Protocols for Laboratory Verification of Performance of the BioFire® FilmArray® Gastrointestinal (GI) 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® Gastrointestinal (GI) 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 GI Panel performance on BioFire® FilmArray® Systems as required by CLIA. Several possible verification schemes, compatible with the BioFire GI Panel, have been designed. Each scheme provides positive and negative tests for each organism detected by the BioFire GI Panel using non-clinical specimens 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 GI 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.

BioFire Intended Use

The BioFire® FilmArray® Gastrointestinal (GI) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with BioFire® FilmArray® Systems. The BioFire GI Panel 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 (including several diarrheagenic *E. coli/Shigella* pathotypes), parasites, and viruses are identified using the BioFire GI Panel:

Technical Note
BioFire Diagnostics, LLC
www.biofiredx.com
FLM1-PRT-0151-06
QS-339E-01

Table 1. Bacteria, Viruses, Diarrheagenic *E.coli/ Shigella*, and Parasites Detected by the BioFire GI Panel

Bacteria	**Viruses**
Campylobacter (C. jejuni/ C. coli/ C. upsaliensis	Adenovirus F 40/41
Clostridiodes (Clostridium) difficile (toxin A/B)	Astrovirus
Plesiomonas shigelloides	Norovirus GI/GII
Salmonella	Rotavirus A
Vibrio (V. parahaemolyticus, V. vulnificus/ V. cholerae)	Sapovirus (Genogroups I. II, IV, and V)
Vibrio cholerae	
Yersinia enterocolitica	
Diarrheagenic *E.coli/ Shigella*	**Parasites**
Enteroaggregative *E. coli* (EAEC)	*Cryptosporidium*
Enteropathogenic *E. coli* (EPEC)	*Cyclospora cayetanensis*
Enterotoxigenic *E. coli* (ETEC) *lt/st*	*Entamoeba histolytica*
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	*Giardia lamblia*
E. coli O157	
Shigella/ Enteroinvasive *E. coli* (EIEC)	

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

Performance Verification: Overview

Two different examples of performance verification procedures are described: (1) a Simple Protocol for the verification the BioFire GI Panel in a synthetic background (Negative) provided with the ZeptoMetrix NATtrol™ control organisms and (2) a Clinical Matrix Protocol that evaluates the performance of each assay on the BioFire GI Panel in a clinical specimen matrix of stool in Cary Blair.

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

A BioFire® 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 entire verification protocol on each individual instrument or module, it is advised that test replicates are evenly distributed among the instruments or modules. An example of a performance verification workflow using 2, 4, or 6 modules is provided in Figure 2.

The procedures have been designed to take advantage of the multiplex nature of the BioFire® FilmArray® GI Panel. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run. The procedures described below will generate multiple positive and negative detections for each of the BioFire GI Panel assays. The procedures were developed using a NATtrol™ GI Verification Panel (NATGIP-BIO) available from ZeptoMetrix LLC, Buffalo, NY.

Clinical/patient specimens may be used in place of or in addition to the verification schemes described here in order to assess clinical sensitivity and sample matrix effects as part of the performance verification of the BioFire GI Panel.

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

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]
Simple Protocol	5 or 6	4	4	16	4 per organism	12 per organism	4
Clinical Matrix 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 BioFire® system configured with one instrument/module.

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 Kit (30 tests)	BioFire Diagnostics, LLC RFIT-ASY-0116
BioFire® FilmArray® Gastrointestinal (GI) Panel Instructions for Use	BioFire Diagnostics, LLC (RFIT-PRT-0143)
BioFire® FilmArray® Gastrointestinal (GI) Panel Quick Guide	BioFire Diagnostics, LLC RFIT-PRT-0141
Control organism[a]	ZeptoMetrix NATGIP-BIO
Cary Blair transport media	Thermo Scientific Part # 23-005-47 (or equivalent)
Stool sample[b]	Various sources
5mL sample tubes	Various manufacturers
Transfer pipettes	VWR Part # 13-711-43 (or equivalent)

[a]Any appropriate source of organism may be used for verification of any or all of the assays in the BioFire GI 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]For use with the Clinical Matrix protocol; the optimal stool specimen will be negative for all analytes tested on the BioFire GI Panel.

Performance Verification: Protocols

Simple Protocol

The Simple Protocol evaluates the BioFire GI Panel performance when sample material is pooled and combined with a synthetic matrix. Sample material (ZeptoMetrix NATGIP-BIO) is pooled and added to the synthetic matrix/negative provided in the control panel. The proposed organism pooling scheme (Table 4) should be followed to obtain the expected positive and negative results for each assay in a time and resource-efficient manner.

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

Figures 1 and 2 (below) illustrate protocol and workflow schemes for testing 4 replicates per pool for 4 pools over multiple days. This produces a total of 16 verification sample test runs and provides 4 positive results and 12 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 more samples per day based on the number of modules in the BioFire® System.

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 technicians may perform testing.

