

Group B Streptococcus Screening in Pregnant Women: A Belgian Multicentre Study Evaluating the Predictive Window of Antenatal Screening With Real-time PCR

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Research Article

Keywords: Group B Streptococcus, Streptococcus agalactiae, real-time PCR, pregnancy, intrapartum screening, neonatal GBS disease

Posted Date: September 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11065358/v1>

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Additional Declarations: Competing interest reported. This study was funded by Hologic Inc. PD is a biostatistician who helped with the statistical analysis of our data.

Abstract

Purpose

Vertical transmission of Group B *Streptococcus* (GBS) during childbirth can cause severe neonatal infections. Vaginal-rectal screening enables intrapartum antibiotic prophylaxis for women with maternal GBS colonisation. Although real-time PCR is highly sensitive, culture remains the recommended method for antenatal GBS screening. Due to dynamic recto-vaginal GBS colonisation in pregnant women, determining optimal timing for antenatal screening remains challenging. This Belgian multicentre study aimed to assess the predictive window of antepartum screening with real-time PCR to reliably predict maternal GBS colonisation at delivery.

Methods

This study first evaluated the Panther Fusion® GBS real-time PCR assay (Hologic®) for antenatal GBS screening at 35–37 weeks of gestation (n = 1353). Specimens were analysed using non-enriched PCR, broth-enriched PCR, and broth-enriched culture. To assess the predictive window of antenatal GBS screening, a subset of women also provided intrapartum samples for PCR and culture analysis (n = 257).

Results

Broth-enriched PCR yielded a higher number of GBS-positive results than non-enriched PCR and culture (positive agreement 68.5% and 95.6%, respectively). At delivery, about 14% of GBS-carrying women were missed by antepartum PCR, while 7.7% received unnecessary intrapartum antibiotic prophylaxis due to loss of GBS colonisation (Cohen's κ = 0.86). Kaplan-Meier analysis revealed GBS status retention > 95% until 3.7 weeks before delivery when antenatal PCR was used.

Conclusion

Broth-enriched PCR identified GBS colonization in a greater number of pregnant women than either broth-enriched culture or non-enriched PCR. A predictive window of 3.5 to 4 weeks before delivery (36–38 weeks of gestation) may optimise antepartum GBS PCR accuracy.

Introduction

Streptococcus agalactiae (Group B *Streptococcus*; GBS) is a frequent coloniser of the female intestinal and vaginal tracts (1). Maternal rectovaginal GBS colonisation poses a significant risk for neonatal early-onset GBS disease, which results from vertical transmission just before or during childbirth. This early-onset disease generally presents within the first week of life as sepsis, pneumonia or meningitis (1–3). In Europe and North America, rectovaginal GBS colonisation in pregnant women ranges from 15–21% (4–6). Vaginal-rectal screening is advised in pregnant women so that intrapartum antibiotic prophylaxis can be administered to all GBS colonised women (3, 7). American guidelines favour culture-based screening at 36–38 weeks of gestation and also propose broth-enriched PCR as an alternative (8–11). European experts recommend universal intrapartum GBS screening using rapid nucleic acid amplification tests (NAATs) (8). Intrapartum screening enables determination of maternal GBS status at delivery. However, sensitivity of rapid NAATs is often suboptimal when performed without enrichment, especially in settings with limited technical expertise (8, 12). Although NAATs generally offer higher sensitivity, shorter hands-on time and faster turnaround time compared to culture, their inability to detect antibiotic resistance markers, the occurrence of technical errors or invalid results, as well as higher costs currently limit their widespread implementation (8, 10, 11, 13, 14). Maternal GBS colonisation is dynamic, meaning women may acquire or lose GBS colonisation between antenatal screening and delivery, compromising the accuracy of antenatal screening results (3, 8, 15–17). The *predictive window* refers to the maximal time interval during which an antepartum screening result reliably predicts maternal GBS colonisation at time of delivery. In this context, loss of GBS colonisation between antepartum screening and delivery is not considered clinically critical, whereas new acquisition of GBS after a negative screening, with consequent omission of intrapartum antibiotic prophylaxis, is regarded as potentially critical. Most guidelines assume a predictive window of antenatal screening of 3 to 5 weeks, based on older studies in which ante- and intrapartum GBS colonisation was compared using only culture (10, 18). To our knowledge, there are no studies on the predictive window of GBS colonisation using molecular methods. The Panther Fusion® GBS assay (Hologic®) is a fully automated CE-IVDR real-time PCR assay for qualitative antenatal detection of GBS DNA in 18-24h enriched LIM broths from vaginal-rectal swabs in pregnant women.

