510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K132726

B. Purpose for Submission:

To obtain a substantial equivalence determination for a new device

C. Measurand:

Clostridium difficile toxin B gene (*tcdB*)

D. Type of Test:

PCR based amplification with capillary electrophoresis based detection of amplification products

E. Applicant:

PrimeraDx

F. Proprietary and Established Names:

ICEPlex® *C. difficile* Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3130, *Clostridium difficile* toxin gene amplification assay

2. Classification:

   II

3. Product code:

   OZN, NSU

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The PrimeraDx ICEPlex® C. *difficile* Assay is for the qualitative detection of the *Clostridium difficile* toxin B gene *(tcdB* gene) in nucleic acids purified from unpreserved liquid or soft human stool specimens from patients suspected of having C. *difficile* infection (CDI).

   The ICEPlex C. *difficile* Assay is intended to be used only on the ICEPlex® System, which integrates PCR-based amplification with capillary electrophoresis (CE) for the detection of amplification products. The assay is intended to aid in the diagnosis of CDI. Results should be considered in conjunction with patient clinical history.

2. Indication(s) for use:

   The PrimeraDx ICEPlex® C. *difficile* Assay is for the qualitative detection of the *Clostridium difficile* toxin B gene *(tcdB* gene) in nucleic acids purified from unpreserved liquid or soft human stool specimens from patients suspected of having C. *difficile* infection (CDI).

   The ICEPlex C. *difficile* Assay is intended to be used only on the ICEPlex® System, which integrates PCR-based amplification with capillary electrophoresis (CE) for the detection of amplification products. The assay is intended to aid in the diagnosis of CDI. Results should be considered in conjunction with patient clinical history.

3. Special conditions for use statement(s):

   For prescription use

4. Special instrument requirements:

   ICEPlex® System

   bioMérieux NucliSENS easyMAG™ System

I. Device Description:

The ICEPlex System combines two functional modules: an amplification module – PCR (Polymerase Chain Reaction) thermal cycler – and an analysis module – CE (Capillary Electrophoresis) system with fluorescent detection. Individual fluorescent PCR products from multiplexed PCR reactions are analyzed by CE through direct electrokinetic

injection into the separating capillaries. The labeled amplicons are separated by size and the dyes are excited by two lasers within the system.

The ICEPlex *C. difficile* Assay kit contains sufficient reagents to perform 100 tests and includes the following components:

• 2X PCR Buffer: Tris buffer, KCl, dNTPs, MgCl2, betaine, BSA, DTT, glycerol and Proclin.

• PCR Enzyme: Hot-start DNA polymerase

• 25X Primers Mix: Oligonucleotide primers specific to the *tcdB* gene of *C. difficile*, Internal Control and Calibration Controls. Each primer is either not labeled or has FAM or TYE-665 fluorescent label. The mix also contains salmon sperm DNA, BSA and Proclin.

• 25X Calibrators Mix: A liquid concentrate containing DNA template for Calibration Controls.

• Internal Control: Non-infectious synthetic DNA.

• 10X Injection Buffer: A liquid used by diluting 1:10 to fill those wells in the PCR plate which are not being used for samples.

• Positive Control: A liquid concentrate containing DNA template for PCR Positive Controls.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   BD MAX C. diff Assay

2. Predicate 510(k) number(s):
   K130470

3. Comparison with predicate:

Similarities		
Item	Device ICEPlex® C. difficile Assay	Predicate BD MAX™ *C.diff* Assay (K130470)
Intended Use	The PrimeraDx ICEPlex® C. *difficile* Assay is for the qualitative detection of the *Clostridium difficile* toxin B gene *(tcdB* gene) in nucleic acids purified from unpreserved liquid or soft human stool specimens from patients suspected of having C. *difficile* infection (CDI). The ICEPlex C. *difficile* Assay is intended to be used only on the ICEPlex® System, which integrates PCR-based amplification with capillary electrophoresis (CE) for the detection of amplification products. The assay is intended to aid in the diagnosis of CDI. Results should be considered in conjunction with patient clinical history.	The BD MAX *C. diff* Assay performed on the BD MAX System is an automated in vitro diagnostic test for the direct, qualitative detection of the *Clostridium difficile* toxin B gene (tcdB) in human liquid or soft stool specimens from patients suspected of having *C. difficile* infection (CDI). The test, performed directly on the specimen, utilizes real-time polymerase chain reaction (PCR) for the amplification of *C. difficile* toxin B gene DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The BD MAX C. diff Assay is intended to aid in the diagnosis of CDI.
Measureand	*Clostridium difficile* toxin B gene (*tcdB)*	Same
Specimen Type	Unformed (liquid or soft) stool	Same
Principle	Real time PCR	Same

