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ProSpecT C. difficile Toxin A/B Microplate Assay

REF R244596 .....96 Tests **EN**

1. INTENDED USE

ProSpecT™ Clostridium difficile Toxin A/B Microplate Assay is a qualitative enzyme immunoassay (EIA) for the detection of *C. difficile* Toxin A and B in human faecal specimens from patients suspected of having *Clostridium difficile* disease. The test is intended for use as an aid in diagnosis of *Clostridium difficile*-associated disease (CDAD).

2. SUMMARY

Clostridium difficile, a gram positive anaerobic spore-forming bacillus, is the most common identifiable cause of antibiotic-associated diarrhoeal disease[1,2]. The disease occurs when treatment with broad-spectrum antibiotics suppresses bacteria in the normal intestinal flora, allowing opportunistic growth of toxigenic strains of *Clostridium difficile*. The toxins produced by *Clostridium difficile*, designated Toxin A and Toxin B, have potent enterotoxic and cytotoxic effects, respectively. The severity of the disease may range from uncomplicated diarrhoea to a condition known as pseudomembranous colitis (PMC), characterized by nausea, abdominal pain, watery diarrhoea, dehydration, low grade fever and the appearance of raised yellow plaques over the colorectal mucosa. Fulminant colitis may be fatal if untreated. Nosocomial outbreaks of *Clostridium difficile* gastrointestinal illness and relapses may occur[11]. Antibiotic treatment directed towards *Clostridium difficile* can help resolve the disease.

Diagnosis is usually performed through the detection of one or both *Clostridium difficile* toxins[3,12]. A cell culture based cytotoxin assay is considered the reference method, but is relatively time-consuming to perform. Immunoassays detecting either Toxin A alone or Toxin A and B together have become established tools in the diagnosis of *Clostridium difficile* disease[4,5]. The existence of non-toxigenic variants of *Clostridium difficile* support the use of toxin-based assays for definitive diagnosis. Nucleic acid probe-based assays have been used experimentally, but may be complicated by the fact that the organism may be present asymptomatically in about 50% of infants, 20-30% of hospitalized patients and in 2-3% of healthy adults[6,7]. The clinical significance of detecting both Toxin A and B is not yet fully understood. While most disease-causing strains produce both toxins, which may act synergistically, documented cases of disease caused by Toxin B only strains of *Clostridium difficile* suggest that it is clinically important to assay for both Toxin A and B[8,9,10].

3. PRINCIPLE OF THE TEST

The ProSpecT Clostridium difficile Toxin A/B Microplate Assay is a solid phase immunoassay for the detection of Toxin A and Toxin B in clinical stool specimens through the use of specific antibodies. A stool specimen can be diluted in Sample Diluent or used directly if pre-diluted in modified Cary Blair medium. Specimens are added to break-away microplate wells on which mouse monoclonal anti-Toxin A and rabbit anti-Toxin B antibodies are bound. If toxins are present, they are 'captured' by the bound antibody. The wells are incubated and then washed to remove unbound material. The enzyme conjugate (goat anti-Toxin A and rabbit anti-Toxin B labelled with horseradish peroxidase enzyme) is added. The wells are incubated and then washed to remove unbound enzyme conjugate. In a positive reaction, the bound toxin binds the enzyme conjugate to the well. The substrate for the enzyme, 3,3',5,5'-tetramethylbenzidine (TMB), is added. In a positive reaction, the enzyme bound to the well converts the substrate to a coloured reaction product. Colour development can be detected visually or spectrophotometrically. In a negative reaction, there is no toxin or an insufficient level of toxin present to bind the enzyme conjugate and no coloured reaction product develops.

4. SYMBOL DEFINITIONS

REF	Catalogue Number
IVD	In Vitro Diagnostic Medical Device
Σ	Contains sufficient for <n> tests
	Consult Instructions for Use (IFU)
2°C 25°C 8°C	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufacturer
DILUTED SAMPLE	Diluted Sample

5. KIT CONTENTS, PREPARATION FOR USE AND STORAGE

The ProSpecT C. difficile Toxin A/B Microplate Assay includes sufficient reagents to perform Σ 96 tests.

See also **Precautions**, section **6**.

The expiration date of each kit is stated on the package label.

Store all components at 2 to 8°C.

Before use, bring all reagents to room temperature (20 - 25°C) and mix gently. Return the unused reagents to the refrigerator after use.

All reagents, except the Wash Buffer, are supplied at working strength. Reagents can be dispensed directly from the dropper bottles or poured out for use with multichannel pipettes. If excess reagent has been poured, the excess should be discarded. Do not pour excess reagent back into the bottle.

