



Clostridium difficile Toxin Reagents for Cytotoxicity Assay

QUIDEL

For the qualitative detection of *Clostridium difficile* toxin in patient samples.

FOR *IN VITRO* DIAGNOSTIC USE



INTENDED USE

Diagnostic Hybrids' Cytotoxicity Assay for *Clostridium difficile* Toxins is intended for use in the qualitative detection of *Clostridium difficile* toxin in patient samples.

SUMMARY

Clostridium difficile toxin (CDT) is the most common cause of antibiotic-associated diarrhea (AAD), a moderate to severe diarrheal disease which may occur after treatment with several antibiotics.^{2,6,8,12,16} Antibiotic-associated colitis (AAC) can result in patient death if not recognized and appropriately moderated through specific treatment and fluid replacement management. A second syndrome which has been associated with the presence of intestinal CDT is pseudomembranous colitis (PMC). In this disease, a reddening of the intestinal mucosa is followed by development of yellowish plaques which coalesce to cover the colonic mucosal lining. *Clostridium difficile* is an opportunistic pathogen which occurs in normally healthy adults, children and infants. It has been reported that 27 to 63 percent of healthy infants may have *Clostridium difficile* toxin within their intestine.^{8,17} When the bacterial flora is modified by antibiotic suppression or other factors which can reduce the normal intestinal flora, *Clostridium difficile* may flourish beyond normal levels and produce CDT disease.^{5,8}

Lower gastrointestinal endoscopy can be used to establish the diagnosis of PMC through direct visualization of changes in the mucosal surface. Endoscopy requires expensive equipment and highly trained personnel and can cause patient discomfort. Culture isolation can be used to detect *Clostridium difficile*, but does not differentiate between toxin producing and nontoxin producing strains. Culturing requires PEA-Cycloserine cefoxitin-egg yolk fructose agar.^{1,16} Isolated colonies have a ground-glass appearance and fluoresce yellow-green under long wave ultraviolet light. However, isolation alone does not indicate that the patient has CDT disease, since *Clostridium difficile* may be a part of the normal intestinal flora.^{1,21,24}

The cytotoxicity test is an ideal method for determining the cause of diarrhea in hospitalized patients treated with antibiotics. By inoculating stool filtrate onto sensitive tissue culture cells, toxins of *Clostridium difficile* can be rapidly detected. Tissue culture tubes, vials or microtiter plates may be used. Human fibroblast cells are commonly used. However, CHO, Vero, HeLa and other epitheloid lines have also been successfully used in cytotoxicity assays.^{3,4,8,13,14,22} Cells affected by toxin will round up and demonstrate a very characteristic (asteroid-like) cytotoxicity.^{3,18,21} Neutralization of this toxin in the cytotoxicity assay by *Clostridium difficile* antitoxin is evidence that cell changes were due to CDT.^{3,4,5}

All toxin-producing strains of *Clostridium difficile* produce both toxin A (enterotoxin) and toxin B (cytotoxin).^{8,10,11,21} Both toxins appear to have a role in human disease and thus the detection of either toxin in

stool samples verifies the presence of the other toxin. Toxin B (cytotoxin) is generally present in a higher concentration (1,000 to 10,000) than toxin A.⁸ Cytotoxicity assay for *Clostridium difficile* toxin is extremely sensitive, as 1 picogram of toxin B is sufficient to cause cell rounding.⁸

Once a patient has been diagnosed with CDT-caused colitis or PMC, antibiotic therapy is usually discontinued or modified and corrective action is taken to replace lost fluids and electrolytes. Relapse can occur, however, after cessation of therapy. A sensitive and specific test is needed to detect CDT in patient fecal specimens, since toxin presence cannot be determined on the basis of clinical grounds alone.¹⁶

Detection of CDT using specific antitoxin and viable cell culture is rapid (2 to 48 hours). Since the cytotoxicity assay is very sensitive to cytotoxin (toxin B), and both toxin A and B are present in toxin positive *Clostridium difficile* specimens, a cell cytotoxicity assay is therefore the optimal laboratory method for diagnosis of AAC and PMC.^{8,27}

