Protocols for Laboratory Verification of Performance of the BIOFIRE® FILMARRAY® Tropical Fever (TF) Panel

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® FILMARRAY® Tropical Fever (TF) Panel has been categorized by the FDA as a CLIA moderate complexity test.

This document provides examples of verification procedures to assist your laboratory in developing a protocol for the verification of the BIOFIRE FILMARRAY TF Panel performance on BIOFIRE® FILMARRAY® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems. A verification scheme compatible with the BIOFIRE FILMARRAY TF Panel has been designed using non-clinical specimens. The methods described provide positive and negative detections for each organism detected by the BIOFIRE FILMARRAY TF Panel 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 operators may test the same sample. In addition, testing patient samples for verification or to evaluate matrix effects on the performance of the BIOFIRE FILMARRAY TF Panel 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® FILMARRAY® Tropical Fever (TF) Panel is an automated qualitative, multiplexed polymerase chain reaction (PCR) test intended for use with BIOFIRE® FILMARRAY® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems. The BIOFIRE FILMARRAY TF Panel detects and identifies selected bacterial, viral, and parasitic nucleic acids directly from EDTA whole blood collected from individuals with signs and/or symptoms of acute febrile illness or recent acute febrile illness and known or suspected exposure to the following target pathogens: chikungunya virus, dengue virus (serotypes 1, 2, 3 and 4), *Leptospira* spp., and *Plasmodium* spp. (including species differentiation of *Plasmodium falciparum* and *Plasmodium vivax/ovale*).

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The complete intended use statement and additional information about the use of the BIOFIRE FILMARRAY TF Panel can be found in the *BIOFIRE® FILMARRAY® Tropical Fever (TF) Panel Instructions for Use.*

Performance Verification: Overview

Examples of performance verification procedures are described for the BIOFIRE FILMARRAY TF Panel. The protocol can be used with whole blood/EDTA (clinical matrix) or with the synthetic matrix/negative provided with the ZeptoMetrix control organisms. The protocols are examples intended to assist your laboratory in developing a verification study for evaluating the performance of each assay on the BIOFIRE FILMARRAY TF Panel.

Note: It is important to characterize clinical matrix specimens for BIOFIRE FILMARRAY TF Panel targets by screening the whole blood/EDTA on the BIOFIRE FILMARRAY TF Panel prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes detected by the BIOFIRE FILMARRAY TF Panel.

The procedures have been designed to take advantage of the multiplex nature of the BIOFIRE FILMARRAY TF Panel. 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 BIOFIRE FILMARRAY TF Panel assays. The procedures were developed using a NATtrol™ Tropical Fever Verification Panel available from ZeptoMetrix, Buffalo, NY (part number NATTFP-BIO).

A BIOFIRE® 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, it is advised that test replicates are evenly distributed among the modules. An example of a performance verification workflow using 2, 3, 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 FILMARRAY TF Panel.

Note: The laboratory should only perform the verification study with analytes that will be reported using the BIOFIRE FILMARRAY TF Panel 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	Expected Positive Results[a]	Expected Negative Results	Approximate Days of Testing[b]
Synthetic Matrix Protocol	4 or 5	2	≥4	8	4 per organism	4 per organism	2
Clinical Matrix Protocol	4 or 5	2	≥4	8	4 per organism	4 per organism	2

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

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® FILMARRAY® Tropical Fever (TF) Panel Kit (6 tests)	BIOFIRE Diagnostics, LLC 424803
BIOFIRE® FILMARRAY® Tropical Fever (TF) Panel Instructions for Use	BIOFIRE Diagnostics, LLC BFR0002-6754
BIOFIRE® FILMARRAY® Tropical Fever (TF) Panel Quick Guide	BIOFIRE Diagnostics, LLC BFR0002-6755
Control organism[a]	ZeptoMetrix, NATTFP-BIO
Whole Blood EDTA[b]	BioIVT, HUMAN Whole Blood K2EDTA (or equivalent, with EDTA)
5mL sample tubes	Various manufacturers
Transfer pipettes	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 FILMARRAY TF Panel. However, when alternate organism sources are used (i.e. not the ZeptoMetrix material), the sample volumes or pooling schemes suggested in the examples below may need to be adjusted.
[b]To be used when evaluating clinical matrix. Whole blood collected in EDTA tubes may be available from various clinical or commercial sources. The optimal blood specimen will be negative for all analytes tested on the BIOFIRE FILMARRAY TF Panel.

