EVALUATION OF THE STREPTO B ID AGAR FOR THE DETECTION OF GROUP B STREPTOCOCCI FROM VAGINAL AND RECTO-VAGINAL SPECIMENS

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P.Melin, S.Bonafé, M.P. Hayette and P. De Mol

Belgian reference laboratory for GBS, Microbiology, University Hospital of Liège, Liège, Belgium

Medical Microbiology, CHU, B-23
Sart Tilman - B 4000 Liège, BELGIUM
Phone: +32-4 366 24 38
Fax: +32-4 366 24 40
Email: Pierrette.Melin@chu.ulg.ac.be

ABSTRACT

Background: Current guidelines for the prevention of GBS perinatal disease are based on prenatal screening culture for recto-vaginal GBS colonization. Use of selective and differential media as Granada type agar improves the sensitivity and workload of these cultures

Objective: To evaluate the Strepto B Id Agar (SBID), formulated by bioMérieux for the selective growth of pink to red colonies of GBS.

Methods: 175 swabs (33 vaginal; 142 recto-vaginal) collected from pregnant women. Each swab : suspended in 2 ml of saline solution and 50 µl-aliquots plated on SBID, modified Granada agar (GRA), Becton Dickinson and blood agar with colistin-nalidixic acid (BA), primary cultures. The remaining suspension: added to a selective Todd-Hewitt broth with antibiotics (STH). After overnight incubation: 50 µl-aliquots of STH plated on SBID, GRA and BA. SBID incubated in air, GRA anaerobically and BA in air + 7% CO2, at 35°C, 24 - 48 h. Positive and negative c ontrol strains (GBS; *E. faecalis*) : cultured with each run. Specific identification of colonies suggestive of GBS (light pink to red on SBID, orange on GRA, β-H on BA) was performed. If conflicting results between the 3 media: colonies not suggestive of GBS, grown on the negative media were identified.

Results: GBS recovered from 38 swabs (21.7 %). 33 from primary cultures and 37 after selective enrichment: characteristic GBS were identified respectively from 32 and 34 SBID, 33 and 36 GRAN, 31 and 35 BA. Non-characteristic GBS were identified from 1 SBID, 0 GRA and 2 BA. Sensitivity and fertility: no significant difference. Characteristic colonies of GBS were not confirmed as GBS : from 11 primary SBID and 8 after selective enrichment, respectively from 2 and 3 BA and from 0 GRA. GRA was significantly more specific. Presumptive GBS were easily observed on SBID and GRA even in low numbers without requiring any subculture. From BA, several subcultures were sometimes necessary to confirm the presence of GBS.

Conclusions: 1) SBID and GRA: very high sensitivity for the detection of GBS 2) GBS easily observed on SBID and GRA without subcultures 3) SBID less specific than GRA 4) SBID incubation in air, no need for CO_2 or anaerobiosis.

BACKGROUND

Group B streptococcus (GBS) or *Streptococcus agalactiae* continue to be a major cause of life-threatening infections 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, further sub-cultured 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.

Based on reported evidence and on experts' opinion, the Belgian guidelines for prevention of perinatal GBS disease recommend to subculture selective enrichment broth onto a Granada medium to improve prenatal screening culture. On this type of agar, β-hemolytic strains of GBS produce red-orange to salmon colonies. Granada medium is very sensitive and highly specific for GBS. In Europe the new chromogenic medium, Strepto B ID from bioMérieux, is now available. This selective and differential agar optimizes the identification of GBS as pale pink to red colonies.

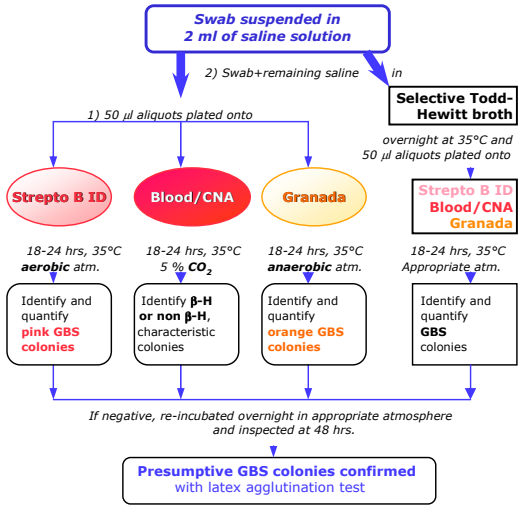
OBJECTIVE

- **To evaluate the performance** (fertility, sensitivity, specificity and selectivity) **of the Strepto B ID, new chromogenic medium (SBID) recently formulated by BioMérieux France, for the selective growth and identification of β-hemolytic (β-H) and non β-H GBS as pale pink to red colonies.**
- **By comparison to culture on Columbia sheep blood agar with colistin/nalidixic acid (BA), and on Granada medium (GRA), a selective differential agar.**

