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Clinical Evaluation of the Walk-Away Specimen Processor and ESwab for Recovery of *Streptococcus agalactiae* Isolates in Prenatal Screening Specimens

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Rectal/vaginal specimens ($n = 97$) were collected in parallel using ESwab and Liquid Stuart (LS) rayon fiber wrapped swab collection devices. Each collection device was used to directly inoculate culture medium and LIM broth. Medium inoculation by ESwab was conducted using the Walk-Away specimen processor (WASP). Medium inoculation by the LS device was conducted manually. The sensitivities of ESwab and LS upon direct plating were 93.8% and 87.5%, respectively, and increased to 96.9% and 90.6%, respectively, following broth enrichment.

Transient colonization of the female urogenital tract by *Streptococcus agalactiae*, or group B *Streptococcus* (GBS), is a recognized risk factor for the development of neonatal infections acquired during the birthing process. These infections are characterized by onset of sepsis, pneumonia, and meningitis within the first 7 days of life and carry a mortality rate of up to 7% (1, 2). While GBS has been associated with bacteremia, soft tissue infections, pneumonia, and meningitis in adults (3), carriage is typically asymptomatic, with a point prevalence of 10 to 30% in pregnant women (2, 4–6). The identification of GBS during routine prenatal screening aids in decreasing the incidence of invasive GBS disease in newborns through the administration of intrapartum antibiotic prophylaxis in women with a positive GBS test result (7). Further, the screening of pregnant women at 35 to 36 weeks gestation has been recommended by Centers for Disease Control and Prevention (2). Therefore, sensitive methods for detecting GBS in routine prenatal screening specimens are a key component in preventing neonatal disease (2).

The use of enrichment broth, selective and chromogenic medium, and molecular diagnostics (6, 8–13) has increased the sensitivity of GBS detection; however, the initial collection and processing of specimens have largely remained unchanged. Advancements in technology and automation in the clinical microbiology lab have made available more efficient methods for specimen collection and processing, which have the potential to improve the sensitivity of screening and recovery of GBS and other pathogens (14–18). The introduction of ESwab (Copan Diagnostics, Murrieta, CA), when used in combination with the Walk-Away specimen processor (WASP) (Copan Diagnostics), has the potential to improve the efficiency and reproducibility of specimen collection, processing, and pathogen recovery through increased sensitivity, specificity, and reduced turnaround time (14–19). Traditional fiber wrapped swabs coupled with nonnutritive transport medium, such as Liquid Stuart or Amies Gel, aid in the preservation of microorganisms during transit to the clinical laboratory; however, the inefficient transfer or release of microorganisms from a traditional fiber swab may reduce the overall sensitivity of the diagnostic procedure. In contrast, flocked swabs are constructed with a solid bulbous head that is covered in fibers protruding perpendicularly to the swab shaft. The combination of a liquid-based transport medium along with a flocked swab, such as the ESwab collection device, may more evenly distribute and release microorganisms and has been demonstrated to increase the recovery of bacteria from wound and other sources compared to use with standard fiber wrapped swabs (14–18). This in turn increases the number of microorganisms inoculated to culture plates and/or other media in a more standardized and reproducible manner, particularly when used with an automated specimen processor, such as the WASP.

In this study, we compare ESwab and the WASP to our standard method of specimen collection and inoculation using the Liquid Stuart CultureSwab (LS) (BBL CultureSwabs; BD, Franklin Lakes, NJ) and manual plating to detect *S. agalactiae* in clinical specimens. Paired rectal/vaginal specimens ($n = 97$) were prospectively collected using the ESwab and LS devices in women undergoing routine prenatal screening for GBS. The current standard-of-care (LS) swab was always collected first, followed by the ESwab. All specimens collected using either the LS or the ESwab were held at room temperature and inoculated within 12 h of collection. Samples collected with LS were inoculated according to the routine laboratory workflow, and qualifying specimens collected with ESwab were inoculated in batch fashion once daily (<12 h from collection). Specimens collected using ESwab were automatically inoculated to Granada agar (Hardy Diagnostics, Santa Maria, CA) and LIM broth by the WASP using the 30-μl inoculating loop. Parallel specimens collected using LS were manually inoculated to Granada agar by rolling the swab across the first quadrant of the agar. The inoculated plates were then streaked for isolation using a sterile loop. The entire fiber wrapped swab was then inserted into an LIM enrichment broth tube and vortexed prior to incubation. The inoculated LIM broths were held at 35°C for 18 to 24 h and were then inoculated to Granada agar

