



SAMPLE PROCEDURE

This “Sample Procedure” is not intended as a substitute for your facility’s Procedure Manual or reagent labeling, but rather as a model for your use in customizing for your laboratory’s needs.

Space has been provided within the document to allow you to update this template with information specific to your facility. It is suggested that a current version of the manufacturer’s directional insert be maintained as a supplement.

I. TEST NAME

OSOM® C. difficile Toxin A/B Test

CLIA Complexity: Non-waived

II. INTENDED USE

The OSOM® C. difficile Toxin A/B Test is an immunochromatographic assay intended for the qualitative detection of *Clostridium difficile* toxins A and/or B in human stool samples. This test is intended as an aid in the diagnosis of C. difficile-associated disease (CDAD) in patients with symptoms of CDAD.

III. SUMMARY AND EXPLANATION OF TEST

Clostridium difficile (*C. difficile*) is a spore-forming anaerobic gram positive bacillus that produces toxins which can cause gastrointestinal infections in humans ranging in severity from asymptomatic carriage, to diarrhea, pseudomembranous colitis (PMC), toxic megacolon and death.¹ While infection rates can vary dramatically depending on geography, across institutions, and from year to year, *C. difficile* is a common nosocomial pathogen in institutions with widespread use of broad spectrum antibiotics. Recent surveillance studies in Europe, Canada and the United States suggest that the incidence of CDAD (now increasingly referred to as “CDI”: *Clostridium difficile* infection) is rising, and some regions have experienced alarming outbreaks associated with higher than expected morbidity and mortality rates.^{6, 7, 11}

Clostridium difficile has been linked to occurrences of both antibiotic-associated and nosocomial diarrhea. Both toxigenic and non-toxigenic strains of the *C. difficile* organism exist, however only toxigenic strains are responsible for causing CDI.⁴ Most pathogenic strains of *C. difficile* produce two toxins, toxin A (enterotoxin) and toxin B (cytotoxin).¹² Although both toxins are generally present in infected individuals, there have been reports of toxin A negative/toxin B positive strains of *C. difficile* that cause disease.^{2,4,13} Because toxin must be present for CDI to occur, culture of the organism from stool samples is not considered sufficient for diagnosis, due to the asymptomatic carriage of *C. difficile* among hospitalized patients.³ The cytotoxicity assay is generally considered the reference method for *C. difficile* toxin tests.⁵ Although accurate, the cytotoxicity assay requires specialized facilities and is labor and time intensive, requiring as much as 48 to 72 hours for results. The OSOM® C. difficile Toxin A/B test is able to quickly detect the presence of toxins A and/or B in stool samples, thereby aiding clinicians in the rapid diagnosis and treatment of CDI, and in the initiation of appropriate measures to control nosocomial spread of the disease.

IV. PRINCIPLE OF THE TEST

The OSOM® C. difficile Toxin A/B Test is a qualitative assay that employs immunochromatographic, dipstick technology. The test format is a sandwich immunoassay, with a single test zone on the nitrocellulose dipstick to detect Toxin A and/or Toxin B (blue/gray line) and a single control line zone to indicate proper sample flow (red line). The test procedure involves binding of *C. difficile* Toxin A and/or Toxin B from a patient stool sample to blue colored latex particles conjugated to a monoclonal antibody against Toxin B or a polyclonal antibody against Toxin A. When Toxin A and/or B is present in the sample, it will form a partial immune complex with the antibody-conjugated colored particles. The OSOM® C. difficile Toxin A/B Test stick, when placed in the sample mixture, initiates sample migration along the nitrocellulose membrane. If *C. difficile* toxin A or toxin B is present, a blue/gray line will appear in the test line region indicating a positive result. A red control line must appear for the results to be valid. If *C. difficile* toxins are not present, only the red control line will appear. An invalid test occurs when no control line appears.