This Belgian multicentre study primarily aimed to evaluate the predictive window of antepartum GBS screening with real-time PCR. To do so, the Panther Fusion® GBS assay (Hologic®) was first assessed using non-enriched and enriched vaginal-rectal specimens and compared to broth-enriched culture. Since broth-enriched PCR is stated in the instructions for use of the Panther Fusion® GBS assay (Hologic®), we used this as the reference method. Finally, the predictive window of antepartum screening, using real-time PCR with the Panther Fusion® GBS assay was evaluated by comparing ante- and intrapartum GBS colonisation.

Methods

Study design, patients and data collection

This prospective cohort study was conducted in three Belgian hospitals (AZ Turnhout, AZ Herentals, Heilig Hart Mol) and approved by the central ethical committee of the University of Antwerp and local ethical committees (2024-6265). This study adhered to the Helsinki Declaration and good clinical practice

guidelines. All pregnant women attending antenatal screening at 35-37 weeks of gestation were included for the evaluation of the Panther Fusion® GBS assay (Hologic®). For this, culture was compared to real-time PCR, both performed on antepartum vaginal-rectal swabs. At the time of partus, and only upon written consent, women provided an additional intrapartum swab before intrapartum antibiotic prophylaxis was given (or 24h preoperatively for elective caesarean section). Participants could withdraw at any time.

Sample Collection and Processing

Vaginal-rectal swabs were inoculated in LIM broth (Copan, Italy) and processed for non-enriched PCR, 18-24h LIM broth-enriched PCR and 18-24h LIM broth-enriched culture. Real-time PCR was performed using the Panther Fusion® GBS assay (Hologic®) and for culture CHROMID® Strepto B agar (bioMérieux, France) was used. Upon arrival in the lab, 250µL of LIM broth was transferred into an Aptima Lysis Tube (non-enriched); the remainder was enriched for 18-24h and then analysed by PCR according to the manufacturer's instructions. Enriched LIM broths were also subcultured on CHROMID® Strepto B agars and incubated at 35°C in 5% CO₂ for 18-24 hours; if negative, agar plates were re-incubated for another 24h. Suspected colonies were identified using mass spectrometry with a Maldi Biotyper Sirius (Bruker, USA). Figure 1 shows an overview of the lab workflow. Clinical decision making was based on broth-enriched PCR. Discrepant results between culture and broth-enriched PCR were resolved using Xpert® Xpress GBS (Cepheid), noting its off-label use on LIM-broth.

Predictive window evaluation

The predictive window of antenatal screening using PCR with the Panther Fusion® GBS assay (Hologic®) was evaluated. The predictive window was defined as the longest interval between sampling and delivery during which the Kaplan-Meier estimate of event-free survival among screening-negative women remained ≥95%. Therefore, the interval between screening and partus was registered for each woman. A 95% threshold was chosen as a clinically conservative criterion, reflecting an upper tolerance of 5% for new GBS acquisition after a negative antenatal screen, beyond which the residual risk of missed intrapartum colonisation was considered unacceptably high.

Statistical analysis

Sensitivity, specificity, Cohen's κ and associated uncertainty measures were calculated (19). Because non-enriched and broth-enriched PCR results were paired within the same specimens, McNemar's test was performed to formally assess whether the difference in positivity rates was statistically significant. A student T-test was performed to compare Ct values with their subcultures. Results were considered significant at the 5% significance level (p<0.05). Kaplan-Meier analysis was performed to plot survival probability from the antepartum screening onwards. All analysis were performed in R4.4.3 (RStudio).

