

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

A. 510(k) Number:

K072138

B. Purpose for Submission:

To obtain substantial equivalence of the device for the detection of *Clostridium difficile* toxins A & B.

C. Measurand:

C. difficile toxins A & B

D. Type of Test:

Qualitative, automated test on the VIDAS instruments using the enzyme linked fluorescent assay (ELFA) technique

E. Applicant:

bioMerieux Inc.

F. Proprietary and Established Names:

VIDAS *C. difficile* Toxin A & B (CDAB) assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
LLH	I	21 CFR 866.2660, reagents, <i>Clostridium difficile</i> toxin	83

H. Intended Use:

1. Intended use:

The VIDAS CDAB assay is an automated test for use on the VIDAS instruments for the qualitative detection of Clostridium difficile toxin A and toxin B in stool specimens using the Enzyme Linked Fluorescent Assay (ELFA) technology.

2. Indication for use:

The VIDAS CDAB assay is an automated test for use on the VIDAS instruments for the qualitative detection of Clostridium difficile toxin A and toxin B in stool specimens using the ELFA technology.

3. Special conditions for use statement:

For prescription use

4. Special instrument requirements:

The VIDAS PC and miniVIDAS instruments

I. Device Description:

The VIDAS BRAHMS CDAB kit consists of 60 ready to use reagent strips and 60 ready to use solid phase receptacles (SPRs) whose interiors are coated with *C. difficile* rabbit polyclonal anti-toxin A and mouse monoclonal anti-toxin B antibodies. It also contains 1 standard (S1), 1 positive control Toxin A (C1), 1 negative control (C2), 1 positive control Toxin B (C3), 1 sample diluent (R1) and 1 MLE card containing the factory master calibration data required to calibrate the test.

J. Substantial Equivalence Information:

1. Predicate device name:

Premier *C. difficile* Toxins A & B

2. Predicate 510(k) number:

K993914

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For the qualitative detection of <i>C. difficile</i> toxins A & B from stool specimens	Same
Assay technology	Enzyme-linked fluorescent assay	Enzyme immunoassay – micro titer well assay
Matrix	Stool	Same

Differences		
Item	Device	Predicate
Technique	Automated	Non-automated
Capture antibodies	Anti-toxin A (rabbit polyclonal); Anti-toxin B (mouse monoclonal)	Anti-toxin A (mouse monoclonal); Anti-toxin B (goat polyclonal)
Detection antibodies	Anti-toxin A & B (mouse monoclonal)	Anti-toxin A & B (goat polyclonal)
Conjugate	Mouse monoclonal anti-toxin A & B antibodies conjugated with biotin	Horse radish peroxidase conjugated to anti-toxins
Sample volume	200 µl	100 µl

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

1. “Review criteria for devices assisting in the diagnosis of *C. difficile* associated diseases” ODE guidance 5/31/1990
2. “Protocols for determination of limits of detection and limits of quantitation.” Approved guideline CLSI EP 17-A, Vol. 24, No. 34

L. Test Principle:

The assay principle combines a two-step immunoassay sandwich method with a final fluorescent detection (ELFA). The SPR, a pipette tip-like device serves as the solid phase as well as the pipetting device for the assay. Assay reagents are ready to use and pre dispensed in the sealed reagent strips. Each of four reaction steps is performed automatically by the VIDAS. The sample/conjugate mixture is cycled in and out of the SPR several times. Each step is followed by a wash cycle which eliminates unbound components. Step 1: Toxin A and/or B present in the sample binds with the anti-toxin A & B antibodies coated on the interior wall of the SPR. Step 2: Binding occurs between toxins and antibodies conjugated with biotin. Step 3: Presence of biotin is detected by incubation with streptavidin conjugated with alkaline phosphatase. Step 4: Two detection steps are performed successively. Alkaline phosphatase catalyzes hydrolysis of the substrate into a fluorescent product. Fluorescence is measured at 450 nm. Results are automatically calculated by the VIDAS. A test value as well as the qualitative result (positive, negative or equivocal) is provided on the result sheet for each sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Six pools of samples; two negative, one equivocal, three positive (low, medium and high) were tested in duplicate in two runs per day over six days at each of three sites. Total precision was 7.4 – 37.6% C.V. intra- assay precision was 2.9 – 26.3% C.V. and inter-assay precision was 6.8 – 26.8% C.V.

b. *Linearity/assay reportable range:*

N/A

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

Stability studies were performed to detect kit stability, specimen, standard and control stability as well as stability of cytotoxicity (CTA) test samples.

Kit stability was evaluated by testing three lots (one verification and two pilot lots used in the clinical trial) for 18 months (real time) at 2-8°C. Currently 12 months of data has been obtained and the data tables support a 12 month kit expiry. Testing is on going until 18 months stability data is obtained.

