



Abstract

Background: Reliable detection of maternal Group B *Streptococcus* (GBS) colonization is essential in preventing neonatal disease. Screening for GBS using a vaginal/rectal swab cultured in Lim broth is commonly used for this purpose. Subculture of Lim broth is typically performed manually but Puritan Medical Products (P) and Copan Diagnostics (C) now offer Lim broth in tubes designed for use with automated plating systems.

Methods: *S. agalactiae* ATCC 12386 and clinical strains of GBS were tested alone or in combination with mixtures of bacteria representing fecal and vaginal flora. P and C standard flocced swabs were inoculated with 100 μ L of approx. 10^7 cfu/mL and placed into Lim broth. After 24h incubation, tubes were vortexed and loaded onto a BD Kiestra InoqulA programmed to dispense 10 μ L of sample onto sheep blood agar with magnetic bead zigzag streaking. After 24h incubation, plates were examined for quality of GBS colony isolation. Quantitative counts were determined for all colony types growing on plates. A one \log_{10} or greater difference in colony count was considered significant when comparing P and C.

Results: When tested alone, P and C yielded equal quantities of well isolated GBS colonies. P and C recovery of GBS was comparable in mixed cultures but GBS colony counts and isolation varied depending on the amount of growth of the other bacterial strains in the mixture. When present in mixed cultures, Enterobacteriaceae growth was comparable for P and C with GBS colony counts and isolation equal to cultures with GBS alone. Growth of *Enterococcus* species in mixed cultures was also comparable for P and C, however, when this organism was present the quantity and isolation of GBS was reduced compared to cultures with GBS alone. Colony counts of *P. aeruginosa* and some *Staphylococcus* and *Corynebacterium* species were significantly higher in C than P in some cases making visualization of isolated GBS colonies difficult. Growth of yeast was significantly higher in P than in C. There were no mechanical problems associated with automated plating using P and C.

Conclusions: P and C Lim broth allowed for efficient and consistent plating on an automated platform. P colony counts of GBS were equivalent to C when tested alone or when tested in combination with mixtures of bacteria representing fecal or vaginal flora. With the exception of *P. aeruginosa* for C, the growth of Gram negative rods was equally suppressed by P and C. For both P and C, overgrowth of Gram positive bacteria in some cases reduced the number of well isolated GBS colonies but some strains of *Staphylococcus* and *Corynebacterium* were better suppressed by P than C.

Introduction

Group B *Streptococcus* (GBS) is a common cause of sepsis, meningitis and pneumonia in newborns. The disease is transmitted during birth from mothers who carry GBS in their rectum or genital tract. To reduce the risk of infection, the Centers for Disease Control and Prevention have published guidelines for prevention of neonatal GBS disease. Reliable detection of maternal GBS colonization is an essential component of this prevention program. Screening for GBS using a vaginal/rectal swab cultured in a selective enrichment broth such as Lim broth is commonly used for this purpose. Subculture of Lim broth is typically performed manually but Puritan Medical Products - Puritan® Lim Broth with Colistin and Nalidixic acid (P) and Copan Diagnostics - LBM™ LIM Broth (C) is now available in tubes designed for use with automated plating systems. The aim of this study was to compare the performance of these two Lim broth products.

Methods

Bacterial strains: *S. agalactiae* ATCC 12386 and 25 clinical strains of GBS were tested alone or in combination with mixtures of bacteria representing fecal and vaginal flora. GBS isolates were suspended in sterile demineralized water to a turbidity equivalent to a 0.5 McFarland standard. Sixty-three bacterial mixtures were created by sweeping a swab through growth on female genital tract culture plates or stool culture plates with suspension of the collected bacteria in sterile demineralized water to a turbidity equivalent to a 0.5 McFarland standard. For GBS alone, a tenfold dilution was performed in sterile demineralized water. For combination studies, equal volumes of the GBS and bacterial mixture suspensions were combined and then diluted tenfold.

