510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION

A. 510(k) Number: K132235

B. Purpose for Submission:

The IMDx *C. difficile* for Abbott *m*2000 assay was submitted to obtain a substantial equivalence determination for the IMDx *C. difficile* assay on the Abbott *m*2000 platform using unpreserved (i.e., fresh) or Cary-Blair preserved stool specimens.

C. Measurand:

DNA target sequences encoding the *tcdA* and *tcdB* genes which are associated with toxigenic strains of *Clostridium difficile*.

D. Type of Test:

Qualitative nucleic acid amplification test using real-time PCR technology to amplify and detect the *tcdA* and *tcdB* genes.

E. Applicant:

Intelligent Medical Devices, Inc.

F. Proprietary and Established Names:

IMDx *C. difficile* for Abbott *m*2000

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.3130 - *C. difficile* Nucleic Acid Amplification Test Assay

2. Classification:

   Class II

3. Product code:

   OZN - Amplification assay for the detection of *Clostridium difficile* toxin genes from stool specimens of symptomatic patients

   OOI - Real-Time Nucleic Acid Amplification System

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The IMDx C. *difficile* for Abbott *m*2000 assay is an *in vitro* diagnostic assay that uses real-time polymerase chain reaction (PCR) amplification for the qualitative detection of nucleic acids encoding the toxin A gene (*tcdA*) and toxin B gene (*tcdB*) sequences of toxigenic strains of *Clostridium difficile* in human liquid or soft stool specimens collected from patients suspected of having symptoms of *Clostridium difficile* infection.

   The assay is intended to be performed on the Abbott *m*2000 System (which comprises the Abbott *m*2000*sp* and *m*2000*rt* instruments) and is indicated for use as an aid in the diagnosis of *Clostridium difficile* infection. The test is intended to be used directly on liquid or soft stool specimens (unpreserved stool, or stool preserved in Cary Blair transport medium). Negative results do not preclude toxigenic *C. difficile* infection and should not be used as the sole basis for treatment or other patient management decisions. The IMDx *C. difficile* for Abbott *m*2000 assay is intended for professional use. The device is not intended for point-of-care use.

2. Indication(s) for use:

   The IMDx C. *difficile* for Abbott *m*2000 assay is an *in vitro* diagnostic assay that uses real-time polymerase chain reaction (PCR) amplification for the qualitative detection of nucleic acids encoding the toxin A gene (*tcdA*) and toxin B gene (*tcdB*) sequences of toxigenic strains of *Clostridium difficile* in human liquid or soft stool specimens collected from patients suspected of having symptoms of *Clostridium difficile* infection.

   The assay is intended to be performed on the Abbott *m*2000 System (which comprises the Abbott *m*2000*sp* and *m*2000*rt* instruments) and is indicated for use as an aid in the diagnosis of *Clostridium difficile* infection. The test is intended to be used directly on liquid or soft stool specimens (unpreserved stool, or stool preserved in Cary Blair transport medium). Negative results do not preclude toxigenic *C. difficile* infection and should not be used as the sole basis for treatment or other patient management decisions. The IMDx *C. difficile* for Abbott *m*2000 assay is intended for professional use. The device is not intended for point-of-care use.

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   Abbott *m*2000*sp*: sample preparation and reagent mixing

Abbott *m*2000*rt*: amplification reaction and detection

I. Device Description:

The IMDx *C. difficile* for Abbott *m*2000 assay is a qualitative, real-time PCR-based assay that targets the genes *tcdA* and *tcdB*; these genes are associated toxigenic strains of *C. difficile*. These toxin A and B genes can be found in human liquid or soft stool specimens from patients with symptoms of *C. difficile* associated disease. The assay is intended for use directly on patient samples and is intended to aid in the diagnosis of *C. difficile*-associated disease. Specimen preparation and reagent mixing occurs on the Abbott *m*2000*sp* instrument, while amplification and detection occurs on the Abbott *m*2000*rt* instrument. The Abbott *m*2000*rt* application software monitors the fluorescence emitted by each fluorescent probe, interprets all data, and provides a final result at the end of the cycling program. Differentiation of *tcdA* from *tcdB* is achieved by labeling the oligonucleotide probes with different colored fluorescent dyes. Assay results are reported as either the presence or absence of the *tcdA* and/or *tcdB* targets

A specimen processing control is introduced into each specimen during the sample extraction. This control is composed of an inactivated Gram-positive bacterium unrelated toxigenic *C. difficile* that is introduced in a defined quantity into each sample during processing; it is co-extracted with the specimen and co-amplified in the same PCR reaction as the *tcdA* and *tcdB* targets and serves to demonstrate that the entire assay process has proceeded within specification. Positive and negative controls are included with each run to ensure the integrity of the system.

IMDx *C. difficile* for Abbott *m*2000 Kit

The IMDx *C. difficile* for Abbott *m*2000 assay consists of two reagent kits packaged together: (1) a separate box for the **Amplification Reagent Kit**, and (2) a separate box for the **Control Kit**:

1) IMDx C. *difficile* for Abbott *m*2000 Amplification Reagent Kit
Each Amplification Reagent Kit contains two types of items:

a) IMDx Process Control-A:
- 4 vials, 0.6 mL per vial of inactivated bacteria in a buffered solution.

b) IMDx Amplification Reagent Packs:
- 4 Amplification Reagent Packs, 24 tests/pack.

*Each Amplification Reagent Pack contains:**
- 1 vial (0.408 mL) IMDx *C. difficile* for Abbott *m*2000 Amplification Reagent consisting of synthetic oligonucleotides in a buffered solution, located in position 1 of the reagent pack.
- 1 vial (0.192 mL) IMDx PCR Reagent-A (DNA polymerase and dNTPs in a buffered solution with ROX™ reference dye), located in position 3 of the reagent pack.

*Each Amplification Reagent Pack, although capable of containing up to three reagent vials, contains only two reagent vials in each pack. These vials are located in positions 1 and 3, as marked on the Reagent Pack; position 2, in the middle of the pack, is empty.

2) IMDx *C. difficile* for Abbott *m*2000 Control Kit *Each Control Kit contains two types of items:*
a) IMDx Negative Control-A: ● 6 tubes (2.5 mL per tube) containing a buffered solution with carrier DNA isolated from *Bacteroides* sp. **b) IMDx C. difficile for Abbott *m*2000 Positive Control** ● 6 tubes (2.5 mL per tube) containing a mixture of synthetic *C. difficile* DNA in buffer.

