



Gastro-Tect™

***Clostridium difficile* Toxin A+B Antigen Detection**

Microwell ELISA Directions For Use

Cat. MCC-CDIF-96, 96 Tests

Intended Use

This microwell enzyme linked immunoabsorbent assay (ELISA) detection kit is an in vitro diagnostic (IVD) immunoassay for the detection of antigen to *C. difficile* A+B toxins in human feces using peroxidase as the indicator enzyme. This assay may be read visually or with an ELISA reader. This ELISA kit is intended to be used with stools that are fresh, frozen, or in Cary-Blair (C&S) transport media. The assay is intended for use as an aid in the diagnosis of *C. difficile*-associated disease.

Summary and Explanation

Clostridium difficile may be part of the normal bacterial flora of the human intestinal tract, but can become an opportunistic pathogen when the intestinal tract has been compromised or altered, as with patients undergoing antibiotic therapy. Hall and O'Toole isolated the bacteria and described its toxigenic characteristics in 1935.¹ Toxin-producing strains of *C. difficile* produce two toxins - toxin A, an enterotoxin, and toxin B, a cytotoxin.

C. difficile was not considered an opportunistic pathogen until the late 1970's when a correlation between the bacteria and pseudomembranous colitis (PMC) was established.^{2,3} PMC is an antibiotic-associated disease that progresses from diarrhea and mucosal inflammation to the formation of colonic pseudomembranes composed of fibrin, mucous, necrotic epithelial cells and leukocytes. Though up to 50% of infants are colonized by toxigenic *C. difficile* and exhibit high levels of toxin A and B, few develop PMC, instead remaining asymptomatic. Hypotheses for this phenomenon include colostrum's ability to neutralize toxin A and B, a diminished sensitivity of toxin A by fetal intestinal cells, and the possible lack of toxin receptors.⁵ A less-studied population exhibiting reduced susceptibility to PMC is cystic fibrosis patients. Rapid methods of isolation and identification of *C. difficile* or its toxin(s) are readily available. The most common clinical diagnostic procedures for *C. difficile* antibiotic-associated colitis are cell culture cytotoxicity and latex agglutination assays.⁵ The cell culture cytotoxicity assay (CTA) detects the presence of toxin B by the observation of cytopathic effect on cell culture. The assay is very sensitive (50 pg/ml toxin B)⁵ but requires a minimum of two days to complete. Latex agglutination is a common stool screening method for detection of proteins associated with *C. difficile*, though cross-reactivity and detection of nontoxigenic *C. difficile* has been reported.^{6,7,8,9,10,11,12}

C. difficile EIA methods have been researched by a number of investigators, with a reported sensitivity to either toxin A or toxin B of 1-10 ng/mL.^{5,13,14,15,16}

Principle of Procedure

This ELISA is an in vitro immunoassay for the qualitative determination of *C. difficile* toxin A+B antigen in human feces. The assay uses rabbit anti-*C. difficile* toxin A+B polyclonal antibodies to capture the antigen from the stool supernatant. The second set of chicken anti-*C. difficile* toxin A+B polyclonal antibodies are then added which sandwiches the captured antigen. The next incubation adds additional antibody conjugated to peroxidase and the chromogen tetramethylbenzidine (TMB). The resulting blue color development is converted to an easily read yellow color by addition of an acidic "stop solution" to end the reaction. The presence of yellow color above 0.15 OD absorbance indicates presence of *C. difficile* antigens.

Materials Provided

Test strips: microwells containing anti-*C. difficile* toxin A+B polyclonal antibodies - 96 test wells.

Test strip holder: One (1).

Reagent 1: One (1) bottle containing 6 mL of chicken anti-toxin A+B polyclonal antibodies with blue dye and Thimerosal.

Reagent 2: One (1) bottle containing 11 mL of anti-chicken antibody conjugated to peroxidase with red dye and Thimerosal.

Positive controls: Two (2) vials containing 2 mL of each (1 vial) toxin A and (1 vial) toxin B in a buffer.

