**PROCEDURE: Immuno*Card®* Toxins A & B**

# PRINCIPLE

ImmunoCard Toxins A & B is a rapid, qualitative, horizontal-flow enzyme immunoassay (EIA) for detecting Clostridium difficile Toxins A and B in human stool. This assay is used as an aid in the diagnosis of C. difficile-associated disease.

ImmunoCard Toxins A & B consists of a membrane held in a plastic frame with two sample ports and two reaction ports. The membrane carries immobilized antibodies to toxins A and B. The Enzyme Conjugate Reagent consists of antibodies to toxins A and B coupled to horseradish peroxidase. To perform the test, patient stool sample is diluted with Sample Diluent and Enzyme Conjugate and the mixture is incubated for five minutes to 24 hours. During the incubation, molecules of toxin, if present, are bound to the anti-toxin antibodies of the conjugate. Following incubation, an aliquot of the mixture is added to each of the two sample ports and the test is incubated for an additional five minutes at 20-26 C. During the second incubation the toxin-conjugate complex is separated from particulate matter as the fluid portion of the sample flows through the membrane to the TEST and CONTROL reaction ports. The toxin-conjugate complexes are then captured at the TEST reaction port by immobilized antitoxin in the reaction membrane. (The second of the two reaction ports serves as an internal control.) Both reaction ports are subsequently washed with ImmunoCard Wash Buffer I to reduce interference by contaminating proteins before ImmunoCard Substrate I is added. The reaction ports are incubated for an additional five minutes during which time the Enzyme Conjugate modifies the ImmunoCard Substrate I. The result is the appearance of a blue color. Reactions are read visually. Development of a blue color in the TEST reaction port indicates a positive test. In the CONTROL port, the anti-toxin antibodies of the conjugate bind directly to the immobilized toxin. The appearance of blue in the CONTROL reaction port indicates that sample was added, that reagents were active at the time of use and that proper sample migration occurred.

# AVAILABILITY

1. This test is performed at least once daily at Rhode Island Hospital on all positive C. difficile PCR stool specimens.

# TEST CODE

1. CDTXF – this test is a reflexed order on C. Difficile PCR Positive specimens

# SPECIMEN

1. Minimum volume of 10mL Fresh Stool specimens stored in 2º and 8ºC that have been identified as Positive by PCR. Specimens should be tested within 96 hours of collection and within 24 hrs of a Positive PCR result.

# MATERIALS and EQUIPMENT

1. Materials provided
2. **ImmunoCard Toxins A & B Test Card:** A membrane pad housed in a plastic frame and enclosed in a foil pouch with a desiccant. The pad carries immobilized monoclonal anti-toxin A and goat polyclonal anti-toxin B at the TEST reaction port and C. difficile toxin at the CONTROL reaction port. Store the test cards at 2-8 C when not in use.
3. **Sample Diluent:** A buffered protein solution containing gentamicin and thimerosal (0.02%) as a preservative. Store at 2-8 C when not in use.
4. **Positive Control\*:** Inactivated crude *C. difficile* toxin in a buffered solution containing thimerosal (0.02%) as a preservative. The reagent is supplied ready for use. Store at 2-8 C when not in use.
5. **Enzyme Conjugate\*:** A blend of goat polyclonal antibodies to toxins A and B conjugated to horseradish peroxidase and suspended in a buffered protein solution containing gentamicin and thimerosal (0.02%). Store at 2-8 C when not in use.
6. **Immuno*Card* Wash Buffer I\*:** A buffered solution containing thimerosal (0.01%) as a preservative. Store at 2-8 C when not in use.
7. **Immuno*Card* Substrate I\*:** A buffered solution containing tetramethyl-benzidine and peroxide. Store at 2-8 C when not in use.
8. Plastic transfer pipettes with measuring marks for 25 μL and 150 μL.
9. Materials not provided
10. Disposable latex Disposable latex gloves that should be used during the handling of the fecal samples as they are considered potentially biologically hazardous material
11. Vortex for suspending of the stool sample in the Sample Diluent
12. Interval timer
13. Applicator sticks
14. Small test tubes, such as 10 x 75 mm or 12 x 75 mm

# STORAGE AND HANDLING

1. The kit expiration date is indicated on the kit label.
2. Store at 2 - 8 C and return the kit promptly to the refrigerator after each use.

# QUALITY CONTROL

## Internal: Each Test Card contains an internal control. The appearance of a blue color in the CONTROL reaction port verifies the assay was active at the time of use, that sample was added and that there was adequate migration of the sample.

