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

IVD

The VIDAS® *C. difficile* Toxin A & B (CDAB) Assay is an automated test for use on the instruments of the VIDAS family for the qualitative detection of *Clostridium difficile* toxin A and toxin B in stool specimens using the ELFA technique (Enzyme-Linked Fluorescent Assay). The VIDAS *C. difficile* toxin A & toxin B (CDAB) assay is an aid for diagnosing *Clostridium difficile* associated disease (CDAD).

SUMMARY AND EXPLANATION OF THE TEST

Normal bacterial flora in the intestine provide an ecological barrier against significant colonization by pathogenic organisms; disruption of this protection by antibiotic use, may permit overgrowth of endogenous or nosocomially acquired pathogens such as *Clostridium difficile*.

C. difficile has been found to be the major etiologic agent of antibiotic-associated pseudomembranous colitis (PMC). PMC is a clinically defined syndrome, associated with a recent history of antibiotic use, where pseudomembranous nodules or plaques form in the distal and sigmoid colon and rectum (1, 2, 3). If unrecognized or untreated, the disease can be fatal. *C. difficile* has also been implicated in antibiotic-associated colitis (AAC) and antibiotic-associated diarrhea (AAD) (4).

Nosocomial acquisition of *C. difficile* is a serious consideration for some institutions, particularly those with high inpatient populations, chemotherapy wards, or long-term patient care (5, 6, 7). Also, infants and cystic fibrosis (CF) patients have been shown to be asymptomatic carriers of toxigenic *C. difficile* with colonization rates as high as 50% in infants (8) and 32% in CF patients (9).

Clinical diagnosis of *C. difficile* associated disease (CDAD) is made using a number of criteria, which usually include the following: patient producing at least 6 unformed stools over a 36-hour period, having received antibiotic therapy within 8 weeks of onset of diarrhea, no other obvious cause for diarrhea, and appropriate response to therapy (10).

C. difficile can either be toxigenic or nontoxigenic. Toxigenic strains of *C. difficile* produce an enterotoxin (toxin A) as well as a cytotoxin (toxin B) in roughly equivalent amounts (2, 11).

However, some strains (serogroup F) produce toxin B but not toxin A (12). It is possible that these strains are under-diagnosed due to the common use of diagnostic methods that detect only toxin A (2, 11).

VIDAS *C. difficile* Toxin A & B (CDAB) allows the detection of *C. difficile* toxin A and toxin B in stool specimens.

The VIDAS *C. difficile* toxin A & toxin B (CDAB) assay is an aid for diagnosing *Clostridium difficile* associated disease (CDAD) (13).

PRINCIPLE OF THE PROCEDURE

The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR)® serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and are pre-dispensed in the sealed reagent strips.

The four reaction steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. Each step is followed by a wash cycle which eliminates unbound components:

- Specific binding of toxin A and/or B present in the sample with anti-toxin A antibodies (rabbit polyclonal) and anti-toxin B antibodies (mouse monoclonal) coated on the interior of the SPR.
- Binding between toxin A and anti-toxin A antibodies (mouse monoclonal) conjugated with biotin and binding between toxin B and anti-toxin B antibodies (mouse monoclonal) conjugated with biotin.
- The presence of biotin is detected by incubation with streptavidin conjugated with alkaline phosphatase.
- Detection : alkaline phosphatase catalyzes the hydrolysis of the substrate (4-Methyl-umbelliferyl phosphate) into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm.

The intensity of the fluorescence increases according to the quantity of toxins A and/or B in the sample.

When the VIDAS *C. difficile* Toxin A & B (CDAB) test is completed, the results are analyzed automatically by the instrument, a test value is generated, and a result is printed for each sample.