Culture method: For each test, 100 μ L of a dilution was placed into each of two tubes. A P standard flocced swab was placed in one tube and a C standard flocced swab was placed in the second tube. The swabs remained in each tube for 20 seconds to allow absorption of the diluted sample. Next the swabs were placed in their respective 2.0 ml Lim broth tubes and incubated at 35-37°C in 5% CO₂ for 24 hours. After incubation, the tubes were vortexed and loaded onto a BD Kiestra™ InoqulA™ programmed in the fully automated mode to dispense 10 μ L of sample onto trypticase soy agar with 5% sheep blood with a zigzag streaking pattern. The subculture plates were incubated for 24 hours and then examined for quantity of growth and quality of GBS colony isolation. Also, quantitative counts to the nearest log₁₀ were determined for all colony types growing on the subculture plates. A one \log_{10} or greater difference in colony count was considered significant when comparing P and C culture results. Isolate identifications were performed using MALDI-TOF.

Figure 1 Group B *Streptococcus* alone



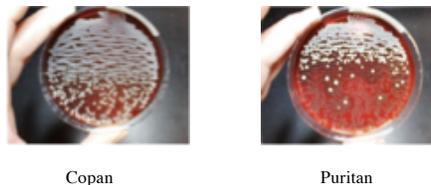
Figure 2 Group B *Streptococcus* with overgrowth of *Enterococcus*



Figure 3 Group B *Streptococcus* with *Pseudomonas aeruginosa*



Figure 4 Group B *Streptococcus* with *Staphylococcus aureus*



Results

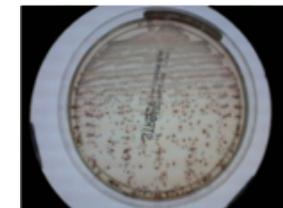
When *S. agalactiae* ATCC 12386 and 25 clinical GBS isolates were tested individually, P and C yielded comparable quantities of well isolated GBS colonies (Figure 1). GBS was recovered from mixtures in C and P but GBS colony counts and degree of isolation varied depending on the amount of overgrowth of the bacterial strains in the mixtures. Enterococci were commonly found in high numbers in tested mixtures. The amount of growth of *Enterococcus* species in mixed cultures was comparable for P and C, however, when this organism was present the quantity and isolation of GBS was significantly reduced compared to cultures with GBS alone or in combination with other fecal or genital tract flora (Figure 2). When present in mixed cultures, the amount of growth of Gram negative rods of the Enterobacteriaceae family was comparable for P and C with GBS colony counts and isolation equal to cultures with GBS alone. When present, colony counts of *P. aeruginosa* were consistently higher in C than P in some cases making visualization of isolated GBS colonies difficult (Figure 3). The same was true for some strains of *Staphylococcus* and *Corynebacterium* species which were in lower numbers in P than in C (Figure 4). For some yeast species colony counts were higher in P than in C. There were no mechanical problems associated with fully automated plating using P and C Lim broth tubes.

Conclusions

P and C Lim broth allowed for efficient and consistent plating on an automated platform. Both culture media performed well as an enrichment step for GBS screening. However, the selectivity of the two media varied and in some cases overgrowth of fecal and genital tract flora reduced the quantity of GBS and the visualization of isolated GBS colonies. With the exception of higher quantities of *P. aeruginosa* for C, the growth of Gram negative rods was equally suppressed by P and C. For both P and C, overgrowth of Gram positive bacteria in some cases reduced the number of well isolated GBS colonies necessitating additional colony isolation steps in order to identify GBS. This was especially true when *Enterococcus* sp were present in the sample. Some strains of *Staphylococcus* and *Corynebacterium* were also problematic and we found that they were often better suppressed by P than C.

The current practice in our hospital is to supply our wards and clinics with a P standard flocced swab and P Lim broth for vaginal/rectal screening for GBS. When received in the lab, the Lim broth tubes containing the flocced swab are incubated 18-24 hrs and then subcultured onto blood agar using the fully automated mode of our sample processing system. As we saw in our comparative study, overgrowth of *Enterococcus* sp can be problematic. This is especially true if the GBS are non-hemolytic. However, we are currently validating the use of GBS chromogenic agar instead of blood agar for the subculturing step. Preliminarily it appears that P Lim broth and this agar will allow for easy distinction between the GBS and enterococci and will significantly reduce extra isolation steps (Figure 5)

Figure 5 GBS chromogenic agar



Red colonies = GBS

Blue colonies = *Enterococcus*

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