Evaluation of the Copan Fecal Swab and Seegene Allplex Gastrointestinal multiplex panels for Accurate Molecular Detection of Stool Pathogens with Operational Efficiency for Implementation in a High Volume Laboratory Setting

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BACKGROUND

Traditional diagnostic methodologies for the detection of pathogens in stool have relied on microscopy for ova/parasites (O/P) or culture for pathogenic bacteria. Microscopy is highly subjective and reliant on technologist expertise. Culture based identification of bacterial pathogens requires plating for growth on various selective media followed by biochemical/phenotypic testing for confirmation. Dynacare Microbiology laboratories in Ontario, Canada receive approximately 200 specimens per day for independent diagnosis of O/P and bacterial pathogens. An accurate diagnostic method with efficient workflow was sought for high volume diagnostic testing for O/P and bacterial pathogens in stool specimens.

METHODS

The Seegene Allplex Gastrointestinal (GI) multiplex PCR panels for Parasites, and Bacteria (I, II) were evaluated against positive and negative specimens both prospectively with "fresh" stool specimens (n=88) and retrospectively with archived specimens (n=115). All specimens underwent diagnostic testing as per current standard operating protocols accredited by CAP and IQMH: (i) detection of O/P was performed on stool collected in sodium acetate formalin fixative via microscopic examination of wet mount and stained smears and (ii) detection of bacterial pathogens was performed on stool collected in Enteric Transport Media. For bacterial detection, stool was plated onto individual selective media for the isolation of *Yersinia enterocolitica*, *E. coli* O157 and *Campylobacter jejuni/coli*. Stool was also inoculated into Selenite broth for enrichment prior to plating for isolation of *Salmonella sp* and *Shigella sp*. Antibiotic susceptibility testing was performed for *Salmonella* and *Shigella* isolates. Serotyping was performed for presumptive identification of O157 strains amongst non-sorbitol fermenting *E. coli* isolates. In addition the limit of detection for bacterial targets was compared in triplicate using "spiked" specimens prepared in Enteric Transport Media and Copan Fecal Swabs. The complete value chain of activities was assessed starting with the pre-analytical phase (specimen collection, transportation, set up for DNA extraction and PCR plates), analytical phase (PCR, reflex test setup for culture and antibiotic susceptibility testing) and post-analytical phase (reporting, referral for confirmatory testing).

RESULTS

The Seegene Allplex GI tests provided superior sensitivity and specificity compared to conventional methods. The limit of detection for bacterial pathogens (*Salmonella sp*, *Shigella sp*, *Campylobacter jejuni/coli*, *Yersinia enterocolitica* and *E. coli* O157) was 10-100 fold improved in Copan Fecal Swab specimens compared to Enteric Transport media. Copan Fecal Swab specimens can be loaded directly onto the Nimbus or Starlet platforms for greater operational efficiency and audit trail during automated DNA extraction and PCR plate setup.

RESULTS

Table 1. Analytical Sensitivity, Limit of Detection for Bacterial Targets

a. Salmonella
b. Shigella
c. E.coli O157 eaeA
d. Yersinia enterocolitica
e. Campylobacter jejuni/coli

Limit of Detection Summary

- Stool samples were spiked with pre-determined colony forming units of the five enteric pathogens of interest.
- Spiked stool samples were "inoculated" into an Enteric Transport Media vial (Bio-Media) and in parallel to a Copan Fecal Swab, as per standard stool sampling instructions.
- PCR detection consistently had greater LOD for all bacterial targets
- Detection of Salmonella was improved in samples processed from Copan Fecal Swab

Parasite detection from Copan Fecal Swab specimens was also verified. 100% Accuracy for all targets with lower Ct values than detection from ETM.

Figure 1. Infection Prevalence in Patient Population; Annual Summary (50,000 per year based on 3 yr cumulative summary)

Figure 2. Test Performance with Clinical Specimens

A. Parasites Results Summary: Prospective "fresh" Stool, N= 115
B. Parasites Results Summary: Archived Stool, N= 195
C. Bacterial Isolates Results Summary

D. Bacterial Detection with "Fresh" Clinical Specimens, N= 88

For 5 Bacterial Pathogens:
100% Sensitivity
100% Specificity*

Additional Identifications:
4 Salmonella
12 Shigella
1 Yersinia

ATCC/Clinical Isolates: Tested in Triplicate
100% Sensitivity; 100% Specificity

Figure 3. Proposed Workflow for Molecular Detection

A. Pre-Analytical Phase: Load & Go DNA Extraction Instrument
Order Entry, Audit Trail from point of specimen receipt with Copan Fecal Swab
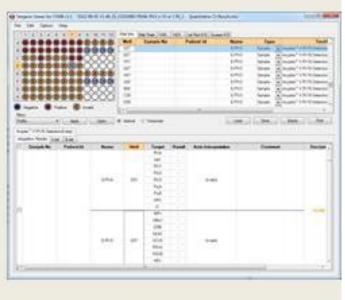Fecal Swab → DNA+Master Mix aliquotted into 96-well Plates for 3 PCR panels

Starlet: 80 Samples/90 min
Nimbus: 48 Samples/90 min

B. Analytical: Load & Go Multiplex PCR and Results Analysis
3 reaction panels; Parasite, Bacteria I, Bacteria II (Ecoli O157 & toxin genes)

CFX96 (Bio-Rad)
Seegene Viewer

96 samples / 2.5 hr / instrument
1 Instrument per Panel

MLT Reviews Positive Bacteria Samples Culture setup
- AST for Salmonella, Shigella
- E coli O157 and Yersinia isolates Reference Lab for surveillance

If Comprehensive O/P ordered → Wet prep for Helminths

C. Post-Analytical: Interface of Seegene software to LIS
Automated merging of PCR results and O/P microscopy and application of appropriate comment codes for reporting

Faster Turn Around Time 2 days vs. 5 days Early Detection = Enhanced Clinical Management Improved Patient Outcomes

CONCLUSIONS

The combination of test performance characteristics, operational parameters (throughput, ease of use) highlight Seegene Allplex GI panels and the Starlet/Nimbus instruments as an attractive testing suite for high-volume diagnostic enteric testing of bacterial and parasitic stool pathogens.

ACKNOWLEDGEMENTS