KIT COMPOSITION (60 TESTS) :

60 CDAB Strips	STR	Ready-to-use.
60 CDAB SPRs (2 x 30)	SPR®	Ready-to-use. *C. difficile* rabbit polyclonal anti-toxin A and mouse monoclonal anti-toxin B antibodies.
Standard 1 x 4 mL (liquid)	S1	Dilution of recombinant toxin A from *C. difficile* in TRIS buffered saline 0.05 mol/L (pH 7.2) + BSA 5% + preservatives. MLE data indicate the confidence interval in "Relative Fluorescence Value ("Standard (S1) RFV Range).
1 Positive Control Toxin A 1 x 4 mL (liquid)	C1	Dilution of recombinant toxin A from *C. difficile* in TRIS buffered saline 0.05 mol/L (pH 7.2) + BSA 5% + preservatives. MLE data indicate the test value (TV) range: Control C1 (+) Test Value Range.
Negative Control 1 x 4 mL (liquid)	C2	TRIS buffered saline 0.05 mol/L (pH 7.2) + BSA 5% + preservatives. MLE data indicate the test value (TV) range: Control C2 (-) Test Value Range.
Positive Control Toxin B 1 x 4 mL (liquid)	C3	Dilution of recombinant toxin B from *C. difficile* in TRIS buffered saline 0.05 mol/L (pH 7.2) + BSA 5% + preservatives. MLE data indicate the test value (TV) range: Control C3 (+) Test Value Range.
Pretreatment Reagent 1 x 61 mL (liquid)	R1	Ready-to-use. Buffer 0.1mol/L (pH 7.2) + foetal calf serum + detergent + preservatives.
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit. or • MLE bar codes printed on the box label.		
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

The SPR ®:

The interior of the SPR is coated during production with anti-*C. difficile* toxin A and toxin B antibodies. Each SPR is identified by the code CDAB. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening.**

The Reagent Strip :

The strip consists of 10 wells covered with a labelled, foil seal. The label contains a bar code which includes the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The eight wells in the center section of the strip contain the various reagents required for the assay.

Description of the CDAB strip :

Well	Reagents
1	Sample Well
2 - 3 - 4	Wash solution :TRIS buffered saline 0.05 mol/L (pH 7.2) + detergent + preservatives (600 µL).
5	Conjugate :Diluted mouse monoclonal anti-*C. difficile* toxin A antibody conjugated with biotin and mouse monoclonal anti-*C. difficile* toxin B antibody conjugated with biotin + preservatives (400 µL).
6	Tracer :Alkaline Phosphatase labeled streptavidin + TRIS* buffered saline 0.05 mol/L (pH 6.0) + preservatives (400 µL).
7 - 8 - 9	Wash solution :TRIS buffered saline 0.05 mol/L (pH 7.2) + detergent + preservatives (600 µL).
10	Reading cuvette with substrate : 4-Methyl-umbelliferyl phosphate (0.6 mmol/L) + diethanolamine DEA** (0.62 mol/L or 6.6%) pH 9.2 + 1 g/L sodium azide (300 µL).

* Signal Word: **WARNING**

Hazard statement

H317 : May cause an allergic skin reaction.

Precautionary statement

P280 : Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352 : IF ON SKIN: Wash with plenty of water.
P333 + P313 : If skin irritation or rash occurs: Get medical advice/attention.

** Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Safety Data Sheet.

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 300 µL, 200 µL, and 1000 µL.
- Powderless disposable gloves.
- Centrifuge capable of ≥ 12,000 *g*.
- Polypropylene or other appropriate centrifuge tubes for specimen dilution and centrifugation (with at least 1.5 mL capacity).
- Sample Transfer Pipets used to measure appropriate quantity of stool sample for processing.
- Applicator stick or loop.
- For other specific materials, please refer to the Instrument User's Manual.
- Instrument of the VIDAS family: VIDAS or miniVIDAS.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **For prescription use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents or disposables from different lots.
- Do not use the SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Use **powderless** gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests (14).
- Use calibrated pipettes to process Standard, Positive, and Negative Controls.
- The optical Cuvette with Substrate (well 10) contains an irritant agent (diethanolamine 6.6 %). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- Store the VIDAS *C. difficile* Toxin A & B (CDAB) Kit at 2-8°C.
- **Do not freeze Reagents.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SPECIMENS

