CAT
Critically Appraised Topic

Group B Streptococcus screening in pregnant women: evaluation of a molecular-based diagnostic strategy

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CLINICAL BOTTOM LINE

Vertical transmission of Group B *Streptococcus* (GBS) during delivery may lead to severe neonatal infections. Intrapartum antibiotic prophylaxis (IAP) can be administered to GBS-carrying women to prevent this transmission. There is an ongoing debate on optimal GBS screening strategies in pregnant women. This Critically Appraised Topic first provides an overview of the current guidelines for GBS screening and their application in Belgian hospitals. Next, a study was conducted to evaluate the analytical performance of the Panther Fusion® GBS assay (Hologic). In this study, 1176 women were screened rectovaginally at 35-37 weeks of gestation (antepartum) and/or at time of labour (intrapartum). Specimens were analysed using broth-enriched culture (golden standard) and (non-) broth-enriched PCR. The overall, positive and negative percent agreement between broth-enriched PCR and culture was 99.2%, 100.0% and 98.9%, respectively (Cohen's κ: 0.97). Although PCR is associated with higher costs, sensitivity of the broth-enriched Fusion® GBS assay was higher compared to culture and facilitates a more optimised lab workflow. Finally, antepartum GBS screening results were compared to GBS colonisation at time of labour. For this, 188 intrapartum screenings were matched with antepartum results. In total, 12.5% of GBS-carrying women at labour did not receive IAP due to negative antepartum screening, while 8.7% of antepartum GBS carriers received unnecessary IAP due to loss of GBS colonisation by the time of delivery. Predictive values of antenatal screening decreased inversely with the interval between antenatal screening and delivery, confirming the dynamic nature of GBS colonisation during pregnancy.

CLINICAL/DIAGNOSTIC SCENARIO

Streptococcus agalactiae, also called 'Group B *Streptococcus'* (GBS), is a β-hemolytic gram-positive coccus and belongs to the group B Rebecca Lancefield streptococci classification (1). Ten different serotypes have been described, all able to colonise the female intestinal and vaginal tract (2,3). GBS colonisation rates during pregnancy vary between geographic regions (e.g. approximately 15-21% in Europe and North America) (4–6). **Maternal rectovaginal colonisation with GBS during pregnancy** is a **primary risk factor** for both **maternal and neonatal morbidity and mortality** and is one of the leading causes of neonatal infections in developed countries (2,3,7). **Maternal complications** range from urinary tract infections to invasive GBS diseases like chorioamnionitis, endometritis and septicaemia. Invasive GBS disease is associated with preterm delivery as well as neonatal and maternal morbidity and mortality (2,7,8). Based on time of onset, **neonatal invasive GBS disease** can be classified in early-onset (EOD), late-onset and late late-onset disease (7–9). GBS EOD is caused by vertical transmission of GBS from mother to child just before or during childbirth (7). Clinical symptoms are manifested within the first week of life, usually between 12-24 hours postpartum. GBS EOD is one of the most common neonatal invasive diseases and most cases present with generalised sepsis, pneumonia or meningitis (7,9,10). Children who survive GBS EOD typically experience long-term morbidity (7). In late-onset GBS disease, GBS is horizontally transmitted to the newborn. Symptoms such as meningitis or fever of unknown origin are developed after the first week of life and within the first three months postpartum (7,10). GBS infections during infancy (that is, after the first 3 months postpartum) are classified as late late-onset GBS disease. Late late-onset GBS disease is commonly manifested as bacteraemia of unknown origin, and less commonly as meningitis, arthritis, cellulitis, or adenitis (9,10). Immunodeficient children and premature children are more prone to develop late-late onset disease (9).

To prevent vertical GBS transmission during labour, **intrapartum antibiotic prophylaxis (IAP)** can be administered (7,10). The Belgian Superior Health Council (BSHC) recommends penicillin G in women without penicillin allergy, whereas cefazolin is preferred for women with a low risk for anaphylaxis (10). In case of severe penicillin allergy, clindamycin should be used (10). Importantly, clindamycin resistance in GBS strains is increasing according to the latest data of the National Reference Centre (NRC) for *Streptococcus agalactiae* (i.e. Centre Hospitalier Universitaire Liège). Approximately 28% of the GBS strains isolated in clinical samples in Belgium in 2021 were resistant to clindamycin, necessitating **antimicrobial susceptibility testing** in case of severe penicillin allergy (11). Vancomycin should be administered in case of colonisation with clindamycine resistant GBS strains (10).

It is not recommended to administer IAP to every pregnant woman since this could result in increased antibiotic resistance, allergic reactions in women at labour, and even more severe adverse events such as necrotising enterocolitis in neonates (10,12,13). Therefore, determination or estimation of rectovaginal GBS colonisation in pregnant women is essential and IAP should only be administered in GBS-carrying women or in women with a high risk of GBS colonisation at delivery (10). Different risk- and microbiology-based strategies have been put forward to estimate and/or determine the GBS colonisation status of pregnant women at labour. Thanks to their implementation, the incidence of GBS EOD globally has decreased to approximately 0.49 per 1000 live births, with notable regional differences (12,14). In the clinical laboratories of AZ Turnhout, AZ Herentals, and HH Mol (hereafter HETUMO), we observed 7 cases of GBS EOD over the past five years. Three of these women showed a negative antepartum GBS screening. In two women GBS was detected antepartum, but no IAP was given. For one woman, no GBS screeningsresult could be retrieved, however no IAP was administered. One woman was GBS-positive antepartum and received IAP, but suffered from prolonged duration of membrane rupture.