Differences		
Item	Device ICEPlex® C. difficile Assay	Predicate BD MAX™ *C.diff* Assay (K130470)
Instrument System	ICEPlex System	BD MAX System
Sample Extraction	bioMérieux NucliSENS easyMAG	Integrated
Assay Amplification/ Detection Format	Real time PCR amplification with Capillary Electrophoresis and direct laser-induced fluorescent detection and size based detection of target	Real time PCR with fluorescent detection of target specific labeled TaqMan probes

Differences		
Item	Device ICEPlex® C. difficile Assay	Predicate BD MAX™ *C.diff* Assay (K130470)
	specific labeled primers	
Time to Result	Four hours for 48 reactions	Under three hours for 24 reactions
Results Interpretation	Positive, Negative, Invalid with error code	Positive, Negative, Unresolved, Indeterminate with error code, Incomplete Run with error code
Assay Controls	Calibration Control, Internal Control, and Positive PCR Control provided. Negative Control and Positive Extraction Control supplied by user	Sample Processing Control provided. Positive and Negative Controls recommended supplied by user

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

ISO 15233-1:2007, Medical devices – Symbols to be used with medical device labels, labeling and information to be supplied

ISO 14971 second edition 2007-0301, Medical devices – Application of risk management to medical devices

AAMI / ANSI / IEC 62304:2006, Medical device software – Software life cycle processes

EN 61326-1:2006 Electrical equipment for measurement, control and laboratory use. EMC requirements.

UL 61010-1:2004, 2nd Ed. Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory User Part 1

CAN/CSA-C22.2 No. 61010-1-04:2001 Safety Requirements for Electrical Eq.1- for Measurement, Control, and Lab. Use Part 1

CAN/CSA-C22.2 No. 61010-2-101-04:2002 Safety Requirements for Electrical Equipment for Measurement, Control, and Lab. Use

EN 55011:2007, Industrial, scientific and medical (ISM) radio-frequency equipment – Electromagnetic disturbance characteristics

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

L. Test Principle:

The ICEPlex *C. difficile* Assay is a molecular diagnostic test for the qualitative detection of toxigenic *C. difficile* nucleic acids isolated and purified from liquid or soft stool specimens. This test targets the *C. difficile* toxin B encoding gene (*tcdB*).

The PrimeraDx ICEPlex system uses Scalable Target Analysis Routine (STAR) technology. STAR employs sequential sampling and analysis of fluorescently end-labeled PCR products (amplicons) by means of Capillary Electrophoresis (CE). Separation of the amplicons is facilitated by appropriate primer design allowing for discrimination by size and fluorescent signal after CE separation. In a molecular diagnostic assay performed on an ICEPlex system, all targets (including sample template, controls and internal calibration standards) are independently assessed within a single reaction well.

Real-time sampling of the amplicons allows for the construction of amplification curves and calculation of threshold cycle (Ct) similar to other real-time PCR methods.

To run the ICEPlex *C. difficile* Assay, nucleic acids are first extracted from stool specimens with the bioMérieux NucliSENS easyMAG system. An ICEPlex system PCR plate is assembled combining PCR buffer, PCR enzyme, primer mix containing fluorescently end-labeled oligonucleotide primers for assay controls and targets, calibration controls mix, and purified DNA from the clinical sample spiked with internal control. The PCR plate is placed in the thermal cycler module of the ICEPlex system and, after inputting sample and assay information into the integrated software, the run is initiated.

At specific PCR cycles, the capillaries and electrodes of capillary electrophoresis module are introduced into the PCR reaction. The ICEPlex system applies voltage for a predetermined time to force negatively charged DNA molecules to enter the capillary ─ a process known as electrokinetic injection. The capillaries and electrodes are then moved and are immersed in a CE buffer, voltage is applied, and capillary electrophoresis separation is performed. The PCR cycling and CE separation are timed to match one complete CE separation within two PCR cycles. All amplicons (including internal calibration controls) are separated, detected and quantified by CE using laser induced fluorescence detection. At the end of the PCR amplification, capillaries and electrodes are chemically decontaminated to remove the residual amplified PCR fragments and to recondition the capillary array for a new assay run.

The ICEPlex system software automatically processes the raw data and reports positive, negative, or invalid results for the detection of the *C. difficile tcdB* gene target.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   The within-laboratory precision was estimated on a panel of samples prepared by

spiking an appropriate amount of *C. difficile* culture strain BAA-1805 into a pooled clinical negative stool matrix. The blinded panel members included: low positive sample (near assay limit of detection, expected positive >95% of the time), moderately positive sample (expected to be positive approximately 100% of the time), negative sample (expected to be negative approximately 100% of the time), and a C20-80 sample (expected to be positive 20 – 80% of the time). The panel also included positive and negative controls.