Specimen type, collection and storage:

Stool specimens should be collected according to standard laboratory procedures and stored at 2 - 8°C until processed.
Transport specimens to the laboratory as quickly as possible, after receipt.
Stool specimens may be held for up to 3 days (from time of collection) at 2 - 8°C prior to processing without large losses in detectable *C. difficile* Toxin A & B. Storage at 2 - 8°C for longer than this is not recommended. The specimen may be stored frozen at -25 ± 6°C for one month and then one month after at - 70°C (or colder) temperature. Avoid repeated freezing and thawing cycles.
A rectal swab will not provide enough specimen for the test and therefore is unacceptable. Avoid containers which may contain detergents, preservatives or media, as they may interfere with the VIDAS *C. difficile* Toxin A & B (CDAB) assay results.

Standard, controls, and specimen preparation for the CDAB test:

Specimens :

Important: it is critical that stool specimens be thoroughly mixed before sample processing is begun. Lack of homogeneity in a stool sample may lead to incorrect results. Thorough mixing of stool specimens is essential to avoid this problem. The diluted sample must be homogeneous after mixing. This will help ensure valid results.

The R1 pretreatment reagents of VIDAS *C. difficile* GDH Assay and VIDAS *C. difficile* Toxin A & B are the same.
Do not use any other pretreatment reagent.

Liquid stools :

1. Homogenize the stool sample by sucking in and out of the transfer pipet.

   Using the same transfer pipet, dispense **200 µL** of liquid stool in a clean centrifuge tube.
2. Using a pipette with disposable tip, add **1 000 µL** of pretreatment reagent (R1) to the centrifuge tube.
3. Homogenize again by sucking in and out of the transfer pipet.

   Then mix using a vortex-type mixer until a homogeneous medium is obtained.

   It is essential that all portions of the sample be uniformly mixed with pretreatment reagent (R1).
4. Centrifuge for 5 minutes at 2-25°C at a minimum of 12,000 *g*.
5. Using a pipette with disposable tip, collect 300 µL of supernatant to perform the VIDAS *C. difficile* Toxin A & B (CDAB) test.

Note : *If particulate matter or lipid material remain at the surface of the supernatant after centrifugation, collect the sample below the surface layer.*

Semi-solid and solid stools:

1. To ensure the precise transfer of **200 mg** of semi-solid or solid stool, dispense **200 µL** of distilled water into a clean centrifuge tube using a pipette with disposable tip; this tube will be used as a "gauged tube".
2. **Vigorously homogenize** the stool sample using a wooden applicator stick.
3. Transfer a quantity of stool (200 mg), equivalent to the volume in place in the gauged tube, into a new clean centrifuge tube identified as the sample tube (see hereafter).
   Deposit the stool sample at **the bottom of the centrifuge tube** so that the quantity of stool is equivalent to the volume of water in the gauged tube (200 µL).

"Gauged tube":
200 µL distilled water

Sample tube:
200 mg stool sample

Note: Discard the gauged tube after transferring each of the solid and semi-solid stool specimens.

4. Add **1000 µL** of pretreatment reagent (R1) to the sample tube using a pipette with disposable tip.
5. **Emulsify** the stool sample using the applicator stick, and then **mix** using a vortex-type mixer until the sample appears homogeneous. It is essential that all **portions of the sample be uniformly mixed with pretreatment reagent (R1)**.
6. Centrifuge for 5 minutes at 2-25°C at a minimum of 12,000 *g*.
7. Using a pipette with disposable tip, collect 300 µL of supernatant to perform the VIDAS *C. difficile* Toxin A & B (CDAB) test.