Negative control: One (1) vial containing 2 mL of buffer

Dilution Buffer: Two (2) bottles containing 30 mL of buffered protein solution with detergent.

Chromogen: One (1) bottle containing 11 mL of the chromogen tetramethylbenzidine (TMB) and peroxide.

Wash Concentrate 20X: Two (2) bottles containing 25 mL of concentrated buffer and surfactant with Thimerosal

Stop solution: One (1) bottle containing 11 mL of IM phosphoric acid.

100 mL calibrated transfer pipettes

Materials Required But Not Provided

Transfer Pipettes

Test Tubes

Squeeze bottle for washing strips (narrow tip is recommended)

Graduated Cylinder

Reagent grade (DI) water
Timer
Waste Container
Cold Sterilant (Wavicide)
Disposable Gloves

Suggested Equipment

ELISA plate reader with 450 and 620-650 nm filters

Warnings/Precautions

For In Vitro Diagnostic Use

All reagents should be warmed to room temperature (15-25° C) and gently mixed before use. Do not use solutions if they precipitate or become cloudy. Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming. Do not add azides to the samples or any of the reagents. Controls and some reagents contain Thimerosal as a preservative. Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of sample. Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, *wash with copious amounts* of water. If acid gets into eyes, wash with copious amounts of water and seek medical attention. Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results. Do not use kit past expiration date. Potential interfering substances such as mucin, blood, fecal fat, and anti-diarrheal products, have not been evaluated in this assay. Improper sample handling may adversely affect the ability to accurately detect *C. difficile* toxins. The clinician should carefully follow their institution's directions for collecting, transporting and testing the specimen. The effect of numerous freeze/thaw cycles has not been tested with this device.

Storage Conditions

Reagents, strips and bottled components:

Store between 2 - 8° C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

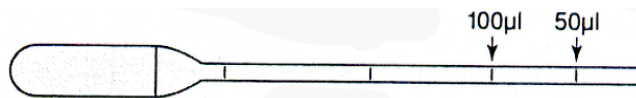
Wash Buffer - Remove cap and add contents of one bottle of Wash Concentrate (20X) to a squeeze bottle containing 475 mL of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings. Working wash buffer may be stored at room temperature (15-25° C) for up to one (1) year.

Collection of Stool (Feces) and Preparation

Stool samples may be used as unpreserved, frozen, or in **Cary-Blair** (C&S) transport media. Samples should be kept at 2 - 8° C and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -15° C or lower until used. Freezing does not adversely affect the test. All dilutions of samples must be made with the dilution buffer provided in the kit or with the MCC "Quick 'N' Easy" fecal dilution device.

Preparation of Fresh/Frozen Stools

Thaw sample if needed. Add sufficient sample diluent to make approximately a 1:4 dilution (0.1 gram of fecal sample to 0.3 mL of sample diluent) and mix well. The effect of numerous freeze/thaw cycles has not been tested with this assay.



1. Use the provided calibrated pipettes to dispense 200 µL (4 drops) of Specimen Diluent into a test tube.
2. For non-solid stool or liquid specimens transfer 50 µL (one drop) of specimen into the test tube containing sample diluent. For solid stools transfer approximately 3mm of sample (small "BB" size) into the Sample Diluent and mix well (vortex).
3. Draw up specimen and diluent into pipette several times and then place pipette back into the test tube.
4. Using the same calibrated pipette in the test tube draw up diluted specimen and dispense 2 drops (100 µL) into the appropriate sample well.

Quick 'N' Easy Fecal Dilution Device (FDD)

DO NOT dilute stools if the Quick 'N' Easy Fecal Sampling device is to be used. This device will do the dilution for you. Please refer to the Quick 'N' Easy procedure for proper use.

