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Evaluating the preservation and isolation of stool pathogens using the COPAN FecalSwab™ transport system and walk away specimen processor

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Abstract

The isolation of stool pathogens is difficult due to their fastidious nature and the rapid overgrowth of fecal flora. In this study, we evaluate the preservation and isolation of enteric pathogens from stool using the automated COPAN Walk-Away Specimen Processor (WASP®) in conjunction with FecalSwab™ and selenite media. Pathogen viability and fecal commensal abundance was stable in FecalSwab™ media under both room-temperature and refrigerated incubation conditions, resulting in a significantly increased number of well-isolated pathogen colonies observed when compared to samples incubated in modified Cary-Blair media. Isolation of individual pathogen colonies was improved via WASP® planting compared to those planted using the Isoplater system. Furthermore, pre-incubation using the newly formulated COPAN selenite media significantly enhanced the yield of *Salmonella enterica* serovar Typhimurium. Together, the automated WASP® system, combined with FecalSwab™ and selenite media, represents a rapid and efficient approach for the processing of stool specimens compared to standard methods.

Keywords

Enteric pathogen; FecalSwab; Stool culture; WASP; Cary-Blair

Introduction

Isolation of diarrheagenic pathogens can be challenging due to the rapid overgrowth of fecal commensals and the gradual decline in viability of fastidious organisms. Additionally, the large burden of commensals present in stool can hinder the ability of laboratory technologists to identify isolated pathogen colonies on solid agar media, potentially introducing delays in identification and susceptibility testing while awaiting pathogen isolation from sub-culture. Furthermore, the large volumes of stool specimens that are processed daily can pose a logistical challenge for many institutions while taking technologists away from more critical interpretive diagnostic duties. The Walk-Away Specimen Processor (WASP®; COPAN Diagnostics, Murietta, CA) is an automated system that provides specimen planting solutions for high-volume microbiology laboratories (Bourbeau and Swartz, 2009, Dauwalder et al., 2016, Bourbeau and Ledeboer, 2013). Use of this technology has streamlined workflow for a growing array of specimen types, including urine, surveillance, screening, and more recently stool (Saegeman et al., 2011, Smismans et al., 2009, Origüen et al., 2016, Buchan et al., 2014). In this study, we compared the pathogen preservation characteristics of COPAN FecalSwab™ and modified Cary-Blair (Bio-Media, Toronto, ON) transport media. In addition, 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 was assessed. 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.

Materials and methods

Viability of Common Enteric Pathogens in FecalSwab™ and Modified Cary-Blair Transport Media.

Viability of seven pathogens (*Salmonella enterica* serovar Typhimurium ATCC 14028, *Shigella flexneri* ATCC 12022, *Yersinia enterocolitica* ATCC 9610, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Campylobacter jejuni* ATCC 33291, *Vibrio parahaemolyticus* ATCC 17802) were tested in both modified Cary-Blair (containing phenol red indicator and 0.16% agar; 15 mL volume) and COPAN FecalSwab™ transport devices (2 mL volume) under refrigerated (4 °C) and room temperature (25 °C) conditions following the Clinical Laboratory Standards Institutes M40-A2 recommendations for evaluating semi-solid and liquid transport media (CLSI, 2014). Bacterial suspensions with final concentrations of approximately 10^4, 10^3, and 10^2 CFU/mL were prepared using 0.85% sterile saline for each organism, and an aliquot was inoculated into each transport device. For both devices, the ratio between media filling volume and inoculum volume was kept consistent to ensure each device received the appropriate amount of pathogen and to facilitate data comparison (the ratio between ‘media filling volume’ and ‘inoculum volume’ was 15/0.75 mL and 2/0.1 mL for Cary-Blair and FecalSwab™ transport devices respectively). Both transport systems were briefly

vortexed

spread plating 100 µL of media from each device at 0, 24, 48, and 72-hour time points and counting the number of colonies present. The percent survival was determined by observing the change in organism abundance for each time-point compared to the initial read at 0 hours. A total of two experimental replicates (with each experiment conducted in duplicate) were performed for this evaluation.

Stability of Enteric Pathogens and Fecal Commensals in Spiked Stool Specimens Incubated in FecalSwab™ and Modified Cary-Blair Transport Media.

The ability of both stool transport systems to maintain pathogen viability while suppressing commensal fecal flora overgrowth was assessed. Unpreserved and unformed human stool specimens were obtained from patients who were tested but negative for *Clostridium difficile* infection. Stool samples were used within 8 hours of collection. These specimens were spiked with *C. jejuni, S. enterica* serovar Typhimurium, *S. flexneri*, and *Y. enterocolitica* reference strains at concentrations of 10^7, 10^6, and 10^5 CFU/mL, and transferred to either FecalSwab™ or modified Cary-Blair transport systems (adjusting for volume differences to preserve inoculum concentration between devices) for incubation for 0, 6, and 24-hours under refrigerated (4 °C) and room temperature conditions. At appropriate time intervals specimens were quadrant streaked for isolated colonies onto appropriate selective media [Hektoen (HEK), Cefsulodin-Irgasan-Novobiocin (CIN), or Karmali (cefoparazone, vancomycin, cycloheximide antibiotics); Oxoid, Nepean, ON], and incubated for 24 (HEK, and CIN) to 48 (Karmali) hours according to manufacturer's instructions. Individual colonies of both pathogen and commensals were identified [pathogen identified initially based on phenotype and then confirmed using VITEK® MALDI-ToF MS (bioMérieux, Saint-Laurent, QC)], enumerated, and compared as a ratio of [#isolated pathogen colonies]:[#isolated commensal colonies]. This comparative approach permitted simultaneous evaluation of both the abundance of isolated pathogen colonies, and overgrowth of commensals which was thought to potentially complicate isolation of pathogens for identification and susceptibility testing. In addition, commensal abundance and potential overgrowth was assessed by plating samples from each transport system and time-point to MacConkey (MAC) media (Oxoid) and enumerating the total number of organisms (pathogen and commensal) present. A total of three experimental replicates (with each experiment conducted in duplicate) were performed.

Comparing WASP® and Isoplater Automated Specimen Planting for Pathogen Isolation.