The precision study consisted of two operators; three lots of ICEPlex *C. difficile* Assay kits; and three ICEPlex instruments. The precision study was run over 12 non-consecutive days with two runs per day and two replicates of each sample per run. One negative sample (out of a total of 144 observations) and one low positive sample (out of a total of 144 observations) produced invalid results during the study. Overall results are summarized in the following table.

Sample Concentration	Overall Agreement		Ct Value		
	n	%	Mean Ct	SD	%CV
Negative [1]	143/143	100%	N/A	N/A	N/A
Low Positive	142/143	99%	30.6	1.4	4.5
Moderate Positive	144/144	100%	28.7	0.8	2.7
C20-80 [2]	105/144	73%	34.6	2.1	6.2

[1] Ct values for negative samples calculated using Internal Control
[2] C20-80 sample percent agreement given as percent positive results

Sample	Lot 1					Lot2					Lot 3				
	Agreement		Ct Value			Agreement		Ct Value			Agreement		Ct Value		
	n	%	Mean	SD	%CV	n	%	Mean	SD	%CV	n	%	Mean	SD	%CV
Negative	84/84	100	N/A	N/A	N/A	36/36	100	N/A	N/A	N/A	23/23	100	N/A	N/A	N/A
Low Positive	83/83	100	30.4	0.9	2.9	36/36	100	30.5	1.2	3.9	23/24	96	31.3	2.5	7.9
Moderate Positive	84/84	100	28.6	0.7	2.5	36/36	100	28.8	1.0	3.3	24/24	100	28.9	0.7	2.5
C20-80	58/84	69	34.6	2.2	6.2	33/36	92	34.1	2.0	6.0	14/24	58	35.1	2.2	6.2

Between lot, between operator, between instrument, and between day repeatability were acceptable.

Reproducibility:

Reproducibility was evaluated at three independent laboratory sites using a study panel with the same strain and concentrations as described for the precision study. Panel samples were tested at each independent laboratory site by two operators for five days with two runs per day and three replicates of each panel member per run. One low positive sample (out of a total of 90 observations) and one moderate positive

sample (out of a total of 90 observations) produced invalid results during the study. Study results are summarized in the tables below.

	n	% Agreement	95% CI
Negative	90/90	100	95.98 to 100
Low Positive	89/89	100	95.98 to 100
Moderate Positive	89/89	100	95.98 to 100
C20-80*	62/90	69	58.26 to 78.23

Site to site reproducibility

	Site A		Site B		Site C	
	n	% Agreement	n	% Agreement	n	% Agreement
Negative	30/30	100	30/30	100	30/30	100
Low Positive	30/30	100	29/29	100	30/30	100
Moderate Positive	30/30	100	30/30	100	29/29	100
C20-80*	23/30	77	23/30	77	16/30	53

Ct values

	Site A			Site B			Site C		
	Average	SD	% CV	Average	SD	%CV	Average	SD	%CV
Negative	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Low Positive	28.1	0.3	1.1	28.5	0.3	1.1	27.7	0.1	0.4
Moderate Positive	24.5	0.3	1.2	24.6	0.3	1.2	24.0	0.3	1.3
C20-80	34.5	1.8	5.1	34.4	1.8	5.3	34.4	2.1	6.1

Between site, between operator, between run, and between day reproducibility results were acceptable.

b. Linearity/assay reportable range:

Not applicable

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

In-reaction controls:

Calibration control: A group of ICEPlex specific elements used to align electropherograms and assign identities of the target peaks. It also controls for the integrity of the kit reagents and the presence of PCR inhibitors.

Internal Control: Non-target nucleic acid that is co-extracted and co-amplified with the *tcdB* target. It controls for nucleic acid extraction efficiency, for the integrity of the reagents and for the presence of PCR inhibitors in a given sample. Detection of the Internal Control is not required for a positive result. The Internal Control needs to be spiked into each sample before extraction.

External controls:

Negative Control: Substitute Stool Transport and Recovery (S.T.A.R., Roche Diagnostics Coporation) buffer for the clinical specimen and process normally through the easyMAG extraction system and on the ICEPlex Instrument.

Positive Control: This control is used for the PCR stage only. It should not be processed for nucleic acids extraction. In the positive control reaction, substitute extracted sample with provided Positive Control. Note: Provided Positive Control must be diluted 1:10 prior to use to ensure target concentration is at the appropriate level.

Known Positive Sample: It is also required to include a previously characterized positive sample or simulated sample with every easyMAG extraction run and include it in the subsequent ICEPlex Instrument run to verify successful lysis.