Currently, there is **no universal recommendation concerning GBS screening strategies**. GBS is known to colonise the rectovaginal tract intermittently, transiently or persistently and often asymptomatically (7,15–18). Therefore, adequate timing and optimal sensitivity of GBS screening is crucial. The BSHC guidelines recommend a **culture-based screening** performed on rectovaginal swabs for all pregnant women at 35-37 weeks of gestation (10). For women who develop GBS bacteriuria at any time during their pregnancy and/or had a previous child with invasive GBS disease, screening is not recommended and IAP should always be administered (10,12). Other guidelines, including the American Society for Microbiology (ASM), also recommend culture-based methods (19). In the last decades, the benefits of **nucleotide acid amplification tests (NAATs)** have been explored for ante- and intrapartum GBS screening. Notably, in 2014, European experts put forward an **intrapartum screening**, using a **fast and sensitive NAAT**, as the preferred screening strategy (15). In **HETUMO**, a **culture-based GBS screening** is applied for all pregnant women at 35-37 weeks of gestation except for women with GBS bacteriuria at any time during the current pregnancy and/or with a previous GBS-infected child.

This **Critically Appraised Topic** (**CAT**) reviewed three guidelines on GBS screening. Next, a national survey was distributed to Belgian clinical laboratories aiming to obtain an overview of the currently used GBS screening strategies. Third, the analytical performance of the Panther Fusion® GBS assay (Hologic) was evaluated using both non-enriched and broth-enriched rectovaginal specimens of pregnant women and compared to broth-enriched culture results. Additionally, the financial impact of a PCR-based antenatal strategy was investigated in HETUMO. Finally, antepartum screening results at 35-37 weeks of gestation were compared to the GBS carriage status of women at time of labour, aiming to better understand the predictive window of antepartum GBS screening.

QUESTION(S)

1. What do guidelines recommend regarding GBS screening in pregnant women?
2. How are GBS screening guidelines implemented in Belgian clinical laboratories?
3. What are the analytical performance and financial impact of the Panther Fusion® GBS assay compared to culture for antenatal GBS screening?
4. What is the predictive value of antenatal GBS screening at 35-37 weeks of gestation for GBS carriage at delivery?

LIST OF ABBREVIATIONS

ACOG	American College of Obstetricians and Gynecologists
ASM	American Society of Microbiology
AST	Antimicrobial Susceptibility Testing
BSHC	Belgian Superior Health Council
CAMP	Christie-Atkins-Munch-Petersen test
CAT	Critically Appraised Topic
CDC	Centers for Disease Control and Prevention
CFU	Colon Forming Units
CHU	Centre Hospitalier Universitaire
CI	Confidence Interval
Ct	Cycle threshold
CV	Coefficient of Variation
EOD	Early-onset Disease
GBS	Group B Streptococcus, *Streptococcus agalactiae*
HOT	Hands-on Time
HETUMO	Clinical laboratories of AZ Turnhout, AZ Herentals and HH Mol
i.a.	inter alia
i.e.	id est, that is
IAP	Intrapartum Antimicrobial Prophylaxis
IFU	Instructions For Use
iv	Intravenously
LIM broth	Todd Hewitt broth with colistin and nalidixic acid
LOD	Limit Of Detection
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight
NA	Not Available
NAAT	Nucleic Acid Amplification Test
No.	Number of
NPA	Negative Percent Agreement
NPV	Negative Predictive Value
NRC	National Reference Centre
OPA	Overall Percent Agreement
po	Per os
PPA	Positive Percent Agreement
PPV	Positive Predictive Value
RIZIV-INAMI	Rijksinstituut voor ziekte- en invaliditeitsverzekering - Institut national d'assurance maladie-invalidité
SD	Standard Deviation
TAT	Turnaround Time

RELEVANT EVIDENCE/REFERENCES

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In **Attachment 1**, a complete list of all references can be found.

Q1: WHAT DO GUIDELINES RECOMMEND REGARDING GBS SCREENING IN PREGNANT WOMEN?

The following guidelines are discussed:

- Preventie van perinatale groep B streptokokkeninfecties, Belgian Superior Health Council (BSHC) (2003) (10)
- Belgian pediatric guidelines on the management of neonates at risk for GBS EOD (2014) (13);
- Guidelines for Detection and Identification of Group B *Streptococcus*, Centers for Disease Control and Prevention (CDC) (2020)/American Society of Microbiology (ASM) (2020) (19,20);
- Prevention of Group B Streptococcal Early-Onset Disease in Newborns, American College of Obstetricians and Gynecologists (ACOG) (2021) (21);
- Intrapartum GBS screening and antibiotic prophylaxis: a European consensus conference (2014) (15).

In general, **three preventive screening strategies** can be used to determine and/or estimate maternal GBS colonisation at delivery: (i) a risk factor-based approach, (ii) an antepartum screening, and (iii) an intrapartum screening. Note that although these GBS screening strategies help to reduce the risk for GBS EOD, they do not prevent (late) late-onset GBS disease or other GBS-related complications such as stillbirth. The Design of a Vaccine Against Neonatal Infections consortium, established in 2008, is a pan-European initiative to assess neonatal GBS disease burden in Europe, to provide clinical and microbiological information for vaccine design, and to improve laboratory performance in diagnosing GBS colonization and infection (14). In the future, GBS vaccines might aid in further reducing the risk for GBS EOD as well as (late) late-onset neonatal GBS disease.

Q1.1 RISK FACTOR-BASED STRATEGY

A **risk factor-based approach** estimates GBS colonisation of pregnant women at delivery based on obstetric parameters. Obstetric factors associated with a higher risk for neonatal GBS EOD include delivery at < 37 weeks of gestation, preterm rupture of membranes, previous pregnancy with a GBS-infected child, fever (> 38°C) during labour, and GBS bacteriuria during the current pregnancy (10,15). If one or more obstetric risk factors are present, IAP should be administered. However, a strategy solely based on risk factors has poor sensitivity and is therefore not recommended by the BSHC nor by the American and European guidelines (10,15,19–22).

