P1873

Abstract (poster session)

A novel concept in diagnosis of Clostridium difficile: rapid functional testing of toxigenic activity in enzyme-linked immunosorbent assay (ELISA) format

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Objectives. Clostridium difficile is the leading cause of nosocomial enteric infections. Pathogenicity is directly attributed to the toxigenic effects of Toxins A and B produced by the bacterium. Diagnosis of C. difficile relies often on detecting the presence of these toxins in stools. However, limited performance in currently-available diagnostic tests yielded a plethora of testing algorithms consisting multiple targets and methodologies. The ultimate gold standard in this context is the cytotoxin assay which detects not only the presence of toxins but their activity. However this test is lengthy (days) and requires maintenance of cell culture. Savyon Diagnostics has developed a novel rapid functional assay in which C. difficile toxigenic activity is detected in ELISA format within less than 2.5 hours. Two alternative approaches have been demonstrated: (a) Rac-Tox assay relying on glucosyltransferase activity of Toxins A/B, which catalyzes monoglucosylation of small GTPases (i.e Rac1). Here toxigenic activity is detected by an antibody which recognizes Rac1 only in its glucosylated form. (b) Gox-Tox assay, which relies on detection of Toxin A/B-dependent release of free glucose following UDP-glucose hydrolysis. Here, Glucosyl oxidase (Gox) – dependent generation of peroxide is measured and is directly correlated to free glucose, and hence to toxigenic activity. Methods. In the Rac-Tox assay, toxin positive or negative samples were added to soluble His-Rac1 in the presence of UDP-Glucose. Following incubation, His-Rac1 was selectively immobilized on Ni^2+ plates. Detection was achieved using a recombinant antibody selected from a combinatorial library for its ability to specifically recognize glucosylated Rac1 but not non-glucosylated polypeptide. In the Gox-Tox assay positive or negative samples were added to immobilized anti-Toxin antibodies in the presence of UDP-Glucose as the substrate. Detection of peroxide generation was achieved following Gox activity using HRP/TMB system. Results. Rac-Tox assay detects activity of less than 1 ng toxin within 2.5 hours. Gox-Tox assay detects activity of less than 10 ng toxin within 2 hours. Both values are of clinical relevance. Conclusions. The two alternative approaches have been shown to have the capability to reliably detect C. difficile toxigenic activity in a rapid ELISA configuration, offering the potential to be used as a stand-alone confirmatory assay for diagnosis of C. difficile.