Protocols for Laboratory Verification of Performance of the BioFire® FilmArray® Gastrointestinal (GI) Panel

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® 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 GI Panel is a multiplexed nucleic acid test intended for use with BioFire® FilmArray® 1.5, BioFire® FilmArray® 2.0, or BioFire® FilmArray® Torch systems for the simultaneous qualitative detection and identification of multiple gastrointestinal viral, parasitic, and bacterial nucleic acid targets in stool samples in Cary Blair transport media obtained from individuals suspected of gastrointestinal tract infections. The following organisms and subtypes are identified using the BioFire GI Panel:

Campylobacter spp. *(C. jejuni, C. coli,* and *C. upsaliensis)*, *Clostridium difficile* toxin A/B, *Plesiomonas shigelloides*, *Salmonella* spp., *Vibrio* spp. *(V. parahaemolyticus, V. vulnificus,* and *V. cholerae)*, *Yersinia entercolitica*, Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC) *lt/st*, Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*, *E. coli* O157, *Shigella*/Enteroinvasive *E. coli* (EIEC), *Cryptosporidium*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*, adenovirus F 40/41, astrovirus, norovirus GI/GII, rotavirus A, and sapovirus (I, II, IV, and V).

FLM1-PRT-0151-05

Technical Note
BioFire Diagnostics, LLC
www.BioFireDX.com

QS-339B-02

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

TECHNICAL ::: NOTE

Performance Verification: Overview

Each procedure described below will generate multiple positive and negative results for each of the organisms targeted by the BioFire® GI Panel. The procedures were developed using a NATtrol™ GI Panel available from ZeptoMetrix Corporation, Buffalo, NY (part number NATGIP-BIO).

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 Organism and (2) a simple Cary Blair Media protocol that evaluates the performance of each assay on the BioFire GI Panel with a stool in Cary Blair sample matrix.

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, it is advised that test replicates are evenly distributed among the instruments or modules.

The procedures have been designed to take advantage of the multiplex nature of the BioFire GI Panel. Verification testing efficiency is maximized by evaluating multiple target organisms in a single test run.

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 in its performance verification of the BioFire GI Panel.

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: Stool in Cary Blair 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 used to perform verification procedures:

Table 2. Materials needed for recommended protocols

Material	Part Number
BioFire® FilmArray® GI Panel Kit (30 tests)	Biofire Diagnostics, LLC RFIT-ASY-0116
Control organism	ZeptoMetrix NATGIP-BIO [a]
Cary Blair transport media	Thermo Scientific Part # 23-005-47 (or equivalent)
5mL sample tubes	VWR Part # 89497-740 (or equivalent)
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.

Performance Verification: Protocols

Simple Protocol

The simple protocol utilizes samples prepared by pooling together either 5 or 6 different organisms (ZeptoMetrix NATGIP-BIO control organism). The proposed pooling scheme (Table 3) 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 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 pouches, providing 4 positive results and 12 negative results per organism. 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 instruments in the BioFire® System.

Table 3. Recommended Organism Pooling Scheme

Organism	Organism Volume	Negative Or Stool in Cary-Blair Media	Approximate Final Volume of Pool
Pool 1			
Enteraggregative *E. coli* (EAEC)	0.25 mL	0.85mL	2.1mL
Adenovirus type 41	0.25 mL		
Cryptosporidium parvum	0.25 mL		
Salmonella typhimurium	0.25 mL		
Sapovirus	0.25 mL		
Pool 2			
Enteropathogenic *E. coli* (EPEC)	0.25 mL	0.85mL	2.35mL
Norovirus GI	0.25 mL		
Norovirus GII	0.25 mL		
Cyclospora cayetanensis	0.25 mL		
Shigella sonnei	0.25 mL		
Astrovirus	0.25 mL		
Pool 3			
Entertoxigenic *E. coli* (ETEC) *lt/st*	0.25 mL	0.85mL	2.35mL
Entamoeba histolytica	0.25 mL		
Clostridium difficile	0.25 mL		
Vibrio cholerae	0.25 mL		
Campylobacter jejuni	0.25 mL		
Campylobacter coli	0.25 mL		
Pool 4			
E. coli O157	0.25 mL	0.85mL	2.1mL
Rotavirus	0.25 mL		
Giardia lamblia	0.25 mL		
Plesiomonas shigelloides	0.25 mL		
Yersinia entercolitica	0.25 mL		

Simple Protocol Example

The estimated total time to completion for this verification example is 4 days for systems configured with one instrument.

TECHNICAL
::: NOTE

Day 1

1. Prepare one sample pool (i.e. pool 1) from ZeptoMetrix NATGIP-BIO control material. An example organism pooling scheme is presented in Table 3.