V. REAGENTS AND MATERIALS PROVIDED

25 Test Sticks

25 Pipettes

10 Applicator Sticks

35 Test Tubes

2 Bottles Sample Diluent: 20 mL each

(buffered solution with protein, surfactant, 0.09% sodium azide and 0.05% ProClin[®] 300)

2 Diluent dropper tops

1 Bottle Reagent: 8 mL

(antibody conjugate, buffered solution with protein, 0.09% sodium azide and 0.05% ProClin[®] 300)

1 Positive control: 1 mL *Clostridium difficile* Toxoid A and Toxoid B (contains 0.09% sodium azide)

1 Negative control: 1 mL (contains 0.09% sodium azide)

1 Directional Insert

1 Result Interpretation Guide

1 Workstation

Note: Two extra Test Sticks have been included in the kit for external QC testing. Extra Test Tubes have been provided for your convenience.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

- A timer or watch
- Vortex mixer (optional)

VII. WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only
- Follow your clinical and/or laboratory safety guidelines in the collection, handling, storage, and disposal of patient specimens, and all items exposed to patient specimens.⁹
- Pipettes, Applicator Sticks, Test Tubes, and Test Sticks are for single use only.
- Discard used Test Tubes and Test Sticks in suitable biohazardous waste container.
- To ensure correct volumes are delivered, hold the Sample diluent and Reagent bottles vertically when dispensing.
- Optimal results are achieved when kit/sample conditions are followed and the test is performed per the specified procedure.
- The Sample diluent and Reagent contain ProClin[®] 300 and sodium azide as preservatives. If solution comes in contact with the skin or eyes, flush with ample volumes of water.
- Solutions that contain sodium azide may react explosively with lead or copper plumbing. Use large quantities of water to flush discarded solutions down a sink.
- Do not interchange components from different kit lots.

VIII. STORAGE CONDITIONS

- Store Test Sticks, Diluent and Reagent bottles tightly capped and refrigerated (2° - 8°C / 36°- 46°F) when not in use. Do not freeze.
- Allow all **COMPONENTS** to come to **ROOM TEMPERATURE** before use.
- Gently **MIX** the OSOM[®] C. difficile Reagent **PRIOR TO EACH USE**.
- Recap the desiccated container immediately after removing a Test Stick.
- Do not use Test Sticks or reagents after expiration date.
- Discard unused Test Sticks that have been removed from the canister after 1 hour.

At this facility, kits are stored: _____.

IX. SPECIMEN COLLECTION, STORAGE & PREPARATION

- Collect specimens in a clean, leak-proof container.
- Specimens that have been concentrated or collected in transport media are not suitable for use with this test.
- Use only fresh, untreated stool specimens. Test specimen as soon as possible after collection.
- Specimens may be held at room temperature for up to 4 hours. Samples may also be stored at 2° - 8°C (36° -46°F) for up to 72 hours or at -20°C (- 4°F) or below for up to 2 months. Multiple freeze-thaw cycles should be avoided.
- Allow sample to come to room temperature before use.
- All specimens must be thoroughly mixed prior to testing (regardless of consistency), to obtain a representative sample for testing.
- Use only the pipettes supplied in the kit.

This facility's procedure for patient preparation is: _____
_____.

This facility's procedure for sample labeling is: _____
_____.

This facility's procedure for transporting specimens is: _____
_____.

This facility's procedure for rejected specimens is: _____
_____.

X. QUALITY CONTROL (QC)

The OSOM[®] C. difficile Toxin A/B Test provides two types of controls:

- Procedural internal controls to aid in determining validity of each individual test
- External positive and negative controls for *C. difficile* Toxin A/B

Internal Procedural Controls

Several controls are incorporated into each Test Stick for routine quality checks.

1. The appearance of the control line in the results window is an internal positive procedural control.
 - **Test System:** The appearance of the control line assures that adequate test volume was present and that adequate capillary migration of the sample has occurred.
 - **Operator:** The appearance of the control line indicates that adequate test volume was present for capillary flow to occur. If the control line does not appear by the read time, the test is invalid.
2. The clearing of the background in the results area may be documented as an internal negative procedural control. It also serves as an additional capillary flow control. When testing the External Controls, the background should appear white to light blue/gray at the read time and not interfere with the reading of the test. When running patient samples, the background may appear light brown at the read time but should not interfere with the reading of the test. Positive samples will have a blue/gray test line and a red control line visible over the background field.