Sample stability:

Sample stability studies were conducted using *C. difficile* strains spiked into negative stool matrix at low positive, moderate positive, and below LoD concentrations. Sample stability was evaluated for fresh sample storage at 2 - 8°C, for fresh versus frozen storage at -70°C +/- 10°C, and for multiple freeze/thaw cycles at -70°C +/- 10°C. The results support sample storage at 2 - 8°C for 48 hours, at -70°C +/- 10°C for 15 months, and for one freeze thaw cycle at -70°C +/- 10°C. More than one freeze thaw cycle is not recommended as it may lead to a higher rate of invalid results.

d. Detection limit:

Analytical sensitivity of the ICEPlex *C. difficile* Assay was determined using a two-fold serial dilution of two *C. difficile* strains that were spiked into qualified negative stool and processed according to ICEPlex *C. difficile* Assay Instructions for Use.

Twenty replicates at each concentration level were tested on three different ICEPlex instruments. Analytical sensitivity of the assay was defined as the lowest concentration at which at least 95% of all replicates were reported positive. The LoD was validated by testing an additional 20 replicates at the target concentration.

LoD of the ICEPlex *C. difficile* assay determined for:

- *C. difficile* strain 43255 (630) (Toxinotype 0): 8CFU/rxn
- *C. difficile* strain BAA-1805 (Toxinotype III): 2CFU/rxn

LoD study results are acceptable.

Analytical reactivity/ inclusivity

To assess the analytical inclusivity of the ICEPlex *C. difficile* Assay, a set of 20 additional toxigenic strains of *C. difficile* were spiked into pooled negative matrix at a level approximately three times above the assay LoD. Spiked samples were processed in accordance with the ICEPlex *C. difficile* Assay Instructions for Use. Two samples out of a total of 81 samples tested produced invalid assay results. The results of the study are as follows:

Strain	Toxinotype	Result
BAA-1382 (630)	A+B+	Positive
BAA-1871 (4111)	0, A+B+binary-, NAP5	Positive
9689 (90556-M6S)	0	Positive
700792 (14797-2)	A+B+	Positive
BAA-1875 (5325)	V, A+B+, NAP7	Positive
51695 (BDMS 18 AN)	A+B+	Positive
43598 (1470)	VIII, A-B+	Positive
43600 (2149)	A+B+	Positive
43599(2022)	A+B+	Positive
43597	A+B+	Positive
43594 (W1194)	A+B+	Positive
43596 (545)	I, A+B+	Positive
17858 (1253)	A+B+	Positive
17857 (870)	A+B+	Positive
BAA-1808	A+B+	Positive
BAA-1806	A+B+	Positive
BAA-1803	III A+B+, NAP1	Positive
BAA-1870 (4118)	III, binary+, NAP1	Positive
BAA-1873 (5283)	0, A+,B+,binary-	Positive

Note: Strain BAA-1814 (Toxinotype XXII) was determined to be non-viable. PrimeraDx cannot claim inclusivity to this strain.

The inclusivity study results are acceptable.

e. Analytical specificity:

Cross reactivity:

A microbial cross reactivity study was performed with the ICEPlex *C. difficile* Assay using a panel of samples spiked into pooled negative clinical matrix or contrived low positive *C. difficile* samples. Three samples produced invalid results on the initial run. The following potential cross-reactive bacteria and viruses were tested at $1x10^7$ organisms/mL and $1x10^6$ $TCID_{50}$/mL, respectively:

Abiotrophia defectiva, Acinetobacter baumannii, Aeromonas hydrophila, Bacillus cereus, Bacteroides fragilis, Bifidobacterium adolescentis, Campylobacter coli, Campylobacter jejuni subsp. jejuni,Candida albicans, Citrobacter freundii, Clostridium beijerinckii, Clostridium bifermentans, Clostridium chauvoei, Clostridium difficile 43593, Clostridium difficile 43601, Clostridium difficile 43602, Clostridium difficile 700057, Clostridium difficile BAA-1801, Clostridium haemolyticum, Clostridium histolyticum, Clostridium novyi, Clostridium orbiscindens, Clostridium perfringens, Clostridium scindens, Clostridium septicum, Clostridium sordellii, Clostridium sporogenes, Clostridium symbiosum, Clostridium tetani, Edwardsiella tarda, Enterococcus dispar, Enterobacter cloacae, Entamoeba histolytica, Enterococcus faecium vanA, Enterococcus faecalis vanB, Escherichia coli, Escherichia coli (serotype O157:H7), Escherichia fergusonii. Escherichia hermannii, Helicobacter pylori, Klebsiella pneumoniae, Lactobacillus acidophilus, Lactococcus lactis, Listeria grayi, Listeria monocytogenes, Peptostreptococcus anaerobius, Plesiomonas shigelloides, Porphyromonas asaccharolytica, Proteus mirabilis, Providencia alcalifaciens, Pseudomonas aeruginosa, Salmonella enterica serovar Typhimurium, *Salmonella enterica* subsp. arizonae, *Salmonella enterica* subsp. enterica, *Serratia liquefaciens, Serratia marcescens, Shigella boydii, Shigella dysenteriae, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Vibrio cholerae, Vibrio parahaemolyticus, Yersinia enterocolitica,* Adenovirus, Rotavirus, Norovirus, Enterovirus, Echovirus, Coxsackie virus, and Cytomegalovirus.