Results

Broth-enriched PCR outperforms culture

Table 1 summarizes the comparative analyses between broth-enriched and non-enriched PCR as well as between broth-enriched PCR and broth-enriched culture. Broth-enriched PCR demonstrated a significant higher sensitivity than broth-enriched culture (sensitivity: 95.6% (95%CI, 91.8-97.7)) and non-enriched PCR (sensitivity: 68.5% (95%CI, 61.8-74.5)). A McNemar's test was performed to confirm that discordant pairs were strongly asymmetric (n = 1223, χ² = 61.0, p < 0.0001). In nine culture-negative specimens, broth-enriched PCR did detect GBS (Ct values ranged between 22.8 and 39.9). Eight of these nine results were confirmed by the Xpert® Xpress GBS assay. However, one specimen (Ct 39.9 with the Panther Fusion® GBS assay) tested negative by the Xpert® Xpress GBS assay. These findings are summarised in Figure 2.

Table 1: Comparison of broth-enriched PCR, non-enriched PCR and culture performed on antepartum swabs using the Panther Fusion® GBS assay (Hologic®). Sensitivity, specificity, overall percent agreement and Cohen's κ are included.

Comparison on antepartum swabs only	N° of specimens				Sensitivity (%)	Specificity (%)	OPA (95%[CI])	Cohen's κ
	TP	TN	FP	FN				
Non-enriched vs. broth-enriched ^R PCR (n=1223)	137	1023	0	63	68.5 [61.8, 74.5]	100 [99.6, 100]	94.8 [93.5, 96]	0.78 [0.73, 0.84]
Broth-enriched culture vs. broth-enriched ^R PCR (n=1239)	195	1035	0	9	95.6 [91.8, 97.7]	100 [99.6, 100]	99.3 [98.6, 99.6]	0.97 [0.96, 0.99]

CI: confidence interval; FN: false negative; FP: false positive; TN: true negative; TP: true positive; OPA: overall percent agreement, PPA: positive percent agreement, NPA: negative percent agreement, R: reference standard.

Ct values as semi-quantitative indicators of GBS density

Although real-time PCR is used for qualitative reporting, semi-quantitative Ct's were correlated to culture. A significant Ct difference ($p < 0.001$) was observed with broth-enriched PCR between culture-positive and culture-negative samples (Figure 3A). Comparing Ct values obtained with ante- and intrapartum broth-enriched PCR showed no correlation of antepartum to intrapartum GBS load (Figure 3B).

A predictive window of 3.7 weeks yielded optimal GBS status retention

A total of 257 matched ante- and intrapartum samples were received. An overview of demographic characteristics is given in Supplementary Table 1. Among the 257 matched specimens 52 women tested GBS-positive antepartum, while 205 were GBS-negative. Figure 4A shows the number of women with GBS retention, loss or acquisition by weekly intervals between ante- and intrapartum screening. First GBS loss was observed in a delivery occurring 2 weeks following antepartum screening. A Cohen's κ of 0.86 was observed between ante- and intrapartum results. However, the observed overall GBS acquisition and loss rates in the window between antenatal screening and delivery were 3.9% and 7.7%, respectively, meaning 7.7% of antepartum GBS-carrying women did not have detectable colonisation at delivery, leading to unnecessary intrapartum antibiotic prophylaxis. Notably, two GBS-carrying women antepartum received antibiotics after screening, which may have contributed to the vaginal eradication of GBS before delivery. Conversely, 14.3% of GBS-carrying women at delivery were missed by antepartum screening, although this did not result in neonatal early-onset GBS disease. Unstratified (overall) Kaplan-Meier analysis revealed that by 3.7 weeks before delivery, GBS status retention begins to decline more noticeably, marking the last time point where the survival probability remains above 95%, with the lower bound of the 95% CI tight at 92.9%. By week 4.3, the survival rate drops to 94.7% and the lower bound of the 95% CI decreases to 90.7%, indicating a progressive loss of status retention and increased uncertainty (Figure 4B). Stratified Kaplan-Meier analysis by initial GBS status confirmed this timepoint as suitable for women with a negative antepartum screening, whose survival curve closely mirrored the overall trend. In contrast, women initially testing positive for GBS showed a steeper decline in retention, from 97.9% at week 2 to 55.0% by week 5.4, with survival falling below 95% by week 3.4 and the lower CI bound dropping to 86.6%. These findings suggest a shorter interval of 2 weeks may be more appropriate for those colonised by GBS at antepartum screening (Figure 4C).

