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Background. Commercially available tests for *Clostridium difficile* infection (CDI) make test selection by the laboratory difficult due to the following unsatisfactory characteristics: long turnaround time, poor sensitivity, and/or poor specificity. The Singulex Clarity® C. diff toxins A/B assay (in development) is a rapid and automated immunoassay for the detection of *C. difficile* toxins A and B in stool, with analytical limits of detection for toxins A and B at 2.0 and 0.7 pg/mL, respectively. In this multicenter study, the clinical performance of the Singulex Clarity C. diff toxins A/B assay was compared with standalone PCR, a multistep algorithm with enzyme immunoassay (EIA) and PCR, and cell cytotoxicity neutralization assay (CCNA).

Methods. Fresh samples from 267 subjects with suspected CDI were tested at two sites (Minneapolis Medical Research Foundation and TriCore Reference Laboratories) with the Singulex Clarity assay, PCR (Xpert® *C. difficile*), and EIA (C. Diff Quik Chek Complete®) for GDH and toxin testing. The performance of the assays and multistep algorithms were evaluated against CCNA (Microbiology Specialists, Inc.).

Results. The overall CDI prevalence was 15.7%. The Singulex Clarity C. diff toxins A/B assay had 90.5% sensitivity and 96.0% specificity, with a 98.2% negative predictive value when compared with CCNA, and the Clarity assay's AuROC was 0.9534. PCR had 90.5% sensitivity and 91.1% specificity. Compared with CCNA, the toxin EIA had 47.6% sensitivity and 100% specificity. Testing with a multistep algorithm using EIA with discordant results reflexed to PCR resulted in 85.7% sensitivity and 94.7% specificity.

Conclusion. The ultrasensitive Singulex Clarity C. diff toxins A/B assay is equivalent to the sensitivity of PCR while providing higher specificity. Compared with a multistep algorithm, the Clarity assay provides higher sensitivity and specificity while providing faster time-to-result in a simpler-to-understand, one-step reporting structure, allowing for a standalone, single-step solution for detection of *C. difficile* toxins in patients with suspected CDI.

Disclosures. **E. Friedland**, Singulex, Inc.: Employee, Salary. **A. Bartolome**, Singulex, Inc.: Employee, Salary. **A. Almazan**, Singulex, Inc.: Employee, Salary. **S. Tam**, Singulex, Inc.: Employee, Salary. **S. Biscocho**, Singulex, Inc.: Employee, Salary. **S. Abusali**, Singulex, Inc.: Employee, Salary. **J. Sandlund**, Singulex, Inc.: Employee, Salary. **J. Estis**, Singulex, Inc.: Employee, Salary. **J. Bishop**, Singulex, Inc.: Employee, Salary.

1089. Analytical Performance of an Ultrasensitive Immunoassay for Detection of *Clostridium difficile* Toxins in Stool

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Background. *Clostridium difficile* infection (CDI) is the main cause for nosocomial diarrhea. Currently available assays for the diagnosis of CDI show deficits in sensitivity, specificity, and/or turnaround time. The Singulex Clarity® C. diff toxins A/B assay, in development for the Singulex Clarity® system, was designed to provide an accurate and automated detection of *C. difficile* toxins A (TcdA) and B (TcdB) in stool. Here, the analytical performance of the assay is reported.

Methods. Limits of detection (LoD) for TcdA and TcdB in stool and buffer was determined, and a preliminary cutoff, as compared with cell cytotoxicity neutralization assay (CCNA), was established. Analytical reactivity against 38 toxigenic and nontoxigenic *C. difficile* strains of eight different toxinotypes was determined. Cross-reactivity against 53 other gastrointestinal pathogens and potential interference by 11 endogenous and exogenous substances were determined. Reproducibility was tested with triplicate samples ($n = 85$), and stability was evaluated in samples stored at room temperature, refrigerated, and frozen conditions, and subjected to three freeze-thaw cycles.

Results. The LoDs for TcdA and TcdB were 0.8 and 0.3 pg/mL in buffer, and 2.0 and 0.7 pg/mL in stool, respectively. Using a preliminary cutoff, the assay demonstrated 96.3% sensitivity and 96.1% specificity compared with CCNA. The Singulex Clarity® C. diff toxins A/B assay detected toxins from all tested strains and toxinotypes. No cross-reactivity or interference were detected. The repeatability was 99%, and samples for *C. difficile* toxin testing were stable up to 8 hours in room temperature, 1 week in 2–8°C, 6 months in −70°C, and up to three freeze–thaw cycles.

