

Evaluation of a New Immunochromatography Test for Rapid and Simultaneous Detection of *Clostridium difficile* Antigen and Toxins

Zmira Samra PhD^{1,4}, Liora Madar-Shapiro PhD³, Mahanez Aziz MSc¹ and Jihad Bishara MD^{2,4}

¹Department of Clinical Microbiology and ²Infectious Diseases Unit, Rabin Medical Center (Beilinson Campus), Petah Tikva, Israel

³Hy Laboratories Ltd., Rehovot, Israel

⁴Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

ABSTRACT: **Background:** *Clostridium difficile* infection is considered the most common cause of nosocomial infectious diarrhea among adults in the developed world. It is responsible for virtually all cases of pseudomembranous colitis. The Tox A/B enzyme immunoassay (EIA) is the most widely used test for the detection of *C. difficile* toxins A and B. However, it is associated with poor sensitivity and an unacceptable high rate of false-negative results.

Objectives: To evaluate the performance of the C. DIFF QUIK CHEK COMPLETE[®] assay, designed to simultaneously detect *C. difficile*-produced glutamate dehydrogenase (GDH) and toxins A and B.

Methods: Using the C. DIFF QUIK CHEK COMPLETE[®] assay, the Tox A/B EIA, and polymerase chain reaction (PCR), we tested 223 stool specimens from hospitalized patients with antibiotics-associated diarrhea. Sensitivity and specificity, and positive and negative predictive values (PPV, NPV) were calculated for the C. DIFF QUIK CHEK COMPLETE test and the Tox A/B EIA against PCR

Results: The C. DIFF QUIK CHEK COMPLETE test had a sensitivity of 83.5% and specificity of 94.3% compared to PCR for Tox A/B, with 93.7% correlation (PPV 98.5%, NPV 91.7%). The Tox A/B EIA yielded corresponding values of 72.1% and 93.1%, with 85.6% correlation (PPV 85.1%, NPV 85.8%).

Conclusions: Given the importance of an early and appropriate diagnosis of *Clostridium difficile*-associated infection, the C. DIFF QUIK CHEK COMPLETE test may be of huge benefit to practitioners.

IMAJ 2013; 15: 441–444

KEY WORDS: *Clostridium difficile*, glutamate dehydrogenase (GDH), polymerase chain reaction (PCR), enzyme immunoassay (EIA), C. DIFF QUIK CHEK[®]

branous colitis [1,2]. The severity of the infection may range from watery diarrhea to pseudomembranous colitis, toxic megacolon, and sepsis [1-3].

The main virulence factors of *C. difficile* are the structurally similar toxin A and toxin B, encoded by the *tcdA* and *tcdB* genes, respectively. Toxin A is enterotoxic to the human colon, and toxin B is both cytotoxic and enterotoxic. Until recently, the tissue culture cytotoxicity assay was considered the gold standard for diagnosis of *C. difficile* infection, with a sensitivity of 94% to 100% and specificity of 97% [4,5]. However, the method is labor and time-consuming, with a turnaround time of more than 48 hours. Furthermore, some authors recommend that the cytotoxic assay be preceded by a bacterial stool culture to increase sensitivity [6-8], making the method even more cumbersome. As a result, the rapid, easy-to-perform enzyme immunoassay has emerged as the most widely used test for the detection of *C. difficile* toxins A and B. Yet its poor sensitivity is problematic. Recent studies reported that none of the six commercially available EIAs was sufficiently reliable for stand-alone use because of sensitivity and specificity limitations [6,8,9].

Polymerase chain reaction, nested PCR, and real-time PCR have been found effective for the detection of *tcdB* in stool specimens [10-12], with good sensitivity and specificity. However, their widespread use in routine clinical microbiology is limited because they require costly equipment, specially trained staff, and a specific DNA extraction procedure to eliminate PCR inhibitors [13-15].

Another method that has been used to diagnose *C. difficile* infection is detection of glutamate dehydrogenase, a metabolic enzyme produced almost exclusively by *C. difficile*. This test has relatively good sensitivity but poor specificity because it merely detects colonization by *C. difficile*. Thus, the rate of false positives is high. To improve specificity, several authors recommended that the GDH test be used for first-

Clostridium *difficile* is the most common cause of nosocomial infectious diarrhea among adults in the developed world. It is responsible for virtually all cases of pseudomem-

EIA = enzyme immunoassay
PCR = polymerase chain reaction
GDH = glutamate dehydrogenase

line screening, followed by cell culture for toxin testing as necessary [8,16,17].

