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

TECHNICAL ::: NOTE

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® FilmArray® Respiratory Panel 2 (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 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 *FilmArray Respiratory Panel 2 Instruction Booklet*.

FLM1-PRT-0232-03

Technical Note
BioFire Diagnostics, LLC
www.BioFireDX.com

QS-339B-02

Performance Verification: Overview

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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).

Two different examples of performance verification procedures are described: (1) a Simple Protocol for the verification of BioFire RP2 performance and (2) a Viral Transport Media (VTM) Protocol that evaluates BioFire RP2 performance when organisms are in a VTM 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	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: Viral Transport Medium (VTM) Protocol	5 or 6	4	4	16	4 per organism	12 per organism	4

[a] Depending on the material used for verification, pooling of organisms may not be appropriate and the values in the table may need to be modified.
[b] The approximate number of days for testing assumes a system configured with one instrument/module.

Performance Verification: Materials

The following materials may be needed to perform verification procedures:

Table 2. Materials needed for recommended verification protocols

Material	Part Number
BioFire® FilmArray® Respiratory Panel 2 (RP2) Kit (30 tests)	BioFire Diagnostics, LLC (RFIT-ASY-0129)
FilmArray® Respiratory Panel 2 (RP2) Instruction for Use	BioFire Diagnostics, LLC (RFIT-PRT-0522)
FilmArray® Respiratory Panel 2 (RP2) Quick Guide	BioFire Diagnostics, LLC (RFIT-PRT-0541)
Control organism	ZeptoMetrix NATRVP2-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 TBD 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). 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 4 positive results and 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 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

Organism	Approximate Organism Volume	Approximate Final Volume of Pool
Pool 1		
Adenovirus type 3	0.3 mL	1.5 mL
Influenza A 2009 H1N1	0.3 mL	
Influenza B	0.3 mL	
Parainfluenza virus 4	0.3 mL	
Coronavirus OC43	0.3 mL	
Pool 2		
Rhinovirus 1 A	0.3 mL	1.5 mL
Influenza A subtype H3	0.3 mL	
Coronavirus 229E	0.3 mL	
Parainfluenza virus 1	0.3 mL	
Parainfluenza virus 2	0.3 mL	
Pool 3		
Adenovirus type 1	0.3 mL	1.8 mL
Influenza A H1N1	0.3 mL	
Parainfluenza virus 3	0.3 mL	
Respiratory syncytial virus A	0.3 mL	
Coronavirus NL63	0.3 mL	
Bordatella parapertussis	0.3 mL	
Pool 4		
Adenovirus type 31	0.3 mL	1.8 mL
Bordetella pertussis	0.3 mL	
Chlamydia pneumoniae	0.3 mL	
Mycoplasma pneumoniae	0.3 mL	
Coronavirus HKU1	0.3 mL	
Human metapneumovirus 8	0.3 mL	

An example Simple Protocol workflow is provided below.

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. Prepare two sample pools (i.e. pools 1 and 2, in Table 3 above) from ZeptoMetrix NATRVP2-BIO control material.

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. Use a transfer pipette to remove 0.3 mL material from the ZeptoMetrix organism vial (draw material to the third line of the transfer pipette) and transfer to a new vial or tube.
b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms into a single vial or tube (approximately 1.5 mL total volume for five organisms or 1.8 mL for six organisms).
c. Cap and vortex prior to testing.

2. Test two samples from a single sample pool. The duplicate samples should be tested in a single day by different users.

Note: For each sample, follow instructions in the *FilmArray® Respiratory Panel 2 Instruction Booklet* and *FilmArray® Respiratory Panel 2 Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

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

4. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

Day 2

To evaluate day-to-day variation, test the remaining volume of the sample pools prepared on Day 1 by repeating Step 2 and 3 above.

Day 3

Prepare 2 new sample pools (i.e. pools 3 and 4) as described in Step 1. Test samples according to Step 2 and 3 above.

Day 4

To evaluate day-to-day variation, test the samples prepared on Day 3 by repeating Step 2 and 3 above.

Note: The remaining material from ZeptoMetrix NATRVP2-BIO may be stored according to manufacturer's instructions for use at a later date.

