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*C. difficile* Toxin A+B Antigen Detection
Microwell ELISA

Cat. No. 8308-3

**Intended Use**

This ELISA is an *in vitro* immunoassay for the qualitative determination of *C. difficile* toxin A+B in feces. It is a double antibody (sandwich) ELISA using an anti-toxin A+B antibodies to capture the antigen from the stool supernatant. A second set of anti-toxin A+B antibodies are added which sandwiches the captured antigen. This reaction is visualized by the addition of an anti-second antibody conjugated to peroxidase and the chromogen tetramethylbenzidine (TMB). The resulting blue color development indicates the presence of *C. difficile* toxin A+B being bound by the antibodies.

**Summary**

*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, mucus, necrotic epithelial cells and leukocytes.\(^4,5\)

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.\(^5\) 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)\(^7\) 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**

During the first incubation, *C. difficile* toxin A+B present in the stool supernatant are captured by antibodies attached to the wells. The second incubation adds additional anti-toxin A+B antibodies that "sandwiches" the antigen. The next incubation adds an anti-second antibody conjugated to peroxidase. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

**Reagents**

- 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 11 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 control: Two (2) vials containing 2 ml each of either toxin A or 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.
- Chromogen: One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.
Wash Concentrate 20X: One (1) bottle containing 25 ml of concentrated buffer and surfactant with Thimerosal.

Stop solution: One (1) bottle containing 11 ml of 1M phosphoric acid.

Precautions
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 samples.

Storage Conditions
Reagents, strips and bottled components:
Store between 2 - 8°C.
Squeeze bottle containing diluted wash buffer may be stored at room temperature.

Preparation
Wash Buffer - Remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

Collection of Stool (Feces) and Preparation
No modification of collection techniques used for standard bacterial examinations is needed. Stool samples may be used as unpreserved or frozen. Make a 1:4 (1 part sample, about the size of a pea, and 3 parts dilution buffer) stool sample dilution using the dilution buffer provided.

Unpreserved 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 -20°C or lower until used. Freezing does not adversely affect the test.

All dilutions of samples must be made with the dilution buffer provided.

Procedure

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

Materials Required But Not Provided
Transfer Pipettes
Squeeze bottle for washing strips (narrow tip is recommended)
Graduated Cylinder
Reagent grade (DI) water

Suggested Equipment
ELISA plate reader with 450 and 620-650 nm filters

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

Test Procedure
1. Break off the number of wells needed (number of samples plus 2 for controls) and place in holder.
2. Add 2 drops (approximately 100 ul) of negative control to well # 1.
3. Add 2 drops (approximately 100 ul) of positive toxin A and toxin B to well # 2 and 3, respectively.
4. Add 2 drops of the sample stool supernatant to each test well and then add 2 drops of Reagent 1 to every well. Mix by pipetting up and down several times.
5. Incubate for 30 minutes at room temperature (15-25°C), then wash.*
6. Add 2 drops of Reagent 2 (red solution) to each well.
7. Incubate for 5 minutes, then wash.
8. Add 2 drops of Chromogen to each well.
9. Incubate 5 minutes.
10. 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. Zero reader on air.

* Washings consist of vigorously filling each well to overflowing and decanting contents three separate times. Controls must be included each time the kit is run.

**Interpretation of Results - Visual**

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

**Non-reactive:** 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.

**Interpretation of Results - ELISA Reader**

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

**Reactive:** Absorbance reading of 0.15 OD units and above indicates the sample contains *C. difficile* toxin.

**Non-reactive:** Absorbance reading less than 0.15 OD units indicates the sample does not contain detectable levels of *C. difficile* toxin.

**Test Limitations**

Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

DO NOT concentrate stool samples. Assay will not give accurate results on a concentrated sample.

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.*

**Expected Results**

Normal healthy individuals should be free of *C. difficile* toxins and should test negative. A positive reaction indicates that the patient is shedding detectable amounts of *C. difficile* antigen. Please refer to the Summary section for references.

**Performance Characteristics**

Data on sensitivity, specificity and cross-reactions are available on request.

**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 absorbance of at least 0.5 OD units and the negative control must be less than 0.15 OD units. Should the value fall below this limit, the kit should not be used.

**Troubleshooting**

**Problem:** Negative control has substantial color development.

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

**References**