Recently, a new, immunochromatography assay, the C. DIFF QUIK CHEK COMPLETE® (TechLab, Blacksburg, VA, USA) was developed for the detection of both GDH antigen and toxin A/B with one easy-to-use cartridge [17,18]. The aim of the present study was to examine the value of the C. DIFF QUIK CHEK COMPLETE assay in initial screening for *C. difficile* infection compared to traditional methods.

MATERIALS AND METHODS

The study was conducted at Rabin Medical Center (Beilinson Campus), a 900-bed university-affiliated tertiary-care facility in central Israel. We collected 223 consecutive, patient-unique stool specimens in July-August 2010 from hospitalized patients with antibiotic-associated diarrhea. The specimens were either tested on the same day or stored at 2–8°C for testing within 48 hours. Three methods were used for *C. difficile* antigen and toxin detection.

- **Tox A/B EIA** (Techlab). Microtiter wells were coated with immobilized affinity-purified polyclonal goat antibody against toxins A and B. The detecting antibody consisted of a mixture of toxin A monoclonal mouse antibody and toxin B polyclonal goat antibody, both conjugated to horseradish peroxidase
- **C. DIFF QUIK CHEK COMPLETE®** (Techlab). For this assay we used 500 µl of a mixture containing 750 µl buffer, 25 µl stool specimen, and one drop of conjugate. Monoclonal antibodies for GDH and toxin A and polyclonal antibody for toxin B were placed in a reaction window with three vertical lines of immobilized antibodies: antigen test line, containing antibodies against *C. difficile* GDH; control line, a dotted line containing anti-horseradish peroxidase antibodies; and toxin A/B test line, containing antibodies against toxin A and B.
- **PCR**. Genomic DNA was extracted from frozen stool samples using an AccuPrep® Stool DNA Extraction Kit (Bioneer, Korea) according to the manufacturer's protocol.

In-house validated PCR assays were performed using three pairs of specific primers targeting the genes encoding triose phosphate isomerase (*tpi*), toxin B (*tcdB*), and toxin A (*tcdA*), as described previously [19]. Each DNA sample was tested for each gene in parallel, and the PCR products were resolved by electrophoresis on 2% agarose gel stained with ethidium bromide. The specimen was considered positive for toxigenic *C. difficile* if both *tpi* and either *tcdA* or *tcdB* were detected. The specificity of the PCR products of the amplified toxins was confirmed by sequencing using the BigDye G Terminator v1.1 Cycle Sequencing Kit on an ABI PRISM 3730 Genetic Analyzer (both from Applied Biosystems, Inc., Foster City, CA, USA). The sequences obtained were aligned and compared with

archived sequences from the National Center for Biotechnology Information. Sensitivity and specificity, and positive and negative predictive values were calculated for the C. DIFF QUIK CHEK COMPLETE test and Tox A/B EIA against PCR.

RESULTS

Of the 223 specimens, 115 (51.6%) tested negative for *C. difficile* by all three methods and 57 (26.6%) tested positive by all three methods [Table 1]. Nine specimens (4%) were positive by PCR and the C. DIFF QUIK CHEK COMPLETE test but negative by Tox A/B EIA, and three specimens (1.35%) were positive by PCR but negative by EIA and the C. DIFF QUIK CHEK COMPLETE test [Table 1].

On the C. DIFF QUIK CHEK COMPLETE test, 66 specimens (29.5%) tested positive for both GDH and toxins A/B, 27 (12%) tested positive for GDH but negative for toxins A/B, and 129 (57.8%) tested negative for both GDH and toxins A/B. Only one specimen tested negative for GDH but weakly positive for toxins A/B [Table 1].