Note:

- If particulate matter or lipid material remain at the surface of the supernatant after centrifugation, collect the sample below the surface layer;
- In cases where semi-solid stools consist more of liquid than of solid, the sample preparation procedure may be performed using the instructions provided for liquid stools (see section "Specimens : Liquid Stools")

Standard and Controls:

The standard and the positive and negative controls must be processed as follows

1. Mix standard, positive and negative controls vigorously using a vortex-type mixer.
2. Collect **200 µL** of standard and controls using a pipette with disposable tip and dispense into a clean tube.
3. Add **1 000 µL**of pretreatment reagent (R1) to the tube using a pipette with disposable tip.
4. Mix using a vortex-type mixer.
5. Using a pipette with disposable tip, collect **300 µL** to perform the VIDAS *C. difficile* Toxin A & B (CDAB) test.

Storage of specimen supernatant and processed standard and controls:

Processed specimen supernatants, standard and controls may be stored up to 48 hours at 2-8°C before being tested with VIDAS *C. difficile* Toxin A & B (CDAB).
After 48 hours processed specimen supernatants, standard and controls must be discarded.

INSTRUCTIONS FOR USE

For complete instructions, see the User's Manual.

Reading VIDAS® PTC (Protocol Test Change) protocol data and MLE data

When using the assay for the first time:

With the external instrument barcode reader,

1. Scan the PTC barcode(s) at the end of the package insert or downloadable from www.biomerieux.com/techlib. This reading allows VIDAS® PTC protocol data to be transferred to the instrument software for its update.
2. Scan the MLE data on the box label.

Note: If the MLE data have been read before the VIDAS® PTC protocol, read the MLE data again.

When opening a new lot of reagents:

Enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.
If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.
Note: The master lot data need only be entered once for each lot.
It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User's Manual).

Calibration

Calibration, using the standard provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration information and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The standard, identified by S1 and processed as defined above, must be tested in **duplicate** (see User's Manual). The standard value must be within the set RFV "Relative Fluorescence Value" range. If this is not the case, recalibrate.

Assay procedure

1. Only remove the required reagents from the refrigerator.
2. Use one CDAB strip and one CDAB SPR from the kit for each processed sample, processed control or processed standard to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
3. The test is identified by the "CDAB" code on the instrument. The processed standard must be identified by "S1", and tested in duplicate. If the processed positive controls are to be tested, they should be identified by "C1" and "C3". If the processed negative control needs to be tested, it should be identified by "C2".
4. **For this test, the processed standard, processed sample supernatant and processed control test portion is 300 µl.**
5. Insert the "CDAB" SPRs and "CDAB" strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
6. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
7. The assay will be completed within approximately 75 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
8. Dispose of the used SPRs and strips into an appropriate receptacle.

RESULTS AND INTERPRETATION :

Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

The test value is calculated for each sample by the instrument as follows :
Test value = patient RFV / standard RFV
This test value as well as the interpretation appear on the result sheet. Interpretation according to the test value is as follows:

Test Value	Result
< 0.13	Negative
≥ 0.13 to < 0.37	Equivocal
≥ 0.37	Positive

In the case of equivocal results, due to the heterogeneity of toxin distribution in some samples, it is recommended to repeat the test using either the original specimen or a fresh specimen. The sample must be collected within two weeks, taking into consideration clinical signs and patient history.

If the result is still equivocal, the sample should be tested with another method.

Invalid results are seen when the background reading for the sample test strip is above a predetermined cut-off, indicating low-level substrate contamination. Specimens with invalid results should be repeated using original processed specimen if possible or by using a fresh sample.
Interpretation of test results should be made taking into consideration the patient history and the results of any other tests performed.

QUALITY CONTROL

Two positive controls and one negative control are included in each VIDAS C. *difficile* Toxin A & B (CDAB) kit. These controls must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using these controls. Test the controls as specified by your laboratory's regulatory guidelines.
Controls are to be processed as defined above and dispensed into the sample wells of the VIDAS *C. difficile* Toxin A & B (CDAB) Reagent Strips.

The instrument will only be able to check the control values if identified as C1, C2 and C3.

