

Comparison of Illumigene, Verigene, and Amplivue for Rapid Molecular Detection of *Clostridium difficile* in Pediatric Stool Specimens

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Abstract

Background: Pediatric *Clostridium difficile* infection (CDI) has been steadily rising over the past decade and is associated with increased morbidity and mortality. Rapid and accurate diagnostic testing is important in medical management. Due to the low sensitivity of enzyme immunoassays, and the complexity of culture based methods, many labs are utilizing newer molecular techniques for direct detection of nucleic acid in stool.

Methods: 59 prospective stool specimens from 57 pediatric patients (aged 4 months to 19 years) with suspected CDI were tested over a 4 month period. Three FDA-cleared molecular platforms, Meridian Illumigene, Nanosphere Verigene, and Quidel AmpliVue, were compared. All samples had a consistency rating (CR) from 1 - 5 (watery - solid) assigned and were refrigerated prior to testing. Those with a CR=5 were excluded. Six positive specimens were frozen for 3 months then retested, and two of those were serially frozen, thawed, and retested 5 more times over 5 months.

Results: All 3 platforms agreed for 55 (93%) specimens. There were 17 positive and 38 negative results. Five positive specimens were called BI/NAP1/027 by Verigene. Of the 4 discrepant results, 3 (2 CR=3, 1 CR=2) were invalid by Illumigene and negative by the other platforms. One sample (CR=4) was Verigene indeterminate, Illumigene positive, and AmpliVue negative. All frozen specimens gave correct results on all platforms. Conclusions: These data suggest that each platform offers a viable solution for rapid diagnosis of CDI in children. Additionally, long term frozen samples can be tested reliably.

Keywords: Clostridium; Difficile; Illumigene; Verigene; AmpliVue; Molecular; Pediatric; Pediatrics; Stool

Introduction

Clostridium difficile causes the majority of antibiotic-associated diarrhea and pseudomembranous colitis, especially in hospitalized patients [1,2]. The prevalence of *C. difficile* infection (CDI) appears to be increasing, and some studies implicate the emergence of a new hypervirulent strain, overuse of antibiotics, and better detection capabilities [3,4]. Some regions of the United States report CDI as the most common nosocomial infection, surpassing methicillin-resistant *Staphylococcus aureus* [5]. There has also been a steady rise in infections of groups previously believed to be "low risk," such as the pediatric population [6]. This increase in pediatric infections has been observed in both inpatient and ambulatory care settings [6-10]. Several studies report an increased risk of death, longer hospital stays, and/or higher healthcare related costs for pediatric patients with CDI [11-15], whereas another study reported a lower correlation with severe outcome when compared to adults [16]. While the exact correlation between age, epidemiology, and clinical outcome is still debated, one fact remains salient: rapid and accurate diagnosis of CDI is crucial for medical treatment. Several methods for the diagnosis of CDI exist, including enzyme immunoassays (EIA), cell culture cytotoxicity neutralization assays, toxigenic culture, and glutamate dehydrogenase detection. Although EIA is fairly rapid, several studies have shown that there is reduced sensitivity [1,17-21]. Culture based assays require special training and results are not available for several days. Molecular techniques, such as direct detection of nucleic acid in stool targeting various *C. difficile* toxin genes, are rapidly becoming the method of choice in many laboratory and clinical settings [22,23]. There have been several studies examining the performance of nucleic acid amplification tests for diagnosis of CDI in adults, but such studies in pediatric patients are very limited. Given the paucity of pediatric

data for rapid diagnosis of CDI, we compared our current FDA 510(k) cleared molecular platform (Illumigene) to two other FDA-cleared assays by parallel testing of prospective pediatric stool samples, both fresh and frozen, and with varying consistencies (watery to semi-solid). To our knowledge, this is the only study comparing these methods in this age group.

The Illumigene *C. difficile* assay (Meridian Biosciences, Inc., Cincinnati, OH) utilizes Loop-Mediated Isothermal Amplification (LAMP) technology in combination with an automated detection platform. The assay targets a 204bp sequence of the *tcdA* region of the Pathogenicity Locus (PaLoc) using loop-mediated isothermal DNA amplification [24,25].