Q1.2 ANTEPARTUM SCREENING

The Belgian and American guidelines currently recommend an **antepartum culture-based strategy** for GBS screening for all pregnant women (10,19–21). Exceptions are pregnant women who develop GBS bacteriuria during the current pregnancy or had a previous child with GBS invasive disease. In these cases, it is recommended to administer IAP regardless of screening results (10,19–21). Notably, European experts favour intrapartum GBS screening over antepartum screening, due to insufficient evidence for culture-based antenatal screening (15). Moreover, several studies reported poor positive (PPV) and negative predictive values (NPV) associated with antepartum culture-based GBS screening, likely due to dynamic colonisation of GBS in pregnant women (16–18).

All guidelines recommend to collect rectovaginal specimens using a single flocked swab, without the use of a speculum (10,15,19–21). The timing is based on a predictive window of GBS antenatal screening of 5 weeks (19,23). Therefore, the BSHC and European experts advise antepartum screening at 35-37 weeks of gestation. On the other hand, the American guidelines recommend screening at 36-37 weeks and six days of gestation. This is based on the study of Martin *et al* who demonstrated that about 7% of American women give birth at or after 41 weeks of gestation (19–21,24). Both the Belgian and American guidelines advise transporting the swabs to the clinical laboratory using a non-nutritive transport medium like Amies (10,20). According to the BSHC guidelines, swabs should be delivered to the lab as soon as possible, and certainly within 48 hours (10). In case of delay, swabs must be stored at 2-8°C (10). The American guidelines, on the other hand, advise to transport swabs to the lab within 24 hours; if not, specimens should be rejected (19,20). Next, swabs should be inoculated in a selective enrichment broth, preferably Todd-Hewitt broth with colistin and nalidixic acid (known as LIM broth), followed by aerobic incubation overnight at 35-37°C (10). This enrichment promotes growth of gram-positive bacteria, while suppressing growth of several gram-negative enteric bacteria. According to the American guidelines and the European consensus, overnight incubation could also be in ambient air or using 5% CO_2 (15,19,20).

In contrast, European experts recommend direct collection of rectovaginal samples in selective enrichment broth (preferably LIM broth) to enhance the yield of GBS (15). LIM broths should be transported to the lab as soon as possible or stored at room temperature for a maximum of 4 days (15). Additionally, inoculation of the rectovaginal samples onto a screening agar before enrichment can be considered. This could enhance sensitivity as the presence of abundant other enteric organisms like *Enterococcus spp.* could cause overgrowth of GBS during enrichment, leading to false negative results (19,20). However, note that direct plating should never replace culture after enrichment (10,19,20).

The Belgian guidelines recommend subculturing on Granada agars, i.e. chromogenic media where GBS colonies appear as orange-red colonies due to their haemolytic properties (10). If unavailable, enriched broths can be subcultured on sheep blood agars (10). The American guidelines recommend different types of selective as well as non-selective media, emphasizing the importance of detecting both haemolytic and non-haemolytic strains (19,20). After 18-24 hours or up to 48 hours of incubation, depending on the culture media, GBS-suspected colonies are identified. The BSHC recommends latex-agglutination tests or similar methods for identification (10). In addition, the American guidelines also put forward the Christie-Atkins-Munch-Petersen (CAMP) test and matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry as identification methods (19,20). They do not recommend rapid immunoassays directly on swabs or enrichment broths due to their low sensitivity (19,20,25–28). Interestingly, the American guidelines point out that MALDI-TOF can differentiate *S. agalactiae* from *S. pseudoporcinus* and *S. halichoeri*, while latex agglutination tests cannot (20). However, the clinical importance of *S. pseudoporcinus* and *S. halichoeri* is not yet fully understood (29,30). One could interpret these results as false positive GBS results, resulting in unnecessary IAP administration (30). Conversely, some studies suggest that rectovaginal colonisation by *S. pseudoporcinus* might also impact pregnancy outcomes (29).

Although culture-based GBS screening strategies are used in many countries, one of their major drawbacks is the variable sensitivity and specificity compared to NAATs (31–36). Operator- and protocol-dependent factors such as storage and transport conditions, subjective interpretation of growth on screening agars, and incubation protocols all contribute to lower sensitivity and specificity of culture compared to NAATs. Patient-related factors such as personal hygiene and recent antibiotic use could also result in false negative cultures (15). Both American and European experts highlight the advantages of using **NAATs on enriched broths for GBS detection**, including increased sensitivity, shorter turn-around-time (TAT) and less hands-on-time (HOT) (15,19,20). However, it is important to note that broth enrichment before PCR analysis remains advisable as it significantly increases sensitivity for GBS detection (37). Consequently, the American guidelines do not currently recommend NAATs directly performed on non-enriched specimens (19,20). Whether a shortened enrichment protocol could be used, remains to be determined (31,37). Despite their advantages, NAATs also have limitations when used for GBS detection in pregnant women. Firstly, there are no PCR assays currently available that can detect susceptibility of GBS strains to clindamycin or other antimicrobial agents. Secondly, genetic variability in the targeted DNA region could lead to false negative results, highlighting the importance of genotypical surveillance of GBS strains (38,39). Therefore, the addition of target genes in PCR assays should regularly be evaluated (36,37). Thirdly, NAATs are costly and require PCR platforms and trained staff. Finally, weak positive results should be interpreted with caution, as they might indicate the presence of dead material or a (very) low bacterial GBS load. It has been shown that a higher bacterial GBS load is associated to an increased risk of vertical transmission (23,40). Nevertheless, even a low GBS load can still result in vertical transmission and GBS EOD in the neonate, particularly in women with prolonged duration of membrane rupture (40).

Both Belgian and American guidelines acknowledge the limitations of a culture-based antenatal screening strategy but continue to recommend this approach (10,19,20). These guidelines do emphasize the importance of optimising measures to enhance sensitivity and specificity of culture-based methods. While the BSHC guidelines do not extensively address NAATs (10), the American guidelines consider broth-enriched NAATs an acceptable alternative to culture (19,20). In contrast, the European consensus recommends intrapartum GBS screening as the preferred strategy (15). It is important to note that at the time these guidelines were published, there was limited evidence regarding intrapartum screening strategies (10,19,20). For a detailed overview of antepartum GBS screening recommendations, see Supplementary Table 1.