Discussion

This multicentre study evaluated the predictive window of antenatal GBS screening using real-time PCR with the Panther Fusion® GBS assay (Hologic®). First, this PCR assay was assessed both with and without broth-enrichment and compared to broth-enriched culture. A McNemar's test was performed indicating that broth enrichment significantly increased PCR detection compared to non-enriched PCR, aligning with previous findings (20, 21). Moreover, broth-enriched PCR out-performed broth-enriched culture, confirming data of Church *et al.* (22) and Shin & Pride (73.5%) (23). We observed nine broth-enriched PCR GBS-positive cases of which cultures remained negative. Eight PCR results were confirmed by the Xpert® Xpress GBS assay. However, one specimen (Ct 39.9) tested negative by Xpert®, likely reflecting the assay's limit-of-detection given the low GBS DNA load (Ct 39.9 with the Panther Fusion® GBS assay). In addition, it should be noted that the comparator assay was not validated for the tested medium, which may have contributed to this discrepancy. Culture negativity in such cases could be attributed to subjective agar interpretation, extensive vaginal hygiene, overgrowth by other enteric organisms or prior antibiotic exposure. In our cohort, three women had Ct values below 27, and none of them had received antibiotics within at least 10 weeks prior to sampling. On the other hand, one woman with a high GBS Ct value received azithromycin at approximately 31 weeks of gestation, which could have contributed to a culture negative result. Although the clinical relevance of samples with high Ct values is still unclear, even low-level GBS colonisation at delivery can result in vertical transmission if intrapartum antibiotic prophylaxis is not administered (16, 18). However, the potential for false-positive Panther Fusion® GBS assay results leading to erroneous classification of non-colonized women as GBS-positive cannot be entirely excluded. We agree with Rallu *et al.* to encourage studies on the predictive thresholds of Ct's for assessing the risk of vertical transmission and development of neonatal early-onset GBS disease (24).

In a next step, the predictive window of antepartum GBS screening was assessed by comparing ante- to intrapartum results. In our study, twelve women either acquired or lost GBS colonisation between ante- and intrapartum screening, emphasising dynamic GBS colonisation in pregnant women (3, 8, 15–17). European experts therefore put forward intrapartum screening to cover this dynamic maternal GBS colonisation. However, our data showed a persistent GBS colonisation rate of 92.3% and 96.1% for antepartum GBS-positive and GBS-negative women, respectively, when the Panther Fusion® GBS assay was used. Unstratified Kaplan-Meier analysis suggests that optimal timing for antepartum screening would be 3.5 to 4 weeks before delivery, based on retention rates remaining above 95% within this timeframe. Clinically, one could reason based on the stratified Kaplan-Meier analysis that adopting a screening interval of 3.5 to 4 weeks would be adequate and clinically justifiable, as GBS colonisation loss poses fewer risk for invasive neonatal early-onset GBS disease than missed GBS acquisition at delivery. This screening window aligns closely with recommendations proposed by other authors (25, 26). Boyer *et al.* were the first to study the predictive window of antenatal GBS screening, using broth-enriched culture. These authors reported an overall PPV and NPV between ante- and intrapartum screening of 67.2% and 91.5%, respectively (18). They observed a 100% NPV for antepartum screening within a 10-week window before delivery. However, in our study, acquisition of GBS colonisations already occurred 2 weeks after antepartum screening. Additionally, we observed loss of GBS colonisation within 2 weeks after antenatal screening, while Boyer *et al.* observed this only after 6 weeks. Other studies confirmed that antenatal screening if performed with PCR closer to the time of delivery improves its accuracy (16, 27). Yancey *et al.* similarly observed improved predictive values with broth-enriched culture-based antenatal screening being performed 5 weeks before delivery, supporting an antenatal screening policy between 35–37 weeks of gestation (25). Valkenburg-van den Berg *et al.* confirmed this screening window, but highlighted limitations of culture-based testing, which showed a PPV and NPV of 69% and 94%, respectively (26). Hussain *et al.* also noted weak agreement (Cohen's $\kappa < 0.60$) between ante- and intrapartum results, using culture-based methods, underscoring the unpredictable nature of GBS colonisation (28). In contrast, we determined a significantly higher agreement (Cohen's $\kappa = 0.86$) between ante- and intrapartum results, using PCR. Differences in sampling methods and laboratory culture protocols may introduce variability across studies, complicating comparisons. Furthermore, unlike previous studies relying solely on culture, we demonstrated superior sensitivity with PCR, so one could reason that PCR might improve diagnostic accuracy of antenatal GBS detection.