The Verigene *C. difficile* Test (Nanosphere Inc., Northbrook, IL) uses a gold nanoparticle probe hybridization array that targets the *tcdA* and *tcdB* genes, binary toxin genes *cdtA/cdtB*, and delta117 in *tcdC* (for detection of the hypervirulent strain BI/NAP1/027) [17].

AmpliVue *C. difficile* Assay (Quidel Inc., San Diego, CA) targets a conserved region of the *tcdA* gene using a Biohelix® isothermal

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amplification technology called helicase dependent amplification (HDA) [25].

Materials and Methods

Specimens: 59 prospective stool specimens from 57 patients (aged 4 months to 19 years) with suspected *C. difficile* disease from January through April, 2013 were included in the study. All samples were assigned an in-house developed consistency rating (CR) from 1 - 5 (1 = liquid; 3 = semi-solid/non-formed; 5 = solid/fully-formed) and then refrigerated for up to 48 hours prior to testing. Samples with a CR=5 were rejected, per ASM guidelines (A Practical Guidance Document for the Laboratory Detection of Toxigenic *Clostridium difficile*; September 21, 2010), and excluded from the study. Specimens with an initial result of invalid or indeterminate were retested within a 24-hour period. If the second test yielded a different result, a third test was performed and the best of 3 was considered the final result. Six randomly selected positive specimens (CR ranged from 1 to 4) were frozen at -20°C for 3 months and retested on all platforms. Two of those specimens (CR of 1 and 3) were then serially frozen, thawed, and retested 5 additional times over a 5 month period (for a total of 7 runs).

Platforms

Testing was performed following manufacturer specifications. Briefly, for the Illumigene illumipro-10 assay, stool was sampled with the manufacturer brush and transferred to diluent, then vortexed. Ten drops were then transferred to a heat treatment tube and placed in a 95oC heat block for 10 minutes. A 50 uL aliquot of heated sample was then transferred to a reaction buffer tube and vortexed. 50 uL of the vortexed buffer was transferred to each chamber of the Illumigene test device and inserted into the Illumipro-10. A qualitative result was printed within 39 minutes.

For the Verigene (Verigene Reader and Processor SP), the stool was collected on the manufacturer supplied swab and transferred to a tube of buffer. The buffer tube was vortexed and then centrifuged for 30 seconds. After loading the extraction tray, tip assembly, and amplification tray into their modules, the test cartridge barcode was scanned and inserted into the Verigene module. 100 µL of the centrifuged buffer was pipetted into the extraction tray and the test processing started. After approximately 90 minutes, the test cartridge was removed from its module and inserted into the Reader for analysis. A printed qualitative result was available within seconds of analysis.

For the AmpliVue, stool samples were collected using the manufacturer's sterile swab, transferred to diluent and vortexed. 50µL of the diluent mixture was transferred to a lysis buffer tube and quickly vortexed. After vortexing, the lysis buffer tube was heat treated in a heat block for 10 minutes at 95°C. 50 µL of the heated sample was then pipetted into reaction tubes and heated for an additional 60 minutes at 64oC in the analyzer. Upon completion, the reaction tube was then placed into the amplicon cartridge and the cartridge was inserted

CR	Number of samples	Number of Specimens Positive by:		
		Verigene	Illumigene	AmpliVue
1	18	4	4	4
2	12	5	5	5
3	21	6	6	6
4	8	2	3*	2
Total:	59	17	18	17

*This sample was initially positive, then negative, then positive upon repeat testing on the Illumigene, Invalid on the Verigene, and Negative on the AmpliVue

Table 1: CR vs. Total Number Positive by Each Platform.

CR	Verigene Result	Illumigene Result	AmpliVue Result	Pt Age	Discrepant
4	No Call-IND x 2	Pos, Neg, Pos*	Neg	3 yr	Yes
2	Neg	Inv x 2	Neg	2 yr	Yes
3	Neg	Inv x 2	Neg	12 yr	Yes
3	Neg	Inv x 2	Neg	1 yr	Yes

*This sample was retested after a positive result due to the discrepancy.

Table 2: Discrepant results.

