EVALUATION OF THE GROUP B STREPTOCOCCAL DIFFERENTIAL AGAR FOR THE DETECTION OF GROUP B STREPTOCOCCI FROM VAGINAL SPECIMENS

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ABSTRACT

Background: Group B streptococci (GBS) are the leading cause of severe perinatal infections. Most current guidelines for the prevention of GBS perinatal disease are based on prenatal screening culture for vaginal GBS colonisation. Use of selective and differential media could improve the sensitivity of these cultures.

Objective: To evaluate the GBS-Differential Agar (GBSDA) recently formulated by Becton Dickinson for the selective growth and production of orange colonies of β-hemolytic (β-H) GBS.

Methods: 283 vaginal swabs (VAG) collected from pregnant women were inoculated in selective Lim broth. After overnight incubation, Lim broth were subcultured on GBSDA, on Granada agar (Biomedics, Spain) and on Columbia blood agar (BA). To evaluate the stability, 99 isolates of GBS (REF) from adult or neonatal infections (Belgian GBS reference laboratory collection) were cultured on GBSDA and Granada at their limit of shelf live, and on BA. GBSDA and Granada were incubated anaerobically and BA aerobically + 7% CO2, at 35°C, 24 to 48 h. Positive and negative control strains (GBS ; *E. faecalis*) were cultured with each run. Specific identification of colonies suggestive of GBS (pale to dark orange on GBSDA and Granada, β-H on BA) was performed.

Results: β-H GBS were recovered from 63 VAG (22.3 %): 62 were easily identified after overnight incubation on GBSDA and 63 on Granada without requiring any subculture. All GBS were also recovered from BA however it was after many subcultures. All orange colonies were confirmed as GBS. Among REF, 3 strains were non hemolytic ; they grew but were not differentiated as orange colonies on GBSDA or Granada. 96 REF were β-H, 94 (97.9%) produced orange to very dark orange colonies on GBSDA, 2 produced white colonies, and on Granada, 74 (77.1 %) produced pale to dark orange colonies and 22 white to white-orange colonies.

Conclusions: 1) GBSDA and Granada: a) very high sensitivity and specificity for the detection of β-H GBS, in a single step b) Results available within 48 h after inoculation in Lim broth, low workload 2) Excellent stability up to expiration date for GBSDA 3) Non hemolytic GBS: grown but not differentiated on GBSDA or Granada.

OBJECTIVE

- **To evaluate the performance and stability of the GBS-Differential Agar (GBSDA) recently formulated by Becton Dickinson for the selective growth and production of orange colonies for β-hemolytic (β-H) GBS.**
- **By comparison to culture on Columbia sheep bood agar, and on Granada agar (Biomedics, Spain), another selective differential agar.**

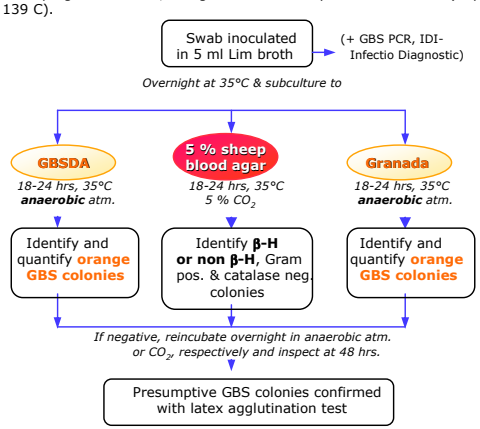
BACKGROUND

Group B streptococcus (GBS) or *Streptococcus agalactiae* continue to be a major cause of life-threatening infections, sepsis, pneumonia and meningitis in neonates. To prevent GBS perinatal diseases, most current guidelines recommend intrapartum antibioprophylaxis for women "at risk"; they are based on prenatal screening culture of all pregnant women at 35-37 weeks of gestation for rectal and vaginal GBS colonization. To provide the highest sensitivity, culture methods must include an enrichment in selective broth like Lim broth, further subcultured on a blood agar plate. However, this enrichment broth is not totally selective for GBS and other Gram positive cocci may as well be enriched by this method, possibly hiding GBS.
Use of selective and differential media could improve the sensitivity of these cultures as well as it could shorten the turn around time.
Recently, modifications of the Islam Agar, a selective growth medium for GBS, were tested for selective and differential growth of GBS, as the new Granada medium. The Group B Streptococcal Differential Agar (GBSDA), Becton Dickinson, is a modified Granada Medium with increased stability and selectivity. On these media, β-hemolytic strains of GBS produce red-orange to salmon colonies.