While our analysis suggests that screening 3.5 to 4 weeks prior to delivery is optimal for GBS retention, translating this into a practical guideline requires consideration of real-world gestational age distributions. National data from Belgium (2022) indicate that approximately 84.0% of women delivered between 37 and 41 weeks of gestation, with 8.1% after 41 weeks of gestation. Assuming all pregnant women were screened, screening results are only reliable for 4 weeks and delivery occurs within 4 weeks following antepartum screening, screening between 35 and 37 weeks of gestation would effectively cover approximately 87.4% of women. However, shifting the screening window to 36–38 weeks of gestation would extend coverage to approximately 92.0% of women. Notably, this one-week adjustment in screening timing would therefore increase population coverage by about 5.2%, enhancing the reliability of antenatal screening. While intrapartum screening would allow GBS detection at time of delivery, its implementation presents logistical and financial challenges and does not allow to perform AST. An important pitfall of current commercially available PCR assays is their inability to determine antimicrobial resistance markers, necessitating reflex culture to perform susceptibility testing.

This study has some limitations. First, there may be a bias in the intrapartum cohort, because women had to consent to be included. This bias may partly be explained by language barriers or other factors affecting willingness or ability to participate. Finally, the intrapartum subgroup is relatively small, and no formal sample size calculation was performed to assess for potential selection bias.

In conclusion, our data indicate antenatal screening at 3.5 to 4 weeks prior to delivery as a more optimal predictive window, especially when used in combination with broth-enriched PCR to improve sensitivity. Screening between 36 and 38 weeks of gestations may offer a more favourable balance between GBS retention and population coverage. Although antenatal screening is not perfect, our data demonstrate that the broth-enriched Panther Fusion® GBS assay effectively identifies the vast majority of GBS carriers at delivery. Broth-enriched PCR identified GBS colonization in a greater number of pregnant women than either broth-enriched culture or non-enriched PCR. However, the potential for false-positive PCR results leading to erroneous classification of non-colonized women as GBS-positive cannot be entirely excluded. Further studies should aim to clarify the clinical significance of positive PCR results by evaluating their association with true maternal GBS colonization, vertical transmission, and early-onset neonatal disease. Additional research should also aim to delineate the predictive window in a larger, more generalisable population. Lastly, future studies should evaluate the cost-effectiveness of PCR-based screening in relation to its clinical benefits, thereby further clarifying the role of PCR in antenatal GBS screening.

Declarations

Conflicts of Interest

This study was funded by Hologic Inc.

Competing Interests

This study was funded by Hologic Inc. PD is a biostatistician who helped with the statistical analysis of our data.

Author Contribution

CW: performed all analysis and basic statistical analysis and wrote the main manuscript text and prepared Figures 1 + 2 + 3 + 4 and Table 1. FDW/HN: provided main feedback on the manuscript text and reviewed the manuscript. FDW also contributed to preparing Figure 3 + 4. PM/CM: provided feedback on study design and reviewed the manuscript text. LC/MG/SVC: reviewed the manuscript text. PD: performed more advanced statistical analysis (constructing Kaplan-Meier etc). SEM: aided in collecting demographic data from included women.

Acknowledgement

We greatly appreciate the contribution of dr. Rosalie Sacheli (University Hospital Centre of Liege, Belgium) and dr. Tom Oosterlaken (Hologic Inc).

Data Availability

The raw data underlying the conclusions of this article are available upon consultation with the corresponding author.