PRINCIPLE OF THE PROCEDURE

The Diagnostic Hybrids' Cytotoxicity Assay for *Clostridium difficile* Toxin consists of two reactions. Tissue culture cells are inoculated with a sterile stool filtrate which has been optimally diluted to provide a screen for presence of toxin. At the same time, a pre-incubated filtrate-antitoxin mixture is inoculated onto a second set of tissue culture cells. Following incubation, cells are observed for toxic effects in the first well and neutralization (no toxic effects) of toxin with specific antitoxin in the second well. In specimens negative for CDT, cell culture will remain normal in appearance, while those specimens positive for CDT will affect the tissue culture cells in a characteristic rounded, asteroid-like cytotoxicity. The lack of specific CPE in the antitoxin-neutralized well confirms CDT. Tissue culture is observed with a brightfield microscope at 30 to 100X total magnification.

REAGENTS

Kit Components

Toxin Control

1 mL

Clostridium difficile toxin is present in MEM with Earles salts, L-glutamine and non-essential amino acids. Fetal bovine serum is present at 2% concentration. Gentamicin sulfate (10 µg /mL) and streptomycin sulfate (50 µg /mL) are the included antibiotics. HEPES and sodium bicarbonate are present as pH buffering agents.

Antitoxin Reagent

3 mL

Purified antisera against *Clostridium difficile* toxin is present in MEM with Earles salts, L-glutamine and non-essential amino acids. Fetal bovine serum is present at 2% concentration. Gentamicin sulfate (10 µg /mL) and streptomycin sulfate (50 µg /mL) are the included antibiotics. HEPES and sodium bicarbonate are present as pH buffering agents.

Specimen Diluent

2 x 50-mL

Phosphate buffered saline consists of 8.5 g/L sodium chloride, 1.14 g/L sodium phosphate dibasic and 0.015 g/L sodium phosphate monobasic with phenol red as a pH indicator. Gentamicin sulfate (10 µg /mL) and amphotericin B (4 µg /mL) are present as antibiotic agents.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Patient specimens which are not refrigerated or frozen after collection and prior to testing may have toxin which has been denatured by mishandling during transport; all specimens must be tested promptly or refrigerated at Store at 2°C to 8°C until tested.

- All specimens should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel.
- Decontamination is most effectively accomplished with a solution of sodium hypochlorite (1:10 final dilution of household bleach) or a standard laboratory disinfectant.
- Although control materials have been shown to contain non-infectious microorganisms, the same precautions should be taken in handling and disposing of reagents and patient fecal samples.
- Microbial contamination of reagents may cause a decrease in sensitivity. Aseptic technique should be used when handling all reagents.
- Do not mouth pipet reagents; use a mechanical pipetting aid.
- Reagents should not be used beyond their expiration dates.
- Cells become less sensitive to the action of CDT as they age; use cell culture cells **only** within a recommended shelf life.
- Rates of reactivity may vary with different cell lines due to their individual sensitivities.
- Incubation at different temperatures and lengths of time can give false results.
- Reagents are supplied at working strength. Any dilution of reagents will decrease sensitivity.
- Do not substitute reagents from other manufacturers.
- Return Toxin, Antitoxin and Specimen Diluent to the refrigerator promptly after use.
- **Do not refrigerate living cell cultures** upon receipt or at any time during the shelf-life of the cells; refrigeration may render the cells nonviable and cause cytotoxicity unrelated to presence of toxin. Store cell cultures under conditions recommended by the manufacturer.
- **Warning to Residents of the State of California:** This product contains an ingredient listed under California Chapter 3, Safe Drinking Water and Toxic Enforcement Act of 1986, Section 12000, Chemicals Known to Cause Cancer or Reproductive Toxicity. The listed ingredient is streptomycin sulfate, which is known to the State of California to be a reproductive toxicant.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