If the Control Line does not appear and/or the background does not clear and interferes with the test result, the test is invalid. Call Sekisui Diagnostics Technical Assistance at (800) 332-1042 (U.S. only) if you experience a problem.

External Quality Control Testing

The OSOM[®] C. difficile Toxin A/B Test kit includes one Positive and one Negative Control vial for external quality control testing. Use the Controls to ensure that the Test Sticks are functioning properly and to demonstrate proper performance by the test operator. Quality Control requirements should be established in accordance with local, state and federal regulations or accreditation requirements. Minimally, Sekisui Diagnostics recommends that positive and negative external controls be run with each new lot, shipment received, and with each new untrained operator.

QC Testing Frequency and Documentation

For this facility, External QC is run: _____

Results of External QC and action(s) taken when control results are unacceptable are documented:

Quality Control Testing Procedures

The Positive Control contains sufficient *C. difficile* A and B toxin to produce a visible positive test result. The positive control is not intended to ensure precision at the analytical assay cutoff. To perform a positive or negative control test, proceed as follows:

1. Add Sample Diluent - Using the supplied dropper, fill it to the line indicated on the barrel (0.75mL) with Sample Diluent. Expel entire contents into Test Tube.
2. Thoroughly mix the control bottle prior to sampling. Add one (1) drop control into the Test Tube. Mix the solution in the Test Tube by vortex mixing the sample tube (or by holding between thumb/forefinger and flicking/tapping the bottom of the tube ~5 times).
3. Perform steps 3-5 of the TEST PROCEDURE FOR LIQUID OR SEMI-SOLID STOOLS

XI. TEST PROCEDURE FOR LIQUID, LOOSE, OR SEMI-SOLID STOOLS

NOTE: If stool sample is NOT liquid or semi-solid, follow the instructions for PREPARING SAMPLES FROM FORMED STOOL SPECIMENS below before proceeding with the TEST PROCEDURE.

NOTE: When opening a kit for the first time, unscrew the cap from the OSOM[®] C. difficile Sample Diluent bottle and replace it with the dropper included in the kit. Discard the original cap.

STEP 1: ADD SAMPLE DILUENT

Using the supplied dropper, fill it to the line indicated on the barrel (0.75mL) with Sample Diluent. Expel entire contents into Test Tube.

Note: To prevent contamination of the Sample Diluent bottle, add Sample Diluent to the Test Tube before adding the specimen.

STEP 2: ADD SAMPLE TO TEST TUBE

Note: Ensure that the specimen is thoroughly mixed prior to adding to the Test Tube.

The addition of too much or too little sample or failure to perform the mixing steps may result in invalid or false negative results.

- Squeeze the bulb of the pipette supplied in the kit and insert the pipette into the sample.
- Release the pressure on the bulb to fill the barrel to the 50µL line.
- Expel the entire contents of the pipette into the Test Tube. Discard the pipette in a suitable biohazardous waste container.
- **Mix the solution by vortex** (or by holding between thumb/forefinger and flicking/tapping the bottom of the tube ~5 times).

STEP 3: ADD REAGENT

Note: The Reagent should be gently mixed before each use by inverting the capped bottle 2-3 times to re-suspend any settled material.

- With the Reagent bottle tip pointed straight down, dispense five (5) drops of Reagent into the diluted sample mixture.
- **Mix the solution by vortex** (or by holding between thumb/forefinger and flicking/tapping the bottom of the tube ~5 times).

STEP 4: ADD TEST STICK AND LET STAND

Remove a Test Stick from the canister and recap the canister immediately. Place the Test Stick with arrows pointing down into the solution in the sample tube. Set a timer for 20 minutes.

STEP 5: READ RESULTS

- At 20 minutes remove Test Stick from the Test Tube and, under adequate lighting, read the results.
- When reading results, the use of the Result Interpretation Guide is recommended.
- Some positive results may be seen and reported earlier. Test is invalid beyond the stated read time.
- See Interpretation of Test Results section below.

XII. PREPARING SAMPLES FROM FORMED STOOL SPECIMENS

NOTE: If testing a formed stool specimen, the sample must be liquefied prior to running in the assay.

1. Using the supplied dropper, add 0.75mL OSOM[®] C. difficile Sample Diluent to a Test Tube by filling the dropper to the line indicated on its barrel and expelling the entire contents into the Test Tube.

