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REAL-TIME PCR DETECTION OF GROUP B STREPTOCOCCI (GBS) FROM PREGNANT WOMEN'S VAGINAL SPECIMENS AT TIME OF DELIVERY: CLINICAL EVALUATION

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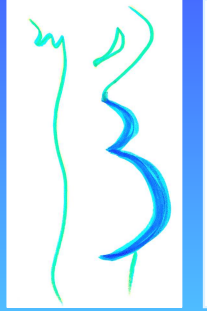


ABSTRACT

Background: GBS are still the leading cause of severe perinatal infections. Current guidelines for prevention recommend intrapartum antimicrobial prohylaxis (IAP) for pregnant women with a positive prenatal culture-based screening for GBS. To improve this strategy, a rapid screening performed at the onset of labor with the IDI-Strep B™ test (IDI), a real time PCR detection (Infectio Diagnostic), may be used.
Objective: To evaluate the performance of the IDI to detect GBS from vaginal specimens collected at time of delivery.
Methods: Intrapartum vaginal specimens from 923 pregnant women were tested to determine the status of GBS colonization, by CDC's recommended culture method (including selective LIM broth) with a Granada agar (GR) added as well as by the IDI and the immunologic StrepB OIA™ test (OIA), BioStar. The performance of the different methods was compared.
Results: GBS were recovered from 16.8% and 23.6% specimens respectively on primary culture plates and overall. The colonization rate for GBS was 18.6 % by IDI and 15.7 % by OIA testing. The sensitivity of IDI for identifying vaginal colonization status at delivery was 92 % or 77.1 % when compared to GR primary cultures or to overall culture results, and for the OIA, it was respectively 65.1 or 52.1 %. The specificity was 99.1 % for IDI and 95.5 % for OIA. The turnaround time for obtaining results was less than one hour for both IDI and OIA.
Conclusions: 1) Strep B-IDI test, performed on intrapartum vaginal specimens, yields relevant results rapidly enough to be used as an efficient diagnostic tool for the identification of GBS colonized women, in order to offer IAP really targeted to GBS carriers. 2) By comparison to the prenatal screening-based strategy, the high sensitivity and specificity of IDI would allow a reduction of useless IAP and of missed opportunities. 3) IDI testing might be implemented "in routine" in some hospitals for further clinical and practical evaluation.

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 (IAP) for women with a positive prenatal culture-based screening at 35-37 weeks gestation for rectal and vaginal GBS colonization. During the elapsed time between screening and delivery, about 10% of patients change their GBS colonization status and about half of those change from GBS-negative to GBS-positive. Furthermore, too many women have still not been screened at time of onset of labor (preterm onset of labor or little prenatal care) or their screening-result is not available. To improve the recommended strategy, as raised in the 2002 CDC's guidelines, *"a rapid test for detection of GBS colonization at the time of onset of labor or rupture of membranes might obviate the need for prenatal culture-based screening, **if** its sensitivity and specificity are comparable to culture in selective broth media and yield results rapidly enough to permit administration of adequate IAP to women detected as carriers."* In November 2002,a real-time PCR test, the IDI-Strep B™ test (IDI), Infectio Diagnostics Inc., Canada, has been cleared by FDA and may be used to determine the status of GBS colonization at time of delivery.

OBJECTIVE

- **To evaluate the performance of the molecular based assay IDI-Strep B™ test and of the automated analyser Smart Cycler™ for the direct detection of GBS in vaginal specimens collected at onset of labor**
  - **By comparison to culture on Granada agar (Biomedics, Spain), a selective differential agar used for primary culture and for sub-culture from enrichment in selective Lim broth**
  - **By comparison to the rapid Strep B OIA test (ThermoBiostar, Co, USA)**
- **To determine if the IDI-Strep B test™ may be integrated in the currently recommended screening strategy.**

MATERIAL & METHODS

Population

923 pregnant women admitted for delivery were recruited for this study in three different sites of the university department of gynecology and obstetrics.

A case report form was used for each patient enrolled in the study in order to record clinical and laboratory information.

Clinical specimens and testing methods

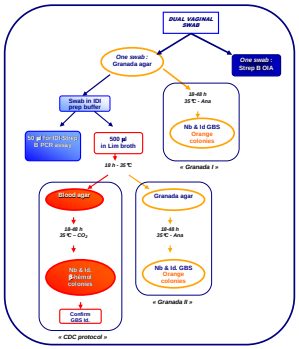
Distal vaginal swabs were collected prior to initiation of any antibiotic treatment from each pregnant woman, using dual-swab with liquid Stuart media (Copan 139 C). Within 48 hours of collection, all specimens were sent to the reference laboratory for determining the status of GBS colonization by (Figure 1):

- **Standard culture method** ("CDC protocol") **improved** by plating onto **Granada agar** (Biomedics, Spain) ("Granada I & II")
- **IDI Strep B™ PCR assay** (Infectio Diagnostics Inc.,Canada) on the SmartCycler® (Cepheid), performed in 45 minutes.
- **Strep B-OIA antigenic test** (BioStar, Co, USA), performed in 30 minutes.

