Poster 950

Evaluation of the Performance of the Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR™) Gastrointestinal Panel vs the Cepheid Xpert® *C. difficile*/Epi Assay for the Detection of Toxigenic *Clostridium difficile*

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Abstract:

Background: *Clostridium difficile* (CD) is one of the most common healthcare associated infections (HAIs). While the gold standard for diagnosis of CD is cytotoxicity assay and toxigenic culture, there are a number of molecular assays for rapid CD detection. This study evaluated the performance of an in-house TEM-PCR™ GI panel with the Cepheid Xpert® *C. difficile*/Epi assay using clinical samples collected in hospital setting.

Methods: Stool samples of patients with suspected CD infections were tested by TEM-PCR GI panel and with Cepheid Xpert® assay. CD detection is the part of the Diatherix multiplex GI panel which can simultaneously detect 3 viral, 8 bacterial, 2 protozoan, and 2 toxins in a single specimen. The Cepheid assay detects the toxin B gene and the O27/NAP1/B1 strain. Two TaqMan® assays targeting the toxin B and *tpi* gene were developed as an alternative approach for confirmation of results. Demographics data, risk factors, and other clinically relevant information was obtained for this study.

Results: We enrolled 80 patients with mean age of 62 years (TaqMan® assays done in 80). 48% females. Known risk factors included: recurrent CD infection (15%), recent antibiotic use (64%), recent hospitalization (55%), proton pump inhibitor use (45%), gastrointestinal disorders (39%) and immunosuppression (19%). There was 98.8% concordance between TEM-PCR CD toxin B, TaqMan® *tpi* gene, and Cepheid Xpert®. Cepheid NAP1/B1 was positive in 8% of patients. TEM-PCR detected positive binary toxin in 14% of patients. All NAP1/B1 positive specimens were positive for binary toxin with TEM-PCR. 17% of patients with positive CD by TEM-PCR had co-detection of other targets (25% Norovirus, 50% Rotavirus and 25% EPEC). Those patients with co-detection, 75% were female and 75% more than 55 years old. Other stool studies done in 63% of patients, 44% had occult blood with 71% positive, 39% had cultures with 6% positive for Gram positives, and 40% had O&P (All were negative).

Conclusion: The performance of TEM-PCR GI panel was comparable to Cepheid assay for CD detection. Further studies are needed to asses the clinical utility of TEM-PCR GI panel to detect multiple pathogens and how this information can be used to improve patient care.

Note: abstract updated to reflect the inclusion of additional patients for negative predictive value calculations.

Introduction:

- *C. difficile* infection (CDI) is a leading cause of nosocomial infectious diarrhea and is emerging as a community acquired pathogen in groups that were previously considered low risk.[5]
- Symptoms of CDI can be caused by other GI pathogens that may be missed if only a *C. difficile* assay is used.
- This study sought to ask the question if a multiplex PCR panel like the Diatherix TEM-PCR™ GI Panel could detect CDI as well as the FDA-cleared Cepheid Xpert® *C. diff*/Epi assay while giving the added benefit of multiplex pathogen detection.
- Patients in this study were inpatients at a local hospital with symptoms suspected to be associated with *Clostridium difficile* infection.
- Stool specimens were split and tested with the Cepheid *C. diff*/ Epi FDA cleared assay and by Diatherix Laboratories on their TEM-PCR™ Gastrointestinal Panel.
- All samples were also tested with a *C. difficile* binary toxin end-point assay, a *C. difficile* toxin B TaqMan® assay, and a *C. difficile* TaqMan® assay that identifies generalized *C. difficile* regardless of toxin presence. A combination of three confirmatory assays were used and a sample was considered positive for *C. difficile* if two out of three of these assays were positive.
- Samples were blinded when tested with the Diatherix panel.

Materials & Methods:

Sample Collection

- Stool samples from inpatients at Huntsville Hospital (Huntsville, AL) with clinical symptoms of CDI and watery stools were submitted for testing to Huntsville Hospital with the Cepheid Xpert® *C. difficile*/ Epi assay (Sunnyvale, CA). The stool sample was split by swabbing the stool and placing the swab in a Copan ESwab™ (Murrieta, CA) liquid transport media tube. This ESwab™ specimen was transported by courier to Diatherix Laboratories (Huntsville, AL) where it was tested using the TEM-PCR™ Gastrointestinal Panel

DNA extraction for TEM™-PCR

- Specimens were extracted using a modified Omega Bio-Tek, Inc. Mag-Bind® kit (Norcross, GA) with additional reagents from MO BIO Laboratories, Inc. (Carlsbad, CA) on a ThermoFisher Scientific, Inc. KingFisher™ Flex (Waltham, MA) semi -automated platform.