MATERIALS PROVIDED

- Toxin Control, *Clostridium difficile*
- Antitoxin Reagent, *Clostridium difficile*
- Specimen Diluent

MATERIALS REQUIRED BUT NOT PROVIDED

- Cell Culture: Human Foreskin fibroblast cells in 96-well plates, 48-wells filled, shell-vials or tubes. All cell culture formats are available from Quidel.
- Cell Culture refeed medium such as Eagle's Minimal Essential medium with 2% fetal bovine serum, 25mM HEPES, 2.2 gm/L sodium bicarbonate and antibiotics (DHI Ref. 10-320100).
- Brightfield or inverted microscope with 30X to 100X total magnification.
- Sterile 1-mL pipets.
- Sodium hypochlorite solution (1:10 final dilution of household bleach).
- Incubator, 35°C to 37°C, CO₂ or non-CO₂.
- Centrifuge capable of attaining 2000 to 6000xg.
- 0.45-micron sterile filter (syringe type). (Optional, 1.2-micron sterile filter)
- Sterile environmental enclosure.

- Micro-pipettors (optional).
- Sterile glass tubes (optional).

REAGENT STORAGE INSTRUCTIONS

Table 1. Reagent Storage Conditions

Toxin Control	Store at 2°C to 8°C.
Antitoxin Reagent	
Specimen Diluent	

STABILITY

- **Reagents:** The reagents will retain potency until the expiration date shown on the label of each item if stored appropriately. Store Toxin, Antitoxin and Specimen Diluent at 2°C to 8°C. Return these reagents to the refrigerator promptly after use. If any reagent appears cloudy discard immediately.
- **Cell Culture:** Important: Living cells cannot be stored at refrigerator temperatures, but must be kept at 34°C to 37°C in condition such that the monolayer is covered by the maintenance medium.

SPECIMEN COLLECTION, TRANSPORT, AND STORAGE

Single or multiple freshly passed fecal specimens are collected into a clean container. Swab specimens are inadequate as the sample is too small and susceptible to variation in storage temperature. Specimens collected after a barium enema or other treatment should be avoided. Other specimens reported as appropriate for cytotoxicity assays include lumen contents, surgical biopsies or autopsy samples of the large bowel.¹ Specimens should be transported in tightly sealed, leak proof plastic containers. If specimens can be processed within 3 to 4 hours after collection, transport at room temperature is adequate. Toxin is acid and heat labile,¹² but stable for at least 24 hours at 2°C to 8°C. Specimens delayed to the laboratory should be promptly cooled and kept at 2°C to 8°C until tested. Ship samples on ice if transported over long distances. Specific requirements for shipping specimens should follow recommendations found in section 42 and 49 of the Code of Federal Regulation, CFR.

PROCEDURE

Microtiter Toxin Assay

Processing the specimen (microtiter)

1. Thoroughly mix stool to obtain homogenous sample.
2. Prepare a 1:5 dilution of stool-fluid by adding 1 mL stool or fecal fluid to 4.0 mL Specimen Diluent.
3. Centrifuge at 2000 to 6000xg for 10 minutes to pellet solid material.
4. Filter this 1:5 dilution of stool supernatant through a sterile 0.45-micron membrane filter.
NOTE: Stools which do not pass through a 0.45-micron filter may either be re-centrifuged and then filtered or may be filtered first through a 1.2-micron non-sterile filter, and then through a sterile 0.45-micron filter. It may be easier to pull the supernatant through the syringe filter. Alternatively, you may aspirate the supernatant into the syringe, attach the filter and needle assembly, then puncture the rubber stopper of a sterile preservative-free vacutainer tube. The vacuum will pull the supernatant through the filter.
5. Using sterile pipets, prepare the following dilutions in empty wells of the microtiter plate:

Table 2. Set up for Microtiter Toxin Assay

Processing	Sample	Toxin	Antitoxin	Diluent	Specimen
Controls	Toxin Control	0.1 mL		0.1 mL	
	Antitoxin Control		0.1 mL	0.1 mL	
	Toxin/Antitoxin	0.1 mL	0.1 mL		
Cells	Cell Control			0.2 mL	
Patient Specimen	Specimen Filtrate			0.1 mL	0.1 mL
	Specimen/ Antitoxin		0.1 mL		0.1 mL

Final volume of above dilutions to be added to microtiter plate is 0.05-mL.

