Protocols for Laboratory Verification of Performance of the BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini

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 the SPOTFIRE R Panel Mini performance on the BIOFIRE® SPOTFIRE® System. A verification scheme compatible with the SPOTFIRE R Panel Mini has been designed using non-clinical specimens. The methods described provide positive and negative tests for each organism detected by the SPOTFIRE R Panel Mini 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 SPOTFIRE R Panel Mini 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.

SPOTFIRE R Panel Mini Intended Use

The BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini (SPOTFIRE R Panel Mini) is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE® SPOTFIRE® System for the simultaneous, qualitative detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19.

Technical Note
BioFire Diagnostics, LLC
www.biofiredx.com
BFR0001-7561-01

QS-339K-01

The following organism types are identified and differentiated using the SPOTFIRE R Panel Mini:

Table 1. SPOTFIRE Respiratory (R) Panel Mini Menu

Viruses
Coronavirus SARS-CoV-2
Human rhinovirus
Influenza A virus
Influenza B virus
Respiratory syncytial virus

The complete intended use statement and additional information about the use of the SPOTFIRE R Panel Mini can be found in the *BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Instructions for Use*.

Performance Verification: Overview

Examples of performance verification procedures are described for the SPOTFIRE R Panel Mini. The protocol can be used with transport media or synthetic background (Negative) provided with the ZeptoMetrix control organisms. The protocols may be expanded to evaluate matrix effects from multiple types of media. Refer to the *BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Instructions for Use* for a complete list of acceptable media types. These protocols are examples intended to assist your laboratory in developing a verification study for evaluating the SPOTFIRE R Panel Mini performance on the SPOTFIRE System.

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

The procedures have been designed to take advantage of the multiplex nature of the SPOTFIRE R Panel Mini. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedures described will generate multiple positive and negative detections for each of the SPOTFIRE R Panel Mini assays. The procedures were developed using a NATtrol™ Respiratory Verification Panel Mini available from ZeptoMetrix, Buffalo, NY (part number NATRMP-BIO).

A SPOTFIRE System is composed of one to four BIOFIRE® SPOTFIRE® Modules connected to a BIOFIRE® SPOTFIRE® Control Station running BIOFIRE® SPOTFIRE® Software. If the Laboratory Director chooses not to perform the entire verification protocol on each individual module of the SPOTFIRE System, it is advised that test replicates are evenly distributed among the modules. Examples of performance verification workflows using 1 to 4 modules are provided in Figures 1 and 2.

Clinical/patient samples may be used in place of, or in addition to the verification schemes described here to assess clinical sensitivity/specificity and sample matrix effects as part of the performance verification of the SPOTFIRE R Panel Mini.

Note: The laboratory should only perform the verification study with analytes that will be reported using the SPOTFIRE R Panel Mini and with media that will be used in their laboratory setting.

Table 2. Overview of Verification Protocol

Verification Protocol	Organisms per Pool	Number of Sample Pools	Replicates per Sample Pool	Pouches Required[a]	Expected Positive Results	Expected Negative Results	Approximate Days of Testing[b]
Pool 1 (Organisms)	6	2	≥4	8	4 per organism	N/A	≥2
Pool 2 (No organisms)	0		≥4		N/A	4 per organism	

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

[b] Two days is shown to meet day-to day testing requirements; the number of days can be expanded, as needed.

Performance Verification: Materials

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

Table 3. Recommended materials for the verification protocols

Material	Part Number
BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Test Kit (30 tests)	BioFire Diagnostics, LLC 424589
BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Instructions for Use	BioFire Diagnostics, LLC BFR0002-1771
BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Quick Guide	BioFire Diagnostics, LLC BFR0002-1770
BIOFIRE® SPOTFIRE® System Operator Manual	BioFire Diagnostics, LLC BFR0001-1641
Control Organism[a]	ZeptoMetrix, NATtrol™ Respiratory Verification Panel Mini: NATRMP-BIO
Transport Media[b] (e.g., BD™ 3 mL Universal Viral Transport or Remel MicroTest™ M4RT 3 mL w/o beads tube)	BD 220220; ThermoFisher R12700 (or equivalent)
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 SPOTFIRE R Panel Mini. 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.