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Figures

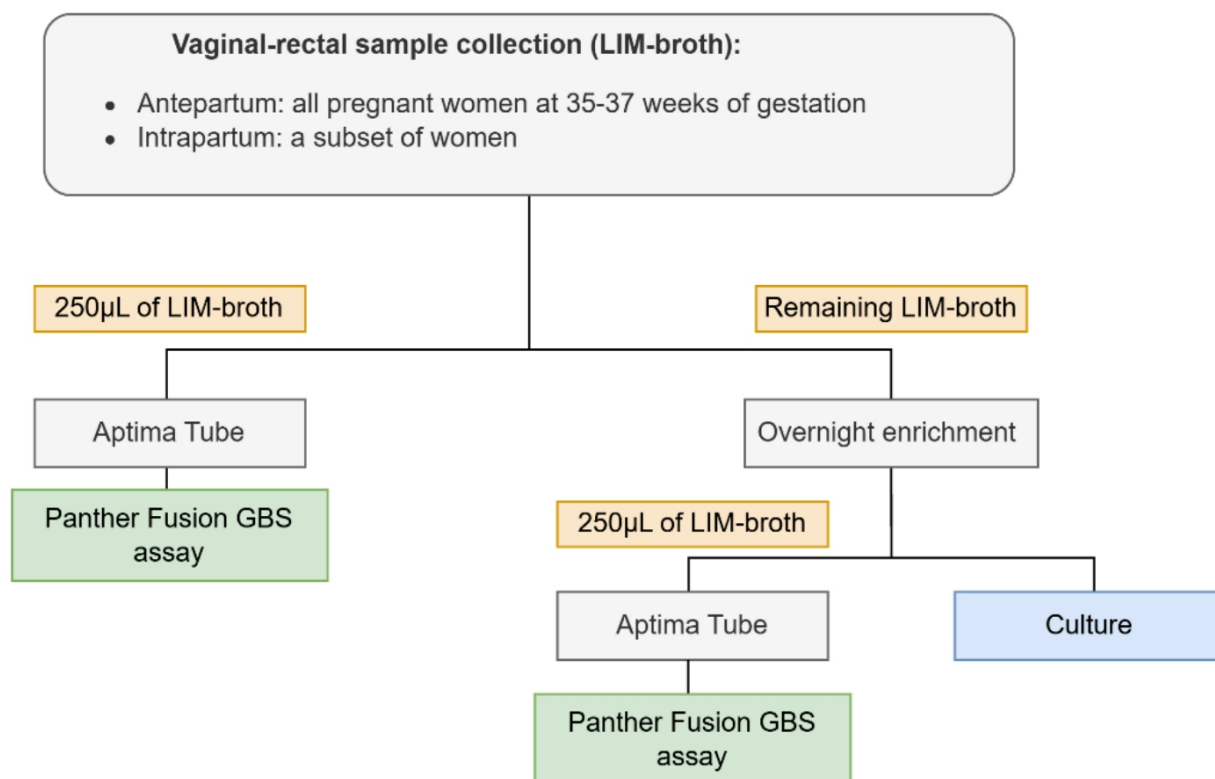


Figure 1

Sample collection and lab workflow.