PCR and Detection for TEM™-PCR

Extracted DNA and RNA samples were amplified using the Target Enriched Multiplex PCR (TEM-PCR™) Gastrointestinal Panel. TEM -PCR™ is a highly multiplexed, nested, end-point PCR technique covered by US patent 7,851,148 B2. The Gastrointestinal Panel contains 59 target specific primers which can identify 13 different gastrointestinal pathogens. The end-point PCR products generated by TEM-PCR™ were detected on a custom microarray (Microarrays, Inc., Huntsville, AL).

PCR and Detection for TaqMan® qPCR Confirmation

- qPCR was performed using a 10µL reaction volume with 5 µL TaqMan® Gene Expression Master Mix (ThermoFisher Scientific, Waltham, MA), 0.5 µL of 20X TaqMan® Assay Mix (ThermoFisher Scientific), 3.5 µL nuclease-free water (ThermoFisher Scientific), and 1 µL of template. Reactions were cycled for 40 cycles in a BioRad CFX96 (BioRad, Hercules, CA). PCR was done in triplicate.

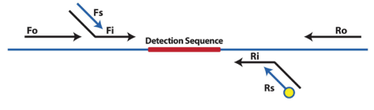

Figure 1. TEM-PCR™ Scheme.
Low concentrations of nested gene-specific primers (Fo – forward out; Fi – forward in; Ri – reverse in; and Ro – reverse out) are designed to enrich the genetic target during the initial PCR cycles. Later in the procedure, a pair of universal SuperPrimers (FS and RS) are used to amplify all targets. The reverse SuperPrimer is labeled with biotin. Target-specific biotinylated PCR products are detected with a complimentary detection probe which is covalently coupled to a glass microarray.

Diatherix TEM-PCR™ Gastrointestinal Panel

Campylobacter jejuni	Enterotoxigenic *E. coli* (ETEC)
Cryptosporidium parvum	Enterohemorrhagic *E. coli* (EHEC)
Giardia lamblia	Shiga-toxin 1 (stx1)
Vibrio parahaemolyticus	Shiga-toxin 2 (stx2)
Enteropathogenic *E. coli* (EPEC)	*Shigella*/ Enteroinvasive *E. coli* (EIEC)
Salmonella enterica	
Clostridium difficile (toxin B gene)	
Adenovirus 40/41	
Norovirus	
Rotavirus	

Results:

Table 1. Oligonucleotide sequences used for confirmatory testing of toxigenic *C. difficile*

Target Gene		Oligonucleotide	Sequence (5'-3')	Position	Amplicon size (bp)	GenBank Accession No.
tcdB	*C. difficile* toxin B	Forward	TGATTGCAGTTGTAGCTGTTGTTAAA	3621-3646	58	AF217292
		Reverse	CGAGTGACCCATTATTAAGACAAGAA	3563-3588		
		Probe	FAM-TTACTGCCATTATACCTATCTT-MGB	3598-3619		
tpi	*C. difficile* triosephosphate isomerase	Forward	AAGCATTAGAAGTAGGAATAGACCCAAT	315-342	76	AY700149
		Reverse	TTTAGTTTTTCCAGCTTCTCTTTGTTC	365-391		
		Probe	FAM-TTATGTGTTGGAGAAACT-MGB	344-361		

Table 2. Comparison of detection results of *C. difficile* between Cepheid, TEM-PCR, and confirmatory methods, such as a combination of TaqMan® *toxB* and *tpi* assays and TEM-PCR based detection of binary toxin

Assay	Cepheid *C. diff* / Epi		Assay performance (95% confidence interval)			
	Detected	Not Detected	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Diatherix TEM-PCR *C. diff* toxin B						
Detected	24	0				
Not Detected	1	55	96 (77.7-99.8)	100 (91.9-1)	100 (82.8-1)	98.2 (89.2-99.9)
Total	25	55				
Confirmatory methods						
Detected	23	0				
Not Detected	2	55	92 (72.4-98.6)	100 (91.9-1)	100 (82.2-1)	96.5 (86.8-99.4)
Total	25	55				

Abbreviations: PPV, positive predictive value; NPV, negative predictive value
CI calculated with efficient score method with continuity correction

Table 3. Results of the confirmatory assays of specimens positive for toxigenic *C. difficile* by Cepheid results including an end-point binary toxin assay (*cdtA, cdtB*), a toxin B TaqMan® qPCR assay, and a *tpi* TaqMan® qPCR assay for general *C. difficile*. The qPCR assays are less sensitive than the TEM-PCR assay and therefore a combination of three confirmatory assays were used.

