C. difficile Toxin A+B
2nd Generation
Cat # 8308-3

<table>
<thead>
<tr>
<th>Test</th>
<th>C. difficile Toxin A+B 2nd Generation ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>Principle</td>
<td>ELISA-Indirect; Antigen Coated Plate</td>
</tr>
<tr>
<td>Detection Range</td>
<td>Qualitative Positive; Negative control</td>
</tr>
<tr>
<td>Sample</td>
<td>1 gm stool sample</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
</tr>
<tr>
<td>Total Time</td>
<td>~ 70 min</td>
</tr>
<tr>
<td>Shelf Life</td>
<td>11 Months</td>
</tr>
</tbody>
</table>

*Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*
Intended Use

The Diagnostic Automation C. difficile toxin A+B 2nd Generation ELISA is an *in vitro* immunoassay for the qualitative determination of *C. difficile* toxins A+B in fecal specimens.

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.²³ 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.⁴⁵

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)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.⁶⁷⁸⁹¹⁰¹¹¹²

*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.⁵¹³¹⁴¹⁵¹⁶

Principle of Procedure

During the first incubation, *C. difficile* toxins A+B antigen present in the stool are “sandwiched” by anti-*C. difficile* toxins A+B antibodies attached to the wells and anti-*C. difficile* toxins A+B antibodies 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. If no antigen is captured, or if there is an insufficient level of antigen, no colored reaction will take place.
Reagents

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Strips</td>
<td>Microwells containing anti-<em>C. difficile</em> toxins A+B antibodies - 96 test wells in a test strip holder.</td>
<td>MT PLATE</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>One (1) bottle containing 6 ml of anti-<em>C. difficile</em> antibodies conjugated to peroxidase with thimerosal.</td>
<td>CONJ</td>
</tr>
<tr>
<td>Positive Control</td>
<td>One (1) vial containing 2 ml of <em>C. difficile</em> toxins A and B in a buffered protein solution with thimerosal.</td>
<td>CONTROL+</td>
</tr>
<tr>
<td>Negative Control</td>
<td>One (1) vial containing 2 ml of Dilution Buffer.</td>
<td>CONTROL-</td>
</tr>
<tr>
<td>Chromogen</td>
<td>One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB) and peroxide.</td>
<td>SUBSTMB</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>Two (2) bottles containing 30 ml of buffered protein solution with thimerosal.</td>
<td>SPECM DIL</td>
</tr>
<tr>
<td>Wash Concentrate (20X)</td>
<td>One (1) bottle containing 25 ml of concentrated buffer and surfactant with thimerosal.</td>
<td>WASH BUF</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>One (1) bottle containing 11 ml of 1 M phosphoric acid.</td>
<td>SOLN</td>
</tr>
</tbody>
</table>

Precautions

- **Do not deviate from the specified procedures when performing this assay.** All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- For In Vitro Diagnostic Use Only.
- Do not interchange reagents between kits with different lot numbers.
- Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture.
- 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, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
- 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 the 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.

Storage Conditions

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

Preparation

- Before use, bring all reagents and samples to room temperature (15-25°C) and mix.

- (20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature (15-25°C) and mixed. **Ensure that (20X) wash concentrate is completely in solution before diluting to working concentration.** To dilute (20X) wash concentrate to working dilution, 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 only. 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. Avoid multiple freeze/thaw cycles. Preserved samples (i.e. in 10% Formalin, SAF or PVA) have not been validated on this assay.

Procedure

Materials Provided

- *C. difficile* Toxins A+B Stool Antigen Microwell ELISA Kit
- Microplate strip holder

Materials Required But Not Provided

- Transfer Pipettes
- Squeeze bottle for washing strips (narrow tip is recommended)
- Graduated Cylinder
- Reagent grade (DI) water
- Micropipette
- Sample Dilution Tubes
• Applicator Stick (recommended) or swabs for sample preparation

Suggested Equipment

• ELISA plate reader with 450 and 620-650 nm filters - DAX800

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

Test Procedure

Notes:

• Ensure all samples and reagents are at room temperature (15-25°C) before use. Frozen samples must be thawed completely before use.
• If needed, prepared samples can be centrifuged at 2000-3000 g for 5-10 minutes. Ensure supernatant is clear before use.
• When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each wash step should help to minimize bubbles in the wells.