Q1.3 INTRAPARTUM SCREENING

To accurately determine the GBS carriage status in pregnant women at labour, rapid diagnostics like NAATs or non-molecular tests are needed. **Non-molecular rapid diagnostic tests** include techniques like latex agglutination and immunoassays, immune-sorbent technology, optical-immunotechnology, and probe hybridisation tests (10,15,19,20). In general, these tests have a variable and low sensitivity (10,25–28,34,41).

The Belgian guidelines mention the Strep B OIA test, a rapid optical immunoassay, as a test to assess GBS carriage of women with an unknown GBS status at time of labour. However, due to its limited sensitivity, only positive results are reliable (10,28,42). Consequently, both European consensus and American guidelines do not recommend the use of rapid antigen tests, emphasizing their high specificity but low sensitivity (15,19,20).

Over the past three decades, there have been significant advancements in **NAAT rapid diagnostics** for GBS detection. In 2003, the IDI-Strep B® real-time PCR assay (Infectio Diagnostics) was introduced. However, studies showed conflicting data regarding its analytical performance compared to antenatal culture (43,44). Most of the GBS assays currently available have been evaluated using broth-enriched rectovaginal specimens (31–36,45). For intrapartum screening, however, point-of-care tests that can be performed directly on specimens without an additional enrichment step are essential (16,46). Relying solely on intrapartum PCR for the prevention of GBS EOD also entails some risks and practical challenges, most importantly the inability to perform AST (42,47). On top of that, implementing rapid PCR tests would imply trained personnel, the need for PCR platforms in the delivery room, and higher costs. Ensuring minimal TAT of these NAATs is of utmost importance, but this could be compromised by invalid results and high error rates in delivery wards (16,48,49).

Regarding rapid PCR tests, the BSHC mentions the ongoing development of real-time PCR tests but emphasizes that both costs and clinical workload should be considered (10). More recent American guidelines and European experts elaborate more on the application of NAATs in both ante- and intrapartum settings. The American guidelines consider broth-enriched NAATs as an acceptable alternative to culture for antepartum screening (19,20). Potential advantages of intrapartum PCR are also recognised, such as low HOT and TAT as well as easy workflow and more accurate representation of GBS colonisation at labour. Nevertheless, they also underline the importance of an initial enrichment step and do not recommend direct-from-specimen NAATs (19,20). On the other hand, European experts recommend intrapartum PCR as the new golden standard, citing its superior PPV and NPV, simplified workflow, lower TAT, the intermittent nature of GBS colonisation, and its advantage to reduce unnecessary and missed IAP administrations (15). Nonetheless, these experts also acknowledge certain limitations of rapid NAATs, including inability to perform AST, potential delay in IAP administration, and high costs (15). As long as it is not feasible to detect clindamycin resistance using NAATs, antenatal screening at 35-37 weeks of gestation remains essential for women with severe penicillin allergies (19,20). An overview of the guidelines on intrapartum GBS screening is provided in Supplementary Table 1.

Q2: HOW ARE GBS SCREENING GUIDELINES IMPLEMENTED IN BELGIAN CLINICAL LABORATORIES?

Q2.1 A NATIONAL SURVEY ON GBS SCREENING POLICIES IN BELGIAN CLINICAL LABORATORIES

To obtain an overview of the currently applied GBS screening strategies in Belgian clinical laboratories, a national survey was conducted in collaboration with the NRC and Sciensano. This anonymous survey was distributed to all Belgian clinical laboratories (n = 116). In total, 68 laboratories participated in the survey (58.6%). Five incomplete responses were excluded, resulting in 63 included responses of which 7 from academic hospitals, 43 from non-academic hospitals and 13 from private laboratories. Of these 63 laboratories, 60 perform GBS screening in their routine practice. Almost all laboratories perform **antepartum GBS screening (95%)** mainly **between 35-37 weeks of gestation (96%)**. Three laboratories offer both ante- and intrapartum culture-based screening. Apart from one laboratory that only performs PCR and one laboratory that uses both culture and PCR, all laboratories perform **culture-based** antepartum screening. Lower costs (87%) and reimbursement of culture methods (78%) were mentioned as the main advantages. Other arguments in favour of culture were the high costs of (intrapartum) PCR (38%), lack of local guidelines recommending molecular (intrapartum) screening (37%), unavailability of PCR techniques at delivery wards (35%) and lack of AST with PCR (27%). However, culture methods are challenged by TAT (68%) and low sensitivity (67%).

The majority of laboratories perform **AST on all GBS-positive samples** (57%) due to an often unknown penicillin allergy status in pregnant women (52%) and/or due to the more convenient workflow (25%). Sixteen laboratories perform AST only in case of penicillin allergy (27%) and seven only on request by gynaecologists (12%). Two laboratories do not perform AST at all. The combination of penicillin, clindamycin and erythromycin (19%) and additionally vancomycin (38%) is most often tested and reported. Eleven laboratories test penicillin, clindamycin and erythromycin together with other antibiotics such as cefotaxime, cefazolin, moxifloxacin, sulfamethoxazole-trimethoprim or gentamicin using AST cards on automated systems. Note that, despite testing multiple antibiotics, they are not always all reported. Almost all centres administer penicillin for IAP (93%) and, in case of penicillin allergy, clindamycin (67%) is most often used. Note that the survey did not make a distinction between low- and high-risk penicillin allergy nor did we inform about which antimicrobial agent to use when GBS strains are clindamycin resistant.