Cross reactivity was observed with *Clostridium sordellii* as expected due to the high sequence similarity between the *C. sordellii* toxin gene and the *C. difficile tcdB.*

The non-toxigenic *C. difficile* strain 43601 tested positive in the ICEPlex *C. difficile* Assay at a value that was just above the assay cutoff in one out of three replicates.

None of the other tested organisms reacted with the ICEPlex *C. difficile* Assay. None of the tested organisms interfered with the detection of low positive toxigenic *C. difficile* samples. The cross reactivity study and microbial interference study results are acceptable.

Interference study:

A chemical interference study was conducted using a panel of contrived samples consisting of pooled negative clinical matrix and matrix spiked with one of two strains of *C. difficile* (ATCC BAA-1805 or 43255) at 9 or 21 CFU/reaction, respectively (approximately three times the assay LoD). The following potentially interfering substances were tested:

Substance	Active Ingredient	Concentration
Anti-Fungal/ Anti-Itch Vaginal	Nystatin	1% (w/v)
Creams/ Ointments/ Suppositories	Hydrocortisone	1% (w/v)
Anti-Hemorrhoid Creams/ Ointments	Phenylephrine	1% (w/v)
Antacids	Calcium Carbonate/ Aluminum Hydroxide/ Magnesium Hydroxide	10% (w/v)
Enemas	Mesalazine/ Mineral Oil	10% (w/v)
Spermicidal Lubricant	Nonoxynol-9	1% (w/v)
Anti-Diarrheal Medication	Loperamide Hydrochloride/ Bismuth Subsalicylate	10% (w/v)
Laxatives	Sennosides	1% (w/v)
Antibiotic	Metronidazole	12.5 mg/ml
Antibiotic	Vancomycin	12.5 mg/ml
Non-Steroidal Anti-Inflammatory Medications	Naproxen Sodium	12.5 mg/ml
Moist Towelettes	Benzalkonium Chloride, Ethanol	0.1% (v/v), 1% (v/v)
Fecal Fat	Lipids, etc.	40% w/v
Whole Blood	Glucose, Hormones, Enzymes, Ions, Iron, etc.	40% v/v
Mucus	Mucin protein	3.5% (w/v)

None of the substances produced a false positive or false negative result with the ICEPlex *C. difficile* Assay at the concentrations tested. Results of the study indicated that samples with high levels of fecal fat may produce elevated rates of invalid results.

The chemical interference study results are acceptable.

Well-to-well Cross Contamination and Run-to-run Carryover Contamination:

Samples for Well-to-well Cross Contamination and Run-to-run Carryover Contamination study were prepared by extracting a series of high positive samples (with analyte concentration exceeding the concentration found in 95% positive samples in the intended use population) alternating with negative samples. Location of positive and negative samples on the extraction instrument was altered run-to-run. On the ICEPlex system, high positive and negative samples were run in a checkerboard fashion. In the consecutive run, the plate map was inverted to allow wells and capillaries that were running negative samples to run positive samples. The study included 6 runs: each run had 24 high positive and 24 negative samples for a total of 144 samples for all runs. The overall invalid rate was 2%. The study identified no well-to-well or run-to run contamination in the negative samples (0 false positives out of 141 valid negatives).

The well-to-well cross contamination and run-to-run carryover contamination study results are acceptable.

f. Assay cut-off:

A panel of 43 *C. difficile* positive and 45 negative clinical samples from the intended use population were collected and tested with the ICEPlex *C. difficile* Assay. Numerical readout data was extracted from the ICEPlex system, and Receiver Operator Curve (ROC) analysis was applied to the data. An ROC specificity/ sensitivity decision plot was used to determine the cut-off level with optimal assay sensitivity and specificity. The cut-off value was determined at 12 copies per reaction.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

A prospective clinical study was conducted at three independent sites to compare performance of the ICEPlex *C. difficile* Assay Kit on the ICEPlex System to toxigenic *C. difficile* direct culture. This study tested specimens from patients suspected of gastrointestinal tract infection with toxigenic strains of *C. difficile* bacteria. Each of

the sites tested the ICEPlex *C. difficile* Assay on specimens collected at the site. The specimens collected from all three sites were tested by toxigenic *C. difficil*e direct culture at a single reference laboratory site. A total of 1103 (806 fresh, 297 frozen) samples were collected and enrolled. 97 frozen samples were excluded from the analysis due to improper storage conditions and temperature excursions. An additional 37 samples were excluded due to study protocol deviations. 969 specimens were compliant and met all protocol requirements. 952 out of 969 (98.2%) gave reportable results and were included in the statistical analysis. 68 out of 969 compliant samples were invalid upon initial testing (7.0% initial invalid rate), and 17 of these 68 samples remained invalid upon retest (1.8% invalid rate). Three out of the 17 unresolved invalids were reported positive by direct culture. Results were reported in percent agreements as performance was compared to direct culture. The Clopper-Pearson exact method was used to calculate confidence intervals.