Incubate above dilutions at room temperature for 30 minutes. During this incubation, antitoxin will neutralize toxin if present. **Do not** add these dilutions to the culture cells prior to the incubation period.

NOTE: Immediately after processing, refrigerate remaining 1:5 stool filtrate and patient sample in case further testing or dilution steps are needed.

Inoculation of Microtiter Plate

The microtiter plates contain 6 columns of microtiter wells with viable tissue culture cells, ready for use. Each column is capped by a plastic strip designed to maintain proper pH conditions and prevent the medium from drying. These should be left in place until the actual testing is to begin.

1. Keep plate right side (cap side) up.
2. Remove the plastic strip caps from sufficient tissue culture wells to perform test using materials in Step 5 above.
3. Using a sterile pipet for each control and specimen filtrate dilution, add 0.05-mL of solution to sequential tissue culture wells. Each well contains 0.15 mL of cell culture maintenance fluid, resulting in a final dilution of 1:40 of fecal supernatant.
4. Replace caps firmly on cell culture wells inoculated with control and patient specimens.
5. Incubate plate at 34°C to 37°C right side up for 2 to 48 hours.
6. Examine for specific CPE development. Positives may be examined as early as 2 to 6 hours after inoculation. Negatives should be held for up to 48 hours.

Culture Tube or Vial Cytotoxicity Assay

Processing the Specimen (culture tube or vial method)

1. Add 1 mL stool or fecal specimen to 3 mL diluent.
2. Centrifuge at 2000-6000xg for 10 minutes to pellet solid material.
3. Remove supernatant fluid and filter through a sterile 0.45-micron membrane filter.
NOTE: Stools which do not pass through 0.45-micron filter may either be re-centrifuged and then filtered or may be filtered first through a 1.2-micron filter, and then passed through a sterile 0.45-micron filter. It may be easier to pull the supernatant through the syringe filter. Alternatively, you can aspirate the supernatant into the syringe, attach the filter and needle assembly, then puncture the rubber stopper of a sterile preservative-free vacutainer tube. The vacuum will pull the supernatant through the filter.
4. Using sterile pipets, prepare the following in sterile tubes without cells (**Do not** add to culture cells at this stage):

Table 3. Set up for Culture Tube or Vial Cytotoxicity Assay

Processing	Sample	Toxin	Antitoxin	Diluent	Specimen
Controls	Toxin Control	0.2 mL		0.2 mL	
	Antitoxin Control		0.2 mL	0.2 mL	
	Toxin/Antitoxin	0.2 mL	0.2 mL		
Cells	Cell Control			0.4 mL	
Patient Specimen	Specimen Filtrate			0.2 mL	0.2 mL
	Specimen/Antitoxin		0.2 mL		0.2 mL

Final volume of above dilutions to be added to tubes is 0.4 mL and 0.2 mL for vials.

Incubate above dilutions at room temperature for 30 minutes. During this incubation, antitoxin will neutralize toxin if present. **Do not** add these dilutions to the culture cells prior to the incubation period.

Inoculation of Cell Culture in Tubes or Vials

NOTE: When received in the laboratory, DHI tissue culture tubes contain 2-mL of maintenance medium; DHI cell culture vials contain 1 mL of maintenance medium. Use of tubes and vials for this modification requires a constant fluid volume of 2 mL in tubes and 1 mL in vials for appropriate dilution of 1:40 of filtrate to culture fluid. Remove maintenance media and add fresh tissue culture refeed medium (*Order Code 10-320100*) to tubes (add 1.6 mL) and vials (add 0.8 mL). Replacement with fresh medium will serve to enhance the activity of toxin.

