Protocols for Laboratory Verification of Performance of the BIOFIRE® FILMARRAY® Gastrointestinal (GI) Panel Mid

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® Gastrointestinal (GI) Panel Mid 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 GI Panel Mid performance on BIOFIRE® FILMARRAY® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems. A verification scheme compatible with the BIOFIRE FILMARRAY GI Panel Mid has been designed using non-clinical specimens. The methods describe provide positive and negative detections for each organism detected by the BIOFIRE FILMARRAY GI Panel Mid 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 GI Panel Mid 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® Gastrointestinal (GI) Panel Mid is an automated qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with BIOFIRE® FILMARRAY® Systems. The BIOFIRE FILMARRAY GI Panel Mid is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from stool samples in Cary Blair transport media obtained from individuals with signs and/or symptoms of gastrointestinal infection. The following bacteria, parasites, and viruses are identified using the BIOFIRE FILMARRAY GI Panel Mid:

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Table 1. Bacteria, Parasites, and Viruses Identified using the BIOFIRE FILMARRAY GI Panel Mid

Bacteria	Viruses
Campylobacter (*C. jejuni/C. coli/ C. upsaliensis*)	Norovirus GI/GII
Clostridioides (Clostridium) difficile (toxin A/B)	
Salmonella	**Parasites**
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	*Cryptosporidium*
*Shigella/*Enteroinvasive *E. coli* (EIEC)	*Cyclospora cayetanensis*
Vibrio (V. parahaemolyticus/V. vulnificus/ V. cholerae)	*Giardia lamblia*
Yersinia enterocolitica	

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

Performance Verification Overview

Examples of performance verification procedures are described for the BIOFIRE FILMARRAY GI Panel Mid. The protocol can be used with stool in Cary Blair transport media (clinical matrix) or with 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 GI Panel Mid.

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

The procedures have been designed to take advantage of the multiplex nature of the BIOFIRE FILMARRAY GI Panel Mid. 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 GI Panel Mid assays. The procedures were developed using a NATtrol™ Gastrointestinal Verification Panel Mid available from ZeptoMetrix, Buffalo, NY (part number NATGIPM-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 GI Panel Mid.

Note: The laboratory should only perform the verification study with analytes that will be reported using the BIOFIRE FILMARRAY GI Panel Mid in their laboratory setting.

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Table 2. 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	6 or 7	2	≥4	8	4 per organism	4 per organism	2
Clinical Matrix Protocol	6 or 7	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 3. Recommended materials for the verification protocols

Material	Part Number
BIOFIRE® FILMARRAY® GI Panel Mid Pouches	BIOFIRE Diagnostics, LLC 424898 (30 tests) or 425089 (6 tests)
BIOFIRE® FILMARRAY® GI Panel Mid Instructions for Use	BIOFIRE Diagnostics, LLC BFR0002-7233
BIOFIRE® FILMARRAY® GI Panel Mid Quick Guide	BIOFIRE Diagnostics, LLC BFR0002-7234
Control organisms[a]	ZeptoMetrix NATGIPM-BIO or NATGIP-BIO[b]
Cary Blair transport media	Thermo Scientific Part # 23-005-47 (or equivalent)
Stool sample[c]	Various sources
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 GI Panel Mid. 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]The NATtrol™ Gastrointestinal Verification panel Mid (NATGIPM-BIO) shares some panel members with NATtrol™ GI Panel (NATGIP-BIO). These shared panel members are equivalent and can be used as a replacement for NATGIPM-BIO, if needed.

[c]To be used when evaluating clinical matrix. Stool in Cary Blair may be available from various clinical or commercial sources. The optimal specimen will be negative for all analytes tested on the BIOFIRE FILMARRAY GI Panel Mid.

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Performance Verification Protocol

The verification protocol evaluates the BIOFIRE FILMARRAY GI Panel Mid performance when sample material (ZeptoMetrix NATGIPM-BIO) is pooled and combined with stool in Cary Blair or synthetic matrix/negative (provided in the control panel) and tested with the BIOFIRE FILMARRAY GI Panel Mid. 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 control organisms beyond levels proposed in these guidelines may lead to inconsistent results and is not recommended.

Note: It is important to characterize stool in Cary Blair clinical specimens for BIOFIRE FILMARRAY GI Panel Mid targets by screening on the BIOFIRE FILMARRAY GI Panel Mid prior to starting the verification procedure. The optimal clinical matrix specimen will be negative for all analytes detected by the BIOFIRE FILMARRAY GI Panel Mid.

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.

To evaluate day-to-day variation: pooled organisms in synthetic matrix/negative or in Cary Blair media (no stool) may be stored overnight or up to 14 days at refrigeration temperature (2–8°C); pooled organisms in stool in Cary Blair may be stored overnight or up to 3 days at refrigeration temperature (2–8°C). To evaluate operator-to-operator variation, multiple laboratory technicians/ staff may perform testing.