## External: The reactivity of each new lot and each new shipment of the ImmunoCard Toxin A & B should be verified on receipt or before use. Positive Control reagent is supplied with the kit. The control is used to verify the reactivity of the other reagents associated with the assay and is not intended to ensure precision at the analytical assay cut-off. Sample Diluent is used for the Negative Control. Additional tests can be performed with the controls to meet the requirements of local, state and/or federal regulations and/or accrediting organizations. The results expected with the controls are described in the section on INTERPRETATION OF RESULTS. The Test Card should not be used if control tests do not produce the correct results. Proper results obtained with the Control Port, Positive Control and Negative Control (Sample Diluent) serve as indicators that the test was performed correctly, that the antibodies embedded in the membrane and the Enzyme Conjugate are active at the time of testing, and that the membrane supports proper sample flow. Failure of the internal and external control to produce the expected results suggests the test was not performed correctly (ie, incorrect volume of reagents added; incorrect incubation temperature or times used or that reagents were not brought to room temperature prior to testing). If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated please contact Meridian’s Technical Services Department at 1-800-343-3858 (US) or your local distributor.

* 1. External controls are run with each new lot or shipment, and/or every 30 days, whichever comes first. Refer to ***IQCP: ImmunoCard Tox A & B*** for additional details.

# STORAGE AND HANDLING

1. Bring all test cards, reagents, and samples to room temperature (20-26 C) before testing. Remove the reagents from the kit box to warm.

**Reagents may take up to 60 minutes to warm following refrigeration.**

**RETURN TO 2-8 C IMMEDIATELY AFTER USE.**

1. Label one test tube for each patient specimen to be tested.
2. Add 200 μL of Specimen Diluent to a test tube using the dropper in the bottle.
3. Add three drops of Enzyme Conjugate (150 μL) to each tube.
4. Immediately add stool or control sample as follows:

**Liquid/Semi-solid stools** – Mix the stool specimen thoroughly. Using a clean transfer pipette (included with the kit), draw the stool to the first mark from the end of the tip (25 μL). Dispense the stool into the Diluent/Conjugate mixture. Use the same pipette to mix the stool by gently aspirating, then dispensing the suspension several times. Vortex the final mixture for 10 seconds. Leave the pipette in the tube for use later in the test.\*

**External control** – Using the container droppers,add 1 drop of Positive Control or Negative Control (Specimen Diluent) (provided with the kit) to the Diluent/Conjugate mixture**.** Vortex for 10 seconds. Add a pipette to the tube and leave for later testing.

**\*NOTE: The transfer of too little sample, or failure to mix and suspend the sample in the Diluent/Conjugate mixture completely may result in a false-negative test result. Addition of too much stool sample may cause invalid results due to restricted sample flow.**

1. Let each diluted sample stand for 5 minutes to 24 hours at 20-26 C. Seal tube containing diluted sample or control if incubating beyond 6 minutes.
2. Use 1 Immuno*Card* Test Card for each sample or control. When ready to perform testing, remove the Immuno*Card* Test Card from its foil pouch. Discard the pouch and desiccant. Label the device with the name of the patient or the control.
3. Vortex each sample or control for 10 seconds before use.
4. Add 150 μL of each diluted sample or control to each of the 2 sample ports on 1 test card (150 μL represents the second mark on the transfer pipette).
5. Incubate for 5 ± 1 minutes at 20-26 C. NOTE: At the end of incubation both reaction ports must appear completely wet. If either reaction port is not completely wet, discard the Test Card and repeat the procedure. Proceed with testing if sample remains in the sample ports yet both reaction ports are completely wetted.
6. Hold the Immuno*Card* Wash Buffer I vial vertically and dispense exactly 3 drops to each of the reaction ports.
7. When the Immuno*Card* Wash Buffer I has been completely absorbed, hold the vial vertically and add exactly 3 drops of Immuno*Card* Substrate I.
8. Incubate the test card at 20-26 C for 5 minutes.
9. **Visually read the results of each card within 30 seconds of the end of incubation.**

# INTERPRETATION OF RESULTS

**READ ONLY UPPER REACTION PORTS TO INTERPRET RESULTS**

 POSITIVE NEGATIVE INVALID

1. Positive
2. Blue color in the TEST (upper right) and CONTROL (upper left) reaction ports. The intensity of the blue color of the TEST port may vary from the bottom to the top of port. A positive test result indicates that toxin A and/or B is/are present in the sample.
3. **PLEASE NOTE:** The TEST port may appear less blue than the CONTROL port. A positive result indicates the presence of *C. difficile* toxin.
4. Negative
5. Blue color in the CONTROL reaction (upper left) port only. The TEST reaction (upper right) port should be colorless. (The wetting of the membrane may make the TEST port appear to be slightly grey.) A negative test result indicates that C. difficile toxins are absent or below the limit of detection of the assay. Occasionally the TEST PORT (upper right) may show evidence of a hint of blue color in the left or right side of the port, with the rest of the port colorless. This should be considered a negative test result.
6. Invalid – **DO NOT REPEAT**
7. No detectable blue color in the CONTROL reaction port. Failure of the CONTROL invalidates any test result. The invalid test may be due to the failure of a reagent or the Test Card to perform properly, failure to add sample, failure of the sample to migrate, or failure to dilute the sample correctly leading to over inoculation of the Test Card. Samples with extremely high levels of toxins may also present with a positive TEST yet negative CONTROL port.
8. A blue ring on the plastic frame surrounding the TEST (upper right) port during the test procedure.
9. **An invalid test will not be repeated.**