   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 instruments connected within a BioFire® System.

   a. Use a transfer pipette to remove the entire contents of a vial of ZeptoMetrix Negative (approximately 0.85mL) and transfer it to a tube large enough (at least 3mL) to hold the entire organism pool volume.
   b. Use a transfer pipette to remove the entire contents of the ZeptoMetrix organism vial (approximately 0.25mL) and transfer to the larger tube containing the Negative diluent.
   c. Repeat step b. for each of the remaining organisms to combine the appropriate organisms for each pool into a single vial or tube.
   d. Ensure the pooled sample is effectively mixed by vortexing prior to removing a sample for testing.
   e. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

2. Prepare and test 2 samples from a single sample pool (i.e. pool 1). The duplicate samples should be tested in a single day by different users.

   Note: For each sample, follow instructions in the *FilmArray® Gastrointestinal (GI) Panel Instruction Booklet* or *GI Panel Quick Guide* for pouch preparation, pouch hydration, sample loading, and sample testing.

3. Repeat steps 1 and 2 for the remaining sample pool (i.e. pool 2) to be tested that day.

Day 2

To evaluate day-to-day variation, test the remaining samples (i.e. samples C and D) from the same sample pools prepared on Day 1 by repeating Step 2 above.

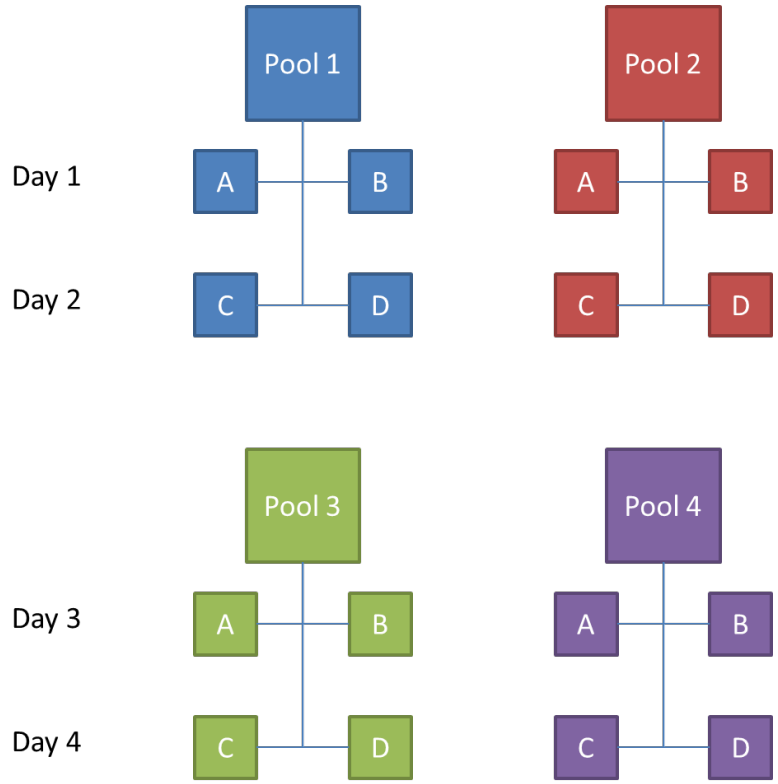
Day 3

Prepare 2 new sample pools (i.e. pools 3 and 4) as described in Step 1. Test samples according to Step 2 (i.e. samples A and B) for each pool.

Day 4

To evaluate day-to-day variation, test the samples prepared on Day 3 by repeating Step 2 (i.e. samples 3 and 4).

Figure 1. Simple protocol workflow



Stool in Cary Blair Protocol

An example organism pooling scheme is presented above in Table 3. For this testing scheme, a stool sample in Cary Blair media will be used as the organism pool background rather than synthetic stool (Negative). The laboratory will require a stool sample in Cary Blair that is negative for all GI pathogens detected by the BioFire® GI Panel. Laboratories may screen stool samples in Cary Blair with the BioFire GI Panel and select samples that yield all negative results as an appropriate background matrix for the pooling scheme. Pooled samples added to negative stool 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.

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

The Stool in Cary Blair Protocol can be followed to test a total of 16 pouches, providing 4 positive results and 12 negative results per organism. 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 instruments in the BioFire® System.

Stool in Cary Blair Protocol Example

The estimated total time to completion for this verification example is 4 days for systems configured with one instrument.

TECHNICAL
::: NOTE

Day 1

1. Prepare one sample pool (i.e. pool 1) by mixing ZeptoMetrix NATGIP-BIO control material with a freshly prepared stool sample in Cary Blair transport media. An example organism pooling scheme is presented in Table 3.