The ability of the WASP® system to plant stool specimens for the isolation of enteric pathogens was compared to that of the currently used Isoplater system. *S. enterica* serovar Typhimurium was utilized as an indicator pathogen for isolation due to the characteristic colony morphology and ease of identification on Hektoen agar plates. Stool specimens were spiked in duplicate with 10^7, 10^6, and 10^5 CFU/mL of *S. enterica* serovar Typhimurium and inoculated into FecalSwab™ transport media. FecalSwab™ transport media were not incubated any further,

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included an initial vortex step (three seconds) followed by planting using a 10 uL loop. A four-quadrant streaking method was selected (4Q Type 6) and included either a loop sterilization step following the inoculation of the first quadrant, or no loop sterilization. Plates planted using the Isoplater were inoculated with 10 uL of vortexed (three seconds) specimen to facilitate comparison to WASP®-planted samples. The number of isolated *S. enterica* serovar Typhimurium and commensal colonies were then enumerated. A total of four experimental replicates (with each experiment conducted in duplicate) were performed for this evaluation.

Evaluation of the Effect of Specimen Pre-Incubation with Selenite Enrichment Broth for the Isolation of S. enterica serovar Typhimurium.

Stool specimens were spiked with various concentrations (10^2 to 10^7 CFU/mL) of *S. enterica* serovar Typhimurium and then either directly planted to Hektoen agar using the WASP® system, or pre-incubated at 37 °C in either COPAN or 'standard' (Oxoid, Nepean, ON) selenite media and then WASP® (COPAN selenite) or Isoplater (Oxoid selenite) planted after 18 hours. The number of isolated pathogen colonies was counted and compared for both conditions. A total of four experimental replicates (with each experiment conducted in duplicate) were performed for this evaluation.

Statistical Methods.

Data were analyzed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA). Significance was determined using two-way ANOVA and Bonferroni's multiple comparison test. The average values of replicates within each experiment were used to perform calculations.

Results

Viability of Enteric Pathogens in FecalSwab™ and Modified Cary-Blair Transport Media.

Storage and transportation characteristics of FecalSwab™ and Cary-Blair devices were first assessed under both room-temperature and refrigerated conditions. After 24 hours at room temperature in the FecalSwab™ device, 86% (6/7 genera) of stool pathogen species remained within +/− 100% of the original inoculum compared to 29% (2/7) when incubated in modified Cary-Blair (Table 1a). There was significant growth (≥ 3 log CFU/mL) of *S. enterica Typhimurium*, *Y. enterocolitica*, and *E. coli*, after 48 hours when incubated in Cary-Blair media, while growth was limited to only ~1 log CFU/mL in the FecalSwab™ device. No viable *C. jejuni* were detected after 48 hours of incubation, and only a marginal amount was observed after 24 hours for both devices. All organisms were relatively stable under refrigerated conditions for both devices, with 100% of stool pathogens surviving for up to 72 hours (Table 1b).

Table

Transport systems at room temperature. Download Share Export

Pathogen	Swab Device	CFU/mL (% change from T0) recovered at: 0 hours	24 hours	48 hours
***Salmonella enterica* serovar Typhimurium ATCC 14028**	FecalSwab	6.5×10^6	9.2×10^6 (41)	2.0×10^7 (208)
	Cary-Blair	6.8×10^6	5.8×10^9 (85752)	5.0×10^9 (73974)
***Shigella flexneri* ATCC 12022**	FecalSwab	7.2×10^6	5.3×10^6 (−26)	2.8×10^6 (−62)
	Cary-Blair	6.8×10^6	1.5×10^7 (118)	2.1×10^7 (211)
***Yersinia enterocolitica* ATCC 9610**	FecalSwab	1.1×10^7	9.0×10^6 (−18)	8.4×10^6 (−23)
	Cary-Blair	8.9×10^6	3.8×10^8 (4188)	5.0×10^9 (56080)
***Escherichia coli* ATCC 25922**	FecalSwab	5.0×10^6	2.7×10^6 (−47)	7.5×10^6 (51)
	Cary-Blair	4.6×10^6	4.0×10^9 (86447)	5.0×10^9 (107814)
***Enterococcus faecalis* ATCC 29212**	FecalSwab	4.3×10^6	6.9×10^6 (61)	5.1×10^7 (1088)
	Cary-Blair	3.9×10^6	5.2×10^6 (33)	1.5×10^7 (289)
***Campylobacter jejuni* ATCC 33291**	FecalSwab	3.2×10^6	3.7×10^5 (−88)	0.0 (−100)
	Cary-Blair	4.8×10^6	2.2×10^5 (−96)	0.0 (−100)
***Vibrio parahaemolyticus* ATCC 17802**	FecalSwab	3.5×10^6	1.9×10^8 (5465)	4.4×10^9 (126503)
	Cary-Blair	2.9×10^6	2.8×10^7 (862)	2.8×10^9 (97807)

Table 1b. Percentage change in organism number after incubation in either FecalSwab or Cary-Blair Transport systems at 4 °C.

Pathogen	Swab Device	CFU/mL (% change from T0) recovered at: 0 hours	24 hours	48 hours	72 hours