Interpretation of Results

The decision algorithm for the IMDx *C. difficile* for Abbott *m*2000 assay is embedded in the Abbott *m*2000*rt* application software (v3.0.338.0). The interpretation of assay results is provided by analyte results and the process control result.

Target	**Reported Target Output**				
tcdA	Detected	Not Detected	Detected	Not Detected	Not Detected
tcdB	Not Detected	Detected	Detected	Not Detected	Not Detected
Process Control	Detected / Not Detected	Detected / Not Detected	Detected / Not Detected	Detected	Not Detected
Result Call	***tcdA* detected**	***tcdB* detected**	***tcdA* and *tcdB* detected**	***tcdA* and *tcdB* not detected**	**Invalid; no result reported**
Indicates presence of toxigenic *C.*	**Yes**	**Yes**	**Yes**	**No**	**N/A**

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Quidel AmpliVue™ C. difficile Assay

2. Predicate 510(k) number(s):

   K123355

3. Comparison with predicate:

Similarities		
Item	IMDx *C. difficile* for Abbott *m*2000 (K132235)	Quidel AmpliVue™ C. difficile Assay (K123355)
Intended Use	The IMDx C. *difficile* for Abbott *m*2000 assay is an *in vitro* diagnostic assay that uses real-time polymerase chain reaction (PCR) amplification for the qualitative detection of nucleic acids encoding the toxin A gene (*tcdA*) and toxin B gene (*tcdB*) sequences of toxigenic strains of *Clostridium difficile* in human liquid or soft stool specimens collected from patients suspected of having symptoms of *Clostridium difficile* infection. The assay is intended to be performed on the Abbott *m*2000 System (which comprises the Abbott *m*2000*sp* and *m*2000*rt* instruments) and is indicated for use as an aid in the diagnosis of *Clostridium difficile* infection. The test is intended to be used directly on liquid or soft stool specimens (unpreserved stool, or stool preserved in Cary Blair transport medium). Negative results do not preclude toxigenic *C. difficile* infection and should not be used as the sole basis for treatment or other patient management decisions. The IMDx *C. difficile* for Abbott *m*2000 assay is intended for professional use. The device is not intended for point-of-care use.	The AmpliVue™ C. difficile Assay is an *in vitro* diagnostic test for the direct, qualitative detection of the *Clostridium difficile* Toxin A gene (*tcdA*) in unformed stool specimens of patients suspected of having *Clostridium difficile*-associated disease (CDAD). The AmpliVue™ C. difficile Assay is intended for use as an aid in diagnosis of CDAD. The assay utilizes helicase-dependent amplification (HDA) for the amplification of a highly conserved fragment of the Toxin A gene sequence and a self-contained disposable amplicon detection device that allows for manual evaluation of assay results.
Sample type	Soft or liquid stool (unpreserved stool, or stool	Unformed stool

Similarities		
Item	IMDx *C. difficile* for Abbott *m*2000 (K132235)	Quidel AmpliVue™ C. difficile Assay (K123355)
	preserved in Cary Blair transport medium).	
Test Principle	Real-time PCR DNA amplification	Isothermal nucleic acid amplification
Analyte	Toxin A gene (*tcdA*) Toxin B genes (*tcdB*, including variant *tcdBv*)	Toxin A gene (*tcdA*)
Controls	Positive Control, Negative Control and Process Control included in the kit	Process Control included in the kit. Positive and Negative Controls not included in kit; separate control kit commercially available

Differences		
Item	IMDx *C. difficile* for Abbott *m*2000 (K132235)	Quidel AmpliVue™ C. difficile Assay (K123355)
Instrument	Assay uses the Abbott *m*2000 System	Self-contained, disposable cassette with an amplicon cartridge and detection chamber
Interpretation of Test Results	Abbott *m*2000*rt* application software.	Visual read
Sample Preparation	Automated by Abbott *m*2000*sp*	Manual

K. Standard/Guidance Document Referenced (if applicable):

Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection of *Clostridium difficile* – Draft Guidance for Industry and FDA Staff (issued on November 29, 2010).

L. Test Principle:

The IMDx *C. difficile* for Abbott *m*2000 assay is a qualitative, *in vitro* diagnostic assay containing reagents for the real-time PCR amplification, detection, and differentiation of nucleic acids from *C. difficile* bacteria harboring *tcdA* and/or *tcdB* genes. Detection of the *tcdA* and *tcdB* genes is measured by the presence of fluorescently-labeled oligonucleotide probes that generate a fluorescent signal when specifically bound to amplified *tcdA* and/or *tcdB* PCR products; differentiation of *tcdA* from *tcdB* is attained by labeling the

oligonucleotide probes with different colored fluorescent dyes. The IMDx *C. difficile* for Abbott *m*2000 assay includes inactivated bacteria as a full process control; this inactivated bacterium is unrelated to *C. difficile* and introduced into each specimen during sample preparation such that it is co-extracted and co-amplified with each specimen. This full process control serves to demonstrate that the entire assay process has proceeded within specification for individual specimens. The Abbott *m*2000 system consists of two instruments: the Abbott *m*2000*sp* (for sample preparation) and the Abbott *m*2000*rt* (for real-time PCR amplification and detection). The assay is intended to be used directly on fresh (i.e., unpreserved), or Cary-Blair preserved stool specimens collected from patients to aid in the diagnosis of *Clostridium difficile* infection.

Fresh/raw or Cary-Blair preserved stool specimens are initially subjected to sample preparation on the Abbott *m*2000*sp*, an automated sample preparation system. Sample preparation lyses bacteria present in the sample, including any toxigenic *C. difficile*, to make the *tcdA* and *tcdB* target nucleic acids accessible for amplification and to remove potential amplification inhibitors. At the completion of the sample processing procedure, the resulting bacterial lysate is transferred to an Abbott 96-Deep-Well. The Abbott *m*2000*sp* also combines the IMDx *C. difficile* for Abbott *m*2000 Amplification Reagent components and dispenses the resulting Master Mix into the Abbott 96-Well Optical Reaction Plate. After manual application of the Abbott Optical Adhesive Cover, the plate is ready for transfer to the Abbott *m*2000*rt*.

