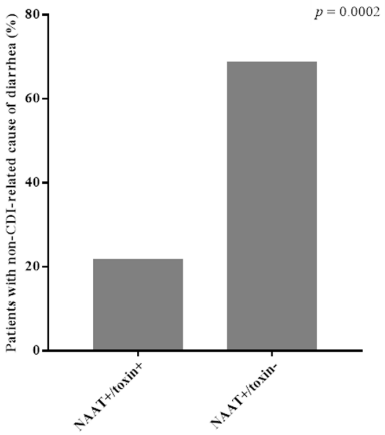

Disclosures. **All authors:** No reported disclosures.

2357. Toxin Detection Using Single Molecule Counting Technology: The Best of Both Worlds?

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Background. Accurate diagnosis of CDI remains challenging as there is no standalone laboratory test with adequate clinical sensitivity and specificity. Thus, many clinical laboratories currently employ a multistep algorithm incorporating a sensitive screening test followed by a specific toxin test. An automated ultrasensitive toxin immunoassay (Singulex Clarity® *C. difficile* toxins A/B assay) has demonstrated excellent performance compared with cell cytotoxicity neutralization assay (CCNA). In this study, the Clarity assay was evaluated relative to glutamate dehydrogenase (GDH), toxin EIA, toxin B gene PCR, multistep algorithms, and *C. difficile* culture with ribotyping.

Methods. Residual clinical stool samples (n = 293) were collected from patients with suspected CDI. The samples were tested on-site with GDH (*C. DIFF CHEK*™-60), PCR (EntericBio realtime® *C. difficile* assay), a membrane-type toxin EIA (Tox A/B Quik Chek®), and culture and ribotyping. In total, 188 samples were tested with GDH and 239 samples were tested by PCR. All PCR-positive samples (n = 148) and prospectively tested GDH samples (n = 97) were tested with the toxin EIA. Culture and ribotyping information were available for 205 samples.

Results. Three of the samples tested gave no result using the Clarity assay and were excluded from the analysis. The Singulex Clarity *C. difficile* toxins A/B assay had high positive percent agreement (PPA) and low negative percent agreement (NPA) compared with toxin EIA and multistep algorithms ending with toxin EIA. The Clarity assay had high NPA and low PPA compared with PCR, GDH, and the multistep algorithm ending with PCR (figure). Less than 70% of the detected *C. difficile* PCR positive samples had toxins present. There was no difference in toxin concentration between the ribotypes.

Conclusion. The Clarity assay had strong PPA compared with toxin EIA and strong NPA compared with PCR. The low NPA and PPA compared with toxin EIA and PCR, respectively, may reflect the poor sensitivity of current toxin EIAs and low specificity of PCR. The Clarity assay detected 30 different ribotype strains, and less than 70% of samples (by PCR) or strains (by ribotyping) had toxins present. The Clarity assay may be considered for use as a standalone test for CDI diagnosis.

Singulex Clarity C. diff toxins A/B assay	Positive (n)	Negative (n)	Total (n)	Positive percent agreement (95% CI)	Negative percent agreement (95% CI)
GDH → Toxin EIA					
Positive	25	39	64	100 (86.3–100)	75.3 (67.8–81.8)
Negative	0	119	119		
Total	25	158	183		
GDH → Toxin EIA → PCR					
Positive	47	9	56	81.0 (68.6–90.1)	91.7 (84.8–96.1)
Negative	11	99	105		
Total	58	108	166		
PCR → Toxin EIA					
Positive	46	65	111	95.8 (85.7–99.5)	65.4 (58.2–72.2)
Negative	2	123	125		
Total	48	188	236		

Singulex Clarity C. diff toxins A/B assay	Positive (n)	Negative (n)	Total (n)	Positive percent agreement (95% CI)	Negative percent agreement (95% CI)
GDH					
Positive	58	6	64	61.1 (50.5–70.9)	91.9 (83.2–97.0)
Negative	37	68	105		
Total	95	74	169		
PCR					
Positive	102	9	111	69.4 (61.3–76.7)	89.9 (81.7–95.3)
Negative	45	80	125		
Total	147	89	236		
Toxin EIA					
Positive	62	69	131	96.9 (89.2–99.6)	49.6 (41.0–58.3)
Negative	2	68	70		
Total	64	137	201		

