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Lack of false-positive results for *Clostridioides difficile* toxins A and B using two commercial enzyme immunoassays in pediatric patients

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Introduction

Identification of an optimal *Clostridioides* (*Clostridium*) *difficile* infection (CDI) diagnostic testing strategy in children and adults remains a conundrum.^{1,2} Although *C. difficile* toxin enzyme immunoassays (EIAs) are highly specific for CDI, concerns regarding potential suboptimal sensitivity have limited their widespread use.^{1,2} Recent data in adults suggest that the diagnostic predictive value of toxin EIAs are better than previously reported.^{3,4} These two large studies have resulted in revised recommendations for toxin EIA use for CDI evaluation in adults, particularly at institutions without preagreed criteria for *C. difficile* testing where use of highly sensitive toxin gene PCR assays would result in frequent misdiagnosis of CDI in patients with *C. difficile* carriage.⁵

Compared to adult patients, studies of *C. difficile* test performance in pediatric patients are relatively limited. A previous pediatric study reported high prevalence (36%) of false-positive toxin EIA results in children.⁶ This anomalous finding has not been corroborated in adult or other pediatric populations. In the absence of evidence challenging this finding, concerns about poor specificity in addition to existing concerns about poor sensitivity have precluded recommendations for use of toxin EIAs in pediatric patients.⁷ Our objective was to assess the frequency of false-positive toxin EIA results using two commercial toxin EIAs in children without *C. difficile* carriage or infection.

Methods

The clinical microbiology laboratory at Lurie Children's Hospital performs *C. difficile* testing on unformed stools from children at least 12 months old. De-identified clinical stool specimens that tested negative by *tcdB* PCR (Xpert *C. difficile*, Cepheid, Sunnyvale, CA) were aliquoted and stored at –80°C for further testing. After a single freeze-thaw cycle, stools underwent two different *C. difficile* lateral flow EIAs: Quik Chek Complete (QCC; Techlab, Blacksburg, VA), which detects both glutamate dehydrogenase (GDH) and toxins A

and B, and ImmunoCard Toxins A&B (Meridian Biosciences, Inc., Cincinnati, OH), which detects toxins A and B only. EIAs were used according to manufacturers' instructions. Stools also underwent anaerobic stool culture, as previously described.⁸ Stool was cultured onto taurocholate-cycloserine-cefoxitin-fructose agar in an anaerobic chamber and incubated at 37°C for 48 hours. *C. difficile* colonies, identified based on typical morphology, were then transferred to trypticase soy broth and incubated anaerobically overnight. The broth culture was then centrifuged at 3000xg for 10 minutes, the supernatant was passed through a 0.45 micron filter, and the filtrate was assessed for presence of GDH and toxin by QCC. Toxigenic strains of *C. difficile* were identified if culture filtrate was GDH- and toxin-positive, and non-toxigenic *C. difficile* strains were GDH-positive and toxin-negative.

Results

All 50 stools were confirmed as unformed (Bristol stool scale types 5-7). Seven of the 50 (14%; 95% confidence interval 0.07-0.26) *tcdB* PCR-negative stools were GDH-positive by QCC, but zero of the 50 (0%; 95% confidence interval 0-0.07) stools tested positive for *C. difficile* toxins A and B by either QCC or ImmunoCard. *C. difficile* grew by anaerobic culture from 2/50 (4%) stools. Both culture-positive stools were GDH-positive, and both *C. difficile* isolates were GDH-positive, toxin-negative, confirming that these were non-toxigenic *C. difficile* strains.

Discussion

In the present study of 50 children with diarrhea and without *C. difficile* carriage or infection (i.e., stools tested negative for toxigenic *C. difficile* by a commercial *tcdB* PCR assay and confirmed negative by toxigenic stool culture), none tested positive for *C. difficile* toxins A or B using two different commercial toxin EIAs. The present study challenges the previously reported finding of frequent (36%) false-positive toxin EIA results in pediatric patients.⁶

The reasons for these discordant findings are unknown. While the prior study⁶ performed EIA on fresh stools, the present study performed EIA on stools thawed once. Recommendations from the manufacturers of both EIAs used in the present study support reliable assay performance after a single freeze-thaw cycle. Differences in false-positive rates among different EIAs have been described.² Our study used two different lateral flow commercial EIAs (TechLab Quik Chek Complete and Meridian ImmunoCard Toxins A&B) that were both different than the microwell commercial EIA used in the previous study (Meridian Premier Toxins A&B).⁶ There may be differences in false-positive rates among assays, particularly related to use of two distinct EIA methodologies between the present and previous studies (i.e., lateral flow vs. microwell, respectively). However, a comprehensive review of *C. difficile* diagnostic assays reported similarly infrequent rates of false positivity among these three assays.² False-positive microwell EIA results may occur if the optical density used to define a positive result deviates from the manufacturer's recommendation,² but it is unclear if that occurred in the previous study. Alternatively, the optical density defining a positive toxin result may not yet be appropriately validated in pediatric patients. Although the predominant specific type of toxin EIA used among pediatric hospitals is unknown, toxin EIAs in general, either as stand-alone tests or part of a

multi-step algorithm, are used at a minority of children's hospitals.^{9,10} Single specimen assays, such as lateral flow EIAs as used in the present study, may be preferred because they do not require specimen batching to be cost-effective.

In summary, our study in children supports previous studies in adults that reported infrequent false positivity rates for various toxin EIAs.² Our data challenge a previous pediatric study that anomalously reported that positive toxin EIA results are unreliable.⁶ However, further work is required to understand the negative predictive value of toxin EIA results in children prior to recommending toxin EIAs as stand-alone tests for diagnosing CDI in pediatric patients.

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