Instructions for Use

Transfer pipettes
Microplate Strip Holder and Cover
Procedure Card

MICROTITRATION PLATE

Microplate* (8 wells / strip)

12 strips coated with mouse anti-Toxin A and rabbit anti-Toxin B. Unused microplate strips should be stored in the foil pouch containing desiccant to exclude moisture.

CONJUGATE

Enzyme Conjugate*

One dropper bottle containing 25 ml of horseradish peroxidase labelled goat anti-Toxin A and rabbit anti-Toxin B and antimicrobial agents.

CONTROL +

Positive Control

One dropper bottle containing 4 ml of *C. difficile* Toxin A and Toxin B culture supernatant, and antimicrobial agents.

CONTROL –

Negative Control

One dropper bottle containing 4 ml of a buffered solution with a red dye, and 0.1% sodium azide

SAMPLE DILUENT

Sample Diluent

One bottle containing 120 ml of a buffered solution, a red dye and 0.1% sodium azide.

WASH BUFFER (x10)

Wash Buffer

One bottle containing 120 ml of (x10) concentrated buffered solution with antimicrobial agents.

Dilute (x10) Wash Buffer concentrate to (x1) by adding 1 part concentrate to 9 parts distilled or deionised water. Diluted Wash Buffer is stable for 1 month when stored at 2 - 8°C.

SUBSTRATE TMB

Colour Substrate

One dropper bottle containing 25 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer.

The Colour Substrate should be stored in and used from the light protected bottle in which it is provided. If an aliquot is removed from the original bottle for any reason, do not return unused Colour Substrate to the original bottle.

STOP SOLUTION

Stop Solution

One dropper bottle containing 12 ml of 0.46 mol/l Sulphuric acid.

***Note:** Do not interchange reagents between kits with different lot numbers.

6. PRECAUTIONS

IVD

The reagents are for *in vitro* diagnostic use only.

For professional use only.

Please refer to the Safety Data Sheet (SDS) and product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

6.1. Reagents are prepared from biological materials and should be handled as potentially infectious material. Discard using appropriate biohazard procedures.

6.2. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.

6.3. Specimens may contain potentially infectious agents and should be handled at Biosafety Level 2 as recommended in the CDC/NIH manual, "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition.

6.4. Wash Buffer contains potential skin sensitiser (< 0.1% v/v). Avoid skin contact. Wear disposable Vinyl or Nitrile gloves.

6.5. Discard used Wash Buffer in appropriate biohazard containers.

6.6. The Sample Diluent and Negative kit control contain 0.1% sodium azide. Azides can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small, nevertheless when disposing of azide containing materials they should be flushed away with relatively large quantities of water.

ANALYTICAL PRECAUTIONS

6.7. Carefully read and follow all instructions in this Instruction for Use.

6.8. Reagents are provided at the necessary working strength, with the exception of the Wash Buffer concentrate. Do not dilute reagents, except where instructed.

6.9. Do not use reagents beyond the expiration dates. Expiration dates are printed on each reagent label. Use of reagents beyond the expiration date may affect the accuracy of results.

6.10. The following common reagents may be used across the ProSpecT product range: Wash Buffer, Colour Substrate and Stop Solution.

6.11. Microbial contamination of reagents may decrease the accuracy of the assay. Avoid microbial contamination of reagents by using sterile disposable pipettes when removing aliquots from reagent bottles.

6.12. Allow all reagents and specimens to reach room temperature (20 - 25°C) before use.

6.13. Microplate strips must be stored in the resealable foil pouch, with desiccant, to protect microplate wells from moisture.

6.14. Stool samples must be thoroughly mixed prior to specimen processing to ensure accurate representation of the specimen. DO NOT CONCENTRATE SPECIMENS BEFORE TESTING.

6.15. Colour Substrate is sensitive to light exposure. If the reagent is exposed to light and develops colour, the reagent must be discarded.

6.16. Persons who are colour blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

6.17. Add reagents to the test wells in the same order throughout the procedure. To avoid contamination do not touch the fluid in the wells with the bottle tips.

6.18. Time each incubation accurately. Start timing after adding reagent to the last well on each microplate being tested. To ensure accurate timing, process no more than three 96 well plates at one time. Deviation from the established procedure may alter the performance of the assay.

6.19. It is important to hold the dropper bottles vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet, a drop of incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.

7. COLLECTION OF FAECAL SPECIMENS

Standard stool specimen collection and handling procedures for each laboratory are appropriate.

FRESH Untreated specimens should be stored at 2 - 8°C and tested within 48 hours.

