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® 2.0 and BIOFIRE® FILMARRAY® TORCH Systems as required by CLIA. Verification schemes compatible with the BIOFIRE GI Panel, have been designed using non-clinical specimens. The methods described positive and negative tests for each organism detected by the BIOFIRE GI 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 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.

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:

- *Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*)
- *Clostridium difficile* (*C. difficile*) toxin A/B
- *Plesiomonas shigelloides*
- *Salmonella*
- *Vibrio* (*V. parahaemolyticus/V. vulnificus/ V. cholerae*), including specific identification of *Vibrio cholerae*
- *Yersinia enterocolitica*

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

- Enteroaggregative *Escherichia coli* (EAEC)
- Enteropathogenic *Escherichia coli* (EPEC)
- Enterotoxigenic *Escherichia coli* (ETEC) *lt/st*
- Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* (including specific identification of the *E. coli* O157 serogroup within STEC)
- *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC)
- *Cryptosporidium*
- *Cyclospora cayetanensis*
- *Entamoeba histolytica*
- *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*)
- Adenovirus F 40/41
- Astrovirus
- Norovirus GI/GII
- Rotavirus A
- Sapovirus (Genogroups I, II, IV, and V)

The complete intended use statement and additional information about the use of the BIOFIRE® System can be found in the *BIOFIRE® FILMARRAY® Gastrointestinal (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 detected on the BIOFIRE GI Panel.

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 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 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, 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.

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

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]
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 module. 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® Gastrointestinal (GI) Panel Kit	BioFire Diagnostics, LLC RFIT-ASY-0116 (30 tests) or RFIT-ASY-0104 (6 tests)
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]To be used when evaluating clinical matrix. Stool in Cary Blair may be available from various clinical or commercial sources. 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/negative (provided in the control panel) and tested with the BIOFIRE GI Panel. The proposed organism 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.

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

Note: Dilution of ZeptoMetrix control 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. 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 3. Proposed Organism Pooling Scheme

Control Organism: NATGIP-BIO	Approximate Organism Volume	Negative or Stool in Cary-Blair	Approximate Final Volume of Pool
Pool 1			
Adenovirus Type 41 (TAK)	0.25 mL	0.85 mL	2.1 mL
Cryptosporidium parvum (Iowa)	0.25 mL		
Escherichia coli (92.0147; EAEC)	0.25 mL		
Salmonella enterica typhimurium (Z005)	0.25 mL		
Sapovirus (recombinant)	0.25 mL		
Pool 2			
Astrovirus (recombinant)	0.25 mL	0.85 mL	2.35 mL
Cyclospora cayetanensis (recombinant)	0.25 mL		
Escherichia coli (7.1493; O84:H28; EPEC)	0.25 mL		
Norovirus GI (recombinant)	0.25 mL		
Norovirus GII (recombinant)	0.25 mL		
Shigella sonnei (Z004)	0.25 mL		
Pool 3			
Campylobacter coli (clinical isolate)	0.25 mL	0.85 mL	2.35 mL
Campylobacter jejuni (clinical isolate)	0.25 mL		
Clostridium difficile (NAP1)	0.25 mL		
Entamoeba histolytica (DS4-868)	0.25 mL		
Escherichia coli (ETEC; ST+, LT+)	0.25 mL		
Vibrio cholerae (Z133; non-toxigenic)	0.25 mL		
Pool 4			
Escherichia coli (EDL933; O157)	0.25 mL	0.85 mL	2.1 mL
Giardia lamblia (H3)	0.25 mL		
Plesiomonas shigelloides (Z130)	0.25 mL		
Rotavirus (Wa)	0.25 mL		
Yersinia enterocolitica (clinical isolate)	0.25 mL		

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

Simple Protocol Example

The estimated total time to completion for this verification example is 4 days for a BIOFIRE® FILMARRAY® System configured with one module.

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 2).

2. Prepare one sample pool (i.e. Pool 1) using the ZeptoMetrix NATGIP-BIO control materials. Organism vials should be mixed vigorously for 5 seconds prior to preparing each pool. Refer to Table 3 for organism pooling schemes and specific volumes for each pool.

    a. Transfer the entire contents of a vial of ZeptoMetrix Synthetic/negative control (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 synthetic/negative matrix.
    c. Repeat with the second (and subsequent) organisms to combine the appropriate organisms for each pool into a single tube.

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

4. Test two 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® 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.

