Improved detection of *Clostridioides difficile* compared to EIA testing using the Singulex Clarity C. diff toxins A/B assay: clinical utility and comparison toxin B gene detection in a symptomatic cohort

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Background: Debates over free-toxin and gene detection for diagnosis of *Clostridioides difficile* infection (CDI) exist. Poor biological stability of *C. difficile* toxins contributes to false-negative toxin enzyme immunoassay (EIA) results and clinical symptoms associated with CDI vary from self-limiting illness to life-threatening colitis. Few studies report CDI testing in context of patient symptoms. We hypothesize that testing defined cases of CDI will stratify performance among CDI assays.

Methods: A multi-disciplinary group examined 88 cases submitted for CDI testing. Inclusion criteria included >2/6 objective determinants of CDI: SrCr 1.5x baseline, loose-stool frequency x3/day and/or persistence >4 days, albumin <3.5 g/dL, WBC >15,000 cells/mL, abdominal cramping, and imaging suggestive of CDI. All samples were tested with Biofire GI panel and six CDI assays: Singulex Clarity® C. diff toxins A/B assay, Cepheid GeneXpert ToxB gene PCR, Roche Liat ToxB gene PCR, Meridian Premier Tox A/B EIA, Alere Tox A/B EIA, and Alere GDH EIA. Samples were tested by a single laboratory, under identical testing conditions.

Results: Percent overall agreement between GeneXpert and Liat was 100%. Percent positive agreement (PPA) between PCR and Premier Tox A/B, Alere Tox A/B, and Alere GDH were 30.5%, 28%, and 99%, respectively. Compared to toxin EIAs, Clarity improved detection of CDI by 28-31%. Initial PPA between PCR and Clarity was 71% (17 PCR+/Clarity- cases). After examining additional infectious and non-infectious causes of diarrhea, 7/17 PCR+/Clarity- cases were determined to be attributable to causes other than *C. difficile*, increasing PPA to 82%. Additionally, 3/17 PCR+/Clarity- cases were assessed as “likely not-attributable” to CDI (PPA 86%). In 7/17 of the PCR+/Clarity- cases, CDI could not be excluded as a diagnosis, but consensus of CDI could be applied to only 4 cases with certainty, further raising the PPA to 96%. The three remaining cases reported toxin concentrations <12 pg/mL, below the threshold of the Clarity assay, and no cases had elevated temperature, WBC, or lactate, suggesting lower severity of CDI.

Conclusion: PPA between Clarity and PCR assays was 96%. The Clarity assay detected acute cases of CDI detected by PCR, providing confidence as a stand-alone, single-step laboratory test for CDI.