[b] The media above was validated for use with the SPOTFIRE R Panel Mini, however, other commercial liquid media may be appropriate. See the Interference section of the BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Instructions for Use for more details.

Performance Verification Protocol

The verification protocol evaluates the SPOTFIRE R Panel Mini performance when sample material (ZeptoMetrix NATRMP-BIO) is pooled and combined with an equal volume of transport media or synthetic matrix/negative (provided in the control panel) and tested with the SPOTFIRE R Panel Mini. 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.

Verification sample tests should be run by following on-screen instructions for patient sample testing. Refer to the *BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Quick Guide* for detailed instructions.

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

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

Figures 1 and 2 (below) illustrate workflow schemes for testing 4 replicates per pool for the 2 pools over multiple days. This produces a total of 8 verification sample test runs and provides 4 positive results and 4 negative results per assay. The number of samples tested per day should be determined by the individual laboratory. This testing scheme can be modified to run fewer samples per day based on the number of modules in the SPOTFIRE System. The pooling scheme provides sufficient volume for testing more replicates if desired.

Pooled samples may 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 operators may perform testing.

Table 4. Proposed Organism Pooling Scheme for Verification of the SPOTFIRE R Panel Mini

Respiratory Verification Panel Mini Organisms	Approximate Organism Volume	Volume Transport Media or Negative	Approximate Pool Volume
Pool 1- Organisms			
Influenza A H1N1pdm (subtype H1-2009)	0.3 mL	1.8 mL	3.6 mL
Influenza A H3 (subtype H3)	0.3 mL		
Influenza B	0.3 mL		
Respiratory syncytial virus A (RSV A)	0.3 mL		
Rhinovirus 1A	0.3 mL		
SARS-CoV-2	0.3 mL		
Pool 2-Negative			
Negative	N/A	1.8 mL	1.8 mL

Verification Protocol Example

The estimated total time to complete this verification example is 2 days for a SPOTFIRE System configured with 1 to 4 modules.

Note: It is important to prepare only the number of sample pools that will be tested within 3 days of preparation. The number of samples prepared may be modified based on the laboratory's work schedule and number of modules connected within a SPOTFIRE System.

Day 1

1. Organize materials needed (Table 3); refer to Table 4 for the pooling scheme. Negativity of transport media may be confirmed by screening on the SPOTFIRE R Panel Mini prior to starting the verification procedure. Negative vials included in the control panel contain 1.8 mL of synthetic matrix; this is sufficient volume to complete the protocol described.

2. Prepare Pool 1 using the ZeptoMetrix NATRMP-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool.
    a. Transfer 0.3 mL of material from the ZeptoMetrix organism vial into a 5 mL tube.
    b. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube. The combined volume of organisms will 1.8 mL.
    c. Add 1.8 mL of transport media or synthetic matrix/negative, as described in Table 4, to the tube containing the organism pool (step b).

Note: If the laboratory verification study will include multiple types of transport media, see the section *Expanding or Modifying the Protocol* below.

3. Pool 2 contains transport media or synthetic matrix/negative, but no organisms. This pool is used to provide negative detections for the SPOTFIRE R Panel Mini targets. Replicate testing can be done directly from the tube of transport media or synthetic matrix/negative, no mixing is required.

4. Test 2 replicates from each sample pool (Figure 1: test replicates A and B from Pools 1 and 2). 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® SPOTFIRE® Respiratory (R) Panel Mini Instructions for Use* and the *BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing. Verification sample tests should be run by following on-screen instructions for patient sample pouch testing.

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

Note: The proposed organism pooling scheme, described in 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 additional replicates from the pools prepared on Day 1 by repeating Step 4 above (i.e., test replicates C and D from Pools 1 and 2).

Note: A SPOTFIRE Respiratory Panel Mini Verification Record is provided and may serve as a template for recording your results.

Figure 1. Verification protocol workflow for testing over two days.