For GDH detection, the C. DIFF QUIK CHEK COMPLETE test had a sensitivity of 92.4% and specificity of 93.9% compared to PCR-*tpi* (PCR-triose phosphate isomerase), with 93.3% correlation (PPV 91.4%, NPV 94.6%), and a sensitivity of 96.2% and specificity of 88.2% compared to PCR-tox [PCR-toxin B (*tcdB*), and toxin A (*tcdA*)], with 91.0% correlation (PPV 81.7%, NPV 97.6%). For toxin detection, the C. DIFF QUIK

PPV = positive predictive value
NPV = negative predictive value

Table 1. Results for 223 stool specimens tested for *C. difficile* by three methods

No. of specimens	QUIK CHEK COMPLETE		TOX A/B EIA	PCR	
	GDH*	Tox		<i>tpi</i> **	Tox
57	+	+	+	+	+
115	-	-	-	-	-
3	+	-	+	-	-
7	-	-	+	-	-
10	+	-	-	+	+
9	+	+	-	+	+
3	-	-	-	+	+
9	+	-	-	+	-
5	+	-	-	-	-
4	-	-	-	+	-
1	-	+	(weak)	-	-
223					

EIA = enzyme immunoassay, PCR = polymerase chain reaction, GDH = glutamate dehydrogenase, Tox = toxin, *tpi* = triose phosphate isomerase

Table 2. Sensitivity and specificity of C. DIFF QUIK CHEK COMPLETE and Tox A/B EIA relative to PCR

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Correlation
Toxin detection					
EIA vs. PCR	72.1	93.1	85.1	85.9	85.6
QUIK CHEK vs. PCR-Tox	83.5	99.3	98.5	91.7	93.7
GDH Detection QUIK CHEK vs. PCR- <i>tpi</i>	92.4	93.9	91.4	94.6	93.3
QUIK CHEK vs. PCR-Tox	96.2	88.2	81.7	97.6	91

PPV = positive predictive value, NPV = negative predictive value, EIA = enzyme immunoassay, Tox = toxin, PCR = polymerase chain reaction, GDH = glutamate dehydrogenase, *tpi* = triose phosphate isomerase

CHEK COMPLETE had a sensitivity of 83.5% and specificity of 99.3% compared to PCR-tox, with 93.7% correlation (PPV 98.5%, NPV 91.7%). The EIA yielded much lower values than PCR: sensitivity 72.1% and specificity 93.1%, with 85.6% correlation (PPV 85.1%, NPV 85.9%) [Table 2].

DISCUSSION

There has been substantial progress in the diagnosis of *C. difficile* infection in recent decades. However, concerns are increasingly being raised that the favored method, EIA for toxin A/B, is not sufficiently sensitive and has an unacceptably high rate of false-negative results. In their extensive review of 45 studies, Crobach et al. [20] reported a sensitivity of 75–80% and specificity of 97–98% for the Tox A/B EIA. Many centers today combine GDH immunoassay with toxin A/B immunoassay.

Our study shows that the C. DIFF QUIK CHEK COMPLETE assay has a high sensitivity and specificity for toxin detection, with high positive and negative predictive values, and a much better correlation with the PCR results than the Tox A/B EIA. These findings are in accordance with previous studies [17,18]. Quinn and co-authors [18] used the C. DIFF QUIK CHEK assay to analyze 174 stool specimens and compared the results to two PCR assays and cytotoxicogenic culture. The reported sensitivity was 78.3% and specificity 100% [18]. The discrepancies in the results of the two studies may be partially explained by the different performance of the two systems.

The C. DIFF QUIK CHEK COMPLETE test is simple to perform and, unlike PCR, requires no special facilities or personnel qualifications that might restrict its use, especially in countries or regions with limited resources [18]. Compared to the PCR, the C. DIFF QUIK CHEK COMPLETE test is much cheaper, costing US\$ 8, while the in-house PCR costs about US\$ 50, and the GeneXpert PCR for *tcdB* costs 70 Euros. It also provides results much more rapidly than PCR and the Tox

A/B EIA; test turnaround time is about 25 minutes compared to 130 minutes for PCR. This is particularly important given that the differential diagnosis of diarrhea includes infection with *Salmonella*, *Shigella*, and *Campylobacter* spp, and empiric antibiotic therapy against these bacteria might be harmful in patients with *C. difficile* infection. Using a reliable and rapid diagnostic test, practitioners could offer appropriate treatment earlier, thereby sparing patients a time-consuming evaluation and unnecessary antibiotic therapy and its complications.