Results cannot be validated if the control values deviate from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

1. **Pretreatment reagents other than VIDAS *C. difficile* Toxin A & B (CDAB) and VIDAS *C. difficile* GDH Assay reagents should not be used.**
2. Meconium may interfere with the VIDAS *C. difficile* Toxin A & B (CDAB) assay - do not test specimens from infants less than two years of age.
3. Stool specimens that appear to have high amounts of fat have not been thoroughly evaluated. Avoid sampling this material in the VIDAS *C. difficile* Toxin A & B (CDAB) assay.
4. Due to sample heterogeneity, thorough mixing of stool specimens is essential to avoid discrepant results. Samples giving results contradictory to the clinical information available should be retested using a fresh specimen.

5. Levels of toxin may vary from one patient specimen to another.
6. A negative VIDAS *C. difficile* Toxin A & B (CDAB) result alone may not rule out the possibility of *C difficile*-associated colitis or diarrhea. It could be the result of inadequate sampling, handling or sample storage. Always evaluate VIDAS *C. difficile* Toxin A & B (CDAB) assay results along with clinical signs and patient history when diagnosing *C. difficile*-related disease.
7. A positive VIDAS *C. difficile* Toxin A & B (CDAB) assay test result alone should not be used to diagnose *C. difficile* associated colitis or diarrhea. VIDAS *C. difficile* Toxin A & B (CDAB) assay results should always be evaluated along with clinical signs and patient history when diagnosing *C. difficile*-related disease.

EXPECTED VALUES

In a European study (15) conducted by the ESGCD (European Study Group on *Clostridium difficile*) the average frequency of stools with positive toxins results collected among 136 hospitals was 9.5 %.

In North America, from a Canadian survey (16) including 380 hospitals, average test positivity rates varied from 13.2% to 17.2% depending on the hospital size (<300 to >500 beds), with an average (*Clostridium difficile*-Associated Diseases) incidence between 23.5 to 40.3 cases per 100,000 patient days. In the SHEA (Society for Healthcare Epidemiology of America) (17) position paper the reported rate was 17 to 60 cases per 100,000 bed-days.

PERFORMANCE

Precision:

A precision study was performed at 3 sites using 6 pools of samples : 2 negative, 1 equivocal, 3 positive (low, medium, and high).

Each sample was tested in duplicate in 2 runs per day with each of 2 reagent lots at each of the 3 sites. Each lot was tested over a period of 6 days (N = 144 per sample).

Intra-assay precision (within-run precision), inter-assay precision (between run, between day, between lot and between site precision), and total precision were calculated based on the recommendations of the CLSI EP5-A2.

Results are given in the table below :

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

		Intra-assay precision		Inter-assay precision		Total precision	
Sample	Mean test value	Standard deviation	CV (%)	Standard deviation	CV (%)	Standard deviation	CV (%)
Negative 1	0.018	0.0049	26.3	0.0050	26.8	0.0069	37.6
Negative 2	0.088	0.0060	6.8	0.0081	9.2	0.0101	11.5
Equivocal	0.197	0.0091	4.6	0.0169	8.6	0.0192	9.7
Low Positive	0.678	0.0256	3.8	0.0622	9.2	0.0673	9.9
Medium Positive	1.340	0.0446	3.3	0.1105	8.2	0.1192	8.9
High positive	2.914	0.0831	2.9	0.1987	6.8	0.2153	7.4

Cross reactivity and interference:

- To test for cross-reactivity, each bacteria or virus was diluted in the VIDAS C. *difficile* Toxin A & B (CDAB) Negative Control, processed, and tested in singlicate with the VIDAS C. *difficile* Toxin A & B (CDAB) assay. To test for interference, each bacteria or virus was diluted in the Positive Controls, processed and tested in singlicate with the VIDAS C. *difficile* Toxin A & B (CDAB) assay. The bacteria were tested at a concentration of 1 x 10^7 CFU/mL (0.033 McFarland) except for *C.sordelli* which was tested at 3 x 10^8 CFU/mL (1 McFarland).
  The following table lists the bacteria or viruses that were tested and showed no cross-reactivity or interference with the VIDAS C. *difficile* Toxin A & B (CDAB) assay.

