Detection Of *Streptococcus agalactiae* Using Thermo Scientific *Brilliance* GBS Agar With And Without LIM Broth Enrichment

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Overview

Purpose: To compare the use of direct plating onto Thermo Scientific™ *Brilliance*™ GBS Agar (Thermo Fisher Scientific), Columbia Blood Agar and chromID™ StreptoB Agar (bioMérieux) with LIM Broth (Todd Hewitt Broth & colistin and nalidixic acid; Thermo Fisher Scientific) enrichment prior to plating.

Methods: Three hundred vaginal swabs were plated onto *Brilliance* GBS Agar, Columbia Blood Agar and chromID StreptoB Agar prior to enrichment in LIM Broth. Post-incubation, LIM Broth was subcultured onto *Brilliance* GBS Agar and Columbia Blood Agar. Colonies on any plate were confirmed using MALDI-TOF (Bruker).

Results: Sensitivity of *Brilliance* GBS Agar was consistently higher than chromID StreptoB Agar and Columbia Blood Agar. LIM broth enrichment allowed detection of more GBS than when samples were plated directly onto the agars.

Introduction

Streptococcus agalactiae [Lancefield group B Streptococcus (GBS)] is the leading cause of sepsis, pneumonia and meningitis in neonates. GBS is a commensal of the genitourinary and gastrointestinal tracts. Vertical transmission to the infant during labour occurs in 50% of deliveries involving colonized women, and 1–3% of colonized neonates go on to develop invasive disease[1].

Common problems with the laboratory detection of GBS include low colony forming units of GBS in some samples, and overgrowth of normal vaginal flora (including Staphylococcus species, lactobacillus, Enterococcus species, α-haemolytic, β-haemolytic and non-haemolytic Streptococcus). To detect low numbers of GBS amongst the normal vaginal flora, swabs can be pre-enriched in either Todd-Hewitt broth or LIM broth, before agar plate culture[2].

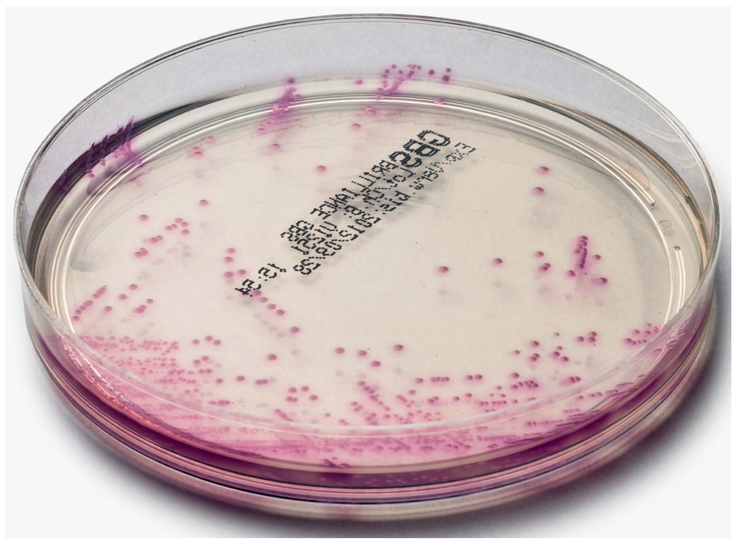
Brilliance GBS Agar (see figure 1.) is a transparent screening media specifically designed for the isolation and presumptive identification of GBS. GBS will grow as pink-coloured colonies on the medium. The inclusion of the Inhibigen™ technology enhances the plate by allowing inhibition of non-target organisms without affecting the growth of GBS. This technology works by targeting organism-specific enzymatic reactions through the uptake and cleavage of inhibitory agents, leading to cell lysis[3].

