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. difficile toxins A/B assay (in development) is a rapid and automated immunofluorescence assay for the detection of C. difficile toxins A and B in stool, with limits of detection for toxins A and B at 2.0 and 0.7 pg/mL, respectively. In this multi-center study, the clinical performance of the Singulex Clarity C. difficile 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 (Xperi C. difficile), and EIAs (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. difficile 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 0.953. PCR had 90.3% sensitivity and 91.4% specificity. Compared with CCNA, the toxin EIAs had 46.7% sensitivity and 100% specificity. Testing with a multistep algorithm using EIAs with discordant results reflected to PCR resulted in 85.7% sensitivity and 94.7% specificity.

**Conclusion.** The ultrasensitive Singulex Clarity C. difficile 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 for a rapid and 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 Ultrasonic Immunoassay for Detection of *Clostridium difficile* Toxins in Stool Amelita Bartolome, PhD, Anna Almazan, BS; Salina Abusali, MS; Stanley Tam, MS; Eric Lee, BS; Amogh Changavi, BS; Wendy Trinh, BS; Kent Chau, BS; Joel Estis, MS; Brian Noland, PhD and Jeffrey Bishop, PhD; Singulex Inc, Alameda, California

**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. difficile 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.** Limit 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 toxotypes was determined. Cross-reactivity against 53 other gastrointestinal pathogens by 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. difficile toxins A/B assay detected toxins from all tested strains and toxotypes. 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. difficile 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.