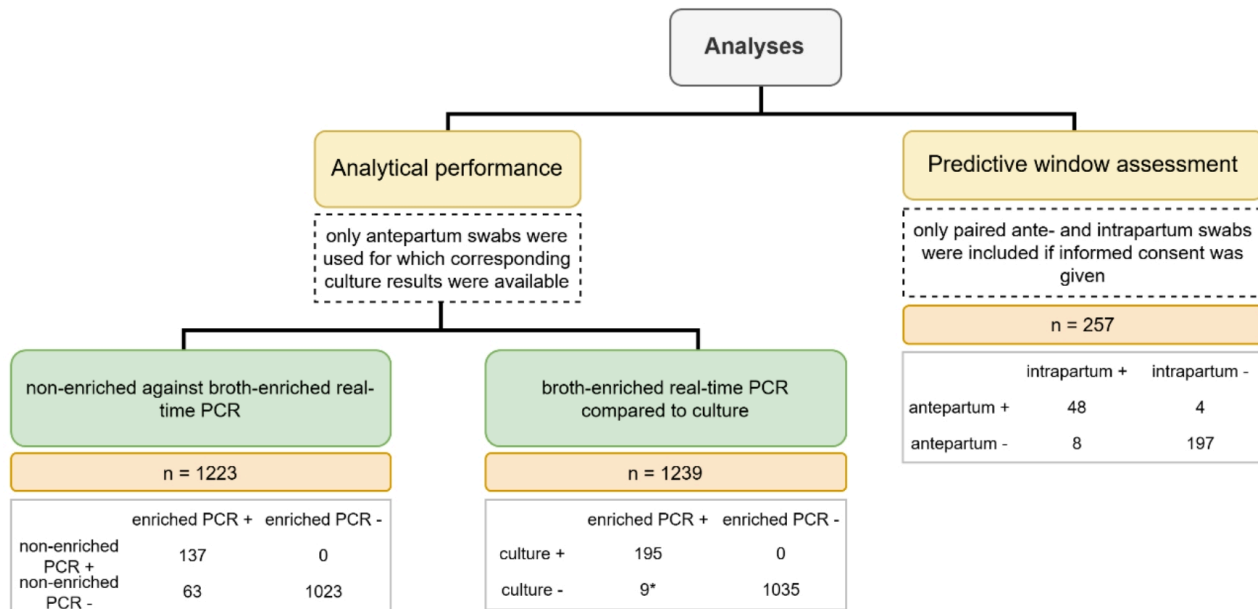


Figure 2

Left: overview of analytical comparison between non-enriched PCR as well as broth-enriched culture to broth-enriched PCR (*9 discordant results resolved using Xpert® Xpress GBS: concordant: n=8, discordant: n=1 (Ct 39.9)). Right: overview of ante- and intrapartum real-time PCR results ('+' = GBS PCR positive; '-' = GBS PCR negative). Real-time PCR was performed using the Panther Fusion® GBS assay (Hologic®)

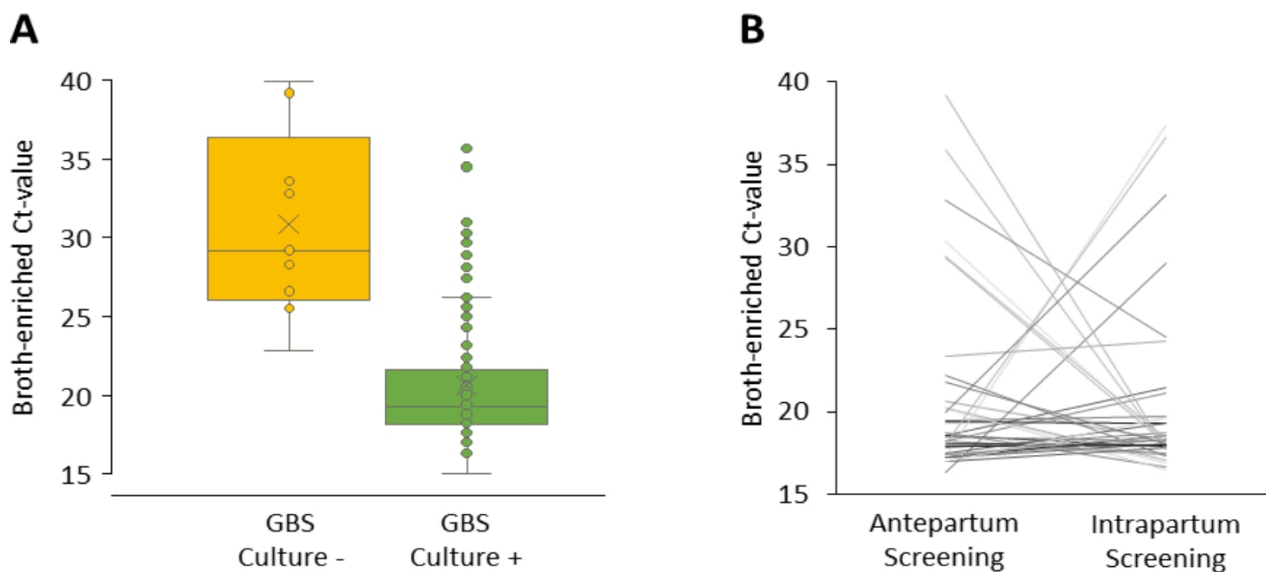


Figure 3

A) Distribution of Ct values of GBS-positive broth-enriched PCR with respect to their broth-enriched subcultures, using only antepartum rectovaginal samples (orange: GBS-negative subcultures, blue: GBS-positive subcultures). B) Comparison of Ct values of GBS- positive broth-enriched PCR both ante- and intrapartum.

