Pathology (October 2015) 47(6), pp. 580–582

MICROBIOLOGY

Evaluation of four chromogenic media for the isolation of Group B *Streptococcus* from vaginal specimens in pregnant women

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Summary

Direct culture onto four commercial chromogenic media, selective for the isolation of Group B *Streptococcus* (GBS), were compared with the conventional pre-enrichment Centers for Disease Control and Prevention (CDC) method for the ability to isolate GBS from 242 pregnant women's self-collected vaginal/perineal swabs. The sensitivities and specificities for direct culture on to chromogenic agar were 92% and 100% for StrepB*Select* (Bio-Rad Laboratories), 96% and 100% for Brilliance GBS (Thermo-Fisher Scientific), 94% and 100% for CHROMagar StrepB (CHROMagar, Dutec Diagnostics), 86% and 100% for ChromID Strepto B (bio-Merieux). CDC recommended broth pre-enrichment then culture on blood containing selective agar had a sensitivity and specificity of 90.0% and 100% respectively. The chromogenic agar tested produced comparable results to the pre-enrichment CDC method.

Key words: Chromogenic agar, GBS, Group B *Streptococcus*, *Streptococcus agalactiae*.

Received 9 February, revised 9 April, accepted 3 May 2015

INTRODUCTION

Group B *Streptococcus* (GBS) can cause serious infections in neonates, specifically early-onset sepsis, which can lead to severe morbidity and mortality. Vaginal/gastrointestinal colonisation during pregnancy increases the risk of neonatal infection; however, screening and prophylactic treatment of the positive mother can reduce the risk of neonatal infection.[1,2] Routine screening of pregnant women at 34–36 weeks gestation for GBS has significantly reduced the occurrence of GBS associated early-onset neonatal sepsis.[3,4] Currently the Centers for Disease Control and Prevention (CDC)[5] recommends placing a vaginal/rectal swab into an enrichment broth [Lim broth with colistin (10 μg/mL) and nalidixic acid (15 μg/mL)] incubated overnight then subcultured onto blood agar with colistin and nalidixic acid (CNA) when screening for GBS. This was the method used in our laboratory at the time of this study.

The manufacturers claim the current newer and improved versions of chromogenic agar selective for GBS have the ability to detect non-haemolytic strains of GBS, which was a problem for the earlier agars. Recent studies comparing performance of chromogenic agar with enrichment culture have shown comparable sensitivity to methods without an enrichment step.[6]

The use of selective chromogenic media, without broth enrichment, may have acceptable sensitivity and specificity while reducing the turnaround time to identification of GBS by up to 24 hours.

Therefore we compared direct culture of vaginal/perineal swabs on to four chromogenic media (StrepB*Select*, CHROMagar, Brilliance GBS, ChromID Strepto B) for the presumptive identification of GBS to the current CDC recommended broth enrichment procedure and subculture on colistin-nalidixic acid agar with 5% sheep blood (CNA).[5] The aim was to find a more efficient method that is at least as sensitive and specific as the CDC recommended method.

MATERIALS AND METHODS

From February to May 2013 self-collected vaginal/perineal swabs from 242 patients were screened for GBS. Dacron swabs were used with Amies gel transport media. When self-collecting the swabs for GBS screening our patients were instructed to swab the vagina, then the perineum using the one swab. Patient swabs were emulsified in 0.5 mL, 0.9% saline and vortexed for 5 s then 50 μL was inoculated onto whole plates of four chromogenic GBS screening media: StrepB*Select* (Bio-Rad Laboratories, USA); Brilliance GBS (Thermo-Fisher Scientific, USA); StrepB (CHROMagar; Dutec Diagnostics, USA); ChromID Strepto B (bioMerieux, France). The remaining 250 μL of saline was transferred into Lim broth (10 μg/mL colistin). The plates and Lim broth were incubated at 35°C in air for 24 h (manufacturer's recommendation), then 50 μL of Lim broth was inoculated onto CNA (Oxoid, Australia), and incubated at 35°C in air for 18 h. All suspect colonies from all media were identified by the Vitek MS MALDI-TOF (bioMerieux). Isolation of GBS from any of the media tested was considered a true positive. The Wilson score method without continuity correction was used to calculate confidence intervals.

To eliminate media inhibition as a cause of false negatives rather than media sensitivity, the isolates were subcultured onto the media on which they had previously failed to grow.

RESULTS

A total of 242 prenatal self-collected vaginal/perineal swabs were tested for the presence of GBS. Of those, 50 (21%) were positive for GBS on at least one of the media tested. Brilliance GBS, StrepB*Select* and CHROMagar agar had sensitivities which were ≥92%, compared to the CDC recommended pre-enrichment/CNA culture of 92% [95% confidence interval (CI) (81.16%, 96.85%)]; ChromIDStrepto B had a lower sensitivity of 86% (95% CI 73.81%, 93.05%) (Table 1). It was noted on Brilliance GBS and CHROMagar that there was a tendency for viridans streptococci to produce small, pale-coloured colonies that required extra testing as per the manufacturer's recommendations.

