Application Note

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Guidelines for Laboratory Verification of Performance of the QIAstat-Dx® Gastrointestinal Panel 2

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Introduction

This document provides a sample protocol for the verification of QIAstat-Dx Gastrointestinal Panel 2 (cat. no. 691413). The protocol provides positive and negative tests for the pathogens detected by QIAstat-Dx Gastrointestinal Panel 2.

Each laboratory is responsible for defining their verification procedure and ensuring that they meet state and federal guidelines.

Materials and methods

The procedure described in Table 1 and below generates multiple positive and negative results for each of the sample control mixes tested. The sample protocol was developed using the NATtrol™ Gastrointestinal 2 Verification Panel available from ZeptoMetrix Corporation (Buffalo, NY; cat. no. NATGIP2-QIA).

Table 1. Overview of sample verification method

Sample protocol	
Organism controls per sample control mix	5-6
Number of sample control mixes	4
Replicates per sample control mix	4
QIAstat-Dx cartridges required	16
Expected number of positive results	92
Expected number of negative results	276
Approximate days of testing	4
Number of operators	2

If testing is being performed using a QIAstat-Dx Analyzer with additional Analytical Modules or a QIAstat-Dx Rise, the laboratory director may choose not to perform the verification protocol on each Analytical Module. If the complete verification protocol is not performed on each Analytical Module, we advise distributing test replicates evenly among the different Analytical Modules of the system.

Table 2. Materials needed for the sample verification method

Material	Catalog number	Quantity
QIAstat-Dx Gastrointestinal Panel 2 kit (6 tests)	691413	3
QIAstat-Dx Operational Module*	9002813	1
QIAstat-Dx Analytical Module(s)	9002814	1–4
NATtrol Gastrointestinal 2 Verification Panel†	NATGIP2-QIA	1
Sample tubes, 2 mL	VWR 525-0131 (or similar)	4
Transfer pipettes	VWR 13-711-43 (or similar)	24

* Or QIAstat-Dx Rise instrument (cat. no. 9003163)

† NATtrol Gastrointestinal 2 Verification Panel includes 4 x 1.0 mL of negative control made of a simulated stool matrix (stool diluent) that serves as sample diluent in the sample verification method. The kit provides 22 organism controls (each tube contains a volume of 0.250 mL).

Sample verification method

This sample verification method describes how to prepare sample control mixes by mixing together either 5 or 6 different organism controls using a combination of pathogens from ZeptoMetrix NATtrol Gastrointestinal 2 Verification Panel. Proposed mixing of organism controls is provided in Table 3. The method tests a total of 16 sample control mixes (4 sample control mixes tested in 4 replicates each). For each assay run, the method provides either 5 or 6 positive results and, correspondingly, 17 or 18 negative results for the 23 targets in total, which are detected and differentiated by QIAstat-Dx Gastrointestinal Panel 2.

Alteration of this protocol should take into account additional lab personnel and number of instruments, based on individual laboratory needs.

Mix the organism controls to create control samples at the beginning of the sample verification method. The sample control mixes can be stored at refrigerated conditions (2–8°C) for up to 3 days.

Note: It is important to prepare only the number of sample control mixes 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 number of QIAstat-Dx Analyzer instruments.

Table 3. Proposed organism-control mixing and expected results (positive or negative)

Mix	Organism	Organism control volume(mL)	Simulated stool matrix* volume (mL)	Final volume of mix (mL)	Expected results	Number of expected negative results
Sample Control Mix 1	*Giardia lamblia*	0.2	0.3	1.5	Positive	17
	Cyclospora cayetanensis	0.2			Positive	
	Enteroaggregative *Escherichia coli* (EAEC)	0.2			Positive	
	Campylobacter jejuni	0.2			Positive	
	Adenovirus Type 41	0.2			Positive	
	Shigella sonnei	0.2			Positive	
Sample Control Mix 2	Astrovirus Type 8	0.2	0.5	1.5	Positive	17
	Entamoeba histolytica	0.2			Positive	
	Clostridium difficile	0.2			Positive	
	Enterotoxigenic *Escherichia coli* (ETEC)	0.2			Positive	
	Shiga-like toxin	0.2			Positive	
	Escherichia coli EDL933†	0.2			Positive	
Sample Control Mix 3	Enteropathogenic *Escherichia coli* (EPEC)	0.2	0.3	1.5	Positive	17
	Vibrio parahaemolyticus	0.2			Positive	
	Yersinia enterocolitica	0.2			Positive	
	Plesiomonas shigelloides	0.2			Positive	
	Sapovirus	0.2			Positive	
	Rotavirus	0.2			Positive	
Sample Control Mix 4	Norovirus GII	0.2	0.5	1.5	Positive	18
	Cryptosporidium parvum	0.2			Positive	
	Vibrio vulnificus	0.2			Positive	
	Salmonella enterica typhimurium	0.2			Positive	
	Vibrio cholerae	0.2			Positive	

*The NATtrol Gastrointestinal 2 Verification Panel includes 4 x 1.0 mL of negative control made of a simulated stool matrix (stool diluent) that serves as sample diluent in the sample verification method.

†Reported as Shiga-like toxin-producing *E. coli* (STEC) serotype O157:H7 which is detected in the QIAstat-Dx Gastrointestinal Panel 2 by a combination of two different signals, one corresponding to the Shiga-like toxin-producing *E. coli* (STEC) *stx1+stx2* assay and the other to the *E. coli* O157 assay.

Protocol

Day 1

1. Prepare Sample Control Mix 1 and Sample Control Mix 2 (refer to Table 3). Vortex and spin each ZeptoMetrix tube before use.
   A. Transfer the appropriate volume of simulated stool matrix (provided as 4 x 1.0 mL negative control in the kit) to a new 2 mL tube.
   B. Transfer 0.2 mL of each of the organism controls in the mix to the 2 mL tube.
   C. Ensure the pooled sample is effectively mixed by vortexing prior to testing.
2. Test two replicates from Sample Control Mix 1. The duplicate samples should be tested in a single day by different operators (see Table 4).
3. Repeat step 2 for Sample Control Mix 2 to be tested on the same day.
4. Store the samples at 2–8°C for up to 3 days for the evaluation of day-to-day variation.

Day 2

To evaluate day-to-day variation, test the remaining volume of the sample control mixes prepared on Day 1 (Sample Control Mix 1 and Sample Control Mix 2) by repeating steps 2 and 3 above.

Day 3

Prepare Sample Control Mix 3 and Sample Control Mix 4 as described in step 1. Test Sample Control Mix 3 and Sample Control Mix 4 according to steps 2 and 3.

Day 4

To evaluate day-to-day variation, test the remaining volume of the sample control mixes prepared on Day 3 (Sample Control Mix 3 and Sample Control Mix 4) by repeating steps 2 and 3 above. Table 4 details a workflow for two operators.

Table 4. Workflow for the sample verification method

	Day 1	Day 2	Day 3	Day 4
Operator 1	Sample Control Mix 1 Sample Control Mix 2	Sample Control Mix 1 Sample Control Mix 2	Sample Control Mix 3 Sample Control Mix 4	Sample Control Mix 3 Sample Control Mix 4
Operator 2	Sample Control Mix 1 Sample Control Mix 2	Sample Control Mix 1 Sample Control Mix 2	Sample Control Mix 3 Sample Control Mix 4	Sample Control Mix 3 Sample Control Mix 4

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