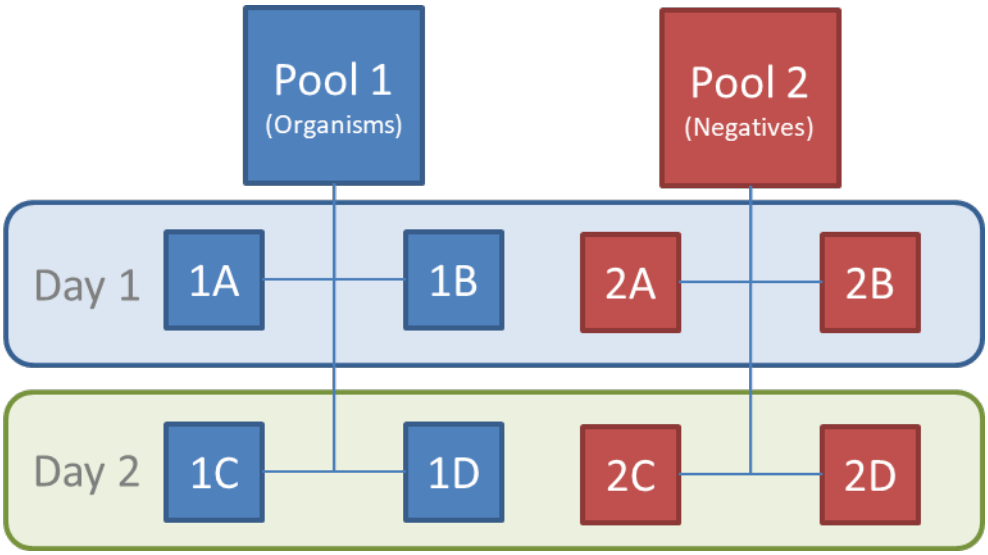

Figure 2. Examples of the Verification workflow testing over two days with different SPOTFIRE System Module configurations.

One Module Verification		
Testing Day	Module 1	
Day 1	Pool 1A/ Operator 1	Pool 2A/ Operator 1
	Pool 1B/ Operator 2	Pool 2B/ Operator 2
Day 2	Pool 1C/ Operator 1	Pool 2C/ Operator 1
	Pool 1D/ Operator 2	Pool 2D/ Operator 2

Two Module Verification				
Testing Day	Module 1		Module 2	
Day 1	Pool 1A/ Operator 1	Pool 2A/ Operator 1	Pool 1B/ Operator 2	Pool 2B/ Operator 2
Day 2	Pool 1D/ Operator 2	Pool 2D/ Operator 2	Pool 1C/ Operator 1	Pool 2C/ Operator 1

Three Module Verification						
Testing Day	Module 1		Module 2		Module 3	
Day 1	Pool 1A/ Operator 1	Pool 2B/ Operator 2	Pool 2A/ Operator 1		Pool 1B/ Operator 2	
Day 2	Pool 1D/ Operator 2		Pool 1C/ Operator 1	Pool 2D/ Operator 2		Pool 2C/ Operator 1

Four Module Verification				
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

Expanding or Modifying the Protocol

The protocol described above can be expanded by increasing the number of tests from Pool 1. Pool 1 contains sufficient volume for testing additional replicates. To test additional media types, prepare additional organism pools using the control material (NATRMP-BIO) and Steps 1-5 above.

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. Refer to the *BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Instructions for Use* for a complete list of acceptable media types. Reference CAP accreditation checklist requirements: MIC.64960 for more information.

Note: The laboratory should perform the verification study with media that will be used with the SPOTFIRE R Panel Mini 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 an appropriate number of specimens and/or proficiency samples (any combination of positives and negatives) previously tested on the SPOTFIRE Respiratory Panel Mini. 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 SPOTFIRE R Panel Mini.

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 any questions or concerns about this process, please contact the BIOFIRE Technical Support team for assistance.

BIOFIRE Technical Support
Email: biofiresupport@biomerieux.com
Phone: +1-801-736-6354, select Option 5

SPOTFIRE R Panel Mini Verification Record

BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini Verification Record

SPOTFIRE R Mini Kit Part # _____ **Module Serial #** _____

SPOTFIRE R Mini Kit Lot # _____ **Module Serial #** _____

Media Type _____ **Module Serial #** _____

Media Lot # _____ **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	# Positives	# Negatives	# Operators	# Days	# Modules	Patient Samples?
Pool 1	**Positive: Multiple Organisms**															
	Coronavirus SARS-CoV-2															
	Human rhinovirus															
	Influenza A virus	subtype H1-2009 subtype H3														
	Influenza B virus															
	Respiratory syncytial virus															
Pool 2	**Negative**															
	No organism detections															

Reviewed by: _____

Signature