Fresh specimens diluted in the Sample Diluent provided in the kit can be stored at room temperature for up to 8 hours or stored refrigerated for up to 72 hours prior to testing.

FROZEN If fresh specimens cannot be tested within 48 hours, they should be frozen at -20°C or below and tested within 2 months. Avoid multiple freeze-thaw cycles, which may result in degradation of toxins.

CARY BLAIR Specimens preserved in modified Cary Blair transport medium with indicator (or equivalent) can be stored at room temperature (20 - 25°C) for up to 5 days prior to testing. Stool specimens that have been concentrated or treated with 10% formalin, SAF or PVA fixatives are not suitable for use.

8. TEST PROCEDURE

REQUIRED MATERIALS PROVIDED

See **Kit Contents**, section **5**

MATERIALS REQUIRED BUT NOT PROVIDED

Stool specimen collection containers
Plastic or glass disposable test tubes
Timer that measures minutes
Wash bottle for Wash Buffer
Distilled or deionised water

OPTIONAL MATERIALS NOT PROVIDED

Microplate reader capable of reading 450/620 to 650 nm
Cotton or rayon tipped applicator sticks
Micropipette to deliver volumes to 200 µl
Vortex mixer with plate adapter or shaker
Modified Cary Blair transport medium

PROCEDURE

8.1. **Specimen Preparation:** Use one of the three preparation methods below to prepare the specimens.

A	**Formed stools**
1.	Label one tube for each specimen.
2.	Add 0.8 ml Sample Diluent (SD) to each tube.
3.	Using a wooden applicator stick, add 0.2 g (small pea-size piece) specimen.
4.	Vigorously stir specimen into SD.
5.	Add a transfer pipette to the tube and mix by drawing up and down once.
6.	Leave transfer pipettes in the tubes.
7.	**PROCEED TO STEP 8.2**

B	**Liquid and semi-solid stools**
1.	Label one tube for each specimen.
2.	Add 0.8 ml SD to each tube.
3.	Mix samples by shaking specimen collection containers.
4.	Using transfer pipettes draw up 0.2 ml (second mark from the tip of the pipette).
5.	Expel sample into SD.
6.	Mix by drawing up and down once.
7.	Leave transfer pipettes in the tubes.
8.	**PROCEED TO STEP 8.2**

C	**Cary-Blair stools**
1.	Invert transport vial several times to thoroughly mix specimen.
2.	No further dilution of the specimen is required.
3.	**PROCEED TO STEP 8.2**

8.2. Open the foil pouch, remove the required number of microplate strips and place into a microplate strip holder. Use one well for the Negative Control and one well for the Positive Control. If using less than 8 wells, break off the required number of wells from a strip and return the unused wells to the foil pouch with desiccant. RESEAL POUCH TIGHTLY TO EXCLUDE MOISTURE AND RETURN TO THE REFRIGERATOR.

8.3. Add **4 drops** (200 µl) Negative Control to well A1. Add **4 drops** (200 µl) Positive Control to well B1.

8.4. Using transfer pipettes add 0.2 ml (second mark from the tip of the pipette) of each specimen to a well. **Note:** Place the opening of the transfer pipettes just inside the wells to avoid splashing into adjacent wells.

8.5. **Cover** microplate and incubate at room temperature (20 - 25°C) for **60 minutes**. Begin timing after the addition of the last specimen.

8.6. Shake out or aspirate the contents of the wells. Wash by completely filling each well with **diluted** Wash Buffer (~350-400 µl/well). Shake out or aspirate all fluid from the wells after each wash. Wash a total of **3 times**. After the last wash remove contents and strike plate on clean paper towels or aspirate. Remove as much Wash Buffer as possible but do not allow the wells to dry out at any time.

8.7. Add **4 drops** (200 µl) of Enzyme Conjugate to each well.

8.8. **Cover** microplate and incubate at room temperature (20 - 25°C) for **30 minutes**.

8.9. Shake out or aspirate and wash each well **5 times** as in step 8.6.

8.10. Add **4 drops** (200 µl) of Colour Substrate to each well.

8.11. **Cover** microplate and incubate at room temperature (20 - 25°C) for **10 minutes**.

8.12. Add **1 drop** (50 µl) Stop Solution to each well. Gently tap or vortex the wells until the yellow colour is uniform. Read reactions within **10 minutes** after adding the Stop Solution. Read visually or spectrophotometrically at 450/620 to 650 nm (dual wavelength).

9. QUALITY CONTROL

Positive and Negative Controls must be included each time the test is performed. The Positive and Negative Controls serve as both reagent and procedural controls. The controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cut-off.