Adenovirus 40 & 41
Rotavirus
Staphylococcus aureus ssp aureus
Staphylococcus epidermidis
Shigella flexneri
Shigella dysenteriae
Shigella sonnei
Salmonella group B (paratyphi B)
Salmonella enteritidis
Salmonella typhimurium
Pseudomonas aeruginosa
Escherichia coli 0157:H7
Escherichia coli
Candida albicans
Enterococcus faecalis
Yersinia enterocolitica
Bacteroides fragilis
Campylobacter jejuni ssp jejuni
Campylobacter coli
Enterobacter aerogenes
Enterobacter cloacae
Helicobacter pylori
Bacillus subtilis
Bacillus cereus

Proteus vulgaris
Klebsiella pneumoniae
Serratia liquefaciens
Vibrio parahaemolyticus
Vibrio cholerae
Aeromonas hydrophila ssp hydrophila
Peptostreptococcus anaerobius
Porphyromonas assacchrolyticus
Clostridium sporogenes
Clostridium bifermentans
Clostridium histolyticum
Clostridium difficile (non-toxigenic) VPI 0210114
Clostridium butyricum
Clostridium subterminale
Clostridium sordelli *
Clostridium perfringens
Clostridium tertium
Clostridium tetani
Clostridium septicum
Clostridium innocuum
Clostridium novyi
Clostridium haemolyticum

* A cross-reactivity could be observed with *C. sordelli* VPI 9048 depending on the culture conditions

- During clinical trials, no interference was observed with stool specimens that appeared to have a high amount of mucus or blood present.
- Drug interference
  During studies conducted in accordance with the CLSI EP7-A2 guidance document, no interference was observed for the therapeutic drugs Vancomycin and Metronidazole or with the anti-diarrhea medications Pepto-Bismol and Imodium or their active ingredients Loperamide, bismuth subsalicylate and salicylate when tested in the assay in low-positive (0.30 to 0.50 TV) and high-positive (>2.5 TV) stool matrix at the concentrations listed below:

Vancomycin	5 mg/mL
Metronidazole	2 mg/mL
Loperamide Hydrochloride	0.08 mg/mL
Bismuth subsalicylate	8.2 mg/mL
Salicylate	4.2 mg/mL
Imodium Tablets	0.12% w/v
Imodium Liquid	12% v/v
Pepto-Bismol Tablets	2.2% w/v
Pepto-Bismol Liquid	48% v/v
Barium sulfate	5% w/w

- The VIDAS C. *difficile* Toxin A & B (CDAB) assay was evaluated using several strains of *C. difficile*. Strains were grown in Yeast Peptone broth and tested for reactivity with the VIDAS C. *difficile* Toxin A & B (CDAB) assay. The results summarized below indicate that VIDAS C. *difficile* Toxin A & B (CDAB) identified toxigenic strains of *C. difficile,* even those producing only toxin B.

C.difficile types	% of VIDAS CDAB positives / total number of strains
A+/B+	100% (23/23)
A-/B+	83% (15/18*)

* Three (3) A-/B + strains gave equivocal results with VIDAS C. *difficile* Toxin A & B (CDAB).

Limit of Detection

VIDAS C. *difficile* Toxin A & B (CDAB) assay will detect Toxin A at a level of ≥ 7.73 ng/mL and Toxin B at a level of ≥ 4.55 ng/mL in human stools.

Clinical Performance

1011 fresh stool specimens were collected and tested at two clinical sites (USA and Europe). Each sample was tested using the VIDAS C. *difficile* Toxin A & B (CDAB) assay on the VIDAS instrument and a commercially available manual EIA. Cellular cytotoxicity assay (gold standard) testing of each sample was centralized and performed at a third site (Internal bioMerieux, SA site). The VIDAS C. *difficile* Toxin A & B (CDAB) assay was compared to cellular cytotoxicity assay and to the commercial EIA tested.