Amplification/detection takes place on the Abbott *m*2000*rt* instrument using real-time PCR techniques; during each round of PCR amplification the fluorescent probes anneal to the amplified target DNA, if present. The probes are labeled with different fluorescent molecules allowing *tcdA*, *tcdB* and the IMDx Process Control-A targets to be distinguished from each other. The probes are single-stranded, linear DNA oligonucleotides modified with a fluorescent moiety covalently linked to one end of the probe and a quenching moiety to the other end. When the probe binds to its complementary sequence in the target during amplification, the fluorophore separates from the quenchers, thus allowing fluorescent emission and detection. Since this fluorescence occurs during every cycle, the PCR reaction can be read in real-time. Positive and negative controls are included with each run to ensure the integrity of the system.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Assay precision was measured in four independent studies:

   - user-to-user reproducibility;
   - lot-to-lot reproducibility;
   - instrument-to-instrument reproducibility, and
   - within-laboratory repeatability.

A seven-member precision panel was comprised of intact organisms from two strains of *C. difficile*: a NAP-1 strain (ATCC BAA-1870), and the *tcdB*-variant 1470 (ATCC 43598). Each strain was formulated at three target levels:

i. positive (corresponding to a concentration associated with ~2-3x LoD),
ii. low positive (1x LoD), and
iii. high negative (~0.05 x LoD, estimated to have a 20 to 80% positivity rate).

Panel members were made fecal matrix buffer to simulate clinical specimens. The seventh panel member was a true negative sample that contained TE Buffer plus fecal matrix alone, without *C. difficile* organism added.

Site-to-Site Reproducibility

For the reproducibility study the panel members were randomized and blinded. Each panel member was tested in replicates of three, for six days, at three study sites, by two technologists at each site for a total of 36 experimental runs; each operator performed one run each day. The entire study was conducted using one instrument system (*m*2000*sp* and m2000*rt*) at each site and one reagent lot of the IMDx *C. difficile* for Abbott *m*2000 assay. The results and percent agreement (observed/tested) at each site, as well as the overall agreement were reported as follows:

Sample ID	SITE						Overall	
	Site #1		Site #2		Site #3			
	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't
ATCC 43598 High Negative	32/36	89%	27/36	75%	27/34	79%	86/106	81%
ATCC 43598 Low Positive	35/35	100%	36/36	100%	36/36	100%	107/107	100%
ATCC 43598 Positive	36/36	100%	36/36	100%	36/36	100%	108/108	100%
ATCC BAA-1870 High Negative	31/35	89%	31/36	86%	30/35	86%	92/106	87%
ATCC BAA-1870 Low Positive	36/36	100%	35/35	100%	35/36	97%	106/107	99%
ATCC BAA-1870 Positive	36/36	100%	37/37	100%	37/37	100%	110/110	100%
Negative	35/35	100%	35/35	100%	35/35	100%	105/105	100%
Overall	241/249	96.8%	237/251	94.4%	236/249	94.8%	714/749*	95.3%

*Of the 758 samples tested 9 samples resulted in an instrument error and were withdrawn from the study. The overall invalid rate was 1%.

Lot-to Lot-Reproducibility

Lot-to-lot reproducibility was assessed using three lots of the IMDx *C. difficile* for Abbott *m*2000 assay. One experiment was run for each of the three lots (for a total of three runs). A single operator performed the study using a single instrument pair (*m*2000*sp* and *m*2000*rt*). Panel members were tested in replicates of six for each run. The expected results and percent agreement at each site, as well as the overall agreement were reported as follows:

Sample ID	IMDx *C. difficile* for Abbott *m*2000 Lot						Overall	
	Lot #1		Lot #2		Lot #3			
	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't
ATCC 43598 High Negative	5/6	83%	5/6	83%	5/6	83%	15/18	83%
ATCC 43598 Low Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
ATCC 43598 Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
ATCC BAA-1870 High Negative	4/6	67%	4/5*	80%	4/6	67%	12/17	71%
ATCC BAA-1870 Low Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
ATCC BAA-1870 Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
Negative	6/6	100%	6/6	100%	6/6	100%	18/18	100%
Overall	39/42	92.9%	39/41	95.1%	39/42	92.9%	117/125	93.6%

*Of the 126 samples tested, one ATCC BAA-1870 sample was invalidated due to an instrument error and was withdrawn from the study. The overall invalid rate was <1%.

Instrument-to-Instrument Reproducibility

Instrument-to-instrument reproducibility was determined independently for both the Abbott *m*2000*sp* and *m*2000*rt* instruments. To measure *m*2000*sp* instrument variability, the test panel was run on three different *m*2000*sp* instruments. Panel members were run in replicates of six. Runs were performed in succession by the same operator, using a single IMDx *C. difficile* for Abbott *m*2000 assay lot. Once the master mix assembly portion of the protocol was executed by the different *m*2000*sp* instruments, the assembled reaction plates were run using a single *m*2000*rt* instrument. The expected results and percent agreement at each site, as well as the overall agreement were reported as follows:

Sample ID	Abbott *m*2000*sp* Instrument-to-Instrument Reproducibility						Overall	
	Instrument #1		Instrument #2		Instrument #3			
	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't
ATCC 43598 High Negative	5/6	83%	6/6	100%	6/6	100%	17/18	94%
ATCC 43598 Low Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
ATCC 43598 Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
ATCC BAA-1870 High Negative	4/6	67%	4/6	67%	4/6	67%	12/18	67%
ATCC BAA-1870 Low Positive	6/6	100%	6/6	100%	5/5*	100%	17/17	100%
ATCC BAA-1870 Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
Negative	6/6	100%	6/6	100%	6/6	100%	18/18	100%
Overall	39/42	92.9%	40/42	95.2%	39/41	95.1%	118/125	94.4%

*Of the 126 samples tested, one ATCC BAA-1870 sample was invalidated due to an instrument error and was withdrawn from the study. The overall invalid rate was <1%.

To measure *m*2000*rt* variability, a single *m*2000*sp* instrument was used in one experiment. Six replicates of each panel member were prepared. The eluted nucleic acids from the sample extractions were automatically transferred to an Abbott 96-well Deep Well Plate by the *m*2000*sp* per normal operational procedures. Aliquots from one deep well plate were then used to assemble three separate, identical Abbott 96-well Optical Reaction Plates, using the same lot of the IMDx *C. difficile* for Abbott *m*2000 assay. Each of the three identical Abbott 96-Well Optical Reaction plates were then run on three separate Abbott *m*2000*rt* instruments, by the same operator. The expected results and percent agreement at each site, as well as the overall agreement were reported as follows:

Sample ID	Abbott *m*2000*rt* Instrument-to-Instrument Reproducibility						Overall	
	Instrument #1		Instrument #2		Instrument #3			
	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't	#observed results / #tested	% Agreem't
ATCC 43598 High Negative	4/6	67%	6/6	100%	6/6	100%	16/18	89%
ATCC 43598 Low Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
ATCC 43598 Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
ATCC BAA-1870 High Negative	5/6	83%	4/6	67%	4/6	67%	13/18	72%
ATCC BAA-1870 Low Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
ATCC BAA-1870 Positive	6/6	100%	6/6	100%	6/6	100%	18/18	100%
Negative	6/6	100%	6/6	100%	6/6	100%	18/18	100%
Overall	39/42	92.9%	40/42	95.2%	40/42	95.2%	119/126	94.4%

Negative samples showed an overall negativity rate of 100%; no false positive results were observed. High Negative samples were expected to provide a negativity rate of 20–80%; the observed negativity rate was 81.2%, and 84.1% for the ATCC BAA-1870 and ATCC 43598 strains, respectively. Low Positive samples were expected to provide a positivity rate of ≥95%; the observed positivity rate was 98%. Positive samples were expected to provide a positivity rate of 100%; the observed positivity rate was 100%. There were 11 instrument errors out of the 1,136 samples tested in these studies (<1%).