# REPORTING

1. Positive Result
2. C. Difficile Free Toxin EIA detected

*Toxigenic C difficile is detected and producing free toxin. These results are consistent with C. difficile infection in the context of the patient’s clinical presentation. Continue with precautions.*

1. Negative Result
2. C Difficile Free Toxin EIA not detected

*No free toxin detected. These results may suggest C difficile colonization, or the toxin is below the limit of detection by EIA. The significance of these results must be interpreted in the context of the patient’s clinical presentation, treat only if clinically indicated. Continue contact precautions.*

1. Indeterminate Result
2. Presence or Absence of toxin cannot be confirmed.

# LIMITATIONS OF TEST

1. All reagents are for *in vitro* diagnosticuseonly.
2. Do not store at temperatures above 2-8 C. Do not freeze.
3. Do not deviate from the established insert method or falsely positive or falsely negative results may occur.
4. Some patient samples contain infectious agents; therefore all patient samples should be handled and disposed of as if they are biologically hazardous.
5. DO NOT interchange the Test Card, Enzyme Conjugate and Positive Control reagents from different kit lot numbers. The other reagents (Sample Diluent, Immuno*Card* Wash Buffer I, Immuno*Card* Substrate I) can be interchanged between kits providing the reagents are within their expiration periods. Do not use any reagent beyond its labeled expiration date.
6. All stool samples must be mixed thoroughly before testing, regardless of consistency, to ensure a representative sample prior to testing.
7. Failure to bring samples and reagents to room temperature (20 - 26 C) before testing may decrease assay sensitivity.
8. Inspect Test Cards before removing the foil pouch. Do not use Test Cards that have holes in the foil pouch or where the pouch has not been completely sealed. . Do not use Test Cards where the desiccant indicator has changed from blue to pink. False-negative reactions may result due to deterioration of the improperly stored Test Cards.
9. Do not use the Sample Diluent or Positive Control if they are discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination. It is normal for the Immuno*Card* Wash Buffer I to appear slightly turbid.
10. The Immuno*Card* Substrate I is light sensitive and should be stored in the dark. The appearance of the reagent should be clear and colorless. If the reagent exhibits any color or is turbid it should be discarded.
11. Hold reagent vials vertically when dispensing drops to ensure consistent drop size and delivery.

# NOTES

1. Reagents from different kits should not be mixed or interchanged. Do not use a kit past the expiration date.
2. Bring all components to ROOM TEMPERATURE BEFORE USE!
3. Caps, tips and dropper assemblies are color-coded; do NOT mix or interchange!
4. Do not freeze the reagents. The kit should be stored between 2°C and 8°C.
5. The pouch containing the *Membrane Device* should be at room temperature before opening and opened just before use. Keep the membrane devices dry before use.
6. Use fecal specimens within 72 hours of collection to obtain optimal results. Specimens that are frozen may lose activity due to freezing and thawing.
7. Specimens that have been preserved in 10% Formalin, merthiolate formalin, sodium acetate formalin or polyvinyl alcohol cannot be used.
8. Hold reagent bottles vertically to dispense reagents to ensure consistent drop size.
9. Specimens and membrane devices should be handled and disposed of as potential biohazards after use. Wear disposable gloves when doing the test.
10. Membrane devices cannot be reused.
11. The test has been optimized for sensitivity and specificity. Alterations of the specified procedure and/or test conditions may affect the sensitivity and specificity of the test. Do not deviate from the specified procedure.
12. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes if removing aliquots from reagent bottles.
13. Be attentive to the total assay time when testing more than one fecal specimen. Add *Diluent* first, then add the *Conjugate* to each tube of *Diluent*. Then add specimen to the tube of *Diluent/Conjugate*. Thoroughly mix all of the diluted specimens, and then transfer to the *Membrane Device*. The 15-minute incubation step begins after the last diluted sample-conjugate mixture has been transferred to the final *Membrane Device*.
14. If the *Substrate* reagent changes to a dark blue/violet color call technical services for replacement.
15. Reagents contain thimerosal as a preservative. Handle reagents according to existing regulations for laboratory safety and good laboratory practice. Safety Data Sheets for this product are available upon request, contact technical support.
16. Follow your national, regional, and local ordinances accordingly for waste disposal regulations. Do not place in trash, dispose of as hazardous waste.
17. For *in vitro* diagnostic use only.

# REFERENCES

1. Refer to Directional Insert – Meridian Bioscience Immuno*Card*® Toxins A & B.

# REVISIONS

1. 5/18/2022 – Updated external control practices to reflect implementation of IQCP