Pathogen	Swab Device	CFU/mL (% change from T0) recovered at: 0 hours	24 hours	48 hours	72 hours
***Salmonella enterica* serovar Typhimurium ATCC 14028**	FecalSwab	5.9×10^6	5.7×10^6 (4)	5.3×10^6 (−10)	4.7×10^6 (−20)
	Cary-Blair	6.2×10^6	7.4×10^6 (20)	7.3×10^6 (18)	6.8×10^6 (9)
***Shigella flexneri* ATCC 12022**	FecalSwab	6.5×10^6	4.0×10^6 (−39)	1.5×10^6 (−78)	9.5×10^5 (−85)
	Cary-Blair	5.7×10^6	5.4×10^6 (−6)	5.5×10^6 (−4)	5.2×10^6 (−9)
***Yersinia enterocolitica* ATCC 9610**	FecalSwab	5.1×10^6	1.8×10^6 (−66)	2.2×10^6 (−57)	7.5×10^5 (−85)
	Cary-Blair	5.0×10^6	1.5×10^7 (210)	1.6×10^7 (222)	2.1×10^7 (323)
***Escherichia coli* ATCC 25922**	FecalSwab	2.8×10^6	1.2×10^6 (−58)	6.8×10^5 (−76)	5.0×10^5 (−82)
	Cary-Blair	2.8×10^6	1.2×10^6 (−58)	1.0×10^6 (−64)	8.0×10^5 (−71)
***Enterococcus faecalis* ATCC 29212**	FecalSwab	1.6×10^6	2.4×10^6 (50)	1.8×10^6 (15)	1.4×10^6 (−12)
	Cary-Blair	1.4×10^6	1.5×10^6 (5)	1.4×10^6 (−1)	1.7×10^6 (15)
***Campylobacter jejuni* ATCC 33291**	FecalSwab	4.5×10^6	8.2×10^5 (−82)	2.8×10^5 (−94)	6.7×10^4 (−99)
	Cary-Blair	4.1×10^6	1.8×10^6 (−57)	1.2×10^6 (−71)	7.5×10^5 (−82)
***Vibrio parahaemolyticus* ATCC 17802**	FecalSwab	1.5×10^6	4.6×10^6 (213)	4.0×10^6 (169)	4.6×10^6 (210)
	Cary-Blair	1.5×10^6	1.4×10^6 (−7)	8.3×10^5 (−43)	4.5×10^5 (−69)

FecalSwab™ Transport Media Preserves Enteric Pathogens While Suppressing Commensal Flora Overgrowth.

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media, especially at room-temperature, stool commensal abundance was assessed using patient stool samples. There was an increase in commensal burden of 289.7% and 506.9% for modified Cary-Blair ($P < 0.01$) versus 11.8% and 144.1% for FecalSwab™ ($P < 0.05$) after 6 and 24 hours of room temperature incubation respectively (Fig. 1). Under refrigerated conditions commensal burden increased by 18.2% (not significant; NS) and 42.4% ($P < 0.05$) for modified Cary-Blair and decreased by 4.3% and 13.0% for FecalSwab™ (NS) after 6 and 24 hours of incubation respectively (Fig. 1). Visible overgrowth of commensal flora in modified Cary-Blair-incubated specimens compared to those incubated in the FecalSwab™ transport system is readily apparent (Supplemental Fig. 1).

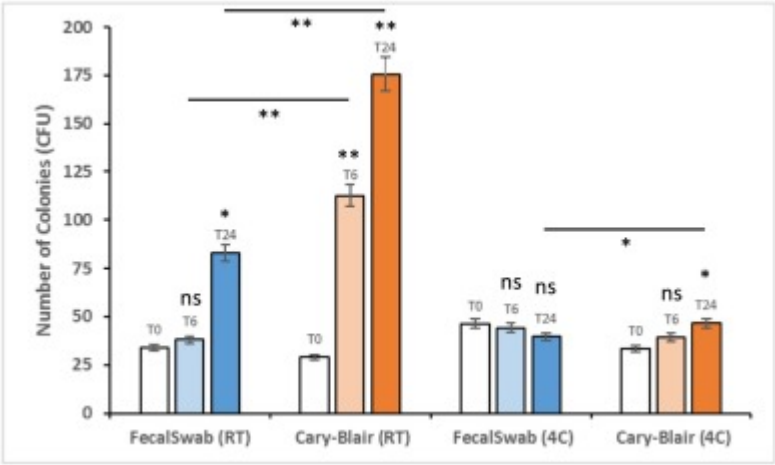

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Fig. 1. The total number of isolated colonies observed on MacConkey agar after incubation of spiked-stool specimens at room-temperature (RT) or 4 °C (4C) in either FecalSwab™ or Cary-Blair transport media for 0, 6, or 24 hours (T0, T6, T24 respectively). Blue bars represent FecalSwab while orange bars represent Cary-Blair. Significant differences were determined by comparing T_6 and T_{24} time points to T_0 within and between transport devices incubated at the indicated temperatures (*, $P < 0.05$; **, $P < 0.01$; ns, not significant).

The effect on pathogen isolation between FecalSwab™ and Cary-Blair transport systems needed to be compared given the significant differences in pathogen and commensal growth characteristics between devices. Enteric pathogens were present as a higher fraction of total isolated colonies (pathogen:commensal) in stools spiked with larger initial inoculum sizes of pathogen (Fig. 2a-f). Specifically, 30–70% of isolated colonies consisted of pathogen at inoculum sizes of 10^7 CFU/mL, while only 0–15% were represented by pathogen at inoculum sizes of 10^5 CFU/mL. For both transport systems, isolated pathogen colonies were readily apparent at inoculum sizes of 10^7 and 10^6 CFU/mL, but were less discernable at inoculum sizes of 10^5 CFU/mL, with most isolated colonies being represented by fecal commensals. The ratio of isolated pathogen:commensal colonies significantly decreased over 24 hours for both transport systems incubated at room-temperature (Fig. 2a,c,e). Notably, changes in the ratio of