Within-Laboratory Repeatability

For the repeatability study, the seven-member panel was tested twice a day for a total of 12 days. Panel members were tested in replicates of three for each run (for a total of 504 data points for the 24 runs). The entire study was conducted by one technician using one instrument pair (*m*2000*sp* and *m*2000*rt*) and one reagent lot of the IMDx *C. difficile* for Abbott *m*2000 assay.

Strain	Category	# expected results/ # tested	Percent Agreement	95% Confidence Interval
***C. difficile* NAP-1 (ATCC BAA-1870)**	**High Negative**	57/70	81%	[0.71, 0.89]
	Low Positive	70/71	99%	[0.92, 1.00]
	Positive	72/72	100%	[0.94, 1.00]
***C. difficile* 1470 *tcdB*-variant (ATCC 43598)**	**High Negative**	61/72	85%	[0.75, 0.91]
	Low Positive	71/72	99%	[0.92, 1.00]
	Positive	72/72	100%	[0.94, 1.00]
True Negative	**Negative**	72/72	100%	[0.94, 1.00]

*Of the 504 samples tested 3 samples resulted in an instrument error and were withdrawn from the study. The overall invalid rate was 1%.

The details regarding the average (Avg), standard deviation (SD), and coefficient of variation (%CV) in cycle number (CN) for *tcdA*, *tcdB* and the Internal Control (IC; Process Control-A) for the repeatability study were reported below. The IMDx *C. difficile* for Abbott *m*2000 assay provided reproducible results.

	***tcdA* CN**			***tcdB* CN**			**IC CN**			**% Agreement**		
	Avg	SD	%CV	Avg	SD	%CV	Avg	SD	%CV	Expect	Total	%
ATCC BAA-1870 High Negative	40.4	1.2	3.0%	42.5	0.2	0.5%	34.3	0.3	0.8%	57	70	81%
ATCC BAA-1870 Low Positive	38.3	1.1	2.8%	40.4	1.3	3.2%	34.5	0.8	2.2%	70	71	99%
ATCC BAA-1870 Positive	37.0	0.7	1.8%	39.3	1.5	3.8%	34.4	0.3	1.0%	72	72	100%
ATCC 43598 High Negative	40.2	1.5	3.7%	41.8	1.1	2.6%	34.4	0.4	1.0%	61	72	85%
ATCC 43598 Low Positive	38.5	0.9	2.4%	40.8	1.3	3.2%	34.4	0.3	0.8%	71	72	99%
ATCC 43598 Positive	37.6	1.1	2.9%	40.2	1.1	2.8%	34.4	0.4	1.1%	72	72	100%
Negative	-1.0	0.0	0.0%	-1.0	0.0	0.0%	34.5	0.3	0.9%	72	72	100%

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The IMDx Process Control-A is composed of inactivated Gram-positive bacteria unrelated to *C. difficile* and is introduced into each sample during specimen processing; therefore, an IMDx Process Control-A result is generated with each specimen result. Positive and negative Quality Controls are run with each IMDx *C. difficile* for Abbott *m*2000 assay run.

There were a total of 5,722 replicates run during analytical verification; a total of 56 (0.98%) errors were observed. Of these 56 errors, 54 were due to instrument failures. There were a total 1,583 evaluable specimens in the clinical study, and 1,718 assay results were generated during testing (including both initial and repeat runs). The reported overall error rate during the clinical study was 3.4% (59/1,718); the majority of these (>82% [47/59]) were due to IMDx Process Control-A (i.e., the internal control) failures. Assay Positive and Negative Controls were tested with each assay run during the trial. Throughout the course of the study there were a total of 90 runs performed; only three runs failed due to quality control failures (in two of these three runs both the positive and negative controls failed). Therefore, the overall QC run-failure rate was 3.3% (3/90), while the total QC failure rate was 0.3% (5/1,718). The remaining failures (0.5%) were due to instrument issues (7/1,718). Eighteen (18) samples yielded an unresolved error message after repeat testing and were eventually categorized as "Invalid." The rate of failure was not excessive; the assay control strategy adequately evaluated the integrity of the result.

Specimen Stability Studies

The following specimen types were tested:

- Unpreserved (fresh/raw) stool
- Cary-Blair preserved stool

The data provided support that each specimen type was stable when stored under the following conditions:

- Unpreserved (fresh/raw) stool:
  - 7 days at 2°C to 8°C.
  - 60 days at -30°C to -10°C.
- Cary-Blair preserved stool:
  - 4 days at 2°C to 8°C.
  - 60 days at -30°C to -10°C.
- Specimens (both unpreserved and preserved) were stable for one (1) freeze thaw cycle.

d. Detection limit:

The limit of detection (LoD) of the IMDx *C. difficile* for Abbott *m*2000 assay was determined using three strains of toxigenic *C. difficile*. These included VPI10463 (ATCC 43255, toxinotype 0), 1470 (ATCC 43598, toxinotype VIII, *tcdB*-variant), and 4118 (ATCC BAA-1870, toxinotype III, BI/NAP1/027). A range finding study was initially performed, and then six serial 2-fold dilutions around the appropriate concentration were tested. A total of 60 replicates of each dilution were tested to determine the LoD of raw/fresh stool, whereas a total of 20 replicates were tested to determine the LoD of Cary-Blair preserved stool specimens. All dilutions were confirmed by colony counting, and three lots of the IMDx *C. difficile* for Abbott *m*2000 assay were used in the raw/fresh LoD determinations (20 replicates for each lot). The concentration of each strain at which ≥95% of the replicates was detected is as follows:

Strain	Raw/Fresh Stool	Cary-Blair Preserved Stool
ATCC 43255	337 CFU/mL	463 CFU/mL
ATCC 43598	256CFU/mL	861 CFU/mL
ATCC BAA 1870	67 CFU/mL	134 CFU/mL

The LoD for fresh/raw stool specimens was determined to be:

- 337 CFU/mL.