Table 4. Proposed Organism Pooling Scheme

Control Organism	Approximate Organism Volume	Negative or Stool in Cary-Blair Media	Approximate Final Volume of Pool
Pool 1			
Adenovirus Type 41	0.25 mL	0.85 mL	2.1 mL
Cryptosporidium parvum	0.25 mL		
Escherichia coli (EAEC)	0.25 mL		
Salmonella enterica typhimurium	0.25 mL		
Sapovirus	0.25 mL		
Pool 2			
Astrovirus	0.25 mL	0.85 mL	2.35 mL
Cyclospora cayetanensis	0.25 mL		
Escherichia coli (EPEC)	0.25 mL		
Norovirus GI (recombinant)	0.25 mL		
Norovirus GII (recombinant)	0.25 mL		
Shigella sonnei	0.25 mL		
Pool 3			
Campylobacter coli	0.25 mL	0.85 mL	2.35 mL
Campylobacter jejuni	0.25 mL		
Clostridium difficile	0.25 mL		
Entamoeba histolytica	0.25 mL		
Escherichia coli (ETEC)	0.25 mL		
Vibrio cholerae	0.25 mL		
Pool 4			
Escherichia coli O157	0.25 mL	0.85 mL	2.1 mL
Giardia lamblia	0.25 mL		
Plesiomonas shigelloides	0.25 mL		
Rotavirus	0.25 mL		
Yersinia enterocolitica	0.25 mL		

Simple Protocol Example

The estimated total time to completion for this verification example is 4 days for a BioFire® FilmArray® System configured with 1 module.

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

Day 1

1. Organize materials needed (Table 3).

2. Prepare one sample pool (i.e. Pool 1) from ZeptoMetrix NATGIP-BIO control material. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 4 for organism pooling schemes and specific volumes for each pool.

   a. Transfer the entire contents of a vial of ZeptoMetrix Negative (approximately 0.85mL) into a 5 mL tube.
   b. Transfer the contents of the ZeptoMetrix organism vial (approximately 0.25mL) into the 5 mL tube containing the Negative diluent.
   c. Repeat for each of the remaining organisms to combine the appropriate organisms for each pool into a single tube.
   d. Ensure the pooled sample is well mixed prior to removing a sample for testing.

3. Repeat Step 2 for the remaining sample pool (i.e. Pool 2) to be prepared on Day 1.

4. Test 2 replicates from a single sample pool (i.e. Pool 1, replicates A and B). The replicate samples should be tested in a single day by different users.

   Note: Follow instructions in the BioFire® *FilmArray® Gastrointestinal (GI) Panel Instructions For Use* or the BioFire® *FilmArray® Gastrointestinal (GI) 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 A and B)

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 4) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more replicates if desired.

Day 2

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 1 (i.e. Pool 1, replicates C and D and Pool 2, replicates C and D) by repeating Step 4 above.

Day 3

Prepare 2 new sample pools (i.e. Pools 3 and 4) as described in Day 1, Steps 2 and 3. Test replicates as described in Step 4 above.

Day 4

To evaluate day-to-day variation, test additional replicates from the pools prepared on Day 3 (i.e. Pool 3, replicates C and D and Pool 4 replicates C and D) by repeating Step 4 above.

Figure 1. Workflow for Simple and Clinical Matrix Verification

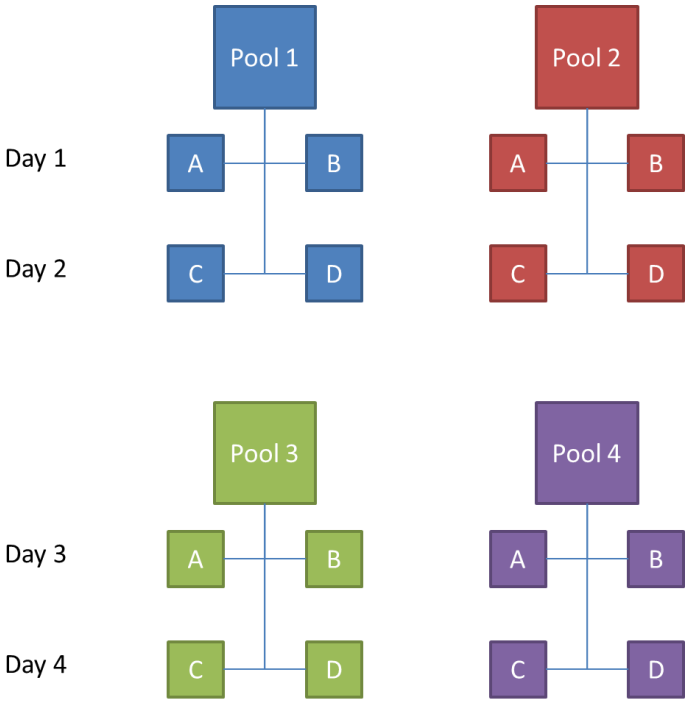

Figure 2. Example of Verification Workflow for Use with Multiple BioFire Modules

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		

Clinical Matrix Protocol using Stool in Cary Blair Media

The Clinical Matrix Protocol evaluates the BioFire GI Panel performance when sample material (ZeptoMetrix NATGIP-BIO) is pooled and combined with stool in Cary Blair media. Multiple stool in Cary Blair specimens may be pooled to meet the volumes described in Table 4. Clinical specimens should be screened on the BioFire GI Panel to characterize the sample before beginning the verification procedure.