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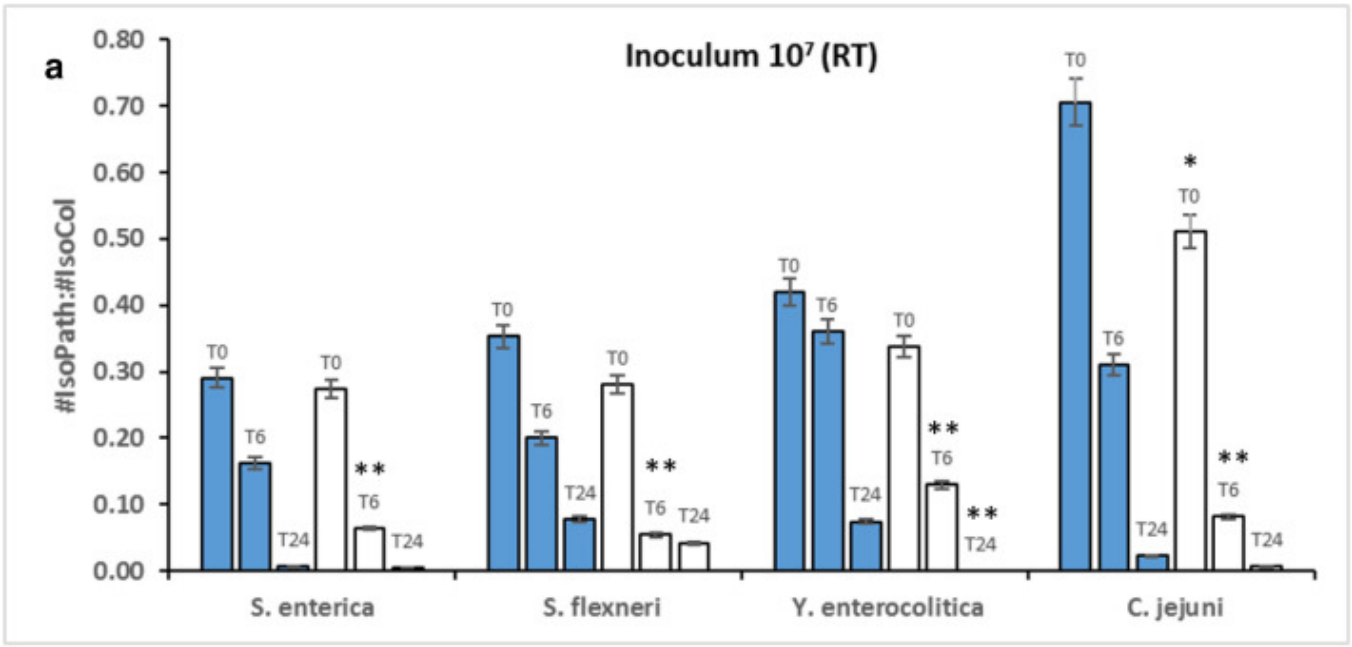
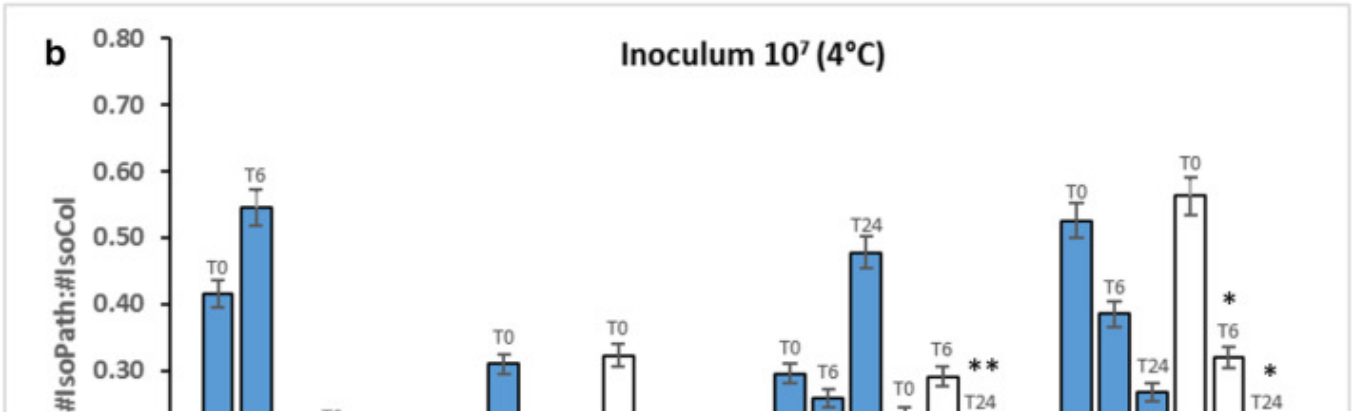
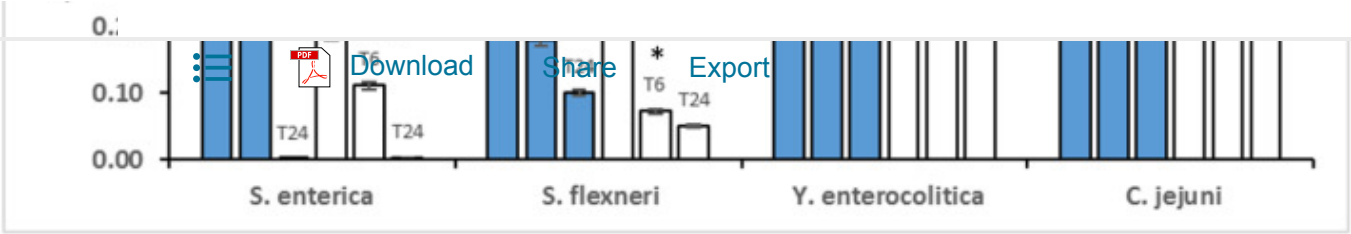
and an increase in the number of isolated commensal colonies. For most pathogens, the ratio of pathogen:commensals was significantly higher at 6 and 24 hour time points when incubated in the FecalSwab™ transport system compared to modified Cary-Blair ($P < 0.05$, $P < 0.01$, $P < 0.001$; Fig. 2). The number of isolated pathogen colonies was also significantly more stable when either transport system was incubated at 4 °C (Fig. 2b,d,f). However, *C. jejuni* and *Y. enterocolitica* were significantly more stable over time in spiked-stool specimens incubated at room-temperature in FecalSwab™ media compared to those incubated in modified Cary-Blair (Fig. 2a; $P < 0.01$). Both *S. enterica* serovar Typhimurium and *S. flexneri* were more stable in FecalSwab™ media compared to modified Cary-Blair when incubated at 4 °C (Fig. 2b,d,e; $P < 0.05$, $P < 0.01$). In two instances, incubation of stool specimens in modified Cary-Blair resulted in no isolated pathogen colonies while isolated pathogen colonies were observed under the same conditions for specimens incubated in FecalSwab™ media (Fig. 2; 10^7 *Y. enterocolitica* incubated at RT after 24 hours, and 10^5 *S. enterica* serovar Typhimurium incubated at RT after 6 hours). The opposite scenario was not observed in this study under any condition (e.g., observation of isolated pathogen colonies in specimens incubated in Cary-Blair with an absence of isolated pathogen colonies for specimens incubated in FecalSwab™).