The LoD for Cary-Blair preserved stool specimens was determined to be:

- 861 CFU/mL.

Analytical Reactivity

Thirty-one (31) *C. difficile* strains and/or clinical isolates were tested for reactivity with the IMDx *C. difficile* for Abbott *m*2000 assay. Strains were reported to originate from at least nine states and four countries (USA, UK, Belgium, and Switzerland); 11 toxinotypes were represented (0, II, III, IV, V, VIII, IX, X, XII, XXI, and XXII). Each strain was diluted in buffer plus fecal matrix at 2-3X LoD; three replicates were tested. All strains were detected by the assay, demonstrating that the IMDx *C. difficile* Assay for Abbott *m*2000 can detect a broad range of toxinotypes.

Name	Strain	Toxin type	Origin
ATCC 17857	870	0	Unknown
ATCC 17858	1253	N/A	Unknown
ATCC 43594	W1194	N/A	Human feces; Belgium
ATCC 43596	545	N/A	Human feces; Belgium
ATCC 43599	2022	N/A	Human feces; Belgium
ATCC 43600	2149	N/A	Human feces; Belgium
ATCC 51695	BDMS 18 AN	N/A	Baltimore, MD, USA
ATCC 700792	14797-2	N/A	Human feces; Michigan, USA,
ATCC 9689	90556-M6S	0	Unknown
ATCC BAA-1382	630	X	Switzerland
ATCC BAA-1805	N/A	III	Unknown
ATCC BAA-1871	4111	0	Human; New Jersey, USA
ATCC BAA-1872	4206	0	Human; Maine, USA
ATCC BAA-1873	5283	0	Human; New York, USA
ATCC BAA-1874	4205	0	Human; Oregon, USA
ATCC BAA-1875	5325	V	Human; Georgia, USA
ATCC BAA-2155	LBM 0801058	N/A	Human; New Mexico, USA
ATCC BAA-2156	LBM 0801040	N/A	Human; Cambridge, UK
CCUG 20309	8864	X	Birmingham, UK
ZeptoMetrix NAP-1	Loyola-02	III	Unknown
278 (II) BZ1	N/A	II	Illinois, USA
464 (IV) AN1	N/A	IV	Illinois, USA
4092 (VIII) CF2	N/A	VIII	Illinois, USA
5572 (VIII) CF4	N/A	VIII	Illinois, USA
3430 (IX) AH1	N/A	IX	Illinois, USA
1753 (XII) AL1	N/A	XII	Illinois, USA
5090 (XXI) V2	N/A	XXI	Illinois, USA
3130 (XXII) BW1	N/A	XXII	Illinois, USA
ATCC BAA-1806	N/A	N/A	Unknown
ATCC BAA-1808	N/A	0	Unknown
ATCC BAA-1811	N/A	0	Unknown

e. Analytical specificity:

Cross reactivity was performed using a panel of 120 test organisms to evaluate any cross-reactivity with the IMDx *C. difficile* for Abbott *m*2000 assay. Bacteria were obtained from commercial sources, checked for purity and tested from fresh cultures at a concentration of $\geq 1 \times 10^6$ CFU/mL. Viruses were acquired commercially and tested from frozen stocks at a concentration of $\geq 1 \times 10^5$ TCID50/mL. Nucleic acids were tested at genomic equivalents 1×10^6 copies/mL for bacteria, and $\geq 1 \times 10^5$ $TCID_{50}$/mL for viruses. Samples were initially evaluated in singlicate; if cross reactivity was observed, the organism was retested in replicates of six. All samples

were prepared by diluting microorganisms or DNA into buffer plus fecal matrix. Cross reactivity of *Clostridium botulinum* was analyzed *in silico*.

Organism	Source	Organism	Source
Abiotrophia defective	ATCC 49176	*Enterococcus faecalis vanB*	ATCC 51299
Acinetobacter baumannii	ATCC19606	*Enterococcus faecium vanA*	ATCC 700221
Acinetobacter lwoffi	ATCC17925	*Enterococcus gallinarum vanC*	ATCC 49573
Adenovirus (Type 40)	ZMC 0810084CF	*Enterococcus hirae*	ATCC 8043
Aeromonas hydrophila	ZMC 0601715	*Enterococcus raffinosus*	ATCC 49427
Alcaligenes faecalis subsp. Faecalis	ATCC 15554	Enterovirus (Type 71)	ZMC 0810047CF
Anaerococcus tetradius	ATCC 35098	*Escherichia coli*	ATCC 23511
Bacillus cereus	ATCC 11778	*Escherichia coli O157*	ZMC 0801622
Bacillus cereus	ATCC 13472	*Escherichia fergusonii*	ATCC 35469
Bacteroides caccae	ATCC 43185	*Escherichia hermannii*	ATCC 33650
Bacteroides stercoris	ATCC 43183	*Fusobacterium varium*	ATCC 8501
Bifidobacterium adolescentis	ATCC 15703	*Gardnerella vaginalis*	ATCC 14019
Campylobacter coli	ATCC 43479	*Gemella morbillorum*	ATCC 27824
Campylobacter jejuni subsp.jejuni	ATCC 33292	*Hafnia alvei*	ATCC 13337
Candida albicans	ATCC 10231	*Helicobacter pylori*	ZMC 0601486; Z040
Candida catenulate	ATCC 10565	*Homo sapiens*	ATCC MGC-15492
Cedecea davisae	ATCC 33431	*Klebsiella oxytoca*	ATCC 33496
Chlamydia trachomatis	ZMC D-UW3; Z054	*Klebsiella pneumonia subsp. pneumoniae*	ATCC 13883
Citrobacter amalonaticus	ATCC 25405	*Lactobacillus acidophilus*	ZMC 0601540
Citrobacter freundii	ATCC 8090	*Lactobacillus reuteri*	ATCC 23272
Citrobacter koseri	ZMC 0601745	*Lactococcus lactis subsp. lactis*	ATCC 11454
Citrobacter sedlakii	ATCC 51115	*Leminorela grimontii*	ATCC 33999
Clostridium beijerinckii	ATCC 8260	*Listeria grayi*	ATCC 19120
Clostridium bifermentans	ATCC 638	*Listeria innocua*	ATCC 33090
Clostridium bolteae	ATCC BAA-613	*Listeria monocytogenes*	ZMC 0801543
Clostridium butyricum	ATCC 19398	Norovirus (Type II)	ZMC 0810087CF
Clostridium chauvoei	ATCC 11957	*Peptoniphilus asaccharolyticus*	ATCC 14963
Clostridium difficile (non-toxigenic)	ATCC 43593	*Peptostreptococcus anaerobius*	ATCC 27337
Clostridium difficile (non-toxigenic)	ATCC 43601	*Plesiomonas shigelloides*	ATCC 14029
Clostridium fallax	ATCC 19400	*Porphyromonas asaccharolytica*	ATCC 25260
Clostridium haemolyticum	ATCC 9656	*Prevotella melaninogenica*	ATCC 25845
Clostridium histolyticum	ATCC 19401	*Proteus mirabilis*	ATCC 25933
Clostridium innocuum	ATCC 14501	*Proteus penneri*	ZMC 0601589
Clostridium nexile	ATCC 27757	*Providencia alcalifaciens*	ATCC 9886
Clostridium novyi	ATCC 19402	*Providencia rettgeri*	ATCC 9250
Clostridium orbiscindens	ATCC 49531	*Providencia stuartii*	ATCC 33672
Clostridium paraputrificum	ATCC 25780	*Pseudomonas aeruginosa*	ATCC 35554
Clostridium perfringens	ZMC 0601585	*Pseudomonas putida*	ZMC 0601722
Clostridium ramosum	ATCC 25582	Rotavirus	ZMC MA-104
Clostridium scindens	ATCC 35704	*Ruminococcus bromii*	ATCC 27255
Clostridium sordellii	ATCC 9714	*Salmonella choleraesuis subsp. choleraesuis*	ATCC 7001
Clostridium sphenoides	ATCC 19403	*Salmonella enterica subsp.*	ATCC 14028