A

Time interval (weeks)	GBS-positive women antepartum (n = 52)			GBS-negative women antepartum (n = 205)		
	No. of GBS retention	No. of GBS losses	PPV (%)	No. of persistent GBS-negative cases	No. of GBS acquisitions	NPV (%)
≤1	2	0	100	7	0	100
≤2	7	1	87.5	36	0	100
≤3	20	1	95.2	86	2	97.7
≤4	30	2	93.8	140	4	97.2
≤5	44	3	93.6	172	7	96.1
≤6	48	4	92.3	195	8	96.1
≤7	48	4	92.3	196	8	96.1
≤9	48	4	92.3	197	8	96.1

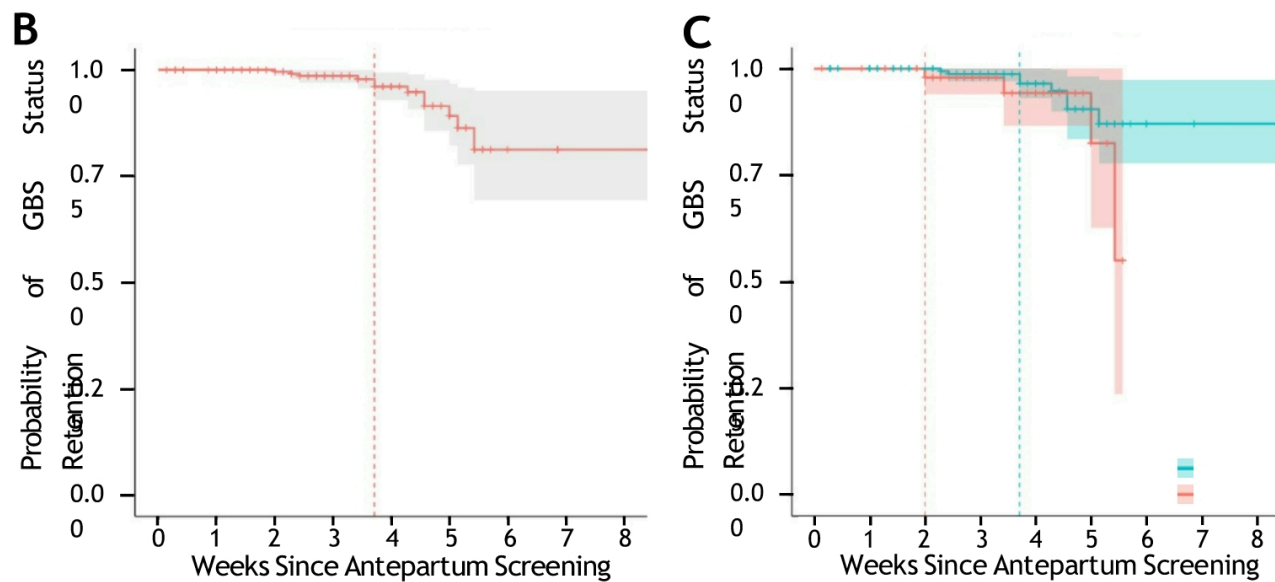


Figure 4

A) Overview of intrapartum GBS-positive and GBS-negative women in relation to antenatal GBS carriage, including positive predictive value (PPV = percentage of GBS-positive women antepartum who remained colonised at delivery) and negative predictive values (NPV = percentage of GBS-negative women antepartum who remained GBS-negative at delivery) per weekly interval, as determined by broth-enriched real-time PCR (n = 257). B) Kaplan-Meier GBS Status Survival Plot. C) Kaplan-Meier GBS status survival plot stratified by antepartum screening result.

Supplementary Files

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- [Supplementaryfilesfinalreview.docx](#)