Cepheid C. difficile Result	TEM-PCR Result	Binary toxin?	ToxB Taqman Result	TPI Taqman Result	Concordance?	Decision Criteria
DETECTED	DET	YES	DET	DET	YES	BT/TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	NOT DET	NOT DET	DET	NO	TPI
DETECTED	DET	YES	DET	DET	YES	BT/TOB/TPI
DETECTED	DET	YES	DET	DET	YES	BT/TOB/TPI
DETECTED	DET	YES	NOT DET	DET	YES	BT/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	YES	DET	DET	YES	TOB/TPI
DETECTED	DET	YES	DET	DET	YES	BT/TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	BT/TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	YES	NOT DET	DET	YES	BT/TPI
DETECTED	DET	YES	DET	DET	YES	BT/TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	YES	DET	DET	YES	BT/TOB/TPI
DETECTED	DET	YES	DET	DET	YES	BT/TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	DET	YES	DET	DET	YES	BT/TOB/TPI
DETECTED	DET	NOT DET	DET	DET	YES	TOB/TPI
DETECTED	NOT DET	NOT DET	NOT DET	NOT DET	NO	N/A

Table 4. TaqMan® assay specificity using high titers of organisms tested in duplicate

Sample	Conc.	tcdB Cq	tpi Cq
NTC	-	ND	ND
Aeromonas hydrophila 39654	1.5e8 cfu/mL	ND	ND
Atopobium vaginae BAA-55	1e7 cfu/mL	ND	ND
Bacillus cereus 10876	1.5e8 cfu/mL	ND	ND
Bacteroides thetaiotaomicron 29741	1.5e8 cfu/mL	ND	ND
Bifidobacterium longum/*B. rectale* 35183	1.5e8 cfu/mL	ND	ND
Campylobacter coli 43133	1.5e8 cfu/mL	ND	ND
Campylobacter jejuni BAA-1153	1.5e8 cfu/mL	ND	ND
Candida albicans 11006	1.5e8 cfu/mL	ND	ND
Candida glabrata 32554	1.5e8 cfu/mL	ND	ND
Chlamydia trachomatis 0801775	1e7 ifu/mL	ND	ND
Citrobacter freundii 8090	1.5e8 cfu/mL	ND	ND
Clostridium difficile 43596	1.5e8 cfu/mL	21.6[A]	21[A]
Clostridium difficile 700057 (non-toxigenic)	1.5e8 cfu/mL	ND	20.8[A]
Clostridium novyi 27606	3e8 cfu/mL	ND	ND
Clostridium sesiculum 8065	3e8 cfu/mL	ND	ND
Clostridium sordelli 9714	1.5e8 cfu/mL	22.3[A]	22.3[A]
Enterobacter aerogenes 13048	1.5e7 cfu/mL	ND	ND
Enterobacter cloacae 13047	1.5e7 cfu/mL	ND	ND
Enterococcus faecalis 700802	1.5e7 cfu/mL	ND	ND
Enterococcus faecium 700221	1.5e7 cfu/mL	ND	ND
Escherichia coli 0111:H8 BAA-2217 (EHEC)	1.5e8 cfu/mL	ND	ND
Escherichia coli O157:H7 43895 (EHEC)	1.5e8 cfu/mL	ND	ND
Escherichia coli O29:NM 43892 (EIEC)	1.5e8 cfu/mL	ND	ND