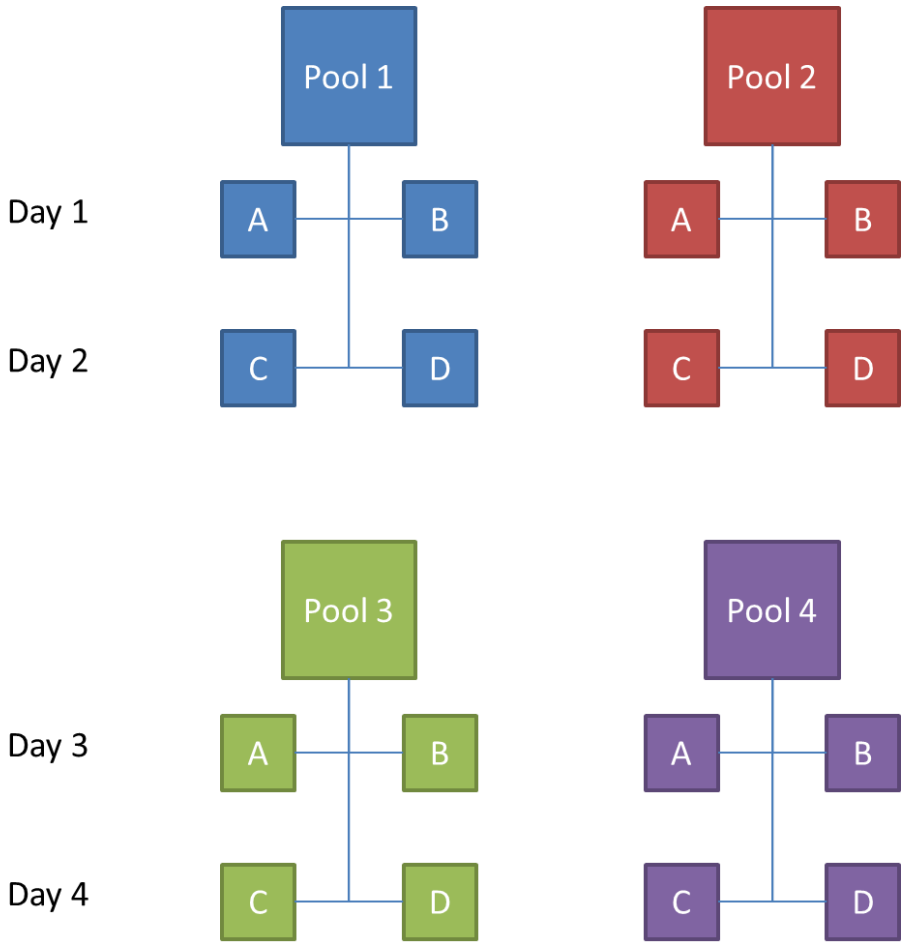

Figure 1. Workflow for Simple Protocol

VTM Protocol

The VTM Protocol evaluates BioFire RP2 performance in a VTM sample matrix. Sample material is pooled and added to an equal volume of Viral Transport Medium (VTM) matrix.

The VTM Protocol can be followed to test a total of 16 pooled samples, providing 4 positive results and 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.

VTM 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 VTM Sample Preparation Scheme

Organism	Approximate Organism Volume	Volume VTM	Approximate Final Volume of Pool
Pool 1			
Adenovirus type 3	0.3 mL	1.5 mL	3.0 mL
Influenza A 2009 H1N1	0.3 mL		
Influenza B	0.3 mL		
Parainfluenza virus 4	0.3 mL		
Coronavirus OC43	0.3 mL		
Pool 2			
Rhinovirus 1 A	0.3 mL	1.5 mL	3.0 mL
Influenza A subtype H3	0.3 mL		
Coronavirus 229E	0.3 mL		
Parainfluenza virus 1	0.3 mL		
Parainfluenza virus 2	0.3 mL		
Pool 3			
Adenovirus type 1	0.3 mL	1.8 mL	3.6 mL
Influenza A H1N1	0.3 mL		
Parainfluenza virus 3	0.3 mL		
Respiratory syncytial virus A	0.3 mL		
Coronavirus NL63	0.3 mL		
Bordetella parapertussis	0.3 mL		
Pool 4			
Adenovirus type 31	0.3 mL	1.8 mL	3.6 mL
Bordetella pertussis	0.3 mL		
Chlamydia pneumoniae	0.3 mL		
Mycoplasma pneumoniae	0.3 mL		
Coronavirus HKU1	0.3 mL		
Human metapneumovirus 8	0.3 mL		

An example VTM Protocol workflow is provided below.

VTM Protocol Example

The estimated total time to completion for this VTM Protocol verification example is 4 days for a BioFire 2.0 or BioFire Torch System configured with 1 module. Refer to Figure 2 for the suggested workflow.

Day 1

1. Prepare two sample pools (i.e. pools 1 and 2, in Table 4 above) from ZeptoMetrix NATRVP2-BIO control material and VTM.

Note: It is important to prepare only the number of samples 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 modules configured on the BioFire System.

a. Pipet 1.5 mL or 1.8 mL of VTM (as described in Table 4) into a sterile tube or vial.
b. Use a transfer pipette to remove 0.3 mL material from the ZeptoMetrix organism vial (draw material to the third line of the transfer pipette) and transfer to the tube containing VTM.
c. Repeat with the second (and subsequent) organisms to combine the appropriate organisms into a single vial or tube (approximately 3.0 mL total volume for five organisms or 3.6 mL for six organisms).
d. Cap and vortex prior to testing.

2. Test two samples (pouches) from a single VTM sample pool. The duplicate samples should be tested in a single day by different users.

Note: For each sample, follow instructions in the *FilmArray® Respiratory Panel 2 Instruction Booklet* and *FilmArray® Respiratory Panel 2 Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

3. Repeat Step 2 for the second sample pool (Pool 2 from Table 3) to be tested that day.

4. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

Day 2

To examine day-to-day variation, test the samples prepared on Day 1 by repeating Step 2 and 3 above.