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2358. Understanding the Clinical Implications of *Clostridium difficile* Detection in the Molecular Age: Colonization vs. Infection in Children Less Than 3 Years of Age

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Background. Infants have a high rate of asymptomatic *Clostridium difficile* (CD) colonization (up to 37%) but can rarely develop true CD infection (CDI). However, currently available polymerase chain reaction (PCR) and enzyme immunoassays (EIA) have suboptimal sensitivity/specificity to distinguish CDI from colonization. Recent data from adults showed that lower cycle threshold (Ct) values of a semi-quantitative CD toxin B gene (*tcdB*) PCR assay in stool correlated with detection of free CD toxin in stool and poor clinical outcomes. We hypothesized that a *tcdB* PCR assay may be utilized to distinguish CDI from colonization in patients < 3 years old.

Methods. Symptomatic patients < 3 years old with CD detected by the BioFire FilmArray Gastrointestinal Panel (FGP) were enrolled 2/2018–3/2019. We performed CD *tcdB* PCR and toxin A/B/GDH EIA on frozen aliquots of stool in Cary Blair. CDI was defined among those that were *tcdB* PCR positive as (1) a consistent clinical syndrome (diarrhea + no current laxative use), (2) CD EIA toxin+, (3) symptomatic improvement with CDI-directed treatment, and (4) no alternative etiology of diarrhea identified. Patients who did not meet criteria for CDI were considered colonized. We compared median *tcdB* PCR Ct values between the CDI and colonized groups using the Mann–Whitney test.

Results. Of 193 FGP CD+ patient samples with charts available for review, 37 (19%) samples were EIA GDH+/toxin+, 121 (63%) were GDH+/toxin– and 35 (18%) were EIA–. 150 (78%) samples had detectable *tcdB* by PCR. Six (4%) patients met criteria for CDI and 144 (96%) for colonization. Median (interquartile range) *tcdB* PCR Ct values were 23.8 (22.0–29.5) and 30.5 (26.3–35.8) in patients with CDI and colonization, respectively (P = 0.03).

Conclusion. Using a strict clinical and laboratory definition, 4% of evaluable patients < 3 years old met criteria for CDI and had significantly lower *tcdB* PCR Ct values than colonized patients. A combination of clinical and laboratory criteria, including semi-quantitative tcdB PCR, may help differentiate colonization from CDI in this patient population.

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2359. Prospective Feasibility Study for Novel Ultrasensitive Multiplexed Immunoassay for *Clostridiodes difficile* Toxins A and B

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Background. The diagnosis of *Clostridiodes difficile* infection is challenging. A wide array of diagnostic tests are used in practice; however, each available test has important limitations. We examined the feasibility and analytical performance of a novel ultrasensitive multiplexed immunoassay designed by Meso Scale Diagnostics (MSD) compared with five current diagnostic assays for detection of *C. difficile* toxin A and B.

Methods. Stool, serum and urine samples from 44 admitted inpatients were collected within 72 hours of a standard of care nucleic acid amplification test (NAAT) result (23 positive, 21 negative). These specimens underwent five standard diagnostic assays: enzyme immunoassay for toxins A and B (EIA), cytotoxin cell assay, bacterial culture isolation, and two different NAATs to determine presence of viable *C. difficile* cells, toxins, and toxin-encoding genes (Table 1). The concentration (fg/mL) of toxin A and toxin B in all stool samples was then quantified using MSD's multiplexed immunoassay (Table 1).

Results. At least one of the five standard diagnostic tests for *C. difficile* was positive in 16 of the 23 clinically positive patients. The MSD multiplex immunoassay detected toxin A and/or toxin B in 15 of these 16 samples and quantified low levels of toxin A in one clinically positive sample that was negative for all other tests. In contrast, only 2 of the 16 positive samples were positive by EIA, demonstrating the benefits of the ultrasensitive assay over standard immunoassay methods. All clinically negative specimens were negative in all tests. Toxin detection in urine and serum samples was negligible. In stool samples, the MSD test had an estimated sensitivity of 93% (95% CI: 70–99%) and specificity of 93% (95% CI: 78–98%) compared with the clinically used NAAT.

Conclusion. The MSD multiplex toxin assay is a feasible test to move forward for further evaluation. Ultimately, future studies should examine the performance of this test compared with standard of care in a prospective randomized trial assessing clinical outcomes.