In conclusion, we believe the dual C. DIFF QUIK CHEK COMPLETE assay, with or without PCR, may serve as a good alternative to the current Tox A/B EIA for the detection of toxigenic *C. difficile* infection. We agree with other authors that specimens that are positive for both GDH and toxins A/B, or negative for both GDH and toxins A/B, may be reported as true positive or true negative, respectively, whereas specimens with discrepant results for GDH and toxins A/B should be retested by PCR or tissue culture cytotoxicity assay.

Corresponding author:

Dr. Z. Samra

Dept. of Clinical Microbiology, Rabin Medical Center (Beilinson Campus), Petah Tikva 49100, Israel

Phone: (972-3) 937-6725

Fax: (972-3) 937-6720

email: zsamra@clalit.org.il

References

- Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. *N Engl J Med* 2002; 346: 334-9.
- McDonald LC, Owingo M, Jernigan DB. *Clostridium difficile* infection in patients discharged from US short-stay hospitals, 1996-2003. *Emerg Infect Dis* 2006; 12: 409-25.
- Frost F, Graun GF, Calderon RL. Increasing hospitalization and death possible due to *Clostridium difficile* diarrhea disease. *Emerg Infect Dis* 1998; 4: 616-25.
- Musher DM, Manhas A, Jain P, et al. Detection of *Clostridium difficile* toxin: comparison of enzyme immunoassay results with results obtained by cytotoxicity assay. *J Clin Microbiol* 2007; 45: 2737-9.
- Turgeon DK, Novicki TJ, Quick J, et al. Six rapid tests for direct detection of *Clostridium difficile* and its toxins in fecal samples compared with the fibroblast cytotoxicity assay. *J Clin Microbiol* 2003; 41: 667-70.
- Arnold A, Pope C, Bray S, et al. Prospective assessment of two-stage testing for *Clostridium difficile*. *J Hosp Infect* 2010; 76 (1): 18-22.
- Bartlett JG, Gerding DN. Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2008; 15; 46 (Suppl 1): S12-18.
- Gilligan PH. Is a two-step glutamate dehydrogenase antigen-cytotoxicity neutralization assay algorithm superior to the premier toxin A and B enzyme immunoassay for laboratory detection of *Clostridium difficile*? *J Clin Microbiol* 2008; 46 (4): 1523-5.
- Planche T, Aghaizu A, Holliman R, et al. Diagnosis of *Clostridium difficile* infection by toxin detection kits: a systematic review. *Lancet Infect Dis* 2008; 8 (12): 777-84.
- Guilbault C, Labbé AC, Poirier L, Busque L, Béliveau C, Laverdière M. Development and evaluation of a PCR method for detection of the *Clostridium difficile* toxin B gene in stool specimens. *J Clin Microbiol* 2002; 40 (6): 2288-90.
- Alonso R, Muñoz C, Gros S, García de Viedma D, Peláez T, Bouza E. Rapid detection of toxigenic *Clostridium difficile* from stool samples by a nested PCR of toxin B gene. *J Hosp Infect* 1999; 41 (2): 145-9.
- Bélangier SD, Boissinot M, Clairoux N, Picard FJ, Bergeron MG. Rapid detection of *Clostridium difficile* in feces by real-time PCR. *J Clin Microbiol* 2003; 41 (2): 730-4.