All positive GBS isolates subcultured onto media where they initially failed to grow, resulted in subsequent growth, indicating the discrepancy was due to low numbers of organisms.

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DOI: 10.1097/PAT.0000000000000299

Table 1 Comparison of chromogenic agar and post-enrichment CNA GBS screening

Media	Positive GBS (n)[a,b]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	95% CI (%)
Brilliance GBS	48/50[c]	96	100[d]	100	98.97	86.54, 98.90
StrepB*Select*	46/50[c]	92	100	100	97.5	81.16, 96.85
ChromID Strepto B	43/50[e]	86	100	100	96.5	73.81, 93.05
CHROMagar	47/50[c]	94	100[d]	100	98.46	83.78, 97.94
CNA[f]	46/50[g]	92	100	100	97.96	81.16, 96.85

CNA, colistin and nalidixic acid; GBS, Group B *Streptococcus*.
[a] Total number of GBS negative samples was 192.
[b] Total number of GBS positive samples detected on all media tested.
[c] All false negatives had ≤4 colonies of GBS on positive media.
[d] Growth of viridans streptococci produced pale colour profiles; the colonies were smaller than GBS but required further work-up as per manufacturer's guidelines.
[e] Four false negatives had ≤4 colonies of GBS on positive media while three had ≥10 colonies of GBS on positive media.
[f] Post-enrichment in Lim broth (10 μg/mL colistin) at 35°C for 24 h.
[g] Three false negatives had ≤4 colonies of GBS on positive media while one had ≥10 colonies of GBS on positive media.

DISCUSSION

CDC[5] guidelines for GBS screening were updated in November 2010. They included mention of new chromogenic agars to aid in the detection of GBS. Prior to this, detection of non-haemolytic strains (approximately 4% of total GBS isolates) were unreliable. As a consequence, the CDC recommended the use of an enrichment broth subcultured onto blood agar with CNA when screening for GBS.

This trial intended to compare our existing method, LIM broth/CNA, which conformed to the CDC guidelines,[5] of screening for GBS from antenatal patients with a proposed new procedure utilising direct inoculation onto four types of chromogenic media. Brilliance GBS had the highest sensitivity and all directly inoculated media except ChromID Strepto B produced similar results compared to pre-enrichment with CNA inoculation. No one method detected all positives.

One patient was GBS positive on subculture after Lim broth enrichment but negative by direct inoculation onto any chromogenic media. This probably reflects very low numbers of GBS present on the initial swab. However, there were four other swabs that were positive on the chromogenic agars but negative by the LIM broth/CNA method. Vaginal swabs were emulsified into 0.5 mL of saline as opposed to direct inoculation (manufacturer's recommendation). Therefore, low numbers of GBS were diluted across the four chromogenic agar plates, reducing recovery rates.

The false negative cultures on Brilliance GBS, StrepB*Select* and CHROMagar were all from low-yield specimens with less than four colonies isolated on positive plates. There were three false negative samples on ChromID Strepto B which had larger yields on all other media tested (>10 CFU). This is consistent with the lower sensitivity of this agar found in this study.

Brilliance GBS suppressed the normal flora in 51% of all samples, which was at least 10% more than the other chromogenic agars tested. It was noted on this agar that *Streptococcus salivarius* and *S. mitis/oralis* produced small, pink to pale-pink-coloured colonies in 4.5% of samples which initially, due to inexperience with the new medium, necessitated further investigation by MALDI-TOF.

Interestingly, there were two non-haemolytic strains of GBS isolated (3.9%) that were detected by all chromogenic media tested.

Despite the introduction of rapid molecular methods such as real-time polymerase chain reaction (PCR)[7] assays, the cost and expertise required limit their use. In addition, the results from these specimens are usually not urgent. Studies[8] indicate comparable performance of a culture-based method to that of PCR-based methods for detection of GBS in antenatal screening swabs can be achieved.

The findings of this study show the inoculation of screening swabs for GBS directly onto chromogenic media is at least as sensitive as the recommended CDC method. Direct plating on chromogenic agar offers the advantage of reducing workload and providing an identification of GBS 24–30 h sooner than the Lim broth enrichment method.

This study provides an indication into the adequacy of new chromogenic media on the market. The four media were equivalent when standard deviations were taken into account; however, Brilliance GBS was subsequently selected for use in the laboratory due to its higher rate of inhibition of normal flora, relatively larger colony size and sensitivity.

Our study was small (study size $n = 242$; total number of positives $n = 50$), therefore we may have missed small differences in rates of detection. However, we believe that direct inoculation onto the new chromogenic agars for GBS screening is at least as sensitive and specific as the CDC method and easier and cheaper, with reduced time to results. Retrospective analysis following Brilliance GBS implementation shows a 1.5% (total $n = 15{,}404$) increase in GBS isolation. Although our study size was small, the retrospective analysis of long term trends using the same patient population demonstrates direct inoculation onto Brilliance GBS produces comparable results to the conventional pre-enrichment CDC method.

Acknowledgments: We would like to thank Thermo-Fisher Scientific, Dutec Diagnostics and bioMerieux Australia for providing the media for this study.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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