• All dilutions of stools must be made with the dilution buffer provided. Do not use dilution buffer from a kit with a different lot number.

Procedure

1. Break off the required number of wells (number of samples plus 2 for controls) and place in holder.
2. Prepare sample dilutions in tubes using 0.3 ml of Dilution Buffer and 0.1 g, about the size of a small pea, of fecal sample using an applicator stick. Mix thoroughly before using.

   -IF USING SWABS, add 0.6 ml of dilution buffer to dilution tube. Coat the swab with a thin layer of specimen and mix into dilution buffer, expressing as much fluid as possible. Mix thoroughly before using.

3. For watery, unpreserved specimens, mix contents, then add 0.1 ml of sample to 0.3 ml dilution buffer in dilution tubes. Mix thoroughly before using.
4. Add 1 drop (50 µl) of conjugate to each well (including control wells).
5. Using a micropipette, add 100 µl of negative control to well # 1
6. Using a micropipette, add 100 µl of positive control to well # 2.
7. Add 100 µl of diluted sample to each well. Mix wells by gently tapping the side of the strip holder with index finger for approximately 15 seconds.
8. Incubate for 60 minutes at room temperature (15-25°C), then wash.* After last wash, slap the wells out on a clean absorbent towel to remove excess wash buffer.
9. Add 2 drops of Chromogen to each well.
10. Incubate for **10 minutes** at room temperature (15-25°C).
11. Add **2 drops** of Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger for approximately **15 seconds**. Read reaction within **5 minutes** after adding stop solution.
12. Read results visually or a dual wavelength of 450/620-650 nm. Zero reader on air.

*Washings consist of vigorously filling each well to overflowing and decanting contents five (5) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.*

**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 0.1 O.D. units and above indicates the sample contains *C. difficile* toxin(s).  
**Non-reactive:** Absorbance reading less than 0.1 O.D. units indicates the sample does not contain detectable levels of *C. difficile* toxin(s).

**Limitation of Procedure**

- 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 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 a 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 (ref. #18) provides a good overview of testing for *C. difficile*. Further information on *C. difficile* and antibiotic colitis can also be found in the Manual of Clinical Microbiology, ASM Press, 7th Edition.

**Study 1:**

A study was performed with fresh/frozen specimens using the *C. difficile* Toxins A+B assay and another commercial available ELISA. The results of the study are show in the table below.

<table>
<thead>
<tr>
<th></th>
<th>DAI ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Other Commercial ELISA</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Positive Agreement: 100% (22/22)
Negative Agreement: 100% (51/51)

**Reproducibility**

The intra-assay (well to well) CV was calculated using 4 positive and 4 negative samples assayed 24 times in a single run. The mean CV was 8.74%.

The inter-assay (run to run) CV was calculated using 4 positive and 4 negative samples assayed on four separate days. The mean CV was 8.03%.

**Cross Reactivity**

No cross-reactions were seen with the following organisms: *Salmonella typhimurium, Salmonella enteritidis, Salmonella infantis, Proteus vulgaris, Campylobacter coli 1114, Campylobacter coli 1111, Campylobacter fetus, Campylobacter lari, Campylobacter jejuni, Campylobacter sputorum, Staphlococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia liquefaciens, Enterobacter cloacae, Citrobacter braakii, Shigella flexneri, Shigella sonnei, Shigella dysenteriae, Escherichia hermanii, Yersinia enterocolitica, Helicobacter pylori, E. coli 25992, E. coli 12014, E. coli 43887, E. coli 33780, E. coli 43890 and E. coli 43893.*
Troubleshooting

**Problem:** Negative control has excessive color after development.

**Reason:** Inadequate washings

**Correction:** Wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel. Do not allow test wells to dry out.

References


