Comparison of Detection Methods for *Clostridium difficile*

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In a recent article, Pancholi et al. (1) reported a comparison study between the Xpert *Clostridium difficile* PCR assay (Cepheid, CA) and Illumigene *C. difficile* loop-mediated isothermal amplification (LAMP; Meridian Bioscience, Inc.) assay. The authors performed two studies: a prospective study performed on 200 sequential samples tested daily (or stored at 4°C for less than 24 h) and a retrospective study performed on 50 samples, previously positive by the cell cytotoxin neutralization assay (CCNA) and stored at −70°C. In both studies, the PCR showed a sensitivity of 100%, while the LAMP showed sensitivities of 81% (prospective study) and 94% (retrospective study).

The number of 027/NAP1/BI specimens studied was small: 9 in the prospective study and 27 in the retrospective study. For the hypervirulent strain, PCR showed a sensitivity of 100%, while the LAMP assay was determined to have sensitivities of 78% (prospective study) and 93% (retrospective study).

One can observe a significant difference between the two studies, with improved sensitivities for the LAMP method in the retrospective study. The main difference between the two groups is that the prospective study was performed on fresh samples, while the retrospective study was performed on samples that had been previously frozen. Since our laboratory introduced this method by validating in part stored, frozen specimens as well as some fresh specimens, we asked the question of whether the results observed during the validation might have been influenced by the freeze-thaw process. Possible inhibitory substances for the LAMP method could have been inactivated during this process. Meridian offered us reagents to test 100 samples, enabling us to answer this question.

The study was conducted on 98 sequential unformed stool specimens, tested daily, within 24 h from collection (maintained at 4°C between collection and testing). Each specimen was aliquoted, and the aliquots were frozen at −70°C. The aliquots were thawed and tested once we reached the number of samples desired (no sample was frozen longer than 21 days). We used the same reagent lot for the entire study and followed the manufacturer's instructions. Our study showed no change between results for the

fresh and freeze-thawed specimens (17 positive results, 1 invalid result, and 90 negative results).

It is important to note that the discrepancy reported in the article between the two groups is not secondary to the sample preparation and that the LAMP method can be used on fresh as well as on previously frozen specimens. Based on data from the literature (2, 3, 4) as well as on our personal experience (after over 1 year of using this test, we had only 3 clinical challenges, and the LAMP results were always confirmed by a PCR method performed by a reference laboratory [5]), a sensitivity of over 90% for the LAMP assay compared to PCR or culture is more plausible. We are also wondering if subsequent to the publication of this article, an explanation for this discrepancy was identified by the authors.

REFERENCES

1. Pancholi P, Kelly C, Raczowski M, Balada-Llasat JM. 2012. Detection of toxigenic *Clostridium difficile*: comparison of the cell culture neutralization, Xpert *C. difficile*, Xpert *C. difficile*/Epi, and Illumigene *C. difficile* assays. *J. Clin. Microbiol.* 50:1331–1335.
2. Norén T, Alriksson I, Andersson J, Akerlund T, Unemo M. 2011. Rapid and sensitive loop-mediated isothermal amplification test for *Clostridium difficile* detection challenges cytotoxin B cell test and culture as gold standard. *J. Clin. Microbiol.* 49:710–711.
3. Lalande V, Barrault L, Wadel S, Eckert C, Petit JC, Barbut F. 2011. Evaluation of a loop-mediated isothermal amplification assay for diagnosis of *Clostridium difficile* infections. *J. Clin. Microbiol.* 49:2714–2716.
4. Bruins MJ, Verbeek E, Wallinga JA, Bruijnesteijn van Coppenraet LES, Kuijper EJ, Bloembergen P. 2012. Evaluation of three enzyme immunoassays and a loop-mediated isothermal amplification test for the laboratory diagnosis of *Clostridium difficile* infection. *Eur. J. Clin. Microbiol. Infect. Dis.* 31:3035–3039.
5. Sloan LM, Duresko BJ, Gustafson DR, Rosenblatt JE. 2008. Comparison of real-time PCR for detection of the *tcdC* gene with four toxin immunoassays and culture in diagnosis of *Clostridium difficile* infection. *J. Clin. Microbiol.* 46:1996–2001.

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