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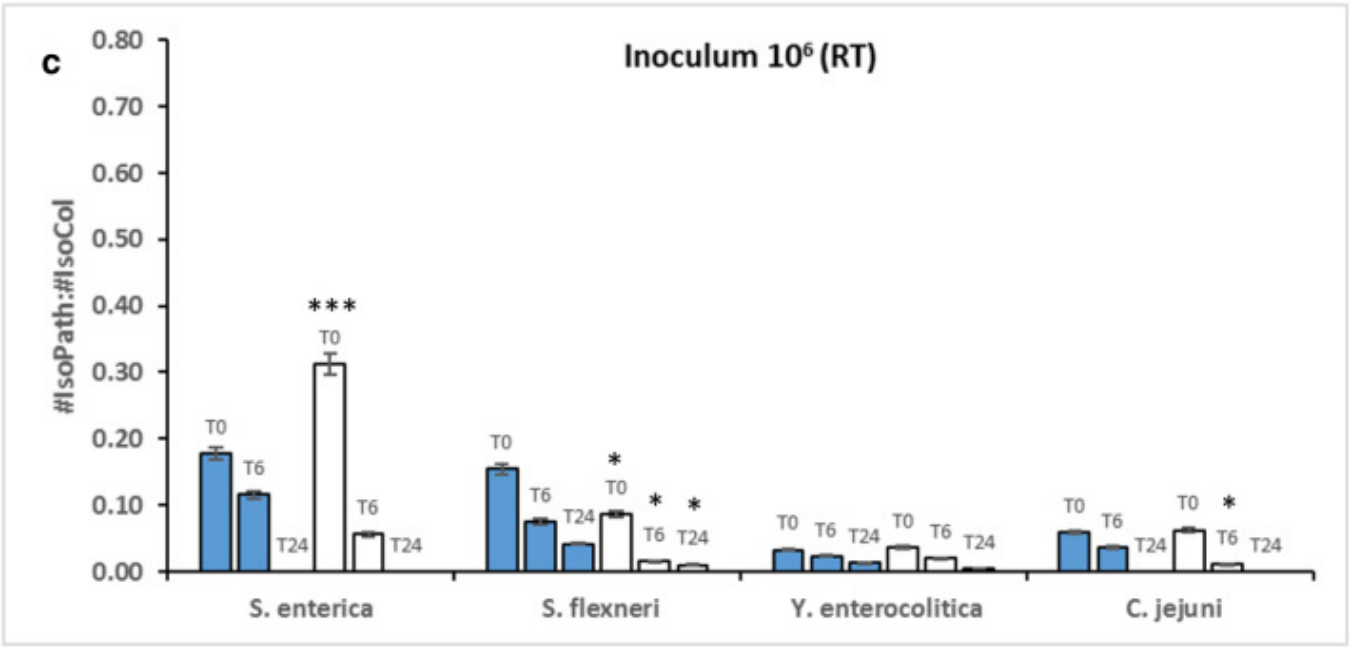

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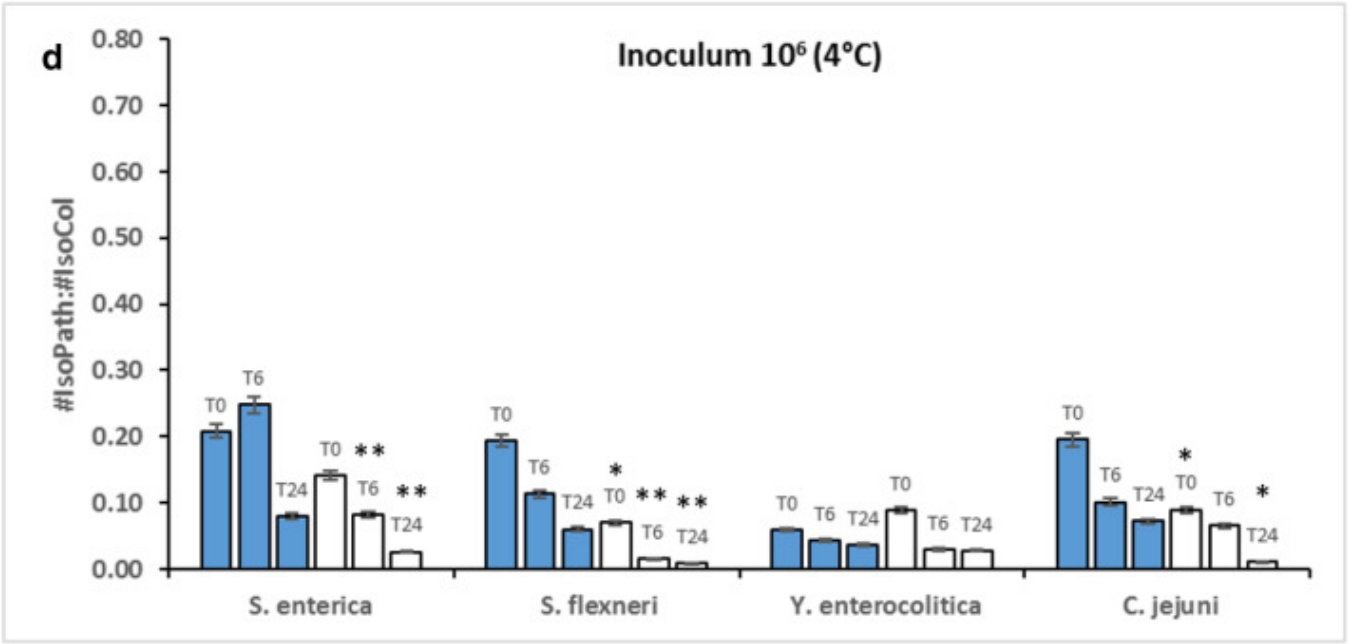

