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TOXIGENIC *CLOSTRIDIUM DIFFICILE*

Clostridium difficile is a Gram-positive anaerobic spore former and the major underlying cause of antibiotic- associated diarrhoea. *C. difficile* infection (CDI) is now one of the most prevalent nosocomial infections and has led to patient isolation, ward closures and even hospital closures in the past. Treatment with clindamycin, as well as cephalosporins and fluoroquinolones has been identified as risk factor for the development of *C. difficile*- associated disease (CDAD), which can range from mild to severe diarrhoea, toxic megacolon and pseudo-membranous colitis. CDI is transmitted via *C. difficile* spores, which are resistant to antibiotics and a large variety of hospital cleaning products. Therefore, spores can reside on hospital surfaces and in healthcare facilities for prolonged periods of time, and may lead to infection of other patients, but also re- and co-infection of the affected individual.

Disease symptoms of CDAD are mainly caused by the large clostridial toxins A and B, which act on the epithelial cells lining the colon. The action of toxin A and B leads to cytoskeletal changes and disruption of the actin cytoskeleton, which ultimately leads to failure of the epithelial barrier. This causes the release of inflammatory mediators that affect enteric nerves and sensory neurons, and increases the influx of inflammatory cells. These factors promote the accumulation of fluid and inflammation of the intestinal tissue, and lead to development of diarrhoea and in severe cases can lead to development of pseudo-membranes. Some strains of *C. difficile* express a third toxin, the binary toxin CDT, but its specific role in CDAD remains unexplained.Treatment of CDAD relies mainly on the administration of antibiotics (metronidazole, vancomycin and fidaxomicin), which have varying success in preventing relapse of disease.

C. difficile detection in the hospital setting is mainly dependent on the detection of the large clostridial toxin proteins in faecal samples using ELISA tests. Those are relatively rapid, but have low sensitivity and may only detect up to 70% of cases. Therefore, combination of ELISA with another form of confirmation is usually recommended. Detection by polymerase chain reaction (PCR) is more rapid and specific than culture for *C. difficile*. PCR is also not influenced by the large amount of other bacterial organisms in faecal samples and can detect the presence of toxin genes even after antibiotic therapy has commenced.

PCR for detection of *C. difficile* is usually based on detection of toxin genes. However, some strains of *C. difficile* only express truncated versions of toxin A and might not be detected using this approach.

At Micropathology Ltd, we have developed a PCR based assay for the detection of toxigenic *C. difficile* using the *tcdR* gene involved in regulation of expression of the large clostridial toxins A and B. This assay will detect toxigenic forms of *C. difficile,* including those expressing only one type of toxin.