Methods

Three hundred vaginal swabs taken from pregnant women at 35-38 weeks gestation were tested. Swabs were streaked onto *Brilliance* GBS Agar, Columbia Blood Agar and chromID StreptoB Agar to ensure individual colonies. Each swab was then inoculated into LIM Broth which was incubated at 35±1ºC for 18-24 hr. Turbidity of the broth was observed and 10 µl of the broth (regardless of turbidity) was then subcultured onto *Brilliance* GBS Agar and Columbia Blood Agar.

All plates were incubated aerobically at 36±1ºC for 18-24 hr. Any presumptive GBS positive colonies (pink colonies on *Brilliance* GBS Agar, white/cream colonies on Columbia Blood Agar and pink-red colonies on chromID StreptoB Agar) and any other coloured colonies were identified using MALDI-TOF.

FIGURE 1. *Brilliance* GBS Agar



Results

Performance of *Brilliance* GBS Agar, Columbia Blood Agar and chromID StreptoB Agar is summarised in tables 1 and 2.

Direct plating

Table 1. performance when swabs were directly plated onto *Brilliance* GBS Agar, Columbia Blood Agar and chromID StreptoB Agar

performance	*Brilliance* GBS Agar	Columbia Blood Agar	chromID StreptoB Agar
Sensitivity	**96.4** (95% CI = 94.3-98.5)	**76.4** (95% CI = 71.6-81.2)	**76.4** (95% CI = 71.6-81.2)
Specificity	**98.0** (95% CI = 96.4-99.6)	**100.0** (95% CI = 100)	**99.6** (95% CI = 98.9-100)

Sensitivity of *Brilliance* GBS Agar (96.4%) was statistically significantly better (P=<0.05) than that of Columbia Blood Agar and chromID StreptoB Agar (both 76.4%). Specificity of *Brilliance* GBS Agar (98.0%) was comparable to both Columbia Blood Agar (100%) and chromID StreptoB Agar (99.6%). Both Columbia Blood Agar and chromID StreptoB Agar showed far more false negative results than *Brilliance* GBS Agar i.e. the two plates failed to detect a greater number of GBS than *Brilliance* GBS Agar.

LIM Broth enrichment

Brilliance GBS Agar detect 17% more GBS when samples were broth enriched (n=62) compared to when samples were plated directly onto the agar (n=53).

Table 2. performance when swabs were enriched in LIM Broth prior to subculture onto *Brilliance* GBS Agar and Columbia Blood Agar

performance	*Brilliance* GBS Agar	Columbia Blood Agar
Sensitivity	**96.9** (95% CI = 94.9-98.9)	**95.3** (95% CI = 92.9-97.7)
Specificity	**97.0** (95% CI = 95.1-98.9)	**100.0** (95% CI = 100)

Sensitivity of *Brilliance* GBS Agar (96.9%) was higher than Columbia Blood Agar (95.3%); Specificity of *Brilliance* GBS Agar (97.0%) was lower than Columbia Blood Agar (100%).

Non-GBS inhibition

Percentage inhibition of organisms other than GBS (i.e. the number of swabs showing no growth of either target or non-target organisms) was higher on *Brilliance* GBS Agar (61.7%) than chromID StreptoB Agar (43.3%) when samples were directly plated. As Columbia Blood Agar is a non-selective plate and grew a high number of organisms other than GBS with similar appearance, percentage inhibition was considerably lower (4.7%) than the other two selective media. The higher the percentage inhibition, the less background growth will be present on the agar, thus improving isolation of GBS colonies. The number of swabs showing no growth on any agar media reduced after LIM Broth enrichment.

Conclusion

Sensitivity of *Brilliance* GBS Agar was consistently higher (statistically significantly higher when samples were directly plated) than the two other agars, regardless of whether samples were broth-enriched or not; *Brilliance* GBS Agar detected more GBS than either chromID StreptoB Agar or Columbia Blood Agar. Specificity of *Brilliance* GBS Agar was slightly reduced. LIM broth enrichment allowed detection of more GBS than when samples were plated directly onto the agars.

References

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