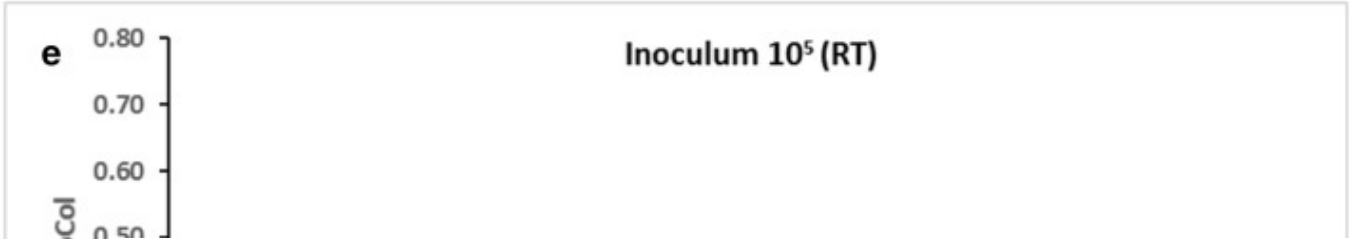

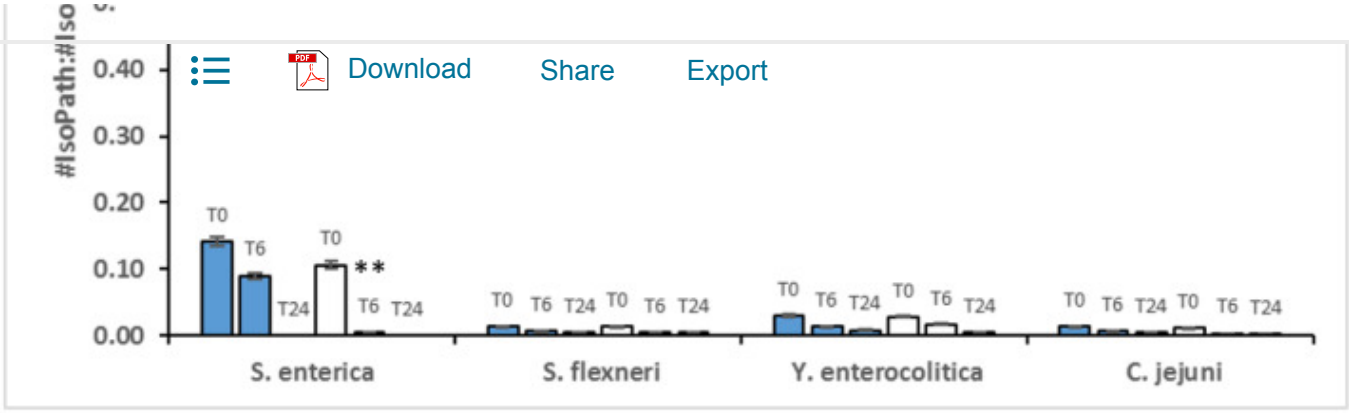

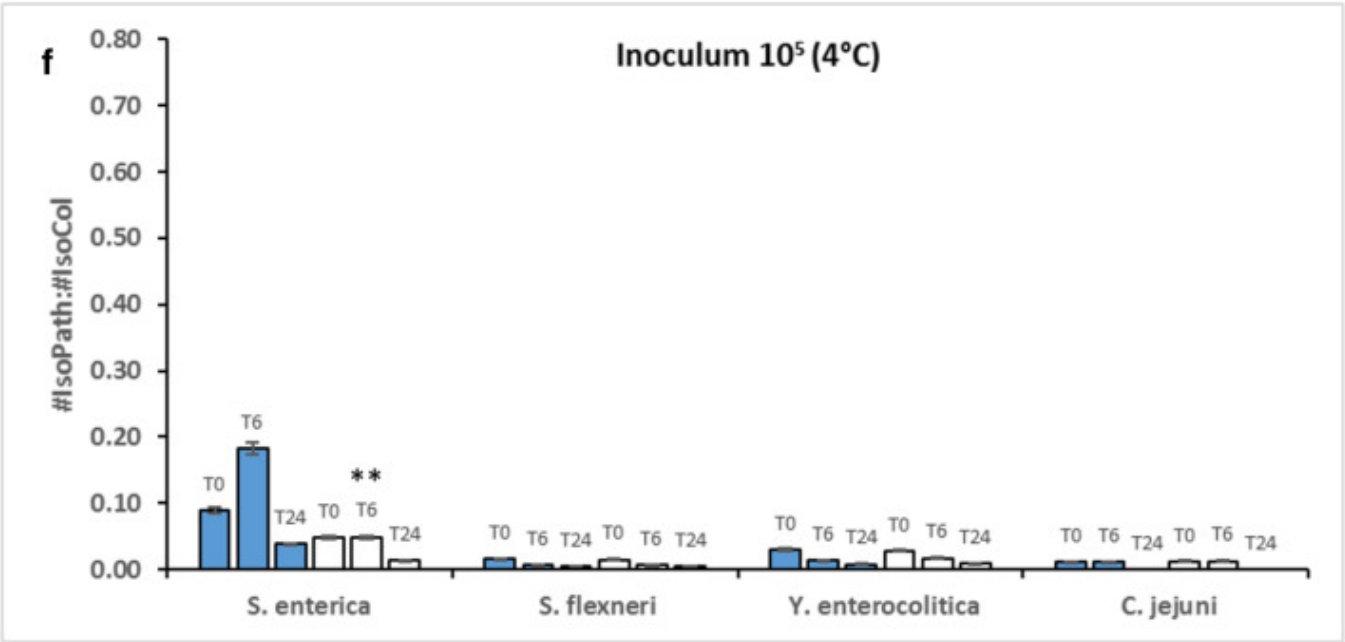

Fig. 2. Comparison of the number of isolated pathogens to the number of isolated commensals as a ratio (#IsoPath:#IsoCol respectively). Blue bars represent FecalSwab™ while white bars represent Cary-Blair. Significant differences for each temperature/inoculum combination was determined by comparing across transport devices at each time point for each fecal pathogen (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Optimization of Stool Specimen Planting Using the WASP® System and Effect on Colony Isolation Compared to the Isoplater Automated System.