Conclusion. The Singulex Clarity C. diff toxins A/B assay (in development) can detect TcdA and TcdB at very low concentrations and it has high sensitivity and specificity compared with CCNA. The assay demonstrates reactivity to common *C. difficile* strains, does not show cross-reactivity to common gastrointestinal pathogens, is robust against common interferents, allows for toxin detection in both fresh and frozen stool samples and up to three freeze–thaw cycles, and provides results with high reproducibility.

Disclosures. **A. Bartolome**, Singulex, Inc.: Employee, Salary. **A. Almazan**, Singulex, Inc.: Employee, Salary. **S. Abusali**, Singulex, Inc.: Employee, Salary. **S. Tam**, Singulex, Inc.: Employee, Salary. **E. Lee**, Singulex, Inc.: Employee, Salary. **A. Changavi**, Singulex, Inc.: Employee, Salary. **W. Trinh**, Singulex, Inc.: Employee, Salary. **K. Chau**, Singulex, Inc.: Employee, Salary. **J. Estis**, Singulex, Inc.: Employee, Salary. **B. Noland**, Singulex, Inc.: Employee, Salary. **J. Bishop**, Singulex, Inc.: Employee, Salary.

1090. Patient Outcomes With Prevented vs. Negative *Clostridium difficile* Tests Using Computerized Clinical Decision Support (CCDS)

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Background. Overtesting and overdiagnosis of *Clostridium difficile* infection (CDI) are increasingly recognized as potentially avoidable causes for unnecessary treatment and cost. Reducing inappropriate testing through diagnostic stewardship may improve *C. difficile* test utilization. However, the safety of these interventions is not well understood, despite the potential risk for missed or delayed diagnosis. A computerized clinical decision support (CCDS) tool was implemented at a 619-bed tertiary care hospital as part of a multifaceted effort to reduce inappropriate *C. difficile* testing. The intervention was associated with reductions in tests (41%) and hospital-onset CDI events (31%). We sought to examine patient outcomes associated with the intervention.

Methods. The CCDS was designed to identify patients with a prevented test if a provider initiated the CCDS and aborted the order. Outcomes of patients with either a prevented or negative nucleic acid amplification test (NAAT) were compared retrospectively. A logistic regression model was created to evaluate the association between a prevented test attempt and serious adverse events. Patients with a subsequent positive result within 7 days of the initial trigger and those treated with CDI-effective antibiotics underwent chart review.

Results. Multivariate analysis of 637 cases (490 negative, 147 prevented) showed that a prevented test was not associated with the primary composite outcome (inpatient mortality or ICU-transfer) compared with a negative test (adjusted odds ratio, 0.912; 95% CI 0.513–1.571). Prevented tests were associated with shorter length of stay and similar rates of CDI-related complications. Eleven (7.5%) had a subsequent positive CDI, four within 30 minutes of the prevented test, suggesting nonsignificant delay in testing. Of the remaining seven patients, case review confirmed that five did not meet testing criteria while two met testing criteria at the time of the prevented test. No serious adverse events attributable to delayed CDI diagnoses or unjustified CDI treatment were identified by individual case review.

Conclusion. CCDS-based diagnostic stewardship for CDI may be both a safe and effective means to reduce inappropriate testing.

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

1091. Algorithmic Release of *Clostridium difficile* PCR Results From a Multiplex Gastrointestinal (GI) Panel in Children <3 Years Old

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Background. Infants have a high rate of asymptomatic *Clostridium difficile* (CD) colonization, up to 37%. Given this, our laboratory does not release CD+ results from the BioFire FilmArray Gastrointestinal Panel (FGP) in patients <3 years, unless requested by a physician. We sought to validate this model by comparing results from FGP to semi-quantitative CD PCRs for toxin B and glutamate dehydrogenase (GDH), enzyme immunoassay (EIA) for toxin A/B/GDH, and physician requests for CD results.

Methods. Retrospective analysis of children <3 years with GI illness and FGP CD+ results between September 2016 and April 2018. CD PCRs for toxin B and GDH, CD EIA for toxin A/B/GDH were performed on convenience samples of frozen aliquots in Cary Blair. Physician request for release of CD results was used as a surrogate of possible role of CD on GI illness.

Results. Of 5,990 FGP, 2,267 (38%) were in children <3 years: 619 (27%) were CD+. Of these 619, 602 (97%) were not reported per algorithm. 62% (386/619) of CD+ samples had copathogens detected; enteropathogenic *Escherichia coli* and norovirus most frequently. For CD PCRs and EIA performed in subset of 49 CD+, mean cycle threshold values (Cts) for toxin B were evaluated (Table 1). Of 48 samples with detectable CD by toxin B PCR, 14 (29%) had both GDH and toxin B detected, 24 (51%) had only GDH detected, and 9 (19%) had neither GDH nor toxin B detected.

