INTENDED USE

ProSpecT™ Clostridium difficile Toxin A/B Microplate Assay is a qualitative in vitro diagnostic test to determine the presence of Clostridium difficile Toxin A and B in human faecal samples from patients suspected of having C. difficile associated disease. ProSpecT™ Clostridium difficile Toxin A/B Microplate Assay is intended for use as an aid in diagnosis of Clostridium difficile-associated disease (CDAD).

1. Clostridium difficile, a gram positive anaerobic spore-forming bacillus, is the most common identifiable cause of antibiotic-associated diarrhoeal disease (AAD). 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 serious condition known as pseudomembranous colitis (PMC), characterised by nausea, abdominal pain, watery diarrhoea, dehydration, low-grade fever, and the appearance of raised yellow plaques over the colonic mucosa. Fulminant colitis may be fatal if untreated. Nosocomial outbreaks of Clostridium difficile gastrointestinal illness and colitis may occur.

2. Antimicrobial treatment directed towards C. difficile infection can help resolve the disease. Diagnosis is usually performed through detection of one or both Clostridium difficile toxins.

3. PRINCIPLE OF THE TEST

The ProSpecT Clostridium difficile Toxin A/B Microplate Assay is a solid phase immunoassay for the detection of Clostridium difficile Toxin A and B in human faecal samples using an enzyme-linked immunosorbent assay (ELISA) technique. The test is based on the principle that the enzyme conjugate will bind the enzyme conjugate and no coloured reaction product will be observed if the sample contains Toxin A and B or an undetectable level of toxin.
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 0.01 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 ≥ 10⁴ colony-forming units per ml. Viral isolates were tested at concentrations of 10⁴ TCID₅₀/ml (Tissue Culture Infectious Dose per milliliter). 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:

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®, and Kaopectate® (active ingredients: bismuth subsalicylate, loperamide HCl and attapulgite respectively). No interference with positive or negative specimens was observed.

14. BIBLIOGRAPHY