Materials Provided

C. difficile Toxin A+B Stool Antigen Microwell ELISA Kit

All incubations are at room temperature (15 to 25° C)

Test Procedure:

1. Break off the number of wells needed (number of samples plus 3 for controls) and place in holder.
 2. Add 2 drops (approximately 100 µL) of negative control to well # 1.
 3. Add 2 drops (approximately 100 µL) of positive toxin A to well # 2 and positive toxin B to well # 3. Then add 2 drops of the diluted specimen into the appropriate well.
 4. Incubate for 30 minutes at room temperature (15-25° C). Discard contents into designated container then wash*
 5. Add 1 (one) drop of Reagent 1 (blue solution) and 2 (two) drops of Reagent 2 (red solution) to each well. Mix for 15-30 seconds.**
 6. Incubate for 10 minutes at room temperature (15-25° C), Discard contents into designated container then wash.*
 7. Add 2 drops of Chromogen to each well
 8. Incubate 10 minutes (15-25° C) at room temperature.
 9. Add 2 drops of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger.
 10. Read results visually or at 450/620-650 nm within 60 minutes after adding Stop Solution. Zero reader on air.
- * Washings consist of vigorously filling each well to overflowing and decanting contents three (3) to (5) separate times. After the final fill, decant the contents into a designated discard container, then turn plate upside down and slap dry against paper towel covered solid surface. Controls must be included each time the kit is run.
- ** The two reagents must be mixed thoroughly. This can be performed by shaking or tapping the plate.

Interpretation of Results

Visual

Positive: Any sample well that is obviously more yellow than the negative control well.

Negative: Any sample well that is not obviously more yellow than the negative control well.

NOTE: The negative control, as well as some samples, may show some slight color. A sample well must be obviously darker than the negative control well to be called a positive result (presumptive detection of either toxin A or toxin B). Please refer to the **Visual Interpretation Card** included in the kit.

ELISA Reader

Zero reader on air. Read all wells at 450/620-650 nm.

Positive: Absorbance reading of 0.15 OD units or greater

Negative: Absorbance reading less than 0.15 OD

A negative result does not rule out the diagnosis of *C. difficile* disease since toxin level may be below the detection limit of the assay.

A positive result indicates that toxin A or toxin B from *C. difficile* (or a closely related toxin from *C. sordellii*) present in the sample.

Troubleshooting

Problem: Negative control has substantial color development.

Correction: Washings were insufficient. Repeat test with more vigorous washings.

Expected Values

A positive reaction indicates that the patient is shedding detectable amounts of *C. difficile* antigen. The frequency of *C. difficile* disease is dependent on various factors such as the type of institution, patient population and potential outbreak status'. Asymptomatic carrier rates have been reported from a low of 2% in Sweden to a high of 15% in Japan. Hospitalized patients taking certain antibiotics are at high risk of acquiring *C. difficile* with infection rates of 21% being reported in one study. A recent article in *Journal of Clinical Microbiology* provides a good overview of testing for *C. difficile*.¹⁸ Further information on *C. difficile* and antibiotic colitis can also be found in the Manual of Clinical Microbiology, ASM Press, 7th Edition.

Performance Characteristics

Data on sensitivity, specificity and cross reactions are on file.

Limitations of Procedure

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves. Do not concentrate patient samples. A negative result can occur from an antigen level lower than the detection limits of this assay. Multiple samples over time may be indicated for those patients that are suspected of being positive for *C. difficile*. *C. difficile* toxins are very labile and may not be stable in stored samples. The level of toxin has not been shown to be correlated with either the presence or severity of disease. Test results should be interpreted by a physician in light of other lab results and clinical findings. *Clostridium sordellii* is known to produce toxins that are structurally and immunologically similar to *C. difficile*. A culture of this species did produce a low positive reaction in the assay

Quality Control

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must have an OD value of greater than 0.5 and the negative control must be less than 0.15. Should the values fall outside these limits, the kit should not be used. Call technical support for further instructions.

References

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Manufactured by:
Medical Chemical Corporation
19430 Van Ness Ave.
Torrance, CA 90501 USA
Telephone: 310-787-6 800, FAX: 310-787-4464

www.med-chem.com