Use of respective positive and negative controls for each assay run.

The performance of the three methods was compared.

Figure 1 : Testing algorithm



Discrepant results for IDI-Strep B/ culture : IDI-Strep B test repeated with the frozen lysate.

RESULTS

Rate of intrapartum colonization among 923 pregnant women

Table 1: Number (%) of positive culture for GBS

From primary culture on Granada I						After selective enrichment: CDC protocol & Granada II	TOTAL
Overall	Density 4+	3+	2+	1+	Very Rare		
155 (16.8%)	54	29	18	30	24	63 (6.8%)	218 (23.6%)

Granada agar : GBS colonies always easily and rapidly identified.
GBS Blood agar (CDC protocol): for numerous specimens, GBS only identified after several subcultures guided by positive PCR or Granada results.
Remark: Guided by PCR results, 2 non-hemolytic strains of GBS were identified from culture either on blood or Granada agar.

Performance of the IDI-Strep B PCR assay by comparison to culture

Table 2:

IDI-Strep B	GBS Positive culture: From primary culture on Granada I — Overall (N=154)	Density 4+ (N=54)	3+ (N=28)	2+ (N=18)	1+ (N=30)	Very Rare (N=24)	After selective enrichment: CDC protocol & Granada II (N=60)	TOTAL (N=214)	GBS Negative culture (N=705)
GBS Positive	133	54	27	16	23	13	26	159	6*
SENSITIVITY :	86.4 %	100%	96.4%	88.9%	76.7%	54.1%	43.3%	74.3%	
Discrepant results repeated Positive				1	3	2			
SPECIFICITY :		99.1 %							
Positive Predictive Value :		96.4 %							
Negative Predictive Value :		92.7 %							

6* : *Among these specimens, 3 were also strongly positive by Strep B OIA. It could reflect false negative culture.*

Performance of the Strep B OIA test by comparison to culture

Table 3:

STREP B OIA	GBS Positive culture: From primary culture on Granada I — Overall (N=152)	Density 4+ (N=53)	3+ (N=29)	2+ (N=17)	1+ (N=29)	Very Rare (N=24)	After selective enrichment: CDC protocol & Granada II (N=63)	TOTAL (N=215)	GBS Negative culture (N=705)
GBS Positive	99	50	22	11	12	4	13	112	32*
SENSITIVITY :	65.1 %	94.3%	75.8%	64.7%	40%	16.7%	20.6%	52.1 %	
SPECIFICITY :		95.5 %							
Positive Predictive Value :		77.8 %							
Negative Predictive Value :		86.7 %							

***Density of GBS growth in primary culture**: Very rare: < 10 CFU; 1+ : if few CFU in the first quadrant; 2+ : if few CFU in the first isolation streaks; 3+ : if few CFU in the secondary isolation streaks and 4+ : if few CFU in the tertiary isolation streaks*

DISCUSSION AND CONCLUSION

- In this study, 23.6% of the 923 pregnant women were GBS carriers based on culture of intrapartum vaginal swabs. Of these patients **16.8% were positive based on primary culture.**
- A rapid non-culture based test for detection of GBS colonization, performed at the admission of pregnant women for delivery, which might obviate prenatal screening cultures **should be at least 85% sensitive** compared to culture methods (CDC 2002).
  - **With an overall sensitivity of 86.5%** compared to improved primary culture on Granada agar, the **IDI-Strep B test,** performed on intrapartum vaginal specimens, yields relevant results **and rapidly enough to be used as an efficient diagnostic tool** for the identification of GBS colonized women, in order to offer IAP really targeted to GBS carriers.
  - By comparison to the prenatal screening-based strategy, as expected, **the high performance of the IDI-Strep B test would allow a reduction of useless IAP and of missed opportunities of IAP.**
  - **IDI-Strep B testing might be implemented "in routine"** in some hospitals for further clinical and practical evaluation.
  - **With an overall sensitivity of 65.1%** compared to improved primary culture on Granada agar, the **Strep B OIA test,** performed on intrapartum vaginal specimens, **doesn't meet the CDC criteria to replace prenatal screening** cultures, except for heavy GBS carriers (Sens.:94.3%). This sensitivity is not too bad and is similar to our previous observations in other studies, but according to other authors it can be as low as 20%.

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