Performance Verification Protocol

The verification protocol evaluates the BIOFIRE FILMARRAY TF Panel performance when sample material (ZeptoMetrix NATTFP-BIO) is pooled and combined with an equal volume of whole blood/EDTA or synthetic matrix/negative (provided in the control panel) and tested with the BIOFIRE FILMARRAY TF Panel. 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.

Note: It is important to characterize human Whole Blood/EDTA clinical matrix specimens for BIOFIRE FILMARRAY TF Panel targets by screening on the BIOFIRE FILMARRAY TF Panel prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes tested on the BIOFIRE FILMARRAY TF Panel.

Figures 1 and 2 (below) illustrate workflow schemes for testing 4 replicates per pool for 2 different 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 BIOFIRE® FILMARRAY® 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 operator-to-operator variation, multiple laboratory technicians may perform testing.

Table 3. Proposed Organism Pooling Scheme

NATTFP-BIO Control Organism	Approximate Organism Volume	Approximate Volume Whole Blood/EDTA or Negative	Approximate Final Volume of Pool
Pool 1			
E. coli with *P. falciparum/vivax* plasmid - recombinant	0.3 mL	1.2 mL	2.4 mL
E. coli with *P. spp/ovale* plasmid - recombinant	0.3 mL		
E. coli with *Leptospira* plasmid - recombinant	0.3 mL		
Chikungunya Virus (R80422)	0.3 mL		
Pool 2			
Dengue Virus Type 1 (Hawaii)	0.3 mL	1.5 mL	3.0 mL
Dengue Virus Type 2 (New Guinea C)	0.3 mL		
E. coli with Dengue Virus Type 2 (Dak Ar A1247) plasmid - recombinant	0.3 mL		
Dengue Virus Type 3 (H87)	0.3 mL		
Dengue Virus Type 4 (H241)	0.3 mL		

Verification Protocol Example

The estimated total time to complete this verification example is 2 days.

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 BIOFIRE FILMARRAY System.

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Day 1

1. Organize materials needed (Table 2); refer to Table 3 for the pooling scheme. Human whole blood/EDTA clinical matrix should be screened on the BIOFIRE FILMARRAY TF Panel to characterize the sample prior to preparing pools. Negative control vials included in the control panel contain 1.0 mL of synthetic matrix. The control panel contains sufficient volume to complete the protocol described, but more than one vial of negative may be needed for preparing the pools.

2. Prepare one sample pool (i.e., Pool 1) using the ZeptoMetrix NATTFP-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 for each pool will be 1.2 mL for Pool 1 and 1.5 mL for Pool 2.

    c. Add human whole blood/EDTA or synthetic matrix/negative (as described in Table 3) to the tube containing the organism pool (step 2b). The volume of blood/ negative should be the same as the organism pool volume. For example: for Pool 1, 1.2 mL of blood/ negative should be added to 1.2 mL of pooled organism. The final volume of Pool 1 will be 2.4 mL.

3. Repeat Step 2 to prepare Pool 2.

4. Test 2 replicates from a single sample pool (Figure 1: Pool 1 replicates 1A and 1B). 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 FILMARRAY Tropical Fever (TF) Panel Instructions for Use* and the *BIOFIRE FILMARRAY Tropical Fever (TF) Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

5. Repeat Step 4 for the remaining sample replicates to be tested that day (i.e., Pool 2 replicates 2A and 2B).

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, described in 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 additional replicates from the pools prepared on Day 1 by repeating Steps 5 and 6 above (Figure 1: Pool 1 and 2 replicates C and D).