1. Using a sterile pipet for each control and specimen filtrate, add solutions to separate culture tubes (add 0.4 mL) or vials (add 0.2 mL). This is a final dilution of patient filtrate/cell culture fluid of 1:40.
2. Replace caps on cell culture tubes or vials inoculated with control and patient test suspensions.
3. Incubate cell culture tubes up to 48-hours at 34°C to 37°C in a position that will allow maintenance fluid to bathe the cells. Slant tube in a stationary position with the reference mark on top. Vials should be placed in an upright position so that fluid constantly bathes the cells.
4. Examine for specific cytotoxicity development. Positives may be observed as early as 2 to 6 hours after inoculation. Negatives should be held for up to 48 hours.

Procedure to Titrate Stool Filtrates

1. If the original 1:5 stool filtrate has been stored at 2°C to 8°C, recover the filtrate and follow Steps 2 through 6 below. If not, prepare a new 1:5 filtrate of the stool following steps in the section under processing the specimen for the Microtiter Toxin Assay. If using culture or vial assay, replace the 1:5 dilution with a dilution of 1:4.
2. Prepare a series of sterile glass or plastic containers to receive further dilutions of filtrate. Clearly label the first tube with patient name, identification number, and the beginning dilution of stool filtrate (1:10 is the beginning dilution described below). Label the remaining containers with the subsequent serial dilutions – the 2nd 1:20, the 3rd 1:40, etc. up to 1:1280 or higher, if necessary.
3. Using a sterile 1.0-mL pipet, add 0.2 mL Specimen Diluent to each tube.
4. From the 1:5 filtrate, aseptically remove 0.2 mL filtrate and add it to the tube marked 1:10. Mix solutions by aspiration and expulsion.
5. Using a new sterile pipet, remove 0.2 mL of the 1:10 dilution and add to the tube marked 1:20, mix, remove 0.2 mL from the 1:20 tube and add to the 1:40 tube and so on until all the dilutions have been made.

6. To rule out high titers of CDT or nonspecific cell rounding, dilutions can now be tested as in previously described inoculation procedures.

IMPORTANT: Two cell culture wells (or tubes or vials) are once again used for each stool dilution, one to contain the new dilution of stool filtrate plus an equal volume of Specimen Diluent and a second to contain the new dilution of stool filtrate plus an equal volume of **non-diluted** antitoxin. The first serial dilution (from the tube labeled 1:10) becomes a 1:80 final dilution when added to the **Test** and **Control** wells of the cell culture, the second is 1:160, etc.

7. For the tubes or vials, use the original 1:4 filtrate to make serial dilutions. Tubes should be labeled 1:8, 1:16, 1:32, etc. Follow the above procedure, using 0.4 mL in place of 0.2 mL of the diluent and filtrate. When the serial dilutions are added to the tubes and vials, the final dilutions become 1:80, 1:160, etc.

Monitoring of Cell Culture

Cell cultures can be monitored either on an inverted microscope or on an upright brightfield microscope using low power objective (30X to 100X total magnification). When using a brightfield upright microscope, cell containers must be positioned so that cells on the surface of the container face the objective. Adjust the illumination contrast by opening or closing the condenser diaphragm.

Upright Microscope Examination of Cells in Plates

1. Ensure all caps on wells are tight, then invert the microtiter plate so that the cell-side is up.
2. Using a low power objective, focus on the cell monolayer.
3. Examine microtiter plate well by well for characteristic cytotoxic effect.
4. If the stage interferes with complete reading of all of the wells in a particular column, turn the plate around to examine the remaining wells. For example, if the reader can easily visualize wells 1-4, but not wells 5-8, reverse the plate on the stage and read wells 5-8.

