26th ECCMID
9-12 April 2016
Amsterdam

Comparison of the CERTEST *Clostridium difficile* GDH+Toxin A+B test with the *C. DIFF QUIK CHEK COMPLETE®* assay

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INTRODUCTION

Clostridium difficile infection (CDI) has become the leading nosocomial infection in hospitals and nursing homes, and is now associated with community-acquired infections. Many laboratories have replaced or supplemented traditional immunoassays for *C. difficile* detection with molecular methods, usually accompanied by a marked increase in the prevalence rate, sparking a debate as to whether or not this patient population (positive for *C. difficile* by molecular testing but lacking detectable toxin in the faeces) have disease or are merely carriers. Recent studies from the United Kingdom show a correlation between detectable faecal toxin by immunoassay and the presence of *C. difficile* disease. One approach is to use a testing algorithm of an immunoassay for glutamate dehydrogenase (GDH) followed by a second, more sensitive test for toxin. In this study we compared two rapid assays that detect *C. difficile* toxins A and B, and GDH antigen. The performance and utility of a new lateral flow test, the CERTEST *Clostridium difficile* GDH+Toxin A+B ("CERTEST"), and the TECHLAB C*. DIFF QUIK CHEK COMPLETE®* ("*COMPLETE*") rapid membrane enzyme immunoassay were evaluated. Both tests were compared to bacterial culture and a cytotoxicity assay, the recognized gold standards for the detection of *Clostridium difficile* and toxin, respectively, in faecal specimens.

METHODS

Both tests were performed according to the Package Insert for testing faecal samples using 158 fecal samples submitted to the clinical laboratory for routine *C. difficile* testing. The presence of *C. difficile* toxin was determined by a cytotoxicity assay (CTA). The presence of *C. difficile* was determined by ethanol shocked culture on cycloserine cefoxitin fructose agar plates (CCFA). Analysis of discrepant samples was performed using a validated in-house qPCR with primer and probe sequences specific for the *C. difficile* toxin B (*tcdB*) gene. For the analytical sensitivity comparisons, serial two-fold dilutions of purified analyte (*Clostridium difficile* GDH, toxin A, or toxin B) were prepared in PBS 2% BSA and tested as a faecal specimen. For culture testing, 72 hr anaerobic BHI cultures were tested as faecal specimens.

RESULTS

CCFA culture identified 33 *C. difficile* positive samples - 30 were detected as GDH+ by the *COMPLETE* (90.9% sensitivity, 96.0% specificity) versus 27 by the CERTEST (81.8% sensitivity, 93.6% specificity). The *COMPLETE* identified an additional 5 GDH+ samples, 4 of which were positive for the *tcdB* gene by qPCR. The CERTEST identified an additional 8 GDH+ samples, 3 of which were positive by qPCR. Cytotoxicity assay (CTA) identified 18 toxin positive samples - 18 were detected (GDH+ and Toxin+) by the *COMPLETE* (100% sensitivity, 100% specificity) versus 12 detected by the CERTEST (66.7% sensitivity, 98.6% specificity). The CERTEST gave a false positive antigen result with a broth culture of *Clostridium sporogenes*.

Clinical Performance Compared to Bacterial Culture

C. DIFF QUIK CHEK COMPLETE®			CERTEST		
N=158	**CCFA +**	**CCFA -**	**N=158**	**CCFA +**	**CCFA -**
GDH +	30	5	**GDH +**	27	8
GDH -	3	120	**GDH -**	6	117
Sensitivity:	90.9%		Sensitivity:	81.8%	
Specificity:	96.0%		Specificity:	93.6%	
PPV:	85.7%		PPV:	77.1%	
NPV:	97.6%		NPV:	95.1%	
Correlation:	94.9%		Correlation:	91.1%	

Clinical Performance Compared to Cytotoxicity Assay

C. DIFF QUIK CHEK COMPLETE®			CERTEST		
N=158	**CTA +**	**CTA -**	**N=158**	**CTA +**	**CTA -**
Toxin +	18	0	**Toxin +**	12	2
Toxin -	0	140	**Toxin -**	6	138
Sensitivity:	100.0%		Sensitivity:	66.7%	
Specificity:	100.0%		Specificity:	98.6%	
PPV:	100.0%		PPV:	85.7%	
NPV:	100.0%		NPV:	95.8%	
Correlation:	100.0%		Correlation:	94.9%	

Broth Culture Cross-Reactivity Testing

	COMPLETE		CERTEST		
Strain	Ag	Tox	Ag	Tox A	Tox B
C. bifermentans ATCC® 638	-	-	-	-	-
P. anaerobius ATCC® 27377	-	-	-	-	-
C. sporogenes VPI 5952	-	-	+	-	-
C. difficile VPI 10463	+	+	+	+	+

Analytical Sensitivity Comparison

ng/mL detected (diluted*/in sample)

Analyte	*COMPLETE*	CERTEST
GDH	(0.05/1.56)	(0.31/3.13)
Toxin A	(0.20/6.25)	(1.25/12.5)
Toxin B	(0.03/0.78)	(0.63/6.25)

*Sample is diluted 1:30 for the *COMPLETE* and 1:10 for the CERTEST

CONCLUSIONS

- Both rapid immunoassays provide a result in less than 30 minutes
- More specimens were identified as falsely positive by the CERTEST than by the *COMPLETE*
- More true positive specimens were identified by the *COMPLETE* than by the CERTEST
- The CERTEST cross-reacts with glutamate dehydrogenase (GDH) produced by *Clostridium sporogenes*
- The *COMPLETE* detects lower amounts of analyte (GDH, toxin A, and toxin B) than the CERTEST

REFERENCES

- Swindells, J., N. Brenwald, N. Reading, and B. Oppenheim.2010. Evaluation of Diagnostic Tests for *Clostridium difficile* Infection. J Clin Microbiol. 48:606-608.
- Planche, T.D., K.A. Davies, P.G. Coen, J.M. Finney, I.M Monahan, K.A. Morris, L. O'Connor, S.J. Oakley, C.F. Pope, M.W. Wren, N.P. Shetty, D.W. Crook, M.H. Wilcox.2013. Differences in Outcome According to *Clostridium difficile* Testing Method: a Prospective Multicentre Diagnostic Validation Study of *C difficile* Infection. Lancet Infect Dis. 13:936–945.