The majority of laboratories showed **interest in molecular tests for GBS screening (81%)**; thirty-seven percent of them were only interested in fast PCR, 12% only in batch testing and 31% in both. Twelve laboratories showed no interest in molecular-based strategies due to high costs (100%), unavailability of AST (90%), the need for additional molecular infrastructure at delivery wards (54%) and the need for trained staff (36%). One laboratory did not have a maternity ward and one laboratory was not BELAC-accredited for molecular biology, hence their disinterest in molecular GBS assays.

Q2.2 AN OVERVIEW OF THE APPLICATION OF NATIONAL GUIDELINES IN HETUMO

The GBS screening strategy used in HETUMO is similar to the one recommended by the BSHC. We receive +/- 2820 rectovaginal samples for GBS screening per year (data from 2022 and 2023) and we observe a positivity rate of +/- 17% (data from 2019 - 2022). All isolated GBS strains were susceptible to penicillin, whereas clindamycin resistance ranged from 18% in 2020 to 15% in 2022 in our centres.

All pregnant women are screened between 35-37 weeks of gestation, unless IAP is required regardless of the GBS screening results (i.e., GBS bacteriuria at any time during the current pregnancy and/or a previous child with invasive GBS disease). Rectovaginal swabs are taken from the lower one-third of the vagina, then across the perineum followed by the insertion through the anal sphincter. After collection, swabs are directly inoculated in LIM broth (Copan, Italy) and transported at room temperature to the clinical laboratory at campus Sint-Jozef in AZ Turnhout. Once arrived in the lab, swabs are aerobically incubated 18-24h at 35°C and subsequently subcultured on chromogenic CHROMID Strepto B agars (bioMérieux, France). The subcultured agars are then incubated for 18-24h at 35°C in 5% CO_2. Hereafter, agars are checked for suspected colonies (i.e., pink-purple-coloured colonies). If none are present, agars are re-incubated for an additional 18-24 hours. Suspected colonies are identified by mass spectrometry, either on Vitek-MS (BioMérieux, France) or on Maldi Biotyper Sirius (Bruker, USA). Results are reported qualitatively, i.e. GBS-positive or negative. AST for penicillin, clindamycin, and erythromycin is performed for each isolated *S. agalactiae* strain using disk diffusion on Mueller Hinton agars with 5% sheep blood. EUCAST clinical breakpoints are used for the interpretation of AST results.

Penicillin (loading dose: 5 million IU intravenously (iv), then 3 million IU/4h until after delivery) is the preferred agent for IAP. In case of penicillin allergy with low risk for anaphylaxis, cefazolin (LD: 2g, then 1g iv/8h until after delivery) is recommended. In case of high risk for anaphylaxis and only if the isolated GBS strain is susceptible, clindamycin (900mg iv/8h until after delivery) is the preferred IAP agent. When the isolated GBS strain is resistant to clindamycin, vancomycin (20mg/kg/8h iv until after delivery) should be used. If amnionitis is suspected, IAP is replaced by broad-spectrum antibiotics that are also effective against GBS.

Q3: WHAT ARE THE ANALYTICAL PERFORMANCE AND FINANCIAL IMPACT OF THE PANTHER FUSION® GBS ASSAY COMPARED TO CULTURE FOR ANTENATAL GBS SCREENING?

A multicentre study was conducted in HETUMO to evaluate the analytical performance and financial impact of the **Panther Fusion® GBS assay** (**Hologic**). During the study period, this PCR assay was used next to culture on all rectovaginal screening swabs taken at 35-37 weeks of gestation, as well as on screening swabs taken intrapartum from a subset of women. PCR results were compared to culture in order to explore the potentially added value of the Panther Fusion® GBS assay for antenatal GBS screening.

Q3.1 CHOOSING A MOLECULAR ASSAY

Two molecular platforms, the **GeneXpert®** from Cepheid and the **Panther Fusion®** from Hologic are available in HETUMO, both offering a molecular assay for GBS detection. TAT, targeted gene sequence(s), potential improvements in lab workflow, laboratory costs, reimbursement, and analytical performances reported in previous studies were all considered when choosing the molecular assay.

Three studies already compared the Panther Fusion® GBS assay to other commercially available PCR assays and to culture (more information on these studies can be found in Supplementary Table 2). **Berry *et al*** conducted a comparative study between the Panther Fusion® and BD MAX® GBS assay performed on broth-enriched rectovaginal specimens, using broth-enriched culture as reference method (45). Both PCR assays showed similar analytical performances, though the Panther Fusion® GBS assay allowed a more high-throughput screening with higher loading capacity, overall shorter TAT and HOT. Also, the Panther Fusion® GBS assay showed a slightly lower limit of detection (LOD) (45). **Shin and Pride** compared the Panther Fusion® GBS, Cepheid Xpert® GBS LB, and Luminex Aries® GBS assay to culture using broth-enriched rectovaginal swabs (32). All NAAT assays showed a high level of agreement and demonstrated a higher sensitivity compared to culture. Note that the identification of GBS-suspected colonies was mainly based on the beta-hemolytic character of the GBS strains, which could also explain the lower performance of culture (32). Furthermore, several studies have pointed towards **genetic variations** at the *cfb* gene, which could result in false negative PCR results when using a single *cfb* gene target assay such as the Xpert GBS LB® (37–39). An advantage of the Panther Fusion® GBS assay is the use of two gene targets *cfb* and *sip*, encoding virulence factors CAMP and surface immunogenic proteins, respectively. In 2022, the Xpert GBS LB XC® assay (Cepheid) was launched, which targets a coding region of the glycosyl transferase family protein and a region in the *LysR* family transcriptional regulator of GBS (51). **Thwe *et al*** compared this new Cepheid assay to the Panther Fusion® GBS assay as well as to culture, using broth-enriched rectovaginal specimens (36). Overall, the Xpert GBS LB XC® assay showed similar sensitivity and specificity to the Panther Fusion® GBS assay as well as to culture. Note that the Xpert GBS LB XC® assay is only available in the USA.