		enterica	
Clostridium spiroforme	ATCC 29900	*Salmonella enterica subsp. arizonae*	ATCC 13314
Clostridium sporogenes	ATCC 15579	*Serratia liquefaciens*	ATCC 27592
Clostridium symbiosum	ATCC 14940	*Serratia marcescens*	ATCC 13880
Clostridium tertium	ATCC 14573	*Shigella boydii*	ATCC 9207
Clostridium tetani	ATCC 19406	*Shigella dysenteriae*	ZMC 0601609
Collinsella aerofaciens	ATCC 25986	*Shigella sonnei*	ATCC 29930
Corynebacterium genitalium	ATCC 33798	*Staphylococcus aureus*	ZMC 0601675
Coxsackie virus (Type B4)	ZMC 0810075CF	*Staphylococcus epidermidis*	ATCC 14990
Cytomegalovirus (AD-169)	ZMC 0810003CF	*Stenotrophomonas maltophilia*	ATCC 13637
Desulfovibrio piger	ATCC 29098	*Streptococcus agalactiae*	ZMC 0601545
Echovirus (Type 11)	ZMC 0810023CF	*Streptococcus dysgalactiae*	ATCC 43078
Edwardsiella tarda	ATCC 15947	*Streptococcus intermedius*	ATCC 27335
Eggerthella lenta	ATCC 25559	*Streptococcus uberis*	ATCC 19436
Enterobacter aerogenes	ATCC 13048	*Veillonella parvula*	ATCC 10790
Enterobacter cloacae	ATCC 13047	*Vibrio cholera*	ATCC 25870
Enterococcus casseliflavus	ZMC 0601565	*Vibrio parahaemolyticus*	ATCC 17802
Enterococcus cecorum	ATCC 43198	*Yersinia bercovieri*	ATCC 43970
Enterococcus dispar	ATCC 51266	*Yersinia rohdei*	ATCC 43380

There were two instances where the initial testing resulted in a detected call: non-toxigenic *C. difficile* (ATCC 43601) and *Proteus penneri* (ZMC 0801589). In both cases, none of the additional six replicates tested resulted in a detected call.

In silico analysis showed that the IMDx *C. difficile* for Abbott m2000 assay had no predicted cross-reactivity for *C. botulinum*. The data indicates that there is no cross reactivity when using the organisms tested.

Microbial Interference

To assess if there was any microbial interference with the IMDx *C. difficile* for Abbott *m*2000 assay, the same panel used to assess analytical specificity was added to tubes, each containing one of three strains of toxigenic *C. difficile* in buffer plus fecal matrix. Each strain was tested in triplicate for each panel member; strains were present in the samples at a concentration corresponding to 2-3x LoD. If interference was observed, the organism was retested in replicates of six. The three strains utilized included:

- NAP-1 (ATCC BAA-1870)
- VPI 10463 (ATCC 43255, toxinotype 0), and
- 1470 (ATCC 43598, toxinotype VIII)

In the microbial interference study 118 of the tested strains showed no obvious interference with the initial test. Two organisms, *Clostridium scindens* and *Porphyromonas asaccharolytica*, required retesting. For *Clostridium scindens*, one of the three replicates was not detected for the *C. difficile* ATCC 43598, and there were two instrument errors in the initial run for *C. difficile* ATCC 43255. When the test was repeated with an additional six replicates using *Clostridium scindens* in

combination with the three *C. difficile* test strains, all replicates were detected. For *Porphyromonas asaccharolytica*, two of three replicates were not detected for *C. difficile* ATCC BAA-1870 and one of three replicates were not detected with the *C. difficile* ATCC 43598. When the test was repeated with an additional six replicates, all replicates were detected.

In silico analysis predicted that interfering PCR products would not be produced by the inclusion of *C. botulinum* organisms in a reaction. In summary, no evidence of microbial interference was observed for any of the 118 test organisms included in the analysis.