Day 3

Prepare 2 new sample pools (i.e. pools 3 and 4) as described in Step 1. Test samples according to Step 2 and 3 above.

Day 4

To evaluate day-to-day variation, test the samples prepared on Day 3 by repeating Step 2 and 3 above.

Note: The remaining material from ZeptoMetrix NATRVP2-BIO may be stored according to manufacturer's instructions for use at a later date.

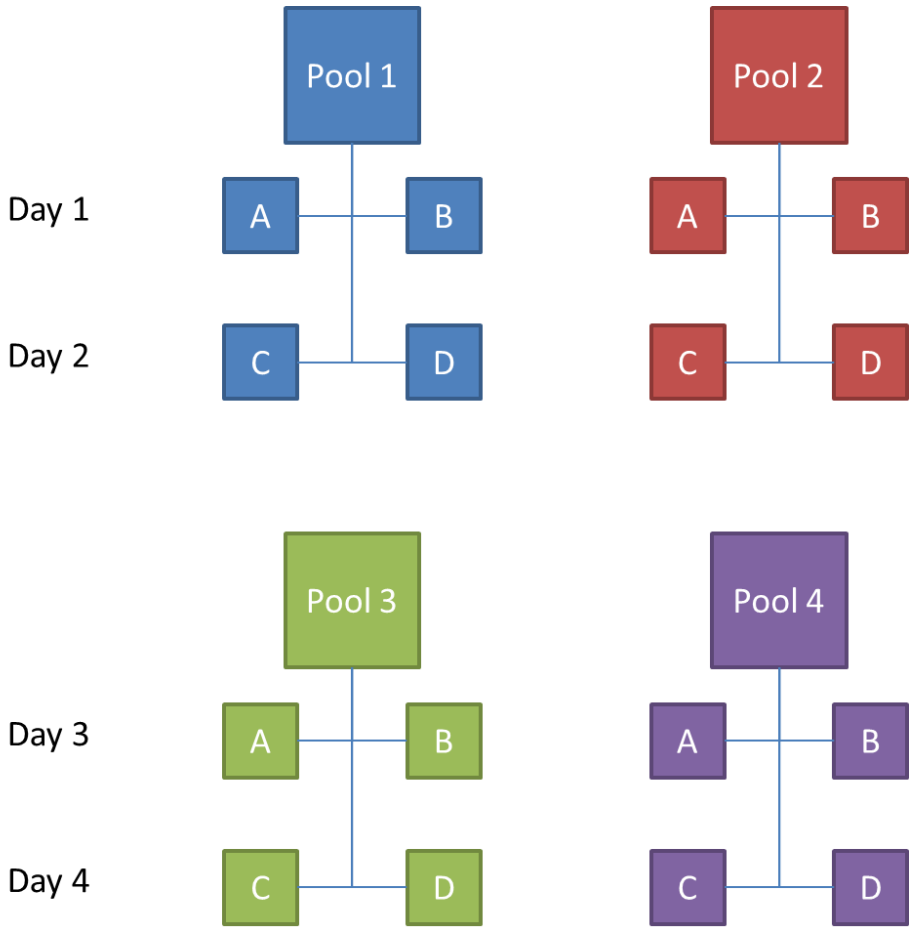

Figure 2. Workflow for VTM Protocol Example

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 RP2 Verification Record

Computer System Serial Number #: _____

BioFire RP2 Kit Part #: _____ Lot #: _____

Organism/Sample Source and Lot #: _____

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Organism Detection	Representative Strains	System Serial #	Was the Organism Detected?	No. Positive	No. Negative	No. Days Tested	No. Users	Patient Samples Tested?
Adenovirus	Type 1 C		☐ Yes ☐ No					
	Type 3 B		☐ Yes ☐ No					
	Type 31 A		☐ Yes ☐ No					
Bordetella parapertussis			☐ Yes ☐ No					
Bordetella pertussis			☐ Yes ☐ No					
Chlamydia pneumoniae			☐ Yes ☐ No					
Coronavirus 229E			☐ Yes ☐ No					
Coronavirus NL63			☐ Yes ☐ No					
Coronavirus OC43			☐ Yes ☐ No					
Coronavirus HKU1			☐ Yes ☐ No					
Human metapneumovirus			☐ Yes ☐ No					
Human rhinovirus/enterovirus			☐ Yes ☐ No					
Influenza A subtype H1			☐ Yes ☐ No					
Influenza A subtype H1-2009			☐ Yes ☐ No					

Organism Detection	Representative Strains	Instrument Serial #	Was the Organism Detected?	No. Positive	No. Negative	No. Days Tested	No. Users	Patient Samples Tested?
Influenza A subtype H3			☐ Yes ☐ No					
Influenza B			☐ Yes ☐ No					
Mycoplasma pneumoniae			☐ Yes ☐ No					
Parainfluenza virus 1			☐ Yes ☐ No					
Parainfluenza virus 2			☐ Yes ☐ No					
Parainfluenza virus 3			☐ Yes ☐ No					
Parainfluenza virus 4			☐ Yes ☐ No					
Respiratory syncytial virus			☐ Yes ☐ No					

Reviewed by: _____
Signature Date