To conclude, the Panther Fusion® and Xpert® GBS assays showed similar analytical performances. For our multicentre study, the **Panther Fusion® GBS assay** was chosen due to its capacity for high-throughput screening, the use of two target genes and lower costs (Panther Fusion® GBS assay, 15.00€ per test excl. VAT *versus* Xpert® Xpress GBS assay 50.90€ per test excl. VAT; note that these are list prices).

Q3.2 A MULTICENTRE STUDY COMPARING THE PANTHER FUSION® GBS ASSAY TO CULTURE

Q3.2.1 Methods

Accuracy, reproducibility, precision and impact of storage conditions were investigated for the IVDR-approved Panther Fusion® GBS assay. For this, both broth-enriched rectovaginal screening samples, as well as commercial GBS-control material from Bio-Rad® (Exact Diagnostics GBS verification & validation panel) and Microbiologics® (inactivated GBS-positive and GBS-negative swabs) were used. Accuracy was determined on 14 screening samples (GBS-positive: n = 7, GBS-negative: n = 7). Additionally, 3 GBS-negative screening samples were spiked with GBS serotypes II, III and V. Reproducibility was determined by analysing a pool of GBS-positive screening samples in triplicate on three different days. Another GBS-positive pool was analysed after storing Aptima Lysis Tubes at 2-8°C for 5 days. The impact of freezing at -80°C and thawing of LIM broths was also evaluated. For all PCR analyses, an Aptima Lysis volume of 0.71 mL was used in the same ratio sample to Aptima Lysis Tube volume as mentioned in the IFU of the Panther Fusion® GBS assay. The use of lower Aptima Lysis volumes showed no analytical impact on GBS detection (data not shown).

Next, a multicentre study was conducted between January 15th and February 9th 2024 and between April 15th and July 22nd 2024. During this study period, rectovaginal specimens were collected from all pregnant women at 35-37 weeks of gestation visiting the outpatient clinics of AZ Turnhout, AZ Herentals or HH Mol (standard of care). Additionally, intrapartum screening was performed for a subset of women between April 25th until July 22nd 2024. For this, all pregnant women screened at 35-37 weeks of gestation at the outpatient clinics were informed about this study. Women who were interested in participating in the study were asked to sign an informed consent. For each woman who signed this form, an additional intrapartum rectovaginal specimen was collected during labour and before the administration of IAP if indicated. In cases of an elective caesarean section, rectovaginal specimens were obtained 24h before the operation at the outpatient clinic. Subjects could withdraw their consent at any time and, in such cases, no intrapartum collection was performed. This study was approved by the Ethical Committee of the University Hospital of Antwerp (EC number: 2024-6265) and the local Ethical Committees of AZ Turnhout, AZ Herentals and HH Mol. Physicians and midwives collected the rectovaginal swabs according to the procedures described in Q2.2.

Upon arrival, 250µL of each LIM broth was added to an Aptima Lysis Tube and stored at 2-8°C until analysis (referred to as 'non-enriched'). The remaining LIM broths were then aerobically incubated at 35°C for 18-24h, after which 250µL of the enriched LIM broth was added to a second Aptima Lysis Tube (referred to as 'broth-enriched') and stored at 2-8°C until analysis. Aptima Lysis Tubes were analysed with the Panther Fusion® GBS assay as soon as possible and within 7 days according to the instructions for use (IFU). In case of invalid results, Aptima Lysis Tubes were reanalysed only once. Additionally, the enriched LIM broth was subcultured on a CHROMID® Strepto B agar (bioMérieux, France) and analysed according to the procedures described in Q2.2. Figure 1 provides an overview of the study workflow for sample collection, processing and analysis.

Sensitivity, specificity, PPV and NPV were determined for non-enriched PCR compared to broth-enriched PCR. Next, the rate of agreement between broth-enriched PCR and culture was evaluated by determining the overall (OPA), positive (PPA) and negative percent agreement (NPA), as well as the Cohen's κ. Samples with discrepant results between PCR and culture were sent to the NRC for confirmation testing. Additionally, sensitivity and specificity of broth-enriched culture were compared to PCR after broth enrichment. Finally, the correlation between Ct values of broth-enriched PCR and their subcultures was analysed, for which a boxplot was constructed and a *p*-value was determined. Pregnant women were considered GBS carriers if GBS was detected in rectovaginal specimens either by culture or broth-enriched PCR, the latter in accordance with the IFU of the Panther Fusion® GBS assay. Physicians were notified in case of culture-negative but PCR-positive results to ensure timely administration of IAP.

