

correspondence

Detection of *Clostridium difficile* as a Routine Diagnosis: Comparison of Real-Time PCR and Enzyme Immunoassay

DOI: 10.1309/AJCPXUM77MFORYSO

To the Editor

We read with great interest the article about the comparison for 3 kinds of toxin enzyme immunoassay (EIA) as a screening test by Reller and colleagues.¹ As described in the article, EIA for toxin A and toxin B has been widely used for the diagnosis of *Clostridium difficile* infection owing to its simplicity and short turnaround time.² However, although the sensitivity of EIA has been reported to be improved in dry and wet tests, problems associated with its relatively low sensitivity still have been reported, and many large laboratories are now replacing or keeping pace with polymerase chain reaction (PCR)-based methods. Therefore, in a study we planned to compare the most popular 2 methods for detecting *C difficile*, EIA and real-time PCR.

This study was conducted at Kyung Hee University Hospital, a tertiary teaching hospital in Seoul, Korea, from October 2010 to February 2011, using 291 fresh diarrheal stools. All specimens were immediately tested by culture and VIDAS *C difficile* toxins A & B assay using an automated VIDAS immunoanalyzer (bioMérieux, Marcy l'Etoile, France), and the remainder was stored at -70°C until required for real-time PCR. The alcohol shock procedure was performed to improve the sensitivity of anaerobic culture.³ Aliquots were directly inoculated on *C difficile* selective agar (CDSA, Becton Dickinson, Sparks, MD) and blood agar (BAP) plates and incubated in an anaerobic jar for 48 to 72 hours at 35°C .² A Rapid ID 32 A test (bioMérieux, La Balme les Grottes, France) was used for identification of the species of the colonies on plates. Real-time PCR for toxin genes was performed by first preparing DNA using a QIAamp mini stool kit (Qiagen, Hilden, Germany). Real-time PCR was performed according to the manufacturer's instructions (AdvanSure CD real-time PCR kit, LG Lifesciences, Seoul, Korea) with the SLAN real-time PCR detection system (LG Lifesciences). An in-house PCR test for toxin A, toxin B, and binary toxin was performed as described previously.⁴

Of 291 clinical samples, 266 showed identical results between EIA and real-time PCR assays, and the concordance rate was 91.4%. To resolve discrepancies, we performed our in-house PCR assay on colonies from a total of 30 culture-positive samples. In-house PCR for toxin A and toxin B detected another 5 positive samples, while repeated real-time PCR assays of these specimens remained negative. However, when real-time PCR was performed using cultured colonies, all 5 specimens were positive. These changes may have been due to differences in the amount of bacteria between the original stool specimens and cultured colonies or the presence of PCR inhibitors in stools.

Feces contain various PCR inhibitors, such as bilirubin, excessive metal cations, and phytic acid.⁵ These PCR inhibitors may result in false-negatives; however, since the internal control was positive, this seems unlikely. Two previous studies have evaluated repeated PCR testing for toxin genes, and they reported a few cases that became positive when serial specimens were tested.^{6,7} These results suggest that the amount of bacteria in a specimen may influence the results of PCR assays.

A partially deleted toxin A gene was found in 3 specimens and resulted in approximately 700-base-pair bands after amplification by in-house PCR. These partially deleted toxin A genes were not detected by real-time PCR for the toxin A gene; therefore, positive EIA on these specimens may be due to the detection of toxin B. The binary toxin gene was identified in only 2 strains, while no ribotype 027 was detected. Finally, 10 specimens resulted in false-negative EIA results, while another 5 specimens resulted in false-negative real-time PCR results.

To ensure detection of toxigenic *C difficile* strains, some other confirmatory method should be applied to indeterminate clinical specimens. Moreover, repeating PCR tests on cultured colonies might increase the sensitivity of PCR-based methods.

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The Authors' Reply

We appreciate the interest of Cho and colleagues in our article.¹ We agree that EIAs for toxin are insensitive for the diagnosis of toxigenic *C difficile* and that molecular detection of toxin in stool is more sensitive than EIAs used alone or as part of 2- or 3-step algorithms that include cell culture cytotoxicity neutralization assays (CCNAs).^{1,2} In addition, several molecular tests have been well-evaluated and are approved by the US Food and Drug Administration. However, molecular assays have not been shown to be more sensitive than culture. Therefore, toxigenic culture is the gold standard to which new assays should be compared, a point highlighted in the IDSA/SHEA guidelines for diagnostic testing for *C difficile* infection.³ Hence, culturing stool for *C difficile* and confirming toxin production by performing PCR on cultured colonies is reasonable in selected cases in which other testing (EIA + CCNA or PCR on stool) is negative and high clinical suspicion of *C difficile* infection remains. We would emphasize the need for communication between clinicians and

laboratory professionals because toxigenic culture is labor-intensive and costly, particularly when added to the cost of molecular testing.

Finally, we read with interest the authors' study comparing PCR with EIA, since we are not aware of any study to date that demonstrates equivalence between the 2 diagnostic approaches. We applaud the authors for additionally performing toxigenic culture on all stool specimens in their study and would be interested in full (n = 291) reporting of the diagnostic performance (sensitivity, specificity, positive predictive value, and negative predictive value) of EIA and real-time PCR relative to that gold standard.

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