MATERIAL & METHODS (I)

Clinical specimens and culture method

In september 2003, a total of 283 distal vaginal swabs were collected from pregnant women, using a swab with liquid Stuart media (Copan 139 C).



Positive, *Streptococcus agalactiae* ATCC 12386, and negative, *Enterococcus faecalis* ATCC 29212, control strains were cultured with each run.

RESULTS

Performance with clinical specimens

- GBS colonization rate : 24 % (68/283)
- β-hemolytic GBS colonization rate : 22.3 % (63/283)

Time to detection on GBS DA and Granada agar

Time to detection	Number of positives		
	GBSDA	Granada	
18-24 hrs	62	63	NS
48 hrs	62 + 1	63	

Detection of GBS from vaginal swabs inoculated in Lim broth and subcultured on GBSDA, Granada agar and blood agar

Media	Number of GBS positive vaginal swabs							
	β-hemolytic					□□□ β-H		Total
	VR	1+	2+	3+	4+	1+	2+	
GBSDA	1	8	10	32	11	0	0	62
Granada	2	8	9	33	11	0	0	63
Blood agar	9	13	6	26	9	2	3	68

- In 18 hours, all orange colonies either on GBSDA or Granada were confirmed as GBS, 98.4 and 100 % !
- On Blood agar, several sub-cultures were often necessary to isolate or even to find the suspected GBS (GBSDA, Granada or GBS PCR Positive) .
- All β-hemolytic colonies on blood agar were not confirmed as GBS

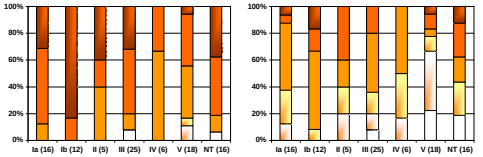
Hemolysis/pigment production of reference strains and stability of GBSDA

- 3 strains/99 were non-hemolytic:
  Grown but not pigmented on GBSDA/Granada

Pigment production on GBS DA and Granada agar at expiration date

Pigment	Number of strains		
	GBSDA	Granada	
Orange	94	74	P < 0.001
White to white orange	2	22	

Distribution of "orange" per serotype (number of strains) on GBS DA and on Granada agar



MATERIAL & METHODS (II)

Reference strains and culture method

A total of 99 clinical isolates from neonatal or adult infections, collection of the Belgian reference laboratory for GBS. All strains were serotyped and stored in skimmed milk at -80°C.

Culture : same algorithm as for clinical specimens, but the Lim broth was replaced by a primary culture on 5 % sheep blood. GBSDA and Granada agar were used within 3 days following their expiration date.

Scheme for semi-quantification of GBS

Observed growth	Reported result
< 10 CFU	Very rare
≥ 10 CFU but in the first quadrant only	1 +
If few colonies present in the first isolating streaks (2nd quadrant)	2+
If few colonies present in the secondary streaks (3rd quadrant)	3+
If few colonies present in the tertiary isolating streaks (4th quadrant)	4+

DISCUSSION AND CONCLUSION

→GBSDA and Granada:
- **→Very high sensitivity and specificity for the detection of β-H GBS.**
- **→Easy to read, even if a few GBS colonies.**
- **→In a single step : all orange colonies are GBS, no need for specific identification tests: low workload.**
- **→More than 98 % of positive results available within 18 h after inoculation in Lim broth.**

→GBSDA :
- **→Excellent stability until expiration date, improved by comparison to Granada agar**

→Blood agar :
- **→in this study, over-estimation of its performance by comparison to its use in routine.**
- **→Lower density of culture by comparison to GBSDA or Granada**

→Non β-Hemolytic GBS : 7 % were isolated from vaginal colonization, but their presence was confirmed only after suggestion by a positive PCR for GBS. Among invasive strains 3 % were non-hemolytic. Are they less virulent?

GBSDA and "fresh" Granada agar are highly sensitive for detecting GBS from vaginal specimens and have the advantage of providing results in a short time with a lower workload by comparison to culture on blood agar.

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