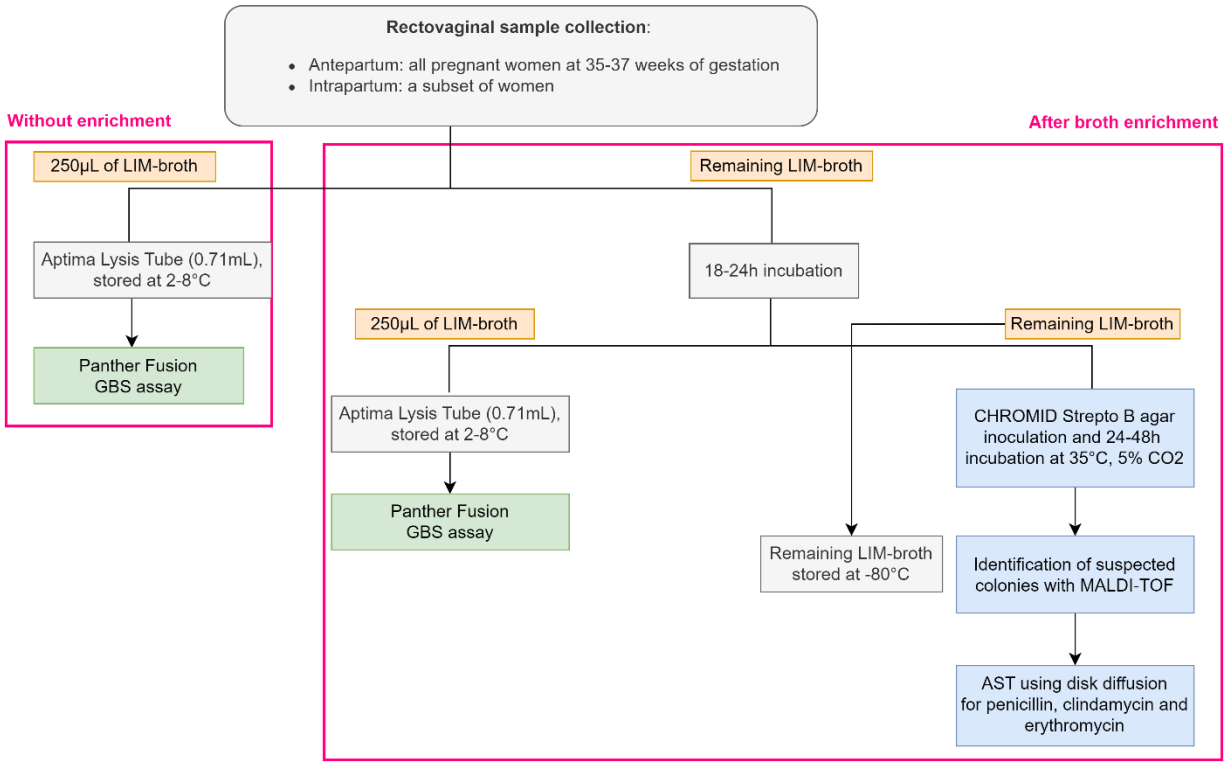

Figure 1: *Workflow for collection, processing and analysis of GBS samples during the multicentre study period.*

Q3.2.2 Results & Discussion

Results of the **analytical performance** of the Panther Fusion® GBS assay are summarised in Table 1. The Panther Fusion® GBS assay showed good reproducibility and accuracy, detecting GBS in all broth-enriched culture-positive screening samples and GBS-spiked samples. Additionally, GBS was detected with PCR in 1 patient sample that was culture-negative; this result was confirmed by the NRC. In total, we found 94.1% concordance between PCR and culture after broth enrichment. No analytical impact was observed when Aptima Lysis Tubes were stored at 2-8°C for 5 days, nor when LIM broths were thawed after storage at -80°C. All inactivated swabs of the Microbiologics GBS panel as well as the GBS control material from Exact Diagnostics (Bio-Rad) were correctly identified. Note that poor reproducibility was observed for most of the positive samples from the Exact Diagnostics panel, most likely due to very low GBS concentrations and further dilution in Aptima Lysis Tubes. The Aptima Lysis Tubes contained 176CFU/mL or 352CFU/mL when loaded on the Panther Fusion®, which is near the LOD of the Panther Fusion® GBS assay.

***Table 1**: Analytical performance of the Panther Fusion® GBS assay: accuracy, reproducibility, precision and impact of storage as well as freezing and thawing.*

Analytical parameter	Results
Accuracy	94.1% concordance between PCR and culture on broth-enriched/spiked screening samples (n = 17)
Reproducibility	Strong PCR-positive GBS samples: mean Ct: 20.07, SD: 0.46 Weak PCR-positive GBS samples: mean Ct: 29.7, SD: 0.54
Precision	GBS control panel Microbiologics® (GBS positive n = 2; GBS negative n = 1): 100% concordance Exact Diagnostics verification & validation panel® Bio-Rad (GBS positive n = 2): 100% concordance
Storage	5-day storage at 2-8°C: mean Ct: 23.18, SD: 0.95
Freeze and thaw	Thawing LIM broths after storage at -80°C: mean Ct: 17.7, SD: 0.00

Next, the **impact of broth enrichment** on PCR sensitivity was explored. For this, 1001 ante- and 238 intrapartum rectovaginal specimens were analysed with and without broth enrichment using the Panther Fusion® GBS assay. Several samples (75 ante- and 29 intrapartum) were excluded because of missing data points (n = 33) or because PCR analysis was performed more than 7 days after inoculation of the Aptima Lysis Tubes (n = 71). Reasons for missing data points were: (i) no PCR result without enrichment (n = 18), (ii) no PCR result after broth enrichment (n = 10), (iii) invalid PCR without enrichment (n = 1) or (iv) invalid PCR after broth enrichment (n = 4). Non-enriched PCR results were compared to broth-enriched PCR results as reference method, since this is in accordance to the IFU of the Panther Fusion® GBS assay. Results are summarised in Table 2. Sensitivity and specificity of non-enriched PCR as compared to broth-enriched PCR were 75.6% and 99.9%, respectively. These results highlight the importance of broth enrichment before PCR testing, as was previously demonstrated in other studies (48,52). Interestingly, one intrapartum sample tested GBS-positive without enrichment (Ct = 39.8), but GBS-negative after broth enrichment. This woman received IAP, but it remains unclear whether IAP was administered before specimen collection. This result might suggest the presence of degraded non-viable GBS or a very low GBS load in the rectovaginal sample.

***Table 2**: Sensitivity, specificity, positive and negative predictive values of non-enriched PCR compared to broth-enriched PCR (n = 1135).*

	Broth-enriched PCR		
Non-enriched PCR	**+**	**-**	
+	155	1	Sensitivity: 75.6%; Specifity: 99.9%; Positive predictive value: 99.4%; Negative predictive value: 94.9%.
-	50	929	

Finally, the **rate of agreement** between **broth-enriched PCR** using the Panther Fusion® GBS assay and **culture** was determined. For this, 1176 rectovaginal specimens were collected either at 35-37 weeks of gestation or intrapartum and analysed with PCR and culture after broth enrichment. OPA, PPA and NPA between broth-enriched PCR and culture were 99.2%, 100.0% and 98.9%, respectively, with a Cohen's κ of 0.97, indicating a high level of agreement (see Table 3). Compared to broth-enriched PCR, sensitivity, specificity, PPV and NPV of **culture** were 95.7%, 100.0%, 100.0%, and 99.1%, respectively (see Table 4). When only antepartum results were considered, sensitivity of culture compared to broth-enriched PCR was slightly higher (96.3%).