Potentially Interfering Substances

To evaluate the potential of substances typically found in fecal specimens to interfere with the IMDx *C. difficile* for Abbott *m*2000 assay, three strains of *C. difficile* were tested in the presence of such substances. The strains used included:

- NAP-1 (ATCC BAA-1870)
- VPI 10463 (ATCC 43255, toxinotype 0), and
- 1470 (ATCC 43598, toxinotype VIII)

Substances were diluted in buffer plus fecal matrix to concentrations that would either replicate or exceed the highest concentration expected to be found in a clinical sample. Each of the three *C. difficile* strains were tested in triplicate at 2-3x LoD in the presence of each substance. No interference was observed with any of the substances tested. The test panel consisted of the 23 substances shown in the table below:

Substance	Active Ingredient(s) in Substance	Concentration of Substance Tested
Miconazole nitrate cream	Miconazole Nitrate	2% w/v
Preparation H®	Hydrocortisone	2% w/v
Zinc Oxide	Zinc oxide	40% w/w paste
Vaseline®	Petroleum Jelly	100%
Hemorrhoid gel	Phenylephrine hydrochloride	2% w/v
	Witch Hazel	N/A*
Gaviscon®	Aluminum hydroxide	0.1 mg/mL
	Magnesium carbonate	0.1 mg/mL
TUMS®	Calcium carbonate	0.5 mg/mL
Tagamet®	Cimetidine	0.5 mg/mL
Prilosec® (delayed release)	Omeprazole magnesium	0.5 mg/mL
Mineral Oil	Mineral Oil	2% v/v
Condoms	Nonoxynol-9	7% v/v
Imodium®	Loperamide HCl	0.00667 mg/mL
Pepto Bismol®	Bismuth Subsalicylate	0.87 mg/mL

Substance	Active Ingredient(s) in Substance	Concentration of Substance Tested
ExLax®	Sennosides	0.1 mg/mL
Vancomycin HCl	Vancomycin	12.5 mg/mL
Metronidazole	Metronidazole	14 mg/mL
Aleve®	Naproxen Sodium	14 mg/mL
Moist Towelettes	Benzalkonium Chloride	0.12% w/v
Whole Blood	Glucose, hormones, enzymes, iron, ions, etc	5% v/v
Mucus	Mucin	3 mg/mL
Palmitic acid (fecal fat)	Palmitic acid	2 mg/mL
Stearic acid (fecal fat)	Stearic acid	4 mg/mL
Barium sulfate	Barium sulfate	5 mg/mL

*N/A = not applicable

Target Carryover Study

Five assay runs were performed with alternating high positive and negative samples to assess the potential for cross-over or carryover contamination. A total of 470 specimens were run: 235 high positive and 235 negative specimens. One strains of *C. difficile* was used in this study. A positive signal for both *tcdA* and *tcdB* was detected in one run for a single negative sample; the carryover rate was 0.4% (1/235).

f. Assay cut-off:

The cut-off for the IMDx C. difficile for Abbott *m*2000 assay was determined using Abbott's proprietary *max*Ratio algorithm for real-time PCR data analysis. The *max*Ratio method identifies a consistent point within or very near the exponential region of the PCR signal. Compared to other analysis techniques that generate only a cycle number, the *max*Ratio method generates several measurements of amplification including cycle numbers (CN), relative measures of amplification efficiency and curve shape. By using these values, the *max*Ratio method can achieve a reliable reactive/nonreactive determination along with quantitative evaluation. For a result to be considered reactive, the fluorescence generated must cross a reactive threshold value (reactive threshold settings are based on "*max*Ratio values"). Initial *max*Ratio threshold parameters were based on analysis of results of characterized strains of toxigenic *C.difficile*. A third set included *tcdB*-variant toxigenic strains; however, since *tcdB* and *tcdBv* are labeled with the same fluorescent, the assay cannot differentiate *tcdB* from *tcdBv*. The assay design allows a positive result call to be made if a signal is detected for either the *tcdA* or *tcdB*/*tcdBv* component or both. Validation of threshold parameters came from analysis of 1,107 results (191 positive, and 916 negative) from the clinical study, compiled from five test sites. The results of this analysis are presented below:

Parameter	*tcdA*		*tcdB*	
	Negative *n* = 916	Positive *n* = 191	Negative *n* = 916	Positive *n* = 191
*max*Ratio mean	0.0016	0.217	0.0037	0.093
*max*Ratio St Dev	0.0009	0.046	0.0027	0.020
*max*Ratio Range	0.000 – 0.009	0.035 – 0.280	0.000 – 0.015	0.016 – 0.124
Threshold set	0.010		0.015	
# standard deviations of threshold from mean of negative samples	17.2		4.8	

2. Comparison studies:

   a. Method comparison with predicate device:

   See clinical studies below.

   b. Matrix comparison:

   Not Applicable.

3. Clinical studies:

The performance of the IMDx *C. difficile* for Abbott *m*2000 assay was evaluated using an IRB approved protocol at seven (7) geographically diverse locations within the United States. Specimens were received as either fresh stool or in Cary Blair transport media (preserved). Results from the IMDx *C. difficile* for Abbott *m*2000 assay were compared to results obtained from direct culture followed by testing using an FDA cleared cell cytotoxicity assay. Reference testing was conducted at two sites.

A total of 1,583 specimens, consisting of 1,204 fresh stool (i.e., unpreserved) stool specimens, and 379 specimens Cary Blair transport media (i.e., preserved), were included in the final data set and analyzed for product performance. Eighteen (18) samples yielded an unresolved error message after repeat testing and were categorized as "Invalid;" the assay invalid rate was 1.1% (18/1,583). Therefore, a total of 1,565 valid specimens were included in the final analyses: 1,186 fresh stool specimens and 379 preserved stool specimens were evaluable. Among fresh stool specimens there were 118 concordant positive and 970 concordant negative results; there were also 79 false positive, and 19 false negative results. Among Cary-Blair preserved stool specimens there were 21 concordant positive and 333 concordant negative results; there were also 23 false positive, and 2 false negative results. Discordant specimens were subjected to further analysis by bi-directional sequencing.

There were a total of 123 discordant specimens. Ninety-one (91) of those specimens (73 false positives, 18 false negatives,) were tested additionally to resolve the discordances. Of the 73 false positives, bidirectional sequencing results confirmed the PCR products present in 52 cases to be toxigenic *C. difficile*, 17 samples remained discrepant, and four were indeterminate. Of the 18 false negatives, bidirectional sequencing confirmed the absence of

PCR products in 14 samples, and four remained discrepant. The clinical validation study results are summarized below:

Fresh/Raw (unpreserved) Stool Specimens

IMDx *C. difficile* for Abbott *m*2000 assay vs. Direct Culture.

		Direct Culture		
		POS	NEG	*Total*
IMDx *C. difficile* for Abbott *m*2000	POS	118	79[b]	197
	NEG	19[a]	970	989
	Total	137	1,049	1,186

	95% CI
Sensitivity	86.1% (79.4% – 90.9%)
Specificity	92.5% (90.7% – 93.9%)
Positive Predictive Value	59.9% (52.9% – 66.5%)
Negative Predictive Value	98.1% (97.0% – 98.8%)
Prevalence	11.6%

[a]16 samples were sequenced: 13 were resolved as negative while 3 remained discrepant.