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 (Table 3) provides sufficient material for running samples as described in Figure. 1. The volume is sufficient for testing more replicates if desired.

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

Day 2

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

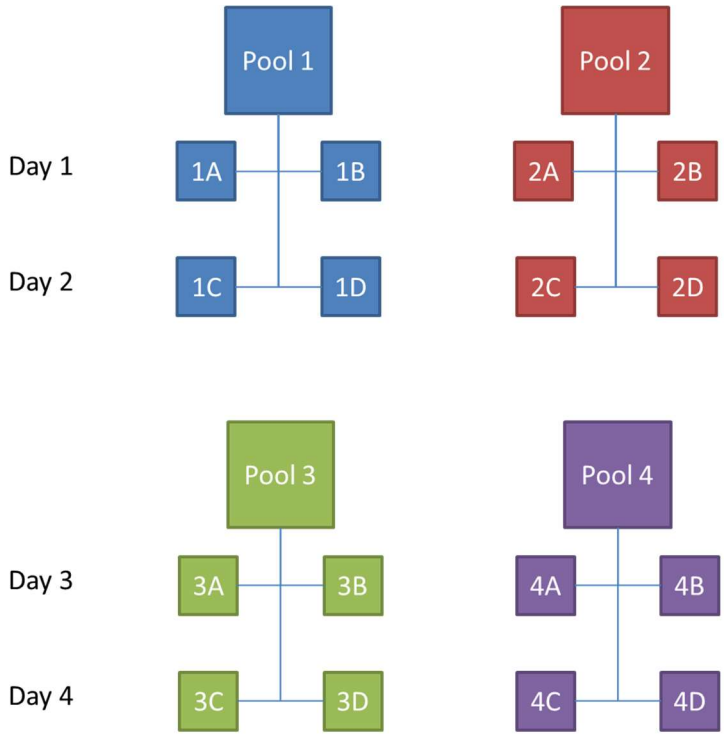
Day 3

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 3C and 3D and Pool 4 replicates 4C and 4D) by repeating Step 4 above.

Figure 1. Workflow for Simple and Clinical Matrix Verification



Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

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
Day 3	Pool 3A / Operator 1	Pool 4B / Operator 1	Pool 3B/ Operator 2	Pool 4A / Operator 2
Day 4	Pool 3D/ Operator 2	Pool 4C / Operator 2	Pool 3C / Operator 1	Pool 4D / 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
Day 3	Pool 3A / Operator 1	Pool 3B/ Operator 2	Pool 4B / Operator 1	Pool 4A / Operator 2
Day 4	Pool 4C / Operator 2	Pool 4D / Operator 1	Pool 3D/ Operator 2	Pool 3C / 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 1C/ Operator 1	Pool 1D/ Operator 2	Pool 2C/ Operator 1	Pool 2D/ Operator 2
Day 3	Pool 3A / Operator 1	Pool 3B/ Operator 2			Pool 4B / Operator 1	Pool 4A / Operator 2
Day 4	Pool 4C / Operator 2	Pool 4D / Operator 1	Pool 3C / Operator 1	Pool 3D/ Operator 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 3. Specimen consistency may make accurate measurement difficult, but care should be taken to try to add the volume indicated. The proposed organism pooling scheme described in Table 3 should be followed to obtain the expected results for each assay in a time and resource-efficient manner.

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 detected on the BIOFIRE GI Panel.

Note: Dilution of ZeptoMetrix control 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.

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

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. 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 one module.

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 2).

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) using the 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 3 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 the second (and subsequent) 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.

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

5. Test two 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: 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 organisms pooled with stool in Cary Blair or 14 days for

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

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 (Table 3) 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 1C and 1D and Pool 2, replicates 2C and 2D) by repeating Step 5 above.

Day 3

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

Day 4

To evaluate day-to-day variation from the pools prepared on Day 3 (i.e. Pool 3, replicates 3C and 3D and Pool 4, replicates 4C and 4D) by repeating Step 5.

Expanding or Modifying 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 sufficient volume for testing additional replicates. 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 Validation or Verification Studies - Specimen Selection.

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

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® FILMARRAY® Gastrointestinal (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

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.

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07

BIOFIRE® FILMARRAY® Gastrointestinal (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 enterocolitica																						

Reviewed by:

Signature **Date**

Attachment 5 of LLDC
069952 - Rev 02.A
Old Document Reference
QS-339M-01
FLM1-PRT-0151-07