Note: A Verification Record for the BIOFORE FILMARRAY Tropical Fever (TF) Panel protocol is provided and may serve as a template for recording your results.

Figure 1. Verification Workflow for the BIOFIRE® FILMARRAY® Tropical Fever Panel

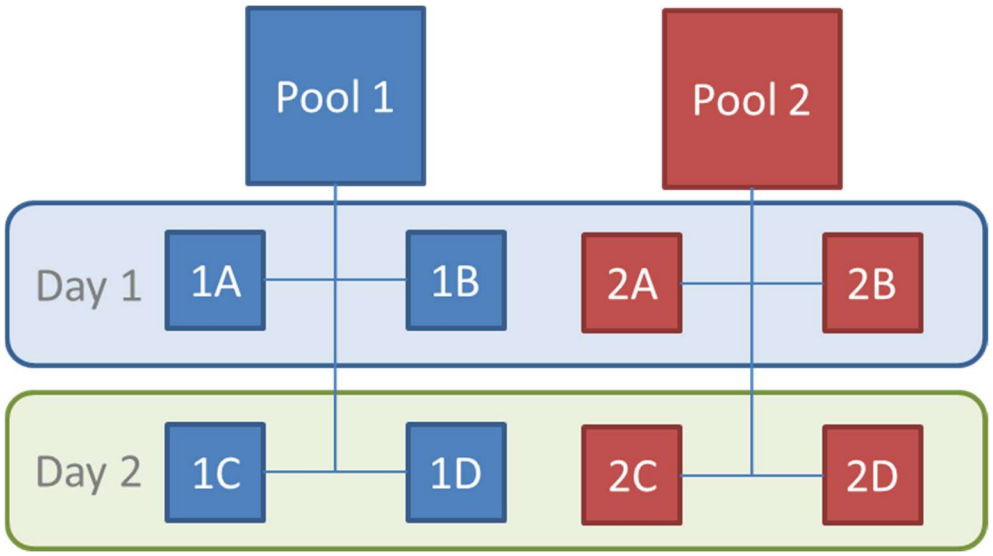

Figure 2. Example of Verification Workflows for use with Multiple BIOFIRE modules.

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**

Verification with 3 modules						
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

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

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 1D/ Operator 2	Pool 1C/ Operator 1	Pool 2C/ Operator 1	Pool 2D/ Operator 2

Expanding or Modifying the Protocol

The protocol described above can be expanded by increasing the number of tests from each of the organism pools. Pools 1 and 2 contain sufficient volume for testing additional replicates. The verification study may use human whole blood/EDTA as a clinical matrix in the pools, as needed. Reference CAP accreditation checklist requirements: MIC.64960 Validation or Verification Studies - Specimen Selection.

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 FILMARRAY Tropical Fever Panel. 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 FILMARRAY Tropical Fever Panel.

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.

BIOFIRE® FILMARRAY® Tropical Fever (TF) Panel Verification Record

BioFire® FilmArray® Tropical Fever (TF) Panel Verification Record

Kit Part # _____ **Module Serial #** _____

Lot # _____ **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	# Positives	# Negatives	# Operators	# Days	# Modules	# Patient Samples
Pool 1	Chikungunya Virus															
	Plasmodium spp.	*E. coli* with P. spp/ovale plasmid														
	Plasmodium falciparum	*E. coli* with P. falciparum/vivax plasmid														
	Plasmodium vivax/ovale	*E. coli* with P. spp/ovale plasmid														
		E. coli with P. falciparum/vivax plasmid														
	Leptospira spp.	*E. coli* with *Leptospira* plasmid														
Pool 2	Dengue virus	Type 1														
		Type 2														
		E. coli with Dengue Virus Type 2														
		Type 3														
		Type 4														

Reviewed by: _____

Signature

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