Age demographics of the study population:

Age Group (years)	n (%)
2 – 5	77 (8.1%)
6 - 21	261 (27.4%)
22 - 59	283 (29.7%)
>=60	331 (34.8%)

The ICEPlex *C. difficile* Assay demonstrated the following performance characteristics:

		Direct Culture		
		Positive	Negative	Total
ICEPlex *C. difficile* Assay	Positive	153	20[a]	173
	Negative	17[b]	762	779
	Total	170	782	952

Positive Percent Agreement, PPA (95% CI)	90.0% (84.5 - 94.1)
Negative Percent Agreement, NPA (95% CI)	97.4% (96.1 - 98.4)

Discordant testing was performed for samples where ICEPlex *C. difficile* Assay and toxigenic *C. difficile* direct culture reported results in disagreement. Discordant analysis included microbiological isolation and PCR targeting of three appropriate regions of the toxin B gene (different recognition sites than the ones used in the ICEPlex *C. difficile* assay) with bi-directional DNA sequencing.

[a] 6 of 20 reported positive by ICEPlex *C. difficile* Assay were reported positive by discordant analysis.

[b] 14 of 17 reported negative by ICEPlex *C. difficile* Assay samples were reported

positive by discordant analysis.

Comparison by Site

	Site A	Site B	Site C
PPA (95% CI)	49/ 55 = 89.1 (77.8 - 95.9)	56/ 64 = 87.5 (76.8 - 94.4)	48/ 51 = 94.1 (83.8 - 98.8)
NPA (95% CI)	281/ 288 = 97.6 (95.1 - 99.0)	256/ 262 = 97.7 (95.1 - 99.2)	225/ 232 = 97.0 (93.9 - 98.8)

The clinical performance study results are acceptable.

*b. Clinical specificity**:***

See section M3a

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

Not applicable

4. Clinical cut-off**:**

See section M1f

5. Expected values/Reference range:

The ICEPlex *C. difficile* Assay clinical study was conducted at three different US clinical site locations with a total of 952 reportable results. The number and percentage of positives by ICEPlex *C. difficile* assay is listed in the following table.

		ICEPlex C. *difficile* Assay		
Groups	Total Number of Specimens	Number Positive	Number Negative	Positive Percentage
Male	458	86	372	18.78% (86/458)
Female	494	87	407	17.61% (87/494)
Total	952	173	779	18.17% (173/952)

N. Instrument Name:

PrimeraDx ICEPlex system

O. System Descriptions:

1. Modes of Operation:

To perform the test, liquid or soft stool is collected in a standard sterile container that can be sealed. Samples can be stored cold for up to 48 hours prior to testing or frozen at -70°C or below if not processed within 48 hours. Sample extraction is performed using bioMérieux NucliSENS easyMAG System as per the ICEPlex *C. difficile* Assay instruction for use. PCR reagent master mix is prepared using primer mix, PCR enzyme, PCR buffer and calibrators. In a designated well of the PCR plate, 40 µL of ICEPlex *C. difficile* Master Mix is aliquoted and 10 µL of extracted sample is added. Negative and positive controls are added in designated wells of the PCR plate. After the PCR plate is prepared, it is placed in the ICEPlex System to execute an instrument run for the detection of the *Clostridium difficile* toxin B gene (*tcdB* gene).

The PrimeraDx ICEPlex system employs sequential sampling and analysis of fluorescently labeled PCR products (amplicons) by means of Capillary Electrophoresis (CE). Separation of the amplicons is facilitated by appropriate primer design allowing for accurate discrimination by size and fluorescent signal after CE separation. This analysis method supports multiplexing in a single reaction well since all targets (including sample template, controls and internal calibration standards) can be detected from a single reaction well.

Real-time sampling of the amplicons is used for the construction of amplification curves and calculations of threshold cycle (Ct) similar to other real-time PCR methods.

To run an assay on the ICEPlex system, a PCR plate is assembled and placed in the thermal cycler module of the ICEPlex system and, after manually inputting sample and assay information into the integrated software, the run is initiated.