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

Control material (ZeptoMetrix NATGIP-BIO) is pooled and combined with clinical matrix (stool in Cary Blair media). The proposed organism pooling scheme described in Table 4 should be followed to obtain the expected results for each assay in a time and resource-efficient manner. Specimen consistency may make accurate measurement difficult, but care should be taken to try to add the volume indicated.

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

Figures 1 and 2 (above) illustrate protocol and workflow schemes for testing 4 replicates per pool for 4 pools over multiple days. This produces a total of 16 verification sample test runs and provides 4 positive results and 12 negative results per assay.

The number of replicates tested per day should be determined by the individual laboratory. This testing scheme can be modified to run more replicates per day based on the number of modules in the BioFire® System. The pooling scheme provides sufficient volume for testing more replicates if desired.
Pooled samples added to clinical matrix can be stored overnight (or up to 3 days) at refrigeration temperature (2–8°C) for subsequent testing to evaluate day-to-day variation. Test replicates should be performed by different users to evaluate user to user variation.

Clinical Matrix Protocol Example

The estimated total time to completion for the Clinical Matrix Protocol verification example is 4 days for a BioFire System configured with 1 module.

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

Day 1

1. Organize materials needed (Table 3).

2. Prepare a fresh stool sample in Cary Blair transport media. Stool in Cary Blair specimens should be screened in the BioFire® FilmArray® GI Panel in order to characterize the sample prior to preparing pools.

3. Prepare one sample pool (i.e. Pool 1) by combining ZeptoMetrix NATGIP-BIO control material with the stool sample in Cary Blair transport media as described below. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 4 for organism pooling schemes and specific volumes for each pool.
   a. Transfer approximately 0.85mL of the characterized stool in Cary Blair specimen into a 5 mL tube. Specimen consistency may make accurate measurement difficult, but care should be taken to try to add the volume indicated.
   b. Transfer the contents of the ZeptoMetrix organism vial (approximately 0.25mL) into the 5 mL tube containing the stool in Cary Blair specimen.
   c. Repeat with each of the remaining organisms to combine the appropriate organisms for each pool into a single tube. The volume of the pool will be approximately 2.1 to 2.35 mL, depending upon the pool.
   d. Ensure the pooled sample is well mixed prior to removing a sample for testing.

4. Repeat Step 2 for the remaining sample pool (i.e. Pool 2) to be prepared on Day 1.

5. Test 2 replicates from a single sample pool (i.e. Pool 1, replicates A and B). The replicate samples should be tested in a single day by different users.

Note: Follow instructions in the BioFire® *FilmArray® Gastrointestinal (GI) Panel Instructions for Use* or the BioFire® *FilmArray® Gastrointestinal (GI) Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

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

7. 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 4) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more replicates if desired.

Day 2

To evaluate day-to-day variation, test additional replicates (i.e. replicates C and D) from the organism pools prepared on Day 1 by repeating Step 5 above.

Day 3

Prepare 2 new sample pools (i.e. Pools 3 and 4) as described in Day 1, Steps 2 -4. Test replicates as described in Step 5 above.

Day 4

To evaluate day-to-day variation, test additional replicates (i.e. replicates C and D) from the organism pools prepared on Day 3 by repeating Step 5.

Expanding the Protocols

The protocols described above can be expanded by increasing the number of test replicates from each of the organism pools. Each organism pool contains enough material to complete up to 10 tests for each pool. The verification study may use stool in Cary Blair transport media as a clinical matrix in the pools, as needed. Reference CAP accreditation checklist requirements: MIC.64960.

Verification of Loaner, Repaired, and Permanent Replacement Instruments or Modules

If it becomes necessary to verify the performance of a loaner, repaired, or permanent replacement instrument or 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® GI 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 GI Panel.

3. Test the selected samples on the loaner, repaired, or permanent replacement instrument or module 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® GI Panel Verification Record

BioFire® FilmArray® Gastrointestinal (GI) Panel Verification Record

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

Lot # _____ **Module Serial #** _____ **Module Serial #** _____

	BioFire GI Panel Detection	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	# Operators	# Days	# Modules	Patient Samples?
Pool 1	Adenovirus F 40/41																						
	Cryptosporidium																						
	Enteroaggregative *E. coli* (EAEC)																						
	Salmonella																						
	Sapovirus																						
Pool 2	Astrovirus																						
	Cyclospora cayetanensis																						
	Enteropathogenic *E. coli* (EPEC)																						
	Norovirus GI/GII																						
	Shigella/ Enteroinvasive *E. coli* (EIEC)																						
Pool 3	*Campylobacter*																						
	Clostridium difficile toxin A/B																						
	Entamoeba histolytica																						
	Enterotoxigenic *E. coli* (ETEC) *lt/st*																						
	Vibro																						
	Vibrio cholerae																						
Pool 4	Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*																						
	E. coli O157																						
	Giardia lamblia																						
	Plesiomonas shigelloides																						
	Rotavirus A																						
	Yersinia entercolitica																						

Reviewed by: _____

Signature **Date**