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TABLE 1 Comparison of sensitivities and specificities of manual/LS swab to WASP/ESwab for the recovery of *S. agalactiae*

Method and swab type	No. with indicated test result				Total no.	Sensitivity (%)	Specificity (%)
	True positive	False positive	True negative	False negative			
Direct plating							
Manual/LS swab	28	0	65	4[a]	97	87.5	100.0
WASP/ESwab	30	0	65	2[b]	97	93.8	100.0
Enrichment culture							
Manual/LS swab	29	0	65	3[c]	97	90.6	100.0
WASP/ESwab	31	0	65	1[d]	97	96.9	100.0

[a] One was positive by manual/LS Swab following LIM enrichment culture and was positive by WASP/ESwab on direct and enriched culture. Two remained negative by manual/LS Swab following LIM enrichment culture and were positive by WASP/ESwab on direct and enriched culture. One remained negative by manual/LS Swab following LIM enrichment culture and was positive by WASP/ESwab on direct culture only.
[b] Two were positive by both manual/LS Swab and WASP/ESwab following LIM enrichment culture.
[c] Three were positive by WASP/ESwab on direct culture.
[d] One was negative by manual/LS Swab following direct and LIM enrichment culture and positive by WASP/ESwab on direct culture only.

either manually using a 10-μl loop or by the WASP using a 30-μl loop. The inoculated Granada agars were incubated at 35°C in an anaerobic environment and were examined for characteristic orange colonies at 24 and 48 h of incubation. Presumptive colonies were identified using the Streptex latex agglutination test (Remel, Lenexa, KS). A specimen was considered to be true positive if GBS was isolated on Granada medium (direct or enriched culture from LS- or ESwab-collected specimens).

Of the 97 specimens tested, 32 (33%) were found to be positive for *S. agalactiae* (Table 1). The use of LS demonstrated 87.5% (28/32) sensitivity following direct manual plating of specimens to Granada medium. Specimens collected using the ESwab and directly plated by the WASP resulted in a sensitivity of 93.8% (30/32). Enrichment culture using LIM broth was more sensitive than direct plating for both processes, increasing the sensitivity of specimens collected using LS to 90.6% (29/32) and those collected using ESwab to 96.9% (31/32). Combined direct and enriched culture methods resulted in 100% (32/32) sensitivity for ESwab and 90.6% (29/32) for LS. Of the specimens that were positive upon direct culture, 85.7% (24/28) were positive following 24 h of incubation using LS compared to 96.6% (29/30) using ESwab. The increased rate of positivity at 24 h observed with specimens collected using ESwab was correlated with a higher concentration of GBS on the Granada medium and may result from a superior release of organisms from the collection device. These data demonstrate increased sensitivity for detecting GBS when using ESwab in combination with the WASP compared to using our standard method.

The single false-negative ESwab result following LIM broth enrichment was positive upon direct ESwab plating. While broth enrichment would be expected to increase culture sensitivity, Dunne and Holland-Staley (8) demonstrated equivalent sensitivity (~85%) for detecting GBS following either direct or enriched culture. The failure of broth enrichment to increase culture sensitivity was correlated with an abundance of *Enterococcus* spp. in the primary specimens, which may have outcompeted GBS during broth enrichment (8). Therefore, the maximum sensitivity for the recovery of GBS was achieved by combining direct- and enriched-culture methods. These results are supported in the current study, in which broth enrichment was only 3% more sensitive than direct plating for both ESwab and LS. For both methods, combined direct and enriched culture was required for maximal recovery of GBS (LS, 90.6% sensitive; ESwab, 100% sensitive).

The degree to which ESwab collection and the WASP plating methods individually contribute to the increase in sensitivity was not investigated; however, these data support the hypothesis that the use of an automated processing system in combination with the ESwab can increase the sensitivity of GBS screening in a clinical laboratory environment. This observation is in agreement with a similar study that demonstrated a 13.1% increase in the recovery of *Staphylococcus aureus* by direct culture and a 10.2% increase in recovery by enrichment culture using ESwab and the WASP compared to specimens collected using traditional fiber wrapped swabs (14). Other clinical evaluations have demonstrated a 6-fold higher total CFU count and recovery of additional microorganisms from wound cultures when using ESwab compared to using traditional swabs (16). In conclusion, while additional studies are needed to assess the use of ESwab and the WASP for screening and recovery of microorganisms from other sources, this study has demonstrated that the flocked swab, when used in conjunction with an automated processing system, may be an effective tool for increasing the sensitivity of culture-based GBS screening in the clinical microbiology laboratory.

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