At specific PCR cycles, the capillaries and electrodes of the capillary electrophoresis module are introduced into the PCR reaction. The ICEPlex system applies voltage for a predetermined time to force negatively charged DNA molecules to enter the capillary ─ a process commonly known as electrokinetic injection. The capillaries and electrodes are then moved and are immersed in a CE buffer, voltage is applied, and capillary electrophoresis separation is performed. The PCR cycling and CE separation are timed to match one complete CE separation within two PCR cycles. All amplicons (including internal calibration controls) are separated, detected and quantified by CE using laser induced fluorescence detection. At the end of the PCR amplification, capillaries and electrodes are chemically decontaminated to remove the residual amplified PCR fragments and to recondition the capillary array for a new assay run.

2. Software:

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

Yes ___X_____ or No _____

Level of Concern:

Moderate

Software Description:

The ICEPlex System is a dual mode system with Combined Functionality that can be run in IVD mode or OPEN mode. The sponsor states that the software architecture, user interface, and instrument design prevent interference of the OPEN functionality with the IVD functionality.

The software designed and distributed by PrimeraDx for operation of the ICEPlex instrument is broken down into 4 core components:

- **User Application (UA):** customer facing software responsible for converting user requests into hardware control.
- **Instrument Control:** responsible for all communication with the hardware. initiating complex automated operations
- **Embedded Software:** responsible for controlling hardware.
- **Data Analysis:** responsible for converting raw data into results.

All software runs natively on a PC embedded within the ICEPlex hardware. This PC runs an OEM copy of Windows XP SP3 with at least 3GB of memory. The software is developed with Windows XP, Java 6, and Microsoft Visual Studio and C++ compiler. The off-the-shelf software runs on an ICEPlex instrument containing a Core 2 quad processor, running at 2.66 GHZ with PC10600 2GB, DDR3, 1333MHZ RAM, and a 320GB, SATA 2.5" disk drive, with a 2x Intel gigabit Ethernet board, and a Critical Link Camera- S7031-1007/28. The instrument peripherals consist of a 104 keys keyboard, an optical mouse, a 19" LCD touchscreen display, and barcode scanner. The end-user is prevented from making any changes to the ICEPlex Instrument software.

The software description is acceptable.

Device Hazard Analysis (DHA): Acceptable

PrimeraDx provided an acceptable DHA for the ICEPlex Analyzer instrument software. Before mitigation, the sponsor identified 10 hazards: one was considered high (potential of serious injury or death); eight were considered medium (potential of injury), and one was considered to be low (little or no potential of injury). All 10 hazards describe appropriate mitigations. The one hazard with a high degree of risk was originally described as having a low probability of occurrence; following mitigation, this was considered very low. The sponsor provided an acceptable tabular description of identified hardware and software hazards, including severity assessment and mitigations.

Architecture Design Chart: Acceptable

PrimeraDx provided acceptable flow charts showing detailed depiction of functional units and software modules.

Software Specifications: Acceptable

Software Requirements Specifications

PrimeraDx provided SRS documentation which includes a software architecture chart that describes the required software structure and content of each software module implemented. A total of 1,422 requirements were identified. These descriptions included Hardware Requirements, Interface Requirements, Software Performance and Functional Requirements for the ICEPlex Analyzer Instrument. The submitted description is acceptable.

Software Design Specifications

PrimeraDx design specification and software development environment are documented. The detailed software description, hardware platform, development environment, and detailed information for software component are acceptable. The sponsor provided adequate SDS descriptions to show how the requirements in the SRS are implemented. The submitted description is acceptable.

Development Environment: Acceptable

The software was developed using Windows XP, Java 6, Microsoft Visual Studio and C++ compiler. The sponsor also described the non-modifiable libraries which are used for the GUI/program interface and object management and creation.

Traceability Matrix: Acceptable

The detailed Software Trace Matrix of the ICEPlex *C. difficile* was provided as well as the System Risk Assessment establishing a relationship between identified software risks and their mitigation actions. PrimeraDx provided comprehensive information showing traceability between Requirements, Design, and Software Validation.

Verification and Validation Testing: Acceptable

PrimeraDx provided sufficient documentation for V&V testing, including all executed software verification procedures and reports for the 10.7.3 software version. The System Level Functioning Testing protocol and report were also provided. Software Validation Activities were completed per System Level Functioning Testing, in accordance with General Principles of Software Validation; Final Guidance for Industry and FDA Staff. These pass/fail criteria and results are acceptable.

Revision Level History: Acceptable

PrimeraDx provided an overview of their RLH, including the details of the RLH. Version 10.5 was first released under Design Control, and version 10.7.3 was released for clinical trial validation. The submitted description is acceptable.