Upright Microscope Examination of Cells in Vials

1. Ensure all caps on vials are tight.
2. Rack the condenser to its lowest position and remove condenser from the condenser holder. Invert vial so that cells which are on the bottom of the vial are now in the up position. Position vial on a clear glass slide placed across the condenser holder.
3. Raise the condenser until the bottom of the vial is flush with the microscope stage.
4. Lower the low power objective until the cells are brought into focus.
5. Examine vial cell culture for characteristic cytotoxic effect.

Upright Microscope Examination of Cells in Tubes

1. Ensure all caps on tubes are tight.
2. Raise the low power objective to maximum height.
3. Place tube on stage so that the white reference mark is in the down position, as the cells will be on the opposite surface.
4. Lower the low power objective until the cells are brought into focus.
5. Examine cell culture for characteristic cytotoxic effect.

Inverted Microscope Examination of Cells

1. Place cell container on stage in same orientation as incubated.
2. Raise low power objective until cells are brought into focus and examine for characteristic cytotoxicity.

QUALITY CONTROL

Examine cells on the day of use for the appropriate morphology. Cells should appear fibroblastic or spindle shaped without rounded, swollen, or refractile cells other than occasional mitotic cells.

CAUTION: Early detection of toxin is largely dependent on cell age and overall quality. Avoid using older cells, since observation of toxicity will be generally delayed on cells which are near expiration. Outdate of cells varies with the individual cell types; fibroblast cells, such as human foreskin cells, are best when used within one week of date of receipt. Cells should not be used for this test if they exhibit undue rounding, “stringiness” of cytoplasm or are detaching from the container. If pH of maintenance fluid on cells is out of range (yellow, indicating an acid condition or pink-purple to dark purple, indicating an alkaline condition), they should not be used for this test without refeeding and subsequent demonstration of stability in pH at normal range (pink to red-orange color).¹¹ To assure cell reactivity, control tests should be performed on cell monolayer with combination of toxin/antitoxin, toxin alone, antitoxin alone, and diluent alone.

- **Toxin Control:** Cells should appear rounded throughout the cell layer. Later stages will have much more rounding than earlier stages. Accompanying these cell changes are increases in vacuolation. Some normal elongated cells are observed among rounded cells in earlier stages. Older fibroblastic cells demonstrate less total cell rounding, but appear with rounded centers and long spindle-like cytoplasm.
- **Antitoxin Control:** Cells should appear normal.
- **Toxin and Antitoxin Control:** Cells should appear normal.
- **Cell Control:** Cells should appear normal.

INTERPRETATION OF RESULTS

Examination of Samples and Controls

Patient specimens can be **reported positive** when characteristic cytotoxicity of CDT occurs in the patient test well (filtrate at a 1:40 dilution), but **no** cytotoxicity is evident in the patient control well (stool filtrate with equal amount of *Clostridium difficile* antitoxin). Cells in the patient test well without antitoxin will be characteristically rounded, show stringy cytoplasm, and will become somewhat refractile. Usually the entire monolayer will become affected. Cells can begin to change with high-titered samples within several hours after specimen is added to cells.

A **negative test** can be reported when **no** cytotoxicity is observed in **either** the patient test well (filtrate) or patient control well (filtrate plus antitoxin) after 48 hours of incubation.

An **equivocal or possible non-specific test** is evident when both the patient test well (filtrate plus diluent) and patient control well (filtrate plus antitoxin) show characteristic cytotoxicity of CDT. This can occur if an overwhelming amount of CDT is present in the sample or when other non-neutralizable bacterial toxins are present. In addition, viral agents or bacteria can also cause cytotoxicity or cytopathic effect. A titration of the stool filtrate can be performed to confirm or rule out the presence of high titer CDT versus a nonspecific reaction.

Results of Titration Test

A positive test for CDT can be reported when a dilution of stool filtrate shows characteristic cytotoxicity in the patient **Test** well (filtrate dilution plus an equal volume of diluent) and no cytotoxicity in the patient **Control** well (filtrate plus an equal volume of antitoxin of the same dilution).