[b]53 samples were sequenced: 40 were resolved as positive, 9 remained discrepant, and 4 had indeterminate results.

Cary-Blair(preserved) Stool Specimens

IMDx *C. difficile* for Abbott *m*2000 assay vs. Direct Culture.

		Direct Culture		
		POS	NEG	*Total*
IMDx *C. difficile* for Abbott *m*2000	POS	21	23[d]	44
	NEG	2[c]	333	335
	Total	23	356	379

	95% CI
Sensitivity	91.3% (73.2% – 97.6%)
Specificity	93.5% (90.5% – 95.7%)
Positive Predictive Value	47.7% (33.8% – 62.1%)
Negative Predictive Value	99.4% (97.8% – 99.8%)
Prevalence	6.1%

[c]2 samples were sequenced: 1 was resolved as negative while 1 remained discrepant.

[d]20 samples were sequenced: 12 were resolved as positive and 8 remained discrepant.

a. Clinical Sensitivity:

See clinical performance studies above.

b. Clinical specificity:

See clinical performance studies above.

c. Other clinical supportive data (when a. and b. are not applicable):

N/A

4. Clinical cut-off:

See discussion in *Assay Cut-off* section above (Section M(1)(f)).

5. Expected values/Reference range:

Expected values by specimen type among prospectively collected specimens:

Age and Gender Distribution of IMDx *C. difficile* for Abbott *m*2000 Assay Positive Results

Age Group	Specimen Type & Gender* # Positive / # Enrolled (Prevalence [%])					
	Raw/Fresh (Unpreserved)			Cary-Blair (Preserved)		
	Male	Female	Total	Male	Female	Total (%)
Unknown age	0/1 (0.0%)	0/1 (0.0%)	0/5[§] (0.0%)	0/0 (0.0%)	0/0 (0.0%)	0/0 (0.0%)
Infant (<2 yrs)	0/4 (0.0%)	2/2 (100.0%)	2/6 (33.3%)	0/3 (0.0%)	0/0 (0.0%)	0/3 (0.0%)
Child (≥2 to <12 yrs)	1/7 (14.3%)	1/9 (11.1%)	2/16 (12.5%)	2/6 (33.3%)	0/3 (0.0%)	2/9 (22.2%)
Adolescent (≥12 to <18 yrs)	0/8 (0.0%)	1/6 (16.7%)	1/14 (7.1%)	0/2 (0.0%)	0/4 (0.0%)	0/6 (0.0%)
Transitional Adolescent (≥18 to ≤21 yrs)	2/7 (28.6%)	3/15 (20.0%)	5/22 (22.7%)	2/4 (50.0%)	1/8 (12.5%)	3/12 (25.0%)
Adult (>21 to 59 yrs)	38/273 (13.9%)	40/297 (13.5%)	78/571[†] (13.7%)	11/82 (13.4%)	8/105 (7.6%)	19/187 (10.2%)
Sr. Adult (> 60 yrs)	47/242 (19.4%)	62/310 (20.0%)	109/552 (19.7%)	9/68 (13.2%)	11/94 (11.7%)	20/162 (12.3%)
Total	**88/542 (16.2%)**	**109/640 (17.0%)**	**197/1,186 (16.6%)**	**24/165 (14.5%)**	**20/214 (9.3%)**	**44/379 (11.6%)**

*Prevalence based on *C. difficile* positives with the IMDx *C. difficile* for Abbott *m*2000 assay.

[§]The gender of three individuals in this age group was not known.

[†]The gender of one individual in this age group was not known.

N. Instrument Name:

Abbott *m*2000 System running *C. difficile* (cDiff) Application Specification (App Spec) Parameter Definitions v1.15:

- Abbott *m*2000sp: sample preparation and reagent mixing (version 3.0.21.0)
- Abbott *m*2000rt: amplification reaction and detection (version 3.0.338.0).

O. System Descriptions:

1. Modes of Operation:

   The Abbott *m*2000 System is an instrument platform that automates steps to perform nucleic acid amplification assays from sample processing through amplification, detection, and data reduction. The Abbott *m*2000 System comprises the *m*2000*sp* and *m*2000*rt* instruments, which are operated with separate System Control Center (SCC) workstations. Each instrument contains an independent software application; one for the *m*2000*sp* and a second for the *m*2000*rt*. The *m*2000*sp* instrument is a floor standing, automated sample preparation system. The *m*2000*rt* instrument is a real-time PCR thermal cycler/reader instrument system. Abbott Molecular is the manufacturer of the *m*2000 System. The principal hardware components that comprise the *m*2000*sp* and *m*2000*rt* were developed by Original Equipment Manufacturer vendors, Tecan Schweiz AG, Mannedorf, Switzerland, and Applied Biosystems, Foster City, CA, respectively. Abbott Molecular developed the software that is uniquely for use with the *m*2000 System. The Abbott *m*2000 System software processes sample preparation and amplification/detection protocols based on pre-determined, assay-specific parameters that are contained in individual assay application specification (App Spec) files that are installed on the SCC. The Abbott *m*2000*sp* reads and processes bar coded primary sample tubes and processes up to 96 specimens, controls, and calibrators in batch mode. The *m*2000 System is capable of processing samples from various matrices, depending on the specific assay application. At the completion of the automated sample preparation protocol, the operator seals and manually transfers the PCR plate to the Abbott *m*2000*rt* for nucleic acid detection. Bar code and *m*2000*sp* data is transferred to the *m*2000*rt* electronically via removable media (i.e., a CD).

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ___X____ or No _____

3. Specimen Identification:

   Specimen identification occurs on the Abbott *m*2000*sp* instrument when it is loaded with specimen tubes.

4. Specimen Sampling and Handling:

   Fresh/raw (i.e., unpreserved) human liquid or soft stool specimens collected and transported to the laboratory are used for testing. Cary-Blair (i.e, preserved) or frozen human liquid or soft stool samples collected and transported to the laboratory in a sterile container and sampled for testing. Specimen processing is automated on the Abbott *m*2000*sp*.

5. Calibration:

   There is no calibration procedure associated with the IMDx *C. difficile* for Abbott *m*2000 assay *per se*. Optical calibration of the Abbott *m*2000*rt* instrument is required for the accurate measurement and discrimination of dye fluorescence during the IMDx *C. difficile* for Abbott *m*2000 assay; the Calibration Procedures section in the Abbott *m*2000*rt* Operations Manual describes how to perform an optical calibration.

6. Quality Control:

   N/A

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

N/A

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.