

BioFire® Respiratory Panel 2 (RP2) Verification
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																							
	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 (IS*1001)*																							
Pool 4	Adenovirus	Type 31																						
	Coronavirus HKU1																							
	Human Metapneumovirus																							
	Bordetella pertussis (ptxP)																							
	Chlamydia pneumoniae																							
	Mycoplasma pneumoniae																							

Reviewed by:

Signature **Date**

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