Specimen#	CR	Verigene Result	Illumigene Result	AmpliVue Result	Pt Age	Discrepant
1*	1	Pos,tcdA,B,Binary,tcdC Mutant, Ribotype 027	Pos	Pos	11 yrs	No
2	2	Pos,tcdA,B,tcdC Wild Type	Pos	Pos	4 m	No
3	2	Pos,tcdA,B,Binary,tcdC Mutant, Ribotype 027	Pos	Pos	8 yrs	No
4*	3	Pos, tcdA,B,tcdC Wild Type	Pos	Pos	3 yrs	No
5	2	Pos,tcdA,B,tcdC Wild Type	Pos	Pos	5 yrs	No
6	4	Pos,tcdA,B,tcdC Wild Type	Pos	Pos	1 yr	No

*Denotes samples that were serially frozen, thawed, and retested over 7 months.

Table 3: Data for frozen samples.

into the detection cassette. Results were visually interpreted from the window of the detection cassette within 10 minutes.

Results

After initial testing of the 59 stool specimens, results of all three platforms agreed for 55 (93%): 17 were positive and 38 were negative. A summary of the specimens tested and their corresponding CR are shown in Table 1. Specimens with a CR of 5 were not tested. Three of the 4 discrepant results (two were CR=3 and one was CR=2) were invalid by the Illumigene and negative by the Verigene and AmpliVue systems. The remaining discrepant result had a CR of 4 and was indeterminate by Verigene, positive by Illumigene (which was positive for 2 out of 3 repeated runs), and negative by AmpliVue testing (Table 2). Another sample that had a CR of 4 was initially invalid by Illumigene, and positive by the other 2 platforms. Repeat testing of the sample on Illumigene yielded positive results on 2 additional runs, and it was reported as a positive result and not considered discrepant.

All frozen specimens showed 100% correlation on all platforms. The 6 positive specimens that were retested after being frozen for 3 months yielded positive results on all platforms. Both of the serially frozen, thawed, and repeat tested specimens yielded positive results on all 7 runs over the 5 month period of testing (which included the initial run, then one run every 25 - 35 days after being thawed, then re-frozen within 1 hour of testing) (Table 3).

Five specimens from 4 patients were called Ribotype 027/NAP1 by Nanosphere. Review of the medical records did not support more severe disease. Three patients, ages 8 years, 11 years, and 4 years, were successfully treated with vancomycin and had no recurrence over the subsequent 12 - 15 months. The fourth patient was a 2-year old girl who was positive on two occasions, 4 weeks apart. She was treated with several courses of vancomycin and has not experienced recurrent disease over the subsequent 12 months.

Discussion

The Illumigene showed reduced sensitivity giving invalid results on 3 samples that were negative by the Verigene and AmpliVue platforms.

This discrepancy did not seem to correlate with sample consistency. However, consistency may have been a factor with the forth discrepant result (CR=4), which was positive, indeterminate, and negative, on Illumigene, Verigene, & AmpliVue, respectively. Clinical review of this patient's chart reveals that he was 3 years-old when treated for *C. difficile*, but no prior history or additional follow-up information is provided to help resolve the discrepancy.

Although Illumigene and Verigene do not recommend freezing samples, and AmpliVue recommends freezing only up to 7 days, all results on frozen samples were concordant over 5 months and 6 freeze/thaw cycles.

Workflow and result reporting was comparable with Illumigene and Verigene, with 10 mins of hands-on time and print-out of results. The AmpliVue required 15 mins and introduced slight subjectivity due to the requirement for line visualization to make the call. Run times are approximately 40 mins, 1.75 hrs, and 1.25 hrs for the Illumigene, Verigene, & AmpliVue, respectively.

The Illumigene illumipro-10 has a footprint of 8.5" x 11", can run up to 10 samples at a time in 2 separate 5-sample chambers, does not require an additional computer or peripheral, and provides a printed result on a receipt-sized piece of paper. The Nanosphere Verigene consists of the processor (7.6" x 22.9") and the reader (11.7" x 20.5"), and runs 1 sample at a time. The system does not require a peripheral computer to be attached, and a printed result is supplied. The Quidel AmpliVue system consists of the amplification block (7.3" x 11.6"), the heat block (5.5" x 7.1"), and a disposable detection device. The AmpliVue does not provide a printed report, however there is no capital investment for this system and it is a good option for low-throughput laboratories. Although both the Illumigene and the Verigene require an investment for the analyzers, they each have options to run additional assays. All three platforms offer better sensitivity and specificity over EIA, faster turn-around-times compared to culture, and each are viable solutions for rapid *C. difficile* testing.

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