Sample	Conc.	tcdB Cq	tpi Cq
Gardnerella vaginalis 0801894	1e6 cfu/mL	ND	ND
Haemophilus influenzae 0801680	1.5e8 cfu/mL	ND	ND
Hafnia alvei 51815	1.5e8 cfu/mL	ND	ND
Helicobacter pylori 0801486	1.5e6 cfu/mL	ND	ND
Klebsiella pneumoniae 35655	1.5e7 cfu/mL	ND	ND
Lactobacillus acidophilus 0801540	1e7 cfu/mL	ND	ND
Listeria monocytogenes 0801534	1.5e8 cfu/mL	ND	ND
Morganella morganii 49948	1.5e7 cfu/mL	ND	ND
Peptostreptococcus anaerobius 27377	1.5e8 cfu/mL	ND	ND
Plesiomonas shigelloides 14029	1.5e8 cfu/mL	ND	ND
Prevotella melaninogenica 25845	1.5e8 cfu/mL	ND	ND
Proteus vulgaris 33420	1.5e8 cfu/mL	ND	ND
Pseudomonas aeruginosa 10145	1.5e7 cfu/mL	ND	ND
Salmonella enterica Enteritidis 13076	1.5e8 cfu/mL	ND	ND
Salmonella enterica Typhimurium 14028	1.5e8 cfu/mL	ND	ND
Serratia marcescens 0801723	1e7 cfu/mL	ND	ND
Shigella flexneri 12025	1.5e8 cfu/mL	ND	ND
Shigella sonnei 11060	1.5e8 cfu/mL	ND	ND
Staphylococcus aureus 29213	1.5e7 cfu/mL	ND	ND
Staphylococcus epidermidis 14990	1.5e8 cfu/mL	ND	ND
Streptococcus agalactiae 49446	1.5e8 cfu/mL	ND	ND
Streptococcus pyogenes 19615	1.5e8 cfu/mL	ND	ND
Veillonella parvula 10790	1.5e8 cfu/mL	ND	ND
Vibrio vulnificus 27562	1.5e8 cfu/mL	ND	ND

The *tcdB* and *tpi* assays were found to cross-react with the closely related organism, *Clostridium sordellii*. *C. sordellii* produces toxins very similar to *C. difficile* toxin A and B. Antibodies to *C. sordellii* toxins will bind *C. difficile* toxins. *C. sordellii* is uncommon in humans.[1,2] [A]Expected result

Results *(continued)*:

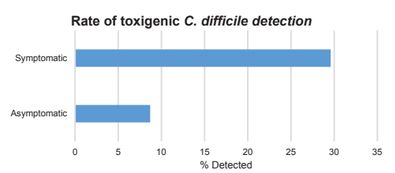

Figure 2. Rate of detection of toxigenic *C. difficile* by TEM-PCR in a community population asymptomatic for diarrhea (n=150) compared to a hospitalized population symptomatic for diarrhea with suspected CDI (n=80).

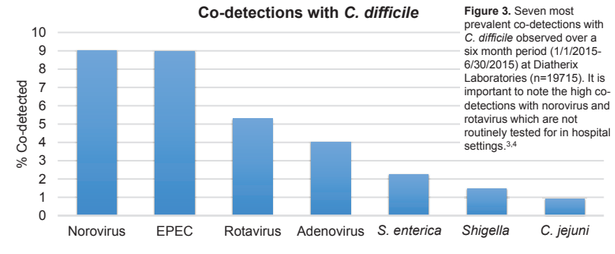

Figure 3. Seven most prevalent co-detections with *C. difficile* observed over a six month period (1/1/2015-6/30/2015) at Diatherix Laboratories (n=19715). It is important to note the high co-detections with norovirus and rotavirus which are not routinely tested for in hospital settings.[3,4]

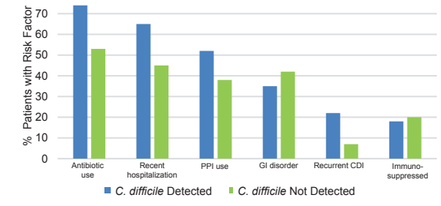

Figure 4. A comparison of the percentage of patients in this study who had risk factors for *C. difficile* infection and who subsequently had *C. difficile* detected or not detected as determined by Cepheid *C. diff*/Epi result and TEM-PCR result. Age is similar in the two groups (66 vs 58 for not detected group)

Abbrev: PPI, proton pump inhibitor

Conclusions:

- The *C. difficile* toxin B target in the Diatherix TEM-PCR™ Gastrointestinal Panel was determined to be 98.8% in agreement with the FDA cleared Cepheid *C. diff*/ Epi real-time PCR assay that targets the toxin B gene (*tcdB*, binary toxin gene, and hypervirulence associated *tcdC* deletion.
- One sample was positive with the Cepheid test but was negative for TEM-PCR toxin B, TaqMan® toxin B, TaqMan® *tpi*, and TEM-PCR binary toxin suggesting a false positive Cepheid result or a mislabeling of the specimen.
- The performance of Diatherix Gastrointestinal Panel for CDI detection is comparable to the FDA cleared Cepheid C. diff/ Epi test while offering simultaneous detection of 12 clinically relevant GI other pathogens.
- Norovirus, rotavirus, and EPEC were found to be the most common co-detections with *C. difficile* in the study population using large dataset of patient samples with suspected gastroenteritis submitted to Diatherix.
- As expected, recent antibiotic use was found to be the most prevalent risk factor for samples positive for CDI detection.

References:

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