Improved Isolation of Salmonella enterica serovar Typhimurium Using the Walk-Away Specimen Processor and FecalSwab Transport System

Laboratory Medicine & Pathobiology
UNIVERSITY OF TORONTO

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ABSTRACT

This study was performed with the main objective of evaluating the isolation of *Salmonella enterica* serovar Typhimurium from stool using the automated Walk-Away Specimen Processor (WASP) and novel COPAN FecalSwab™ and selenite media. WASP® automated planting improved colony isolation compared to those planted using the Isoplater, while COPAN selenite broth increased the limit of *Salmonella* detection in spiked-stool specimens. These features would improve discrimination and isolation of enteric pathogens, increasing the likelihood of establishing the etiology of diarrheal illness due to bacterial pathogens.

BACKGROUND

The isolation of stool pathogens can prove difficult due to their fastidious nature and the rapid overgrowth of commensal flora. Furthermore, the processing of stool specimens in some laboratories can be tedious and time consuming due to high specimen volumes and the large number of selective media that are required for pathogen isolation. The Walk-Away Specimen Processor (WASP®; COPAN Diagnostics, Murietta, CA) is an automated system that provides pre-analytical specimen planting solutions for high-volume microbiology laboratories. In this study, we evaluated the ability of the WASP® system to isolate enteric pathogens directly from FecalSwab™ transport media, or following a pre-incubation step with COPAN selenite enrichment broth media. The ability of the WASP® system to streak for isolated colonies from stool was also optimized and compared to the widely used semi-automated Isoplater (Vista Technology, Edmonton, AB) streaking platform.

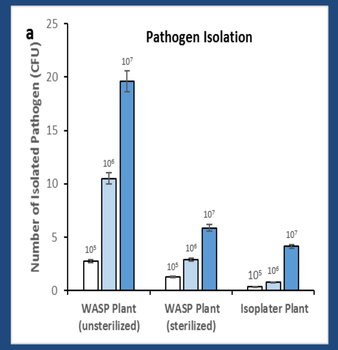
METHODS

The ability of the WASP® and Isoplater systems to plant for isolated colonies was compared. Stool specimens were spiked with 10^7, 10^6, or 10^5 CFU/mL *S. enterica* serovar Typhimurium and inoculated into FecalSwab™ transport media then processed using either planting system. Isolated colonies of pathogen and commensals were enumerated. Pathogen enrichment was assessed by spiking stool with 10^2-10^7 CFU/mL of *S. enterica* serovar Typhimurium and then directly planting to Hektoen agar, or pre-incubating at 37°C in COPAN selenite media followed by WASP® planting after 18 hours. The number of isolated pathogen colonies was enumerated and compared.

RESULTS

The number of isolated colonies of *S. enterica* serovar Typhimurium planted using the WASP® system was 5-10 times higher when compared to those planted using the Isoplater (Figure 1a; $P < 0.001$). Isolated colonies of *S. enterica* serovar Typhimurium were not observed for specimens inoculated with the lowest test concentration (10^5 CFU/mL) of pathogen and planted using the Isoplater, but were present in those planted using the WASP® system (Figure 1b). Incubation of spiked-stool specimens in COPAN selenite further increased the number of isolated *S. enterica* serovar Typhimurium colonies approximately four-fold (Figure 2; $P < 0.001$). This step increased the sensitivity of *S. enterica* serovar Typhimurium colony isolation to initial pathogen inoculum concentrations as low as 10^2 CFU/mL compared with 10^5 CFU/mL for directly plated specimens.

Figure 1. The number of isolated colonies of *S. enterica* serovar Typhimurium (a) and total number of isolated colonies (b) of spiked-stool specimens planted using the WASP (with and without loop sterilization following the first quadrant streak) and Isoplater automated systems. The initial pathogen inoculum size is indicated above each bar (10^5, 10^6, or 10^7 CFU/mL).



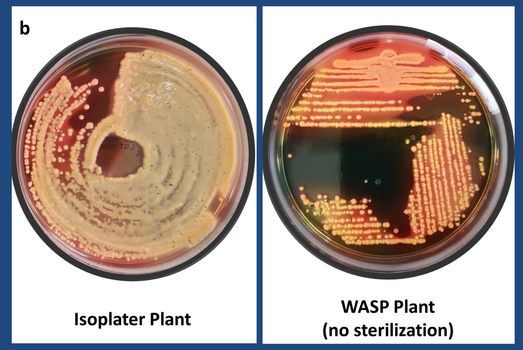
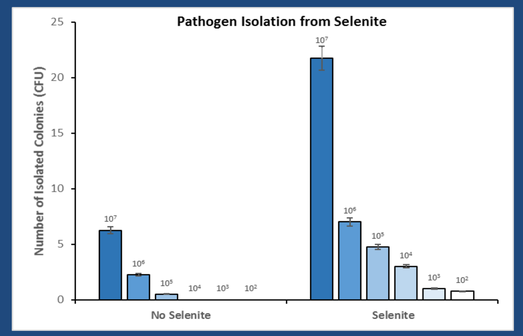


Figure 2. The number of isolated colonies of *S. enterica* serovar Typhimurium from spiked-stool specimens planted either directly from FecalSwab™ media or following an 18-hour incubation in selenite media. All specimens were planted using the WASP system. The initial pathogen inoculum size is indicated above each bar (10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 CFU/mL).



DISCUSSION

- Streaking for isolated colonies was optimized for the WASP®, and compared to the semi-automated Isoplater system.
- Overall, the WASP® outperformed the Isoplater in terms of number of isolated colonies generated (pathogen plus commensal), and also specifically for the number of isolated pathogen colonies.
- Early identification of enteric pathogens as the etiological agent of acute episodes of diarrhea is critical for rapid therapeutic intervention and the prevention of secondary transmission. We believe these results demonstrated that the WASP® is well-suited to simultaneously increase specimen processing speed and the quality of cultures generated, and that the potential exists for a positive clinical impact by decreasing time to culture resulting. However, further studies are required to demonstrate this.
- COPAN selenite enrichment broth significantly increased the detection of *Salmonella* compared to specimens directly plated without enrichment. Importantly, selenite increased the limit of *S. enterica* serovar Typhimurium detection from ~10^5 CFU/mL to ~10^2 CFU/mL with only a moderate incubation delay.
- There is a possibility that without selenite incubation a portion of patients with active salmonellosis, or asymptomatic carriers, would be missed, especially if specimens are delayed in transit and viability is impacted.

CONCLUSION

- WASP® automated planting improved colony isolation compared to those planted using the Isoplater.
- COPAN selenite broth increased the limit of *Salmonella* detection in spiked-stool specimens.
- These features would improve discrimination and isolation of enteric pathogens, increasing the likelihood of establishing the aetiology of diarrheal illness due to bacterial pathogens.

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