MATERIAL & METHODS (I)

Clinical specimens and culture method

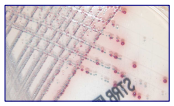
From October to December 2005, a total of 175 swabs, 33 distal vaginal and 142 recto-vaginal swabs, were collected from pregnant women, using a swab with liquid Amies media (Copan)



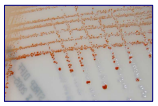
RESULTS

GBS colonization rate: 21.7 % (38/175)

Strepto B ID:



Granada :



Fertility - Sensitivity

Detection of GBS from vaginal/recto-vaginal swabs

+/- Enrichment & Incubation (hrs)		Positive cultures : number (% of positive swabs) Strepto B ID	Granada	BA/CNA**	
Without enrich.	24 hrs	26 (68.4)	30 (78.9)	31 (81.6)	NS
	48 hrs	32 (84.2)	33 (86.8)	31 (81.6)	NS
With Enrich.	24 hrs	34 (89.5)	35 (92.1)	36 (94.7)	NS
	48 hrs	35* (92.1)	36 (94.7)	37 (97.4)	NS

* Growth of 35 isolates of GBS, **fertility = 92.1%** ; 34/35 presented characteristic colonies, **sensitivity = 89.5%**.

** On BA, several sub-cultures were often necessary to isolate or even to find the suspected GBS (when SBID or GRA Positive).

Density of GBS (after 24 hours incubation)

		0	1+	2+	3+	4+	Total 1/2/3/4+	Nb Pos. swabs
Without enrich.	SBID	6	1	12	11	2	26	32
	GRA	2	5	11	10	4	30	
With Enrich.	SBID	1	0	0	20	14	34	36
	GRA	1	0	1	20	14	35	

Specificity

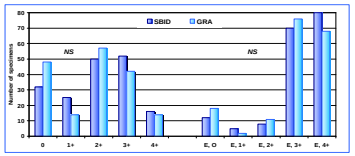
A total of 137 specimens were GBS-negative.

False + : "characteristic" colonies not confirmed as GBS

+/- Enrichment & Incubation (hrs)		Strepto B ID False + (N)	Sp. (%)	Granada False + (N)	Sp. (%)	
Without enrich.	24 hrs	5	96.4	0	100	NS
	48 hrs	11	92.0	0	100	P <0.01
With Enrich.	24 hrs	3	97.8	0	100	NS
	48 hrs	8	94.2	0	100	P <0.01

Selectivity

Density of non GBS bacterial flora (+/- Enrichment): no difference



MATERIAL & METHODS (II)

Media

Strepto B ID, bioMérieux, pilot batch (SBID)

Granada medium: BD GBS Differential Agar, Becton Dickinson (GRA)

Blood agar/CNA: Columbia sheep blood agar + colistin / nalidixic acid (BA), bioMérieux

Control strains

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

Scheme for semi-quantification of GBS

Observed growth/quadrant	Reported result
No growth	0
Colonies in the first quadrant only	1
Colonies present in the first two	2
Colonies present in the first three	3
Colonies present in the four	4

Statistics

Non-parametric Mac Nemar test or Binomial test

DISCUSSION AND CONCLUSION

- **SBID, GRA and BA:**
  - **Very high sensitivity for growth and detection of GBS** *(NS).*
- **SBID and GRA :**
  - **Easy to read, even if a few GBS colonies.**
  - **Reduction of consecutive sub-cultures to isolate presumptive GBS.**
  - **≥ 90 % of positive results available overnight after sub-culture from Lim broth.**
- **SBID :**
  - **Aerobic conditions for incubation**
  - **Not 100 % specific, presumptive GBS to be confirmed.**
- **GRA :**
  - **100% specific, no need for GBS confirmation tests: low workload.**
- **Blood agar :**
  - **in this study, over-estimation of its performance by comparison to its use in routine.**
  - **Not 100 % specific, presumptive GBS to be confirmed**

SREPTO B ID, a new chromogenic medium, demonstrated very good performance for GBS prenatal screening from recto-vaginal swabs. It provided results in a short time with a lower workload by comparison to culture on BA.

REFERENCES

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- Evaluation of the Granada agar plate for detection of vaginal and rectal group B streptococci in pregnant women. Garcia E, et al. J Clin Microbiol 1999; 37:2648-51

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