Conclusion. Only 3% of FGP CD results in children <3 years were released per physician request, suggesting limited clinical significance. A copathogen was detected in 62% of CD+ samples that may explain illness. Among evaluable samples, only 28.6% of CD+ had both GDH and toxin detected by EIA, possibly indicating low specificity of CD PCR. Ongoing testing and prospective studies are warranted to determine the validity of our algorithm and if semi-quantitative PCR or EIA can be useful to identify when CD detection by FGP in children <3 years is clinically significant.

Table 1: CD Toxin B PCR Ct Values Compared with FGP Result Release, Co-Pathogen Detection, and EIA Results

	Median Ct		Median Ct	*P*-value
FGP-CD+ result released (*n* = 1)	20.64	FGP-CD+ result not released (*n* = 47)	28.77	NS
FGP-CD+ only (*n* = 24)	26.83	Co-pathogen (*n* = 24)	29.34	NS
EIA toxin+ (*n* = 14)	23.23	EIA toxin− (*n* = 34)	31.11	0.0005

Disclosures. **A. Leber**, Nationwide Children's Hospital: Research Contractor, Research support.

1092. Tuning Down *Clostridioides difficile* PCR Sensitivity Reduces Treatment for *C. difficile* Infection in Toxin-Negative Patients With No Increase in Adverse Outcomes

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Background. Studies have shown that toxin detection identifies those who require treatment for *C. difficile* infection (CDI) and free toxin can be predicted with high negative predictive value from PCR cycle threshold (CT). CT-toxin was introduced at our institution in two phases: from October 2016 to October 2017, CT-toxin was reported with the PCR result (split reporting) and CDI therapy was discouraged if CT-toxin was negative (PCR+/CTtox−). Interim analysis showed that CDI treatment had no effect on outcomes in these CTtox− patients, so starting November 2017, only CT-toxin was reported. Outcomes in PCR+/CTtox− patients treated during split reporting and untreated during the toxin-only period are detailed here.

Methods. Patients tested from October 2016 to February. 2018 with a positive Xpert *tcdB* PCR (Cepheid, Sunnyvale, CA) and CTtox− result were included. Clinical data were collected by retrospective chart review in the split reporting period and prospective review in the toxin-only period and analyzed using SPSS at $\alpha = 0.01$.

Results. Of 186 unique PCR+/CTtox- patients during split reporting, 99 (53%) were treated, compared with 6 (12%, $n = 51$) in the toxin-only period ($P < 0.001$). In comparing treated patients during split reporting to untreated patients during toxin-only reporting ($n = 45$), there were no significant differences in age, sex, prior antibiotic use, CDI in the previous 6 months, Charlson Comorbidity Index, patient location, immune status, or data at testing, including WBC count, creatinine, albumin, and stools/day. There were no cases of fulminant CDI in either group and no difference in outcomes (table).

Conclusion. Reporting of CT-toxin alone significantly reduced treatment for CDI compared with split reporting in CTtox− patients with no increase in adverse outcomes in short-term follow-up. Further study is needed to confirm these findings in a larger cohort.

Table: Outcomes in Patients With PCR+/CTtox− Result by Treatment Status and Reporting Period. Categorical Variables Are Denoted as n (%) and Continuous Variables as Mean [Standard Deviation]

Outcomes	Split Reporting Treated (*n* = 99)	CT-Toxin Only Reporting Untreated (*n* = 45)	*P*-value
Days to diarrhea resolution (<3 stools/day)	2.8 [1.9]	2.2 [2.1]	0.2
CTtox+ CDI within 8 weeks	8 (8.1)	4 (8.9)	0.9
30-Day all-cause mortality	9 (9.1)	3 (6.7)	0.8

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

1093. Single Molecule Counting Technology for Ultrasensitive Quantification of *Clostridium difficile* Toxins A and B

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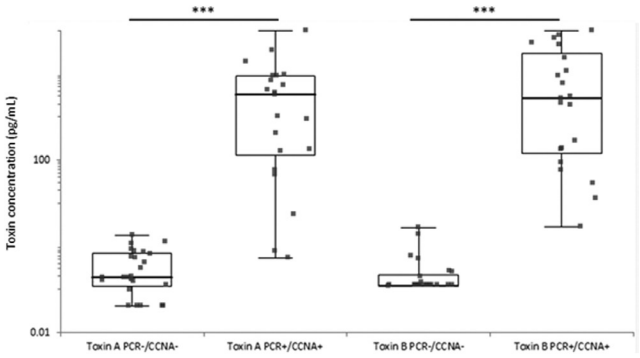
Background. *Clostridium difficile*, a spore-forming, anaerobic, Gram-positive bacterium, is the leading cause of nosocomial diarrhea. *C. difficile* infection (CDI) is mediated by two toxins, A (TcdA) and B (TcdB), and the role of each toxin in CDI pathogenesis remains unclear. Many assays used in CDI diagnostics, such as most NAATs and cell cytotoxicity neutralization assay (CCNA), detect presence of only *tcdB* or TcdB. In this study, an ultrasensitive immunoassay (UIA) powered by Single Molecule Counting technology was used for quantification of TcdA and TcdB, to assess toxin dynamics in CDI.