13. Terhes G, Urbán E, Sóki J, Nacsá E, Nagy E. Comparison of a rapid molecular method, the BD GeneOhm C. diff assay, to the most frequently used laboratory tests for detection of toxin-producing *Clostridium difficile* in diarrheal feces. *J Clin Microbiol* 2009; 47 (11): 3478-81.
14. Van den Berg RJ, Vaessen N, Endtz HP, Schülin T, van der Vorm ER, Kuijper EJ. Evaluation of real-time PCR and conventional diagnostic methods for the detection of *Clostridium difficile*-associated diarrhoea in a prospective multicentre study. *J Med Microbiol* 2007; 56 (Pt 1): 36-42.
15. Van den Berg RJ, van Coppenrate LSB, Gerritsen HJ, Endtz HP, van der Vorm ER, Kuijper EJ. Prospective multicenter evaluation of a new immunoassay and real-time PCR for rapid diagnosis of *Clostridium difficile*-associated diarrhea in hospitalized patients. *J Clin Microbiol* 2005; 43: 5338-40.
16. Ticehurst J, Arid DZ, Dam MD, Boreff AP, Hargrove JT, Carroll KC. Effective detection of toxigenic *Clostridium difficile* by a two-step algorithm including test of antigen and cytotoxins. *J Clin Microbiol* 2006; 44: 1145-9.
17. Sharp SE, Ruden LO, Pohl JC, Hatcher PA, Jayne LM, Ivie WM. Evaluation of the C. Diff Quik Chek Complete Assay, a new glutamate dehydrogenase and A/B toxin combination lateral flow assay for use in rapid, simple diagnosis of *Clostridium difficile* disease. *J Clin Microbiol* 2010; 48 (6): 2082-6.
18. Quinn CD, Sefers SE, Babiker W, et al. C. Diff Quik Chek complete enzyme immunoassay provides a reliable first-line method for detection of *Clostridium difficile* in stool specimens. *J Clin Microbiol* 2010; 48 (2): 603-5
19. Lemee L, Dhalluin A, Testelin S, et al. Multiplex PCR targeting tpi (triose phosphate isomerase), tcdA (Toxin A), and tcdB (Toxin B) genes for toxigenic culture of *Clostridium difficile*. *J Clin Microbiol* 2004; 42 (12): 5710-14 .
20. Crobach MJ, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendation for diagnosing *Clostridium difficile* infection (CDI). *Clin Microbiol Infect* 2009; 15: 1053-66.

Capsule

The shaping and functional consequences of the microRNA landscape in breast cancer

MicroRNAs (miRNAs) show differential expression across breast cancer subtypes, and have both oncogenic and tumor-suppressive roles. Dvinge and co-authors report the miRNA expression profiles of 1302 breast tumors with matching detailed clinical annotation, long-term follow-up and genomic and messenger RNA expression data. This provides a comprehensive overview of the quantity, distribution and variation of the miRNA population and provides information on the extent to which genomic, transcriptional and post-transcriptional events contribute to miRNA expression architecture, suggesting an important role for post-transcriptional regulation. The key clinical parameters and cellular pathways related to the miRNA landscape are characterized, revealing context-dependent interactions, for example with regards to cell adhesion and Wnt signaling.

Notably, only prognostic miRNA signatures derived from breast tumors devoid of somatic copy-number aberrations (CNA-devoid) are consistently prognostic across several other subtypes and can be validated in external cohorts. The authors then use a data-driven approach to seek the effects of miRNAs associated with differential co-expression of mRNAs, and find that miRNAs act as modulators of mRNA-mRNA interactions rather than as on-off molecular switches. They demonstrate such an important modulatory role for miRNAs in the biology of CNA-devoid breast cancers, a common subtype in which the immune response is prominent. These findings represent a new framework for studying the biology of miRNAs in human breast cancer.

Nature 2013; 497: 378

Eitan Israeli

Capsule

Synthetic generation of influenza vaccine viruses for rapid response to pandemics

During the 2009 H1N1 influenza pandemic, vaccines for the virus became available in large quantities only after human infections peaked. To accelerate vaccine availability for future pandemics, Dormitzer et al. developed a synthetic approach that very rapidly generated vaccine viruses from sequence data. Beginning with hemagglutinin (HA) and neuraminidase (NA) gene sequences, the authors combined an enzymatic, cell-free gene assembly technique with enzymatic error correction to allow rapid, accurate gene synthesis. Then they used these synthetic HA and NA genes to transfect Madin-Darby canine kidney (MDCK) cells that were qualified for vaccine manufacture with

viral RNA expression constructs encoding HA and NA and plasmid DNAs encoding viral backbone genes. Viruses for use in vaccines were rescued from these MDCK cells. The authors performed this rescue with improved vaccine virus backbones, increasing the yield of the essential vaccine antigen, HA. Generation of synthetic vaccine seeds, together with more efficient vaccine release assays, would accelerate responses to influenza pandemics through a system of instantaneous electronic data exchange followed by real-time, geographically dispersed vaccine production.

Sci Transl Med 2013; 5: 185

Eitan Israeli

“The difference between fiction and reality is that fiction has to make sense”

Tom Clancy (born 1947), American author best known for his technically detailed espionage and military science storylines set during and in the aftermath of the Cold War, along with video games