A nonspecific reaction (confirmed negative test for CDT) is evidenced by cytotoxicity in both patient test and patient control wells.

Reporting Results

When specimen filtrates show evidence of specific CDT, report “Patient specimen positive for *Clostridium difficile* toxin.” Data indicates that less than 15% of positive specimens may produce a titer of 1:1250 or higher. When specimen filtrate shows no evidence of cytotoxicity at 48 hours, report “No *Clostridium difficile* toxin detected.” When specimen filtrates show evidence of cytotoxicity which is nonspecific (not neutralized

by *Clostridium difficile* antitoxin) report “Nonspecific reaction in patient sample, not characteristic of *Clostridium difficile* toxin. A repeat specimen may be helpful to rule out patient disease caused by *Clostridium difficile* toxin.”

NOTE: Infrequently, specimens contain both *Clostridium difficile* toxin and another agent which may also cause cells to show cytotoxic effect. In this case, a true neutralization end-point reaction may be greater than 1:40 and a dilution of specimen (titration test) should be performed to establish true cytotoxicity versus nonspecific factors. This type of specimen will show cytotoxicity in both test and control wells through dilutions equal to or greater than 1:80. When a dilution of specimen eventually shows cytotoxicity in the test well but **not** in the control well (diluted specimen plus antitoxin), toxin is confirmed present. Do not report the titer of toxin (dilution of stool which can be neutralized by antitoxin) since the actual titer of toxin present in stool is not related to severity of patient disease. The titer can be affected by factors such as the action of fecal pH on the toxin, unknown fecal components and changes in specimen temperature during transportation. Qualitative results should be reported rather than quantitative results, i.e., toxin is present or absent from the sample tested.

LIMITATIONS OF PROCEDURE

- Detection of *Clostridium difficile* toxin will vary depending on the specimen quality and its subsequent handling. A negative result does not exclude the possibility of the presence of *Clostridium difficile* toxin.
- Detection of *Clostridium difficile* toxin will vary depending on the type and quality of cell culture used. The observation of toxicity will generally be delayed on cells which are near outdate.
- Performance of the kit can only be assured when components used in the assay are supplied by Diagnostic Hybrids, Inc.
- Reagents are supplied at working strength and any alterations of the reagents or deviation from the procedure may produce suboptimal results.
- Infrequently, specimens contain both *Clostridium difficile* toxin and another agent which may also cause cells to show cytopathic effect. In this case, a true neutralization endpoint reaction may be greater than 1:40 and a dilution of specimen (titration test) should be performed to establish true cytotoxicity versus nonspecific factors.

EXPECTED VALUES

Cytotoxicity Assay on stool filtrate from patients with colitis and/or diarrhea yielded the following results:

Table 4. Cytotoxicity Assay results

Results	# of Specimens
Negative	54 (72.9%)
Positive	11 (14.9%)
Cytotoxicity due to other agents/factors	9 (12.2%)
TOTAL	74 (100%)

PERFORMANCE CHARACTERISTICS

In a study in the Western region of the U.S., 253 stool specimens were tested to compare the Diagnostic Hybrids' *Clostridium difficile* Cytotoxicity Assay with another procedure using WI38 cells. Forty specimens (17%) were positive by both assays, for a sensitivity of 100% and specificity of 100%. A second comparison of specimens obtained in the Northeastern region of the U.S. also yielded a sensitivity of 100% (11/11) and specificity of 100% (63/63). The DHI *Clostridium difficile* Cytotoxicity Assay was compared with conventional tissue culture using WI38 cells for this study. Additional testing was done on 37 specimens which were known positive for *Clostridium difficile* toxin. The reagents from DHI *Clostridium difficile* Cytotoxicity Assay were

compared to those from another supplier according to specified instructions for each system. Each assay yielded a positive result on all specimens for a sensitivity of 100%.

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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