The ability of the WASP® system to plant spiked-stool specimens for isolated colonies of *S. enterica* serovar Typhimurium was assessed. First, the optimal processing protocol was determined using a 10 uL volumetric loop with and without loop sterilization. Loop sterilization did not yield colonies beyond the first quadrant of the streaked specimen, and only a moderate number of isolated colonies were observed (Supplemental Fig. 2a). However, removal of loop sterilization following the streaking of the first quadrant inoculum increased the yield of well-isolated colonies (Supplemental Fig. 2b). Streaking of an equivalent 10 uL of specimen using

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(Supplemental Fig. 2). Specifically, the number of isolated colonies of *S. enterica* serovar Typhimurium planted using the WASP® system without loop sterilization was 2–4 times higher than those planted with loop sterilization, and 5–10 times higher when compared to those planted using the Isoplater (Fig. 3a; $P < 0.001$). In addition, the overall number of isolated colonies (combined pathogen and commensals) was higher for specimens planted using the WASP® (Fig. 3b; $P < 0.001$). 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 (Fig. 3a).

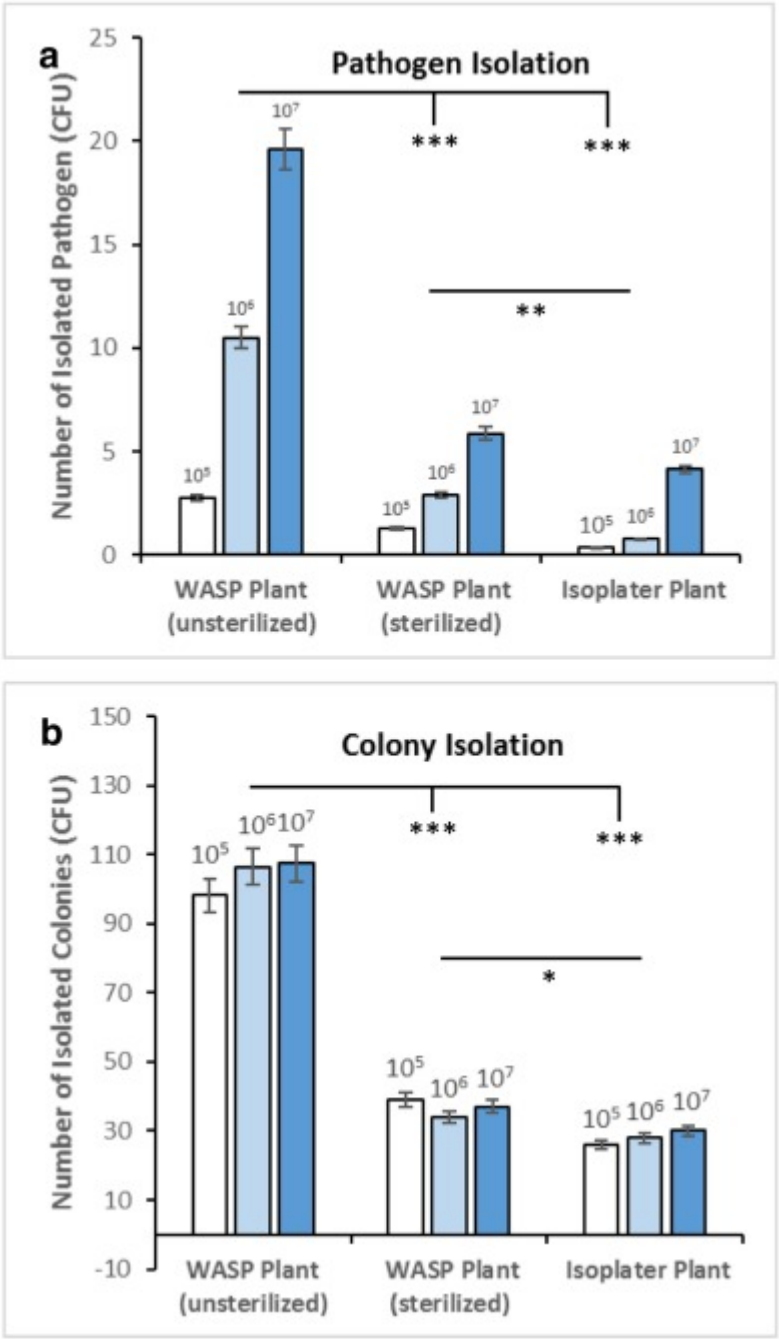

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Fig. 3.

colonies (b) of spiked-pork specimens planted using the EWAS P (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). Significant differences were determined by comparing across planting techniques for each inoculum size (***, $P < 0.01$; ns, not significant).

Incubation of Spiked-Stool Specimens in COPAN Selenite Increases the Yield of S. enterica serovar Typhimurium.

Pre-incubation of spiked-stool specimens for 18 hours in both 'standard' and COPAN selenite media significantly increased the number of isolated *S. enterica* serovar Typhimurium colonies (Fig. 4; $P < 0.001$). Additionally, selenite pre-incubation increased the sensitivity of *S. enterica* serovar Typhimurium colony isolation to initial pathogen inoculum concentrations as low as 10^2 CFU/mL. Conversely, the lowest concentration yielding isolated colonies in the directly planted specimens (no selenite pre-incubation) was 10^5 CFU/mL. However, there was no significant difference in pathogen isolation between cultures incubated in COPAN or 'standard' selenite media (not significant; NS).