Note: To prevent contamination of the Sample Diluent bottle, add Sample Diluent to the Test Tube before adding the specimen.

2. Collect a pea-sized (approximately this diameter ●) portion of stool with the applicator stick and transfer to the sample tube containing Sample Diluent. **Do not remove the applicator stick from the Test Tube.**

3. Thoroughly mix the sample on the applicator stick in the Sample Diluent with a vortex mixer (or by holding between thumb/forefinger and flicking/tapping the bottom of the tube ~5 times) to create an even mixture. Remove applicator stick **after** mixing.
4. Use the diluted stool mixture as the liquid stool sample in the procedure above beginning at STEP 1.

XIII. INTERPRETATION OF TEST RESULTS

NOTE: Only Test lines in any shade of blue or gray should be read as positive.

The appearance of a red Control Line at the read time, with or without a blue/gray Test Line, indicates a valid result. A Test Line or Control Line that appears uneven in color shading is still considered a valid line. In cases of moderate or high positive specimens, some color behind the Test Line may be seen. As long as the Test Line and the Control Line are visible the results are valid.

POSITIVE RESULTS

A blue/gray Test Line and a red Control Line is a positive result for the detection of *C. difficile* A and/or B toxin.

NOTE: the red and blue/gray lines can be any shade of those colors. The lines may be lighter or darker than the lines in the picture. Any visible blue/gray Test Line should be considered positive.

NEGATIVE RESULTS

A red Control Line but no blue/gray Test Line is a negative result. A negative result means that either no *C. difficile* A and/or B toxin is present in the sample, or the level of the toxin in the sample is below the detection limit of the assay.

INVALID RESULTS

If no red Control Line appears or background color makes reading the Control Line impossible, the result is invalid. If this occurs, repeat the test using a new Test Stick or contact Sekisui Diagnostics Technical Assistance.

In the event this test becomes inoperable, this facility's course of action for patient samples is:

XIV. LIMITATIONS

- The OSOM[®] *C. difficile* Toxin A/B Test is only for the qualitative detection of *C. difficile* A and B toxins in human stool samples. Results should be used in conjunction with other diagnostic procedures and in the context of the patient's clinical information to establish a diagnosis. A negative result may warrant additional patient follow up.
- The test yields qualitative results. No quantitative or semi-quantitative interpretation should be made based on the intensity of the Test Line color when reporting positive results.
- The performance of this test with specimens other than human stool samples has not been established.
- The performance of this test in pediatric patients has not been evaluated.
- A negative result may occur if the specimen collection is inadequate or if the toxin concentration is below the sensitivity of the test.

- Although not recognized as an important human pathogen, *C. sordellii* produces toxins similar to *C. difficile* that may cause cross-reactivity in diagnostic tests that detect *C. difficile* toxin A and/or B.⁵

XV. EXPECTED RESULTS

Clostridium difficile is the most frequently identified cause of nosocomial diarrhea, accounting for 15%-25% of cases of antibiotic-associated diarrhea and more than 95% of cases of pseudomembranous colitis.^{1,3} CDI is primarily a nosocomial disease, and therefore the rate of infection may vary from location to location. Risks for infection include length of hospitalization, patient age, antibiotic use, severity of underlying disease and gastrointestinal surgery or procedures.⁸ Test results should be used in conjunction with the patient's clinical information as asymptomatic colonization with *C. difficile* and its toxins can be seen in some healthy adults, up to 50% of cystic fibrosis patients and up to 50% of infants.⁴

In a prospective clinical study involving the OSOM[®] *C. difficile* Toxin A/B Test at 5 independent sites, an overall prevalence rate of 8.0% (102/1274) was observed in freshly acquired diarrhea samples submitted to the laboratory for CDI testing.

XVI. PERFORMANCE CHARACTERISTICS

Refer to directional insert – OSOM[®] *C. difficile* Toxin A/B Test

XVII. REFERENCES

Refer to directional insert – OSOM[®] *C. difficile* Toxin A/B Test

XVIII. ASSISTANCE

For assistance contact Sekisui Diagnostics Technical Assistance at (800) 332-1042.