The optical density (O.D.) of the Negative Control should be < 0.080 at 450/620 to 650 nm and should be colourless (< 1+ intensity) when read visually. If yellow colour equal to 1+ or greater on the Procedure Card is present in the Negative Control, the test should be repeated with careful attention to the wash procedure.

The O.D. of the Positive Control should be ≥ 0.500 at 450/620 to 650 nm and should be equal to or greater than the 2+ reaction when read visually. If yellow colour less than 2+ on the Procedure Card is present in the Positive Control, call for technical assistance.

10. RESULTS

Refer to the enclosed Procedure Card for colour interpretations.

VISUAL

10.1. Read the test results by comparing with the reaction colours on the Procedure Card.

Positive: yellow colour of at least 1+ intensity
Negative: colourless

10.2. Interpretation of visual results:

Positive: If yellow colour of at least 1+ intensity develops in the test well, the sample contains Toxin A or B or both and the test is positive.
Note: Tests with faint yellow colour (less than 1+) should be repeated.
Negative: A colourless reaction is a negative result and indicates that no Toxin A or B or an undetectable level of toxin is present in the sample tested.

SPECTROPHOTOMETRIC

10.3. Read results at dual (450/620 to 650 nm) wavelength.

10.4. Read the optical density (O.D.) of the Negative Control. The O.D. should be < 0.080 for 450/620 to 650 nm. If the O.D. is greater than 0.080, the results are invalid and the test should be repeated with careful attention to the wash procedure.

10.5. The O.D. for the Positive Control should be ≥ 0.500 at 450/620 to 650 nm.

10.6. Read the test results:

Positive: O.D. of ≥ 0.080
Negative: O.D. of < 0.080

10.7. Interpretation of spectrophotometric results:

Positive: Positive for *C. difficile* Toxin A and/or B. A positive test does not define the presence of disease. Results should be used in conjunction with other clinical findings to establish a diagnosis.
Negative: Negative for *C. difficile* Toxin A and/or B. Infection cannot be ruled out since the toxin present in the specimen may be below the detection limit of the test.
***Note:** Any wells that are visually clear but give an O.D. reading that is inconsistent with the visual interpretation should be considered a discrepant reading and examined for the presence of bubbles, small particles in the wells, or an opaque film on the bottom of the well. To remove the film, wipe the underside of the wells and read the O.D. again. If the discrepancy between visual and O.D. readings persists, repeat the test.

11. PERFORMANCE LIMITATIONS

The validity of results with the ProSpecT Clostridium difficile Toxin A/B Microplate Assay depends on the control reaction performing as expected. See **Quality Control**, section **9**.

A positive test does not define the presence of disease. The test detects the presence of Toxin A and/or B in faecal specimens. Results should be used in conjunction with other clinical findings to establish a diagnosis.

A negative test result does not exclude the possibility of the presence of *C. difficile* Toxin A or Toxin B, and may occur when the toxin level in the sample is below the detection level of the test.

The level of toxin has not been shown to be correlated with either the presence or severity of disease. As with all IN VITRO diagnostic tests, results should be interpreted by the clinician in conjunction with clinical findings and/or other laboratory results.

Proper specimen collection and processing are essential to achieve optimal performance of the assay. Optimal test results are obtained from specimens tested within 48 hours of collection. See **Collection of Faecal Specimens**, section **7**.

The Performance Characteristics of this test have not been evaluated in pediatric populations.

12. EXPECTED VALUES

C. difficile colitis occurs at a much higher frequency in patients who are hospitalized and is the fourth most common nosocomial disease reported to the Centres for Disease Control and Prevention. *C. difficile* is responsible for 20-30% of antibiotic-associated diarrhoea and more than 90% of pseudomembranous colitis. The incidence rate of nosocomial CDAD may vary with hospital populations and is influenced by the presence of predisposing factors, such as increased patient age, type and duration of antimicrobial therapy, severity of underlying illness(es), and length of hospital stay. *C. difficile* is found in 3-5% of healthy adults and up to 50% of infants and young adults asymptomatically carry both the bacteria and its toxins[13].

13. PERFORMANCE CHARACTERISTICS

COMPARED TO TISSUE CULTURE CYTOTOXICITY ASSAY

Testing was conducted at three clinical laboratories in North America. For all specimens evaluated, the overall sensitivity of the ProSpecT Clostridium difficile Toxin A/B Microplate Assay when compared to Tissue Culture Cytotoxicity Assay (CTA) was 90.3% (149/165), and the overall specificity when compared to CTA was 96.2% (576/599).