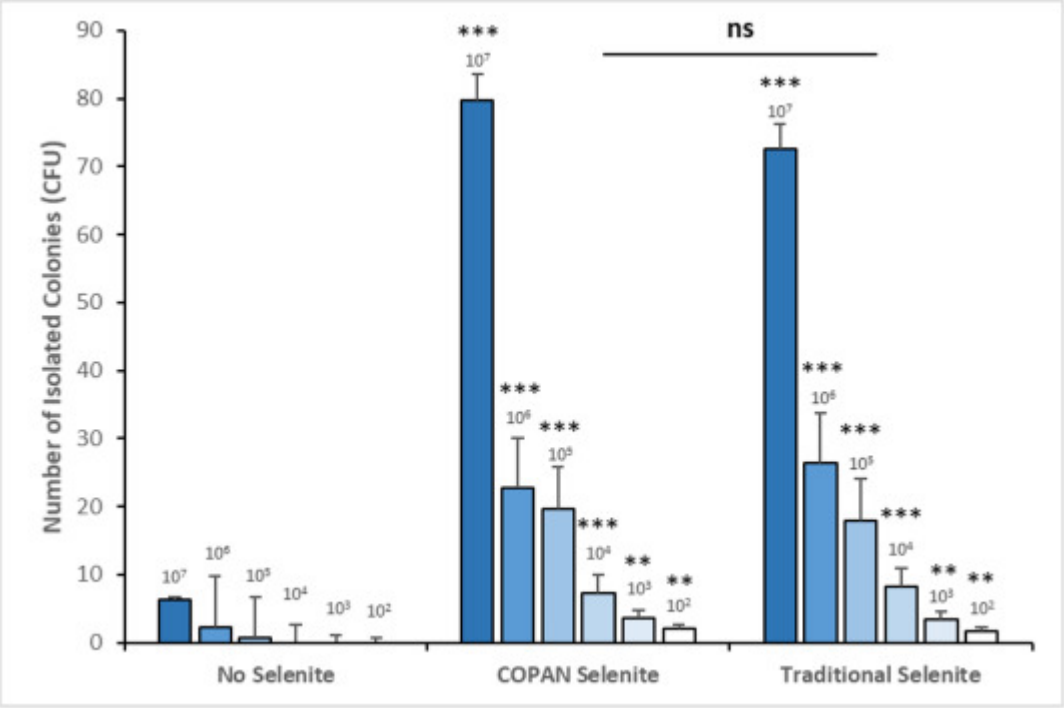

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Fig. 4. The number of isolated colonies of *S. enterica* serovar Typhimurium from spiked-stool specimens planted either directly from FecalSwab™ media or following an 18-hour incubation in COPAN or 'standard' selenite media. The initial pathogen inoculum size is indicated above each bar (10^2, 10^3, 10^4, 10^5, 10^6, or 10^7 CFU/mL). Significant differences were determined by comparing across inoculum size for samples

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***, $P < 0.00$

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Discussion

In the current study we assess enteric pathogen isolation from stool specimens using the WASP® planting system and accompanying FecalSwab™ and selenite enrichment media to modified Cary-Blair and a conventional automated planting method using the Isoplater. A comparative evaluation of FecalSwab™ and modified Cary-Blair transport devices revealed that pathogens were stable in either media under refrigeration conditions, with all strains maintaining detectable levels for up to 72 hours. These findings reflect those from a previous study which compared pathogen viability in FecalSwab™ and ESwab transport devices (Hirvonen & Kaukoranta, 2014). Importantly, most pathogens remained viable in FecalSwab™ and Cary-Blair devices even when incubated at room-temperature. Stability under these conditions would be beneficial in scenarios where refrigeration or cold storage is not immediately available, or for when the specimen is delayed prior to transport. Furthermore, significant pathogen overgrowth was observed under room-temperature incubation conditions in modified Cary-Blair media. Although pathogen growth would be a desirable characteristic from the stand-point of increasing analytical sensitivity, it was thought that the same overgrowth might be observed in fecal commensals which would impede primary pathogen isolation. Thus, the ability of both transport devices to preserve pathogens while suppressing commensal overgrowth was assessed. Fecal commensals were quite robust in modified Cary-Blair media, growing significantly during 6- and 24-hour room-temperature incubation periods, thereby decreasing the relative number of isolated pathogen colonies. The relative abundance of isolated pathogen colonies compared to commensals was more stable in spiked-stool specimens incubated in FecalSwab™ transport devices, with well isolated colonies observed under both room-temperature and refrigerated conditions for most pathogens. In two cases, isolated colonies of *S. enterica* and *Y. enterocolitica* were not observed for spiked-stool specimens incubated in Cary-Blair at RT, but were observed when the samples were incubated under the same conditions in FecalSwab™ media. Lack of well-isolated colonies limits the ability of laboratory personnel to extract pathogens for further characterization, necessitating the picking of colonies from the main inoculum for further sub-culture with potential delays in identification and antimicrobial susceptibility testing. Importantly, statistically significant increases in pathogen:commensal ratio at T0 were observed between transport devices for 5 of 24 experiments (4 of which occurred for FecalSwab and 1 of which occurred for Cary-Blair). However, the majority of experiments did not demonstrate initial differences in pathogen abundance and thus the overall conclusion that FecalSwab increases the number of isolated pathogen colonies compared to those samples incubated in Cary-Blair remains valid for most experimental scenarios.

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 (Guerrant et al., 2001). 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.

One limitation of this study is that these automated methods were not compared to manual plating; however, other studies have demonstrated that the WASP is at least non-inferior to manual methods for urine, while providing more reproducible results (1, 11). An additional limitation is the use of spiked-stool specimens rather than clinically-collected samples, which would incorporate all sources of variability during transport and storage. Importantly, spiked-stool specimens represent an acceptable proxy to clinically-collected samples, and have been used as the basis of several similar study designs in the past (12, 13).

COPAN selenite performed as well as ‘standard’ selenite, with either enrichment broth significantly increasing 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. Although there is lack of human data describing *Salmonella* fecal burden during infection and carriage, recent porcine data suggests that *Salmonella* fecal shedding in asymptomatic pigs ranges from 10^3 to 10^6 CFU/g of feces (Pires et al., 2013). If this translates to humans, it is possible 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. Additionally, the PCR methods used in the porcine model study had a limit of detection of ~10^3 CFU/g stool, suggesting that selenite broth culture sensitivity may exceed that of molecular assays which have gained traction in recent years.

In conclusion, FecalSwab™ transport media demonstrated comparable pathogen viability storage characteristics to that of modified Cary-Blair, but was superior in limiting the overgrowth of commensal flora, especially at room-temperature conditions. Furthermore, the chance of isolating single colonies of pathogen was greater in specimens stored in FecalSwab™ transport media compared to modified Cary-Blair, while the WASP® system was found to be superior to the semi-automated Isoplater method for providing a relatively higher number of isolated pathogen colonies compared to commensal colonies, which might improve the likelihood of isolating pathogen for immediate identification without further sub-culture. Lastly, COPAN selenite significantly increased the limit of *Salmonella* detection in spiked-stool specimens, with

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FecalSwab™, selenite and WASP® system features would allow easier discrimination and isolation of enteric pathogens increasing the likelihood of establishing the etiology of diarrheal illness due to bacterial pathogens, enhancing the ability to manage these patients accordingly.

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Informed Consent: Not applicable.

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