Specimen stability study was performed to determine the storage conditions of stool specimens prior to processing and stool specimens after processing at various temperatures and storage times. Thirty one samples (14 negative and 17 positive) were used for the “prior to processing” study. Twenty five samples (8 negative and 17 positive) were used for the fresh vs. frozen stability storage studies. Twenty samples (10 negative and 10 positive); standard (S1), controls (C1, C2 and C3) were evaluated in the “after processing” study. Results showed that prior to processing, at 2- 8 °C, storage up to 3 days was acceptable; at $-25 \pm 6^{\circ}\text{C}$ storage up to one month was acceptable and at -70°C storage for one additional month is acceptable. After processing, specimen, standard and controls are stable up to 48 hrs. at 2-8°C.

d. *Detection limit:*

A negative stool pool was spiked with toxin A or toxin B at different levels. Two samples with levels of 7 ng/ml and 8 ng/ml of toxin A and 2 samples with levels of 4 & 5 ng/ml of toxin B were used for the study. These low positive samples and a negative sample were tested on the VIDAS according to the following protocol. For negative sample: 30 repetitions x 2runs i.e. 60 measurements; for each low positive sample: 4 repetitions x 7 runs and 2 repetitions x 1 run, so 60 measurements per toxin. The limit of detection study followed CLSI EP 17-A document. Results showed that the limit of blank of VIDAS CDAB assay is 0.03. For toxin A, LoD is 7.73 ng/ml while for toxin B LoD is 4.55ng/ml

e. *Analytical specificity:*

Specimens were spiked with relevant microorganisms and cross reactivity and interference studies were performed using the VIDAS assay. All organisms were tested at a concentration of 1×10^7 cfu/ml except for *Clostridium sordelii* tested at a concentration of 3×10^8 cfu/ml. No cross reactivity nor interference was detected in the 33 organisms tested. The organisms are as follows:

Staphylococcus aureus, *Shigella flexneri*, *Shigella dysenteriae*, *Shigella sonnei*, *Salmonella* group B, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *E. coli* O157:H7, *E. coli*, *Candida albicans*, *enterococcus faecalis*, *Yersinia enterocolitica*, *Bacteriodes fragilis*, *Campylobacter jejuni*, *Campylobacter coli*, *Vibrio cholerae*, *Aeromonas hydrophila*, *Peptostreptococcus anaerobius*, *Porphyromonas assacharolytica*, *Clostridium sporogenes*, *Clostridium bifermentans* and 12 other *Clostridium* species.

f. *Assay cut-off:*

The assay cut off is as follows:

Test value <0.13 is a negative result; test value ≥ 0.13 to <0.37 is equivocal and test value ≥ 0.37 is a positive result. The test value is equal to the patient relative fluorescence value/ standard relative fluorescence value.

2. Comparison studies:

a. *Method comparison with predicate device:*

The VIDAS CDAB assay was compared to a commercial EIA at 2 clinical testing sites. At site one, 623 samples were tested and at site two, 388 samples were tested. Combined results from both sites were as follows:

Positive agreement 81.3% (73.4 – 87.6 % C.I.)

Negative agreement 99.5% (98.8 – 99.9% C.I.)

Overall agreement 97.1% (95.9 – 98.1% C.I.)

A total of 42 samples were equivocal and calculations were done with the equivocal results considered first as negative and then as positive. Results from both sites were as follows:

With equivocal results considered as negative, positive agreement was 75.9% (67.9 – 82.8% C.I.). and negative agreement was 99.5% (98.8 - 99.9 % C.I.). Overall agreement was 96.3% (95.0 – 97.4 % C.I.). With equivocal results considered as positive, positive agreement was 82.5% (75.1 – 88.4 % C.I.) and negative agreement

was 95.8% (94.2 – 97.0 % C.I.). Overall agreement was 94.0% (92.3 – 95.4% C.I.).

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

N/A. Cytotoxicity assay testing was not presented only percent agreement comparison was done.

b. Clinical specificity:

See (3a).above

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

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

Data provided from the literature showed that the frequency of stools with positive toxins was 9.5% from 136 hospitals in North America and 13.2 – 17.2 % from 380 hospitals in Canada. The Society for HealthCare Epidemiology of America (SHEA) reported rates of 17 to 60 cases per 100,000 bed-days.

N. Instrument Name:

The VIDAS PC and the miniVIDAS

O. System Descriptions:

1. Modes of Operation:

The VIDAS PC instrument cleared in 1989 under K891385 is attached to a computer and a printer. Each instrument has 5 independent sections allowing 5 different assays to be run simultaneously. Each section can process up to six samples. When fully loaded it can process 30 samples. The miniVIDAS is a smaller compact version of the VIDAS PC and was cleared under K923579 in 1993. It has a built in computer, keyboard and printer and is popular in physicians' office laboratories. Two independent sections each can process

six tests so twelve samples can be processed simultaneously. The VIDAS assay can be run on either instrument.

2. Software:

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

Yes _____ or No

3. Specimen Identification:

All assay steps are controlled automatically by the instrument. The sample is transferred into the wells. The CDAB strip consists of ten wells covered with labeled foil seal. The label comprises a bar code which indicates the assay code, kit lot number and expiration date.

4. Specimen Sampling and Handling:

The solid phase receptor (SPR) serves as both the solid phase and the pipetting device. The foil of the 1st well is perforated to allow introduction of the sample into well 1. The last well (well 10) of each strip is a cuvette in which the fluorometric reading is performed. The center wells of the strip contain the various reagents required for the assay.

5. Calibration:

The kit contains a standard S1. It is a dilution of recombinant toxin A from *C. difficile* in TRIS buffered saline and BSA 5% and preservatives. It also contains 3 controls. C1 is positive control Toxin A, C2 is the negative control and C3 is positive control Toxin B.

6. Quality Control:

The Master curve is established at time of manufacture for each lot of reagents. It is provided with each test kit and is entered into the VIDAS instrument using the Master Lot Entry card (MLE) included in the kit. Data from the MLE card is entered once for each lot of reagents. Each lab establishes its own calibration curve (recalibration) based on the mathematical master curve data and the test results of two calibrators tested in duplicate by the lab. Recalibration serves to control for minor variations in assay signal from one VIDAS instrument to another and is therefore specific for each instrument.

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