Unresolved Anomalies: Acceptable

PrimeraDx provided an adequate list of Unresolved Anomalies for review. The sponsor reports 10 unresolved anomalies in version 10.7.3; all 10 unresolved anomalies have a severity of 1 ("little or no potential injury"), none of which impact the device safety or performance. The sponsor outlined the impact and mitigation required for each; no plans or timeframes for correcting the problems were indicated.

Off the Shelf Software (OTS): Acceptable

The sponsor described 24 OTS software and firmware applications along with the OS including descriptions of the version, manufacturer, part number, function, testing performed, known defects, reasons for acceptability, how the OTS links to other software and any maintenance information relevant to updates. User driven software updating is disabled by the Windows Steady state configuration. The firmware is installed by the manufacturer and updates are effectively disabled due to the need for special equipment to interface with the instrument. No user updating of third-party executables incorporated into the ICEPlex operational software are possible. Upgrading any third party software requires evaluation and testing at the manufacturer before incorporation into the released software image.

EMC and Electrical Testing: Acceptable

The instrument was tested and evaluated to demonstrate compliance with the following standards:

- European EMC Directive 2004/108/EC in accordance with emissions product specific standard
- En 55011:2007 immunity product specific standard EN 61326-1:2006.
- UL 61010-1 :2004 Safety Requirements for Electrical Equipment for Measurement,
- Control, and Laboratory Use- Part **1:** General Requirements
- CAN/CSA C22.2 No. 61010-2-101-04 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use -Part 2-101: Particular Requirements for
- In Vitro Diagnostics (IVD) Medical Equipment

CAN/CSA C22.2 No. 61010-1-04 Safety Requirements for Electrical Equipment for

Measurement, Control, and Laboratory Use - Part 1: General Requirements

3. Specimen Identification:

The user first must verify that the onboard consumables are adequate for the run. Adequate supplies are indicated by the appearance of the buttons shown in the status bar, or by touching the System navigation menu to display the Run Status screen. The Run Status screen provides status information about the current run, the onboard consumables, and instrument messages. The user then registers an IVD assay kit from a barcode label that is included with a PrimeraDx assay IVD kit. The barcode scanner is used to register the IVD assay kit and make a corresponding assay definition available on the instrument. The assay definition needs to be updated for every new lot of the IVD assay kit. The user manually defines the following types of wells:

- Sample, a sample well containing a clinical specimen or test sample;
- Negative control;
- Positive control;
- Buffer, a well without any sample containing only 1X Injection buffer;
- Buffer/Lock, a well without any sample containing only 1X Injection buffer that has been locked by the system due to a well-specific quality evaluation of the installed cartridge.

In the software UI, the user selects a plate map location, for example A1, and enters the sample ID or Accession number or designates plate map locations as Negative, Positive control or Buffer. Each sample or control well must have a unique accession number/identification number.

4. Specimen Sampling and Handling:

Appropriate Specimen type for testing

This assay is intended for use with liquid or soft raw stool specimens. Do not use on well-formed stools or on other types of specimens.

Collecting the specimen

Standard stool collection and handling procedures are appropriate to obtain raw stool. Obtained sample must be placed in a sterile container that can be adequately sealed.

Storing Specimens

Samples should be tested as soon as possible. It is possible to store specimens refrigerated (2-8˚C) for up to 48 hours prior to testing. If sample cannot be processed within 48 hours, store frozen at -70˚C or below.

5. Calibration:

Each individual PCR reaction run for the ICEPlex *C. difficile* Assay is internally controlled by utilizing the included controls. In-reaction Controls (provided as a separate kit component or included as a part of reagent) consist of the following:

- Calibration Control – a group of ICEPlex specific elements used to align electropherograms and assign identities of the target peaks. It also controls for the integrity of the kit reagents and the presence of PCR inhibitors in a given sample.
- Internal Control – Non-target nucleic acid that is co-extracted and co-amplified with the *tcdB* target. It controls for nucleic acid extraction efficiency, for the integrity of the reagents and for the presence of PCR inhibitors in a given sample. The Internal Control needs to be spiked into each sample before extraction.

6. Quality Control:

The ICEPlex system reports the reason for a sample or control result to be called "Invalid". If a control sample is invalid due to a reason unrelated to its function as a control, it is excluded from consideration. To monitor run validity, the user is required to include the following external controls (additional control PCR reactions) with the run (duplicates are recommended):

- Negative Control, substitute S.T.A.R. buffer for the clinical specimen and process normally through the extraction system and on the ICEPlex Instrument.
- Positive Control, this control is used for the PCR stage only, it should not be processed for nucleic acids extraction. In the positive control reaction, substitute extracted sample with provided Positive Control.
- Known Positive Sample, is required to include previously characterized positive sample or simulated sample with every easyMAG extraction run and include it in the subsequent ICEPlex Instrument run to verify successful lysis.

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

Not applicable

Q. Proposed Labeling:

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

R. Conclusion:

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