VIDAS C. *difficile* Toxin A & B (CDAB) Assay Compared to Cellular cytotoxicity assay

		Site 1			**Site 2**			**All sites**		
		Cellular cytotoxicity assay			**Cellular cytotoxicity assay**			**Cellular cytotoxicity assay**		
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
VIDAS	Positive	68	2	70	38	0	38	106	2*	108
	Equivocal	6	20	26	6	10	16	12	30	42***
	Negative	9	518	527	5	329	334	14**	847	861
Total		83	540	623	49	339	388	132	879	1011

Performance	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**
Sensitivity	88.3	79.0-94.5	88.4	74.9-96.1	88.3	81.2-93.5
Specificity	99.6	98.6-99.9	100.0	98.9-100.0	99.8	99.2-99.9
Positive Predictive Value (PPV)	97.1	90.1-99.7	100.0	90.7-100.0	98.1	93.5-99.8
Negative Predictive Value (NPV)	98.3	96.8-99.2	98.5	96.5-99.5	98.4	97.3-99.1

* 2 samples were found to be positive with the VIDAS C. *difficile* Toxin A & B (CDAB) and negative with the Cytotoxicity test, of which both were found to be negative with a commercial EIA.

** 14 samples were found to be negative with the VIDAS C. *difficile* Toxin A & B (CDAB) and positive with the Cytotoxicity test, of which 10 were found to be negative and 4 positive with a commercial EIA.

*** 4.2% (42/1011) samples were found to be equivocal with the VIDAS C. *difficile* Toxin A & B (CDAB) assay, and were not taken into account for the sensitivity, specificity, PPV and NPV calculations.

Performance compared to Cellular cytotoxicity with VIDAS equivocal results considered as VIDAS negative results

	Site 1		Site 2		All sites	
Performance	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**
Sensitivity	81.9	72.0-89.5	77.6	63.4-88.2	80.3	72.5-86.7
Specificity	99.6	98.7-100.0	100.0	98.9-100.0	99.8	99.2-100.0
Positive Predictive Value (PPV)	97.1	90.1-99.7	100.0	90.7-100.0	98.1	93.5-99.8
Negative Predictive Value (NPV)	97.3	95.6-98.5	96.9	94.4-98.4	97.1	95.8-98.1

Performance compared to Cellular cytotoxicity with VIDAS equivocal results considered as VIDAS Positive results

	Site 1		Site 2		All sites	
Performance	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**
Sensitivity	89.2	80.4-94.9	89.8	77.8-96.6	89.4	82.8-94.1
Specificity	95.9	93.9-97.4	97.1	94.6-98.6	96.4	94.9-97.5
Positive Predictive Value (PPV)	77.1	67.4-85.0	81.5	68.6-90.7	78.7	71.2-84.9
Negative Predictive Value (NPV)	98.3	96.8-99.2	98.5	96.5-99.5	98.4	97.3-99.1

VIDAS C. *difficile* Toxin A & B (CDAB) Assay Compared to Commercial EIA

		Site 1			Site 2			All sites		
		Commercial EIA			**Commercial EIA**			**Commercial EIA**		
		Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
VIDAS	Positive	67	3	70	37	1	38	104	4*	108
	Equivocal	7	19	26	2	14	16	9	33	42***
	Negative	18	509	527	6	328	334	24**	837	861
Total		92	531	623	45	343	388	137	874	1011

Performance	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**
Positive agreement	78.8	68.6 - 86.9	86.0	72.1 - 94.7	81.3	73.4 - 87.6
Negative agreement	99.4	98.3 - 99.9	99.7	98.3 - 99.9	99.5	98.8 - 99.9
Global agreement	96.5	94.7 - 97.8	98.1	96.2 - 99.2	97.1	95.9 - 98.1

* 4 samples were found positive with the VIDAS C. *difficile* Toxin A & B (CDAB) assay and negative with a commercial EIA of which 2 were found positive with the Cellular Cytotoxicity Assay.