   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 instruments connected within a BioFire® System.

   a. Use a transfer pipette to transfer approximately 0.85mL of a prepared stool in Cary Blair sample to a tube large enough (at least 3mL) to hold the entire organism pool volume.
   b. Use a transfer pipette to remove the entire contents of the ZeptoMetrix organism vial (approximately 0.25mL) and transfer to the larger tube containing the stool sample.
   c. Repeat with each of the remaining organisms to combine the appropriate organisms for each pool into a single vial or tube.
   d. Ensure the pooled sample is effectively mixed by vortexing prior to removing a sample for testing.
   e. Refrigerate samples (2–8°C) for up to 3 days for the evaluation of day-to-day variation.

2. Prepare and test two samples from a single sample pool (i.e. pool 1). The duplicate samples should be prepared consecutively and tested in a single day. For each sample:

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

3. Repeat Step 1 and 2 for the remaining sample pool (i.e. pool 2 to be tested that day.

Day 2

To evaluate day-to-day variation, test the remaining samples (i.e. samples C and D) from the same sample pools prepared on Day 1 by repeating Step 2 above.

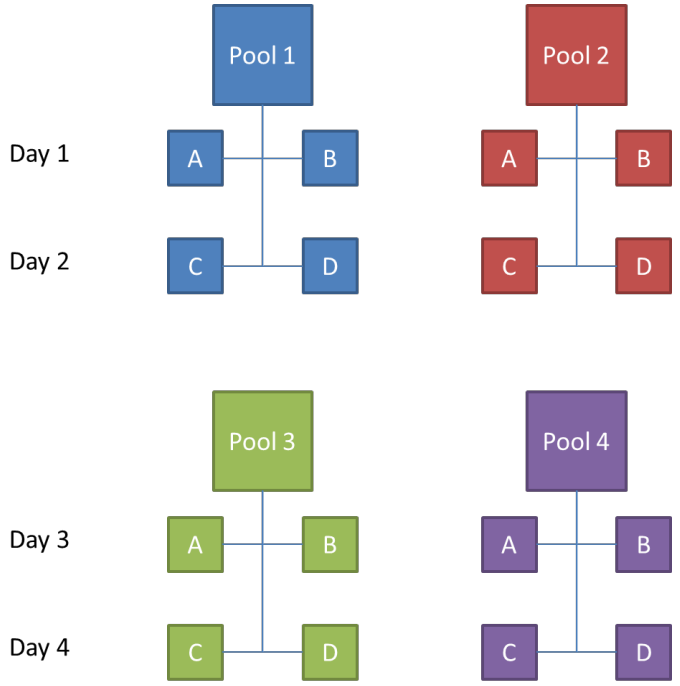
Day 3

Prepare 2 new sample pools (i.e. pools 3 and 4) as described in Step 1. Test samples according to Step 2 (i.e. samples A and B) for each pool.

Day 4

To evaluate day-to-day variation, test the samples prepared on Day 3 by repeating Step 2 (i.e. samples C and D).

Figure 2. Stool in Cary Blair media protocol workflow



Expanding the protocols

The protocols described above can be expanded by increasing the number of tests from each of the organism pools. Each organism pool contains enough material to complete up to 8 tests for each pool, doubling the number of tests described in the example protocols.

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 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 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

Computer System Serial #: _____

BioFire GI Panel Kit Part #: _____ Lot #: _____

Organism/Sample Source and Lot #: _____

TECHNICAL ::: NOTE

Organism	System Serial #	Was the Organism Detected?	No. Positive	No. Negative	No. Days Tested	No. Users	Patient Samples Tested?
Enteroaggregative *E. coli* (EAEC)		☐ Yes ☐ No					
Adenovirus F 40/41		☐ Yes ☐ No					
Cryptosporidium		☐ Yes ☐ No					
Salmonella		☐ Yes ☐ No					
Sapovirus (I, II, IV, and V)		☐ Yes ☐ No					
Enteropathogenic *E. coli* (EPEC)		☐ Yes ☐ No					
Norovirus GI/GII		☐ Yes ☐ No					
Cyclospora cayetanensis		☐ Yes ☐ No					
Shigella/ Enteroinvasive *E. coli* (EIEC)		☐ Yes ☐ No					
Astrovirus		☐ Yes ☐ No					
Entertoxigenic *E. coli* (ETEC) *lt/st*		☐ Yes ☐ No					
Entamoeba histolytica		☐ Yes ☐ No					
Clostridium difficile toxin A/B		☐ Yes ☐ No					
Vibrio (V. parahaemolyticus, V. vulnificus, and *V. cholerae)*		☐ Yes ☐ No					
Campylobacter (C. jejuni, C. coli, and *C. upsaliensis)*		☐ Yes ☐ No					
Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2		☐ Yes ☐ No					
E. coli O157		☐ Yes ☐ No					
Rotavirus A		☐ Yes ☐ No					
Giardia lamblia		☐ Yes ☐ No					
Plesiomonas shigelloides		☐ Yes ☐ No					
Yersinia entercolitica		☐ Yes ☐ No					

Reviewed by: _____
Signature Date