Table 4. Proposed Organism Pooling Scheme

Control Organism NATGIPM-BIO or NATGIP-BIO[1]	Approximate Organism Volume	Media: Negative Or Stool in Cary-Blair	Approximate Final Volume of Pool
Pool 1			
Cryptosporidium parvum	0.2 mL	0.85 mL	2.25 mL
Cyclospora cayetanensis (recombinant)	0.2 mL		
Norovirus GI (recombinant)	0.2 mL		
Norovirus GII (recombinant)	0.2 mL		
Salmonella enterica Typhimurium	0.2 mL		
Shigella sonnei	0.2 mL		
Vibrio cholerae	0.2 mL		

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Pool 2			
Campylobacter coli	0.2 mL	0.85mL	2.35mL
Campylobacter jejuni	0.2 mL		
Clostridium difficile	0.2 mL		
Escherichia coli (EDL933; O157)	0.2 mL		
Giardia lamblia	0.2 mL		
Yersinia enterocolitica	0.2 mL		

[1]The NATtrol™ Gastrointestinal Verification panel Mid (NATGIPM-BIO) shares some panel members with NATtrol™ GI Panel (NATGIP-BIO). These shared panel members are equivalent and can be used as a replacement for NATGIPM-BIO, if needed.

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 for organisms pooled with stool in Cary Blair or 14 days for organisms pooled with synthetic matrix/negative or Cary Blair media (no stool). 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.

Day 1

1. Organize materials needed (Table 3); refer to Table 4 for the pooling scheme.

2. If using clinical specimens in the Verification study, prepare a fresh stool sample in Cary Blair transport media. Stool in Cary Blair specimens should be screened on the BIOFIRE FILMARRAY GI Panel Mid in order to characterize the sample prior to preparing pools. Synthetic matrix/negative is included in the NATGIPM-BIO control panel package.

3. Prepare one sample pool (i.e., Pool 1) using the ZeptoMetrix NATGIPM-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool.

    a. Transfer approximately 0.85mL of the characterized stool in Cary Blair specimen or synthetic matrix/ negative into a 5 mL tube. When using stool in Cary Blair, specimen consistency may make accurate measurement difficult, but care should be taken to try to add the volume indicated.

    b. Transfer 0.2 mL of material from the ZeptoMetrix organism vial into the 5 mL tube containing the stool in synthetic matrix/ Cary Blair specimen.

    c. 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 approximately 2.25 mL for Pool 1 and 2.35 mL for Pool 2.

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4. Repeat Step 3 to prepare Pool 2.

5. Test 2 replicates from each sample pool (see Figure 1: Pool 1 replicates 1A and 1B and Pool 2 replicates 2A and 2B). 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 GI Panel Mid Instructions for Use* and the *BIOFIRE FILMARRAY GI Panel Mid Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

6. Refrigerate samples (2–8°C) for up to 3 days for organisms pooled with stool in Cary Blair or 14 days for organisms pooled with synthetic matrix/negative or Cary Blair media (no stool) 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 5 above (Figure 1: Pool 1 replicates 1C and 1D and Pool 2 replicates 2C and 2D).

Note: A Verification Record for the BIOFIRE FILMARRAY GI Panel Mid protocol is provided and may serve as a template for recording your results.

Figure 1. Verification Workflow for the BIOFIRE FILMARRAY GI Panel Mid

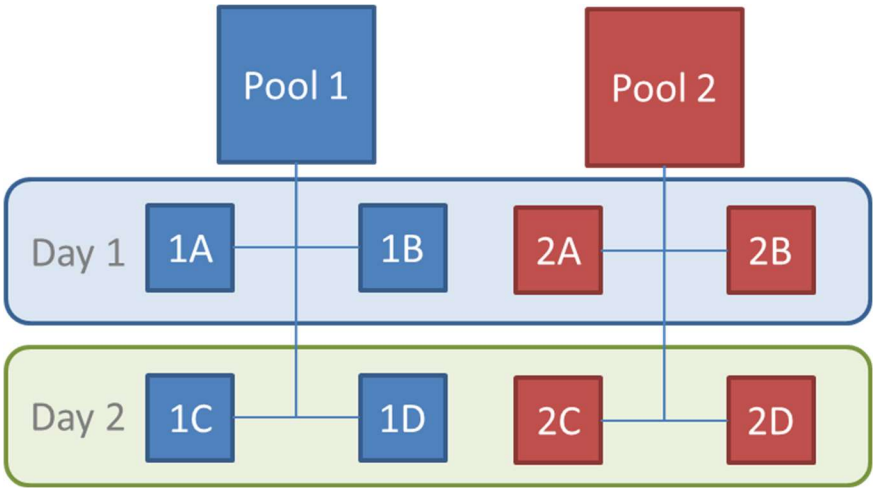

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Figure 2. Example of Verification Workflows for use with Multiple BIOFIRE modules.

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 stool in Cary Blair as a clinical matrix in the pools, as needed. Reference CAP accreditation checklist requirements: MIC.64960 Validation or Verification Studies - Specimen Selection.

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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 GI Panel Mid. 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 GI Panel Mid.

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.

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BIOFIRE® FILMARRAY® Gastrointestinal Panel Mid (GI Mid) Verification Record

BIOFIRE® FILMARRAY® Gastrointestinal Panel Mid (GI Mid) Verification Record

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

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	***Salmonella***															
	Vibrio (V. parahaemolyticus/ V. vulnificus/ V. cholerae)	*V. cholerae*														
	***Shigella/*Enteroinvasive *E. coli* (EIEC)**	*S. sonnei*														
	Cryptosporidium	*C. parvum*														
	Cyclospora cayetanensis	Recombinant														
	Norovirus GI/GII	Norovirus GI (recombinant)														
		Norovirus GII (recombinant)														
Pool 2	***Campylobacter* (*C. jejuni/C. coli/ C. upsaliensis*)**	*C. coli*														
		C. jejuni														
	***Clostridioides (Clostridium) difficile* (toxin A/B)**															
	Yersinia entercolitica															
	Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	*E. coli* (EDL933; O157)														
	Giardia lamblia															

Reviewed by:

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Signature

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