** 24 samples were found negative with the VIDAS *C. difficile* Toxin A & B (CDAB) assay and positive with the commercial EIA of which 20 were found negative with the Cellular Cytotoxicity Assay.

*** 4.2% (42/1011) samples were found to be equivocal with the VIDAS *C. difficile* Toxin A & B (CDAB) assay, and were not taken into account for the positive, negative and global agreement calculation.

Performances compared to Commercial EIA with VIDAS equivocal results considered as VIDAS negative results

	Site 1		Site 2		All sites	
Performance	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**
Positive Agreement	72.8	62.6 - 81.6	82.2	67.9 - 92.0	75.9	67.9 - 82.8
Negative Agreement	99.4	98.4 - 99.9	99.7	98.4 - 100.0	99.5	98.8 - 99.9
Global Agreement	95.5	93.6 - 97.0	97.7	95.6 - 98.9	96.3	95.0 - 97.4

Performances compared to Commercial EIA with VIDAS equivocal results considered as VIDAS positive results

	Site 1		Site 2		All sites	
Performance	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**	**Value (%)**	**95% confidence interval**
Positive Agreement	80.4	70.9 - 88.0	86.7	73.2 - 94.9	82.5	75.1 - 88.4
Negative Agreement	95.9	93.8 - 97.4	95.6	92.9 - 97.5	95.8	94.2 - 97.0
Global Agreement	93.6	91.4 - 95.4	94.6	91.8 - 96.6	94.0	92.3 - 95.4

Additional performance compared to cellular cytotoxicity

An additional study was performed to compare the VIDAS® C. *difficile* Toxin A & B (CDAB) assay to the cellular cytotoxicity performed at a reference laboratory external to bioMérieux. 90 frozen stool specimens were tested at external clinical sites (USA). Each sample was tested using the VIDAS C. *difficile* Toxin A & B (CDAB) assay on the VIDAS instrument at site 1 and using the cellular cytotoxicity assay (gold standard) at site 3.

VIDAS C. *difficile* Toxin A & B (CDAB) Assay Compared to Cellular cytotoxicity assay

			Site 3		
			Reference Laboratory Cellular Cytotoxicity Assay		
			Positive	Negative	Total
Site 1	VIDAS	Positive	22	3	25
		Equivocal	1	3	4
		Negative	3	58	61
		Total	26	64	90

Performance: VIDAS Compared to the External Site Cytotoxicity Test Method

Performance with VIDAS Equivocal Results Excluded*		
Performance	**Value**	**95% confidence interval**
% Sensitivity	88.0%	68.8-97.5
% Specificity	95.1%	86.3-99.0
% Total agreement	93.0	85.4-97.4

*4.4% (4/90) of the samples were found to be equivocal by VIDAS C. *difficile* Toxin A & B (CDAB), and were not taken into account for the sensitivity, specificity, and total agreement calculations.

Performance with VIDAS Equivocal Results Considered as VIDAS Positive

Performance	**Value**	**95% confidence interval**
% Sensitivity	88.5%	69.8-97.6
% Specificity	90.6%	80.7-96.5
% Total agreement	90.0	81.9-95.3

Performance with VIDAS Equivocal Results Considered as VIDAS Negative

Performance	**Value**	**95% confidence interval**
% Sensitivity	84.6%	65.1-95.6
% Specificity	95.3%	86.9-99.0
% Total agreement	92.2	84.6-96.8

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

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INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user
Note:	*Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	14019I	Administrative	REVISION HISTORY INDEX OF SYMBOLS
		Technical	KIT COMPOSITION (60 TESTS) WARNINGS AND PRECAUTIONS
2015/06	14019J	Technical	KIT COMPOSITION (60 TESTS) INSTRUCTIONS FOR USE

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