Methods. Banked samples from 46 patients with suspected CDI were tested with PCR (BD MAX™ Cdiff Assay) and CCNA, and TcdA and TcdB were quantified using the UIA (tested in triplicate). The limits of detection (LoDs) for the TcdA and TcdB assays are 0.04 and 0.12 pg/mL, respectively.

Results. There were 21 PCR+/CCNA+ and 25 PCR−/CCNA− samples. Both toxins were measured above LoD in all PCR+/CCNA+ samples, ranging up to 100,000 pg/mL. The average CV for the PCR+/CCNA+ samples was 9%. The median TcdA concentrations in PCR−/CCNA− and PCR+/CCNA+ samples were 0.19 pg/mL (IQR 0.12–0.67) and 3,301 pg/mL (125–8,737), respectively. The median TcdB concentrations in PCR−/CCNA− and PCR+/CCNA+ samples were 0.12 pg/mL (0.12–0.21) and 2,690 pg/mL (145–30,307), respectively. In the PCR+/CCNA+ samples, TcdA was one or more logs higher than TcdB in two samples, one or more logs lower than TcdB in six samples, and within one log of TcdB in 13 samples. In one sample (4.8% of PCR+/CCNA+ samples), TcdA was at moderately high concentration while TcdB was below a provisional cutoff, indicating that only TcdA was expressed. There was a significant correlation between TcdA and TcdB (Spearman $r = 0.753$).

Conclusion. The UIA allows for toxin quantification over a concentration range of ≥5 logs, suggesting that the quantitative TcdA and TcdB assays could be of value in CDI characterization and clinical decision making. The TcdA/TcdB ratio varied, and toxin quantification could be a useful tool in further understanding their individual roles in CDI. The TcdA concentration was not lower than TcdB (trended higher), indicating that detection of *tcdB* or TcdB alone may not be sufficient for accurate CDI diagnostics.



Disclosures. **P. Katzenbach**, Singulex, Inc.: Employee, Salary. **G. Dave**, Singulex, Inc.: Employee, Salary. **A. Mukherjee**, Singulex, Inc.: Employee, Salary. **J. Todd**, Singulex, Inc.: Employee, Salary. **J. Bishop**, Singulex, Inc.: Employee, Salary. **J. Sandlund**, Singulex, Inc.: Employee, Salary. **J. Estis**, Singulex, Inc.: Employee, Salary.

1094. Performance of Toxin Enzyme Immunoassays and PCR Cycle Threshold for Differentiating *Clostridium difficile* Infection From Colonization in Children With Diarrhea

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Background. *Clostridium difficile* colonization is common in children. PCR does not distinguish infection (CDI) from colonization. Toxin enzyme immunoassay (EIA) and PCR cycle threshold (Ct) may predict CDI in PCR+ adults, but assay performance in children is poorly understood.

Methods. Stools from children aged 2–21 years with laboratory-identified (labID) CDI (*tcdB* PCR+; GeneXpert) underwent: toxin EIA (QUIK CHEK Complete [QCC] and Immunocard [IC]); cell culture cytotoxicity neutralization assay (CCCNA); and *C. difficile* stool culture (Cx). Children were determined to have clinical CDI (cCDI) by chart review and/or parent communication if all were noted: at least three unformed stools (Bristol type 5–7) in 24 hours; response to CDI treatment within 5 days; and no other likely diarrheal etiology. EIA and PCR Ct performance were measured for various reference standards (RefStd) based on stool assay results and/or cCDI classification.

Results. A total of 253 PCR+ stools were included. All stools underwent QCC; 218 (86%) were quantity sufficient for IC. Discordant EIA results occurred in 19/218 (8.7%) stools. Table 1 lists EIA sensitivity (Sn), EIA specificity (Sp), and median PCR Ct for each RefStd. Figure 1 shows the receiver operating characteristic (ROC) curve for PCR Ct to identify PCR+/CCCNA+/cCDI+ children (area under curve = 0.76). The difference between sensitivity (71%) and specificity (72%) was minimized at Ct < 23.5.

Conclusion. Only a minority of PCR+ children meets strict clinical and laboratory CDI criteria. More stringent CDI definitions are associated with increasing toxin EIA Sn and lower PCR Ct (i.e., greater stool *C. difficile* inoculum). However, both toxin EIA and PCR Ct perform suboptimally as stand-alone tests to distinguish CDI from colonization in PCR+ children.