Performance of toxin enzyme immunoassays and PCR cycle threshold for differentiating *Clostridium difficile* infection from colonization in children with diarrhea

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**Background**

- *C. difficile* infection (CDI) and *C. difficile* colonization frequently occur in children.
- Teen gene PCR (cDNA) commonly used to diagnose CDI does not differentiate CDI and colonization leading to frequent misdiagnosis of true CDI.
- While detection of toxin in stool is more specific for CDI, toxin enzyme immunoassays (EIA) are infrequently used as stand-alone tests for CDI because of perceived low sensitivity (≤44%) and specificity.
- Underestimation of toxin in stool can result from inadequate study design, such as failing to establish CDI in study subjects (asymptomatic bias) and/or use of an inappropriate gold standard (i.e., comparing toxin EIA for culture positive cases).
- Recent data in adults suggest clinical value of toxin EIA and potential for PCR cycle threshold to differentiate CDI and colonization.

**Methods**

**Objective**

To determine the sensitivity and specificity of two commercial toxin EIAs when:

- Assessed in a cohort of stool (true EIA-positive) children in whom CDI or colonization was determined.

**Primary objective:**

Determine the sensitivities and specificities of two commercial toxin EIAs when assessed in a cohort of stool (true EIA-positive) children in whom CDI or colonization was determined.

**Secondary objective:**

Reliability of PCR cycle threshold to differentiate CDI and colonization.

**Patients and Setting:**

Children (2-3) years old diagnosed with CDI by stool (true EIA-positive) between April 15, 2019, and February 10, 2020, at the Ann & Robert H. Lurie Children’s Hospital of Chicago.

**Laboratory Evaluation of Patients with CDI:**

- Clinical stool specimens tested positive by true EIA (Phenotype) in clinical laboratory as diagnostic and frozen at −80°C.
- PCR cycle threshold (CT) extracted from PCR instrument.
- Detection of free toxin in stool (performed after initial stool).
- Stool culture cytotoxicity neutralization assay (CCNA) (Corynebacterium Rileyi) - Gold standard for stool toxin detection.
- QUET CHEK Complete (ForchEIA) for glutaraldehyde dehydrogenase (GDH) and toxin.
- Immunocor (Monaldi) ELISA for toxin.
- Detection of toxicogenic strains of *C. difficile* in stool.
- Tangential stool culture.
- FilmArray Gastrointestinal MultiPath PCR Panel (BioFireAssays)

**Establishing Clinical CDI (clin CDI) in Children with true CDI:**

- Charts of children with lab CDI reviewed and/or patient/patient deemed to call])
- Clinical symptoms and antimicrobial use for potential confounding factors.
- Alkaline stools visualized and classified by stool Scale Score (patterned: Bristol 5–7).
- C. difficile (CDI) A, Persistent diarrhea (≥2) lasted at least 3 stools in preceding 24 hours; (B) Bristol stool score 5–7; (C) other diarrhea etiologies (per CDC classification).

**Assessment of true EIA EIAs to differentiate true CDI and Colonization**

- Teen EIA performance associated in comparison to various CDI reference standards not incorporating various laboratory and clinical characteristics that contribute to true CDI (false positives).
- While the above clin-CDI definition may exclude children with true CDI, the primary outcome was to measure true toxin EIA in a cohort of children with highest likelihood of true CDI.