		CTA Results	
OVERALL		+	-
ProSpecT	+	149	23
EIA Results	-	16	576
Total		165	599

90.3% Sensitivity (149/165); 95% CI = 84.7% - 94.4%
96.2% Specificity (576/599); 95% CI = 94.3% - 97.5%

VISUAL INTERPRETATION OF TEST

Visual read data was collected at two of the three laboratories for a total of 586 specimens. The overall sensitivity when compared to CTA was 85.0% and the overall specificity was 95.5%. The visual read results were in 99.0% (580/586) agreement with the spectrophotometric results obtained for each specimen.

		CTA Results	
		+	-
ProSpecT	+	85	22
EIA Visual Results	-	15	464
Total		100	486

85.0% Sensitivity (85/100); 95% CI = 76.5% - 91.4%
95.5% Specificity (464/486); 95% CI = 93.2% - 97.1%

PERFORMANCE COMPARED TO PREDICATE DEVICE

The ProSpecT Clostridium difficile Toxin A/B Microplate Assay was

compared to two commercially available Enzyme Immunoassays (predicate devices). The performance of the ProSpecT Clostridium difficile Toxin A/B Microplate Assay and the predicate devices when compared to a CTA (using the same specimens) are as follows:

EIA	Performance versus CTA			
	Sensitivity		Specificity	
	#	%	#	%
ProSpecT	33/40	82.5	263/268	98.1
Predicate 1	33/40	82.5	260/268	97.0
ProSpecT	115/124	92.7	302/320	94.4
Predicate 2	98/124	79.0	309/320	96.6

ANALYTICAL SENSITIVITY

The ProSpecT Clostridium difficile Toxin A/B Microplate Assay detects Toxin A at levels of ≥ 0.20 ng/ml and Toxin B at levels of ≥ 0.61 ng/ml.

REPRODUCIBILITY

Reproducibility testing was conducted at three sites on three separate days with four blinded samples. Each site tested eight replicate wells of each specimen on each day of testing (n=288). The specimens included one negative specimen and three positive specimens with varying levels of reactivity. The average inter-assay or run-to-run coefficient of variation (CV) for a mid-range sample was 18.9%.The average intra-assay within-run CV for a mid-range sample was 7.7%.

CROSS-REACTIVITY

Forty microorganisms were evaluated with the ProSpecT Clostridium difficile Toxin A/B Microplate Assay. Bacteria and yeast isolates were tested at ≥ 10^8 colony-forming units per ml. Viral isolates were tested at concentrations of 10^4 $TCID_{50}$/ml (Tissue Culture Infectious Dose per millilitre). No cross-reactivity was observed. There was no cross-reactivity to the strain of *Clostridium sordellii* (ATCC® 9714) tested. However, published literature indicates that certain strains of *C. sordellii* can produce toxins which may be cross-reactive with antibodies to *C. difficile* Toxins A and B. The following organisms were tested in the ProSpecT Clostridium difficile Toxin A/B Microplate Assay.

Adenovirus Type 40
Adenovirus Type 41
Aeromonas hydrophilia
Bacillus cereus
Bacillus subtilis
Bacteroides fragilis
Campylobacter coli
Campylobacter jejuni
Candida albicans
Clostridium beijerinckii
Clostridium difficile (non-toxigenic)
Clostridium haemolyticum
Clostridium histolyticum
Clostridium novyi (toxin A)
Clostridium perfringens (type A)
Clostridium septicum
Clostridium sordellii
Clostridium sporogenes
Clostridium tetani
Enterobacter aerogenes
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli
Klebsiella pneumoniae
Peptostreptococcus anaerobius
Porphyromonas asaccharolytica
Proteus vulgaris
Pseudomonas aeruginosa
Rotavirus
Salmonella choleraesuis
Serratia liquefaciens
Shigella dysenteriae
Shigella flexneri
Shigella sonnei
Staphylococcus aureus
Staphylococcus aureus (Cowan)
Staphylococcus epidermidis
Vibrio cholerae
Vibrio parahaemolyticus
Yersinia enterocolitica

INTERFERING SUBSTANCES

The following substances were tested with the ProSpecT Clostridium difficile Toxin A/B Microplate Assay: Vancomycin (12.5 mg/ml), Metronidazole (12.5 mg/ml), blood, mucous, faecal fat and the following over-the-counter anti-diarrhoeal products: Pepto-Bismol®, Imodium® A-D, Kaopectate® (active ingredients: bismuth subsalicylate, loperamide HCl and attapulgite respectively). No interference with positive or negative specimens was observed.

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