Our data align with those of Church *et al* (53). However, Shin & Pride found a much lower sensitivity for culture compared to PCR (**73.5**%) (32). As mentioned in Q1, it is challenging to compare these different studies, given the variety of protocols used for GBS culture and/or PCR (different sampling methods, with or without broth enrichment, different agar media, various incubation and identification methods, …).

Table 3: *Agreement between broth-enriched PCR using the Panther Fusion® GBS assay and culture (n = 1176).*

	Broth-enriched culture		
Broth-enriched PCR	**+**	**-**	Overall Percent Agreement: 99.2% (95%CI, 0.986-0.996); Positive Percent Agreement: 100.0% (95%CI, 0.981-1.00); Negative Percent Agreement: 98.9% (95%CI, 0.983-0.995); Cohen's κ: 0.97.
+	203	9	
-	0	964	

In total, 9 culture-negative samples (antepartum: n = 6; intrapartum: n = 3) tested GBS-positive with PCR. False negative cultures could be a consequence of specimen collection after IAP administration (for intrapartum samples) or due to recent antibiotic treatment. If these data are extrapolated, it is likely that about 22 antepartum GBS carriers would have been missed annualy in HETUMO when relying solely on culture (based on 2819 deliveries annually in HETUMO). Previous research has shown that approximately 50% of GBS-carrying women vertically transmit GBS and, in the absence of IAP, approximately 1-3% of these neonates develop GBS EOD (4,12,21). By using broth-enriched PCR instead of culture for antenatal screening in HETUMO, we could potentially prevent an estimated 0.2 GBS EOD per year.

Table 4*: True GBS-positive/negative samples and false GBS-positive/negative samples obtained with culture and PCR using the Panther Fusion® GBS assay on broth-enriched samples taken ante- or intrapartum (n = 1176).*

	Ante- and intrapartum results (n = 1176)			
Method	True GBS-positives	True GBS-negatives	False GBS-positives	False GBS-negatives
Broth-enriched culture	203	964	0	9
Broth-enriched PCR	212	964	0	0

Table 5 provides an overview of all nine cases with discrepant results between broth-enriched PCR (GBS-positive) and culture (GBS-negative). **Six antepartum specimens** were GBS-positive only after broth enrichment, with Ct values ranging from 25.5 to 39.9. Note that only the very weak GBS-positive sample of case 4 (Ct 39.9 with the Panther Fusion® GBS assay) tested GBS-negative by the NRC. Theoretically, this could be a false positive PCR result due to aspecific reaction. More likely, however, this result could be due to DNA degradation and/or weak reproducibility of PCR in samples with a GBS load near the LOD. Furthermore, the negative culture result in case 9 was unexpected, as the PCR after enrichment showed a Ct of 25.5 and the woman did not receive antibiotics at the time of antenatal screening. Possible explanations for this false negative culture result include subjective interpretation of agar media and/or abundant presence of other enteric organisms overgrowing GBS. The other PCR-positive and culture-negative antepartum cases showed higher Ct values, which could suggest a low bacterial GBS load. None of these women received antibiotics at the time of antenatal screening. Additionally, extensive female hygiene before sampling could also account for these weak positive PCR results. It is difficult to assess the clinical relevance of such weak positive PCR results, as low-level antenatal colonisation could still result in high-density GBS carriage during labour and thus potentially result in vertical transmission (17,23). To our knowledge, the correlation between Ct values and viability of PCR-detected GBS has not been extensively studied, nor are studies available that describe the relationship between Ct values and risk for vertical transmission and/or GBS EOD.

Three intrapartum discrepancies between broth-enriched PCR and culture were observed (see Table 5). All these women received IAP based on GBS bacteriuria and/or a positive GBS antepartum screening. Several factors could explain these false negative intrapartum cultures but weak positive PCR results after broth enrichment, including intrapartum specimen collection after IAP, recent administration of antibiotics before labour, and/or the low sensitivity of culture methods. The timing of specimen collection was specified for only one woman (case 7), i.e. after IAP was administered. In this case and also for case 6, weak GBS-positive PCR results were observed without significant differences in Ct values between broth- and non-enriched PCR. This makes it difficult to conclude whether viable or non-viable GBS was present in these rectovaginal samples. Interestingly, for case 8, we observed significantly lower Ct values after broth enrichment, suggesting the presence of viable GBS.

Although the Panther Fusion® GBS assay is used for qualitative reporting, it is worth taking a closer look at **Ct values** of GBS-positive PCR results and relating them to their subcultures. Figure 2 displays the Ct values of GBS-positive broth-enriched PCR, categorised into two groups, i.e. PCR with positive (orange) or negative (blue) subcultures. A significant difference in Ct values between these two groups was observed ($p < 0.001$). Whether Ct values could help interpret PCR results or serve as predictive thresholds for assessing the risk of vertical transmission, the development of neonatal GBS EOD and the need for IAP remains to be studied. Rallu *et al* recommended against using broth-enriched PCR until quantitative studies of GBS carriage in rectovaginal specimens established a predictive threshold for neonatal disease (34). Negative subcultures in PCR-positive specimens could suggest the presence of non-viable GBS. However, as mentioned earlier, the lower sensitivity of culture compared to PCR can be attributed to several factors including overgrowth of GBS by other enteric organisms, low bacterial GBS loads, extensive female hygiene, recent antibiotic use and subjective interpretation of culture results (10,15,20). Further studies exploring the clinical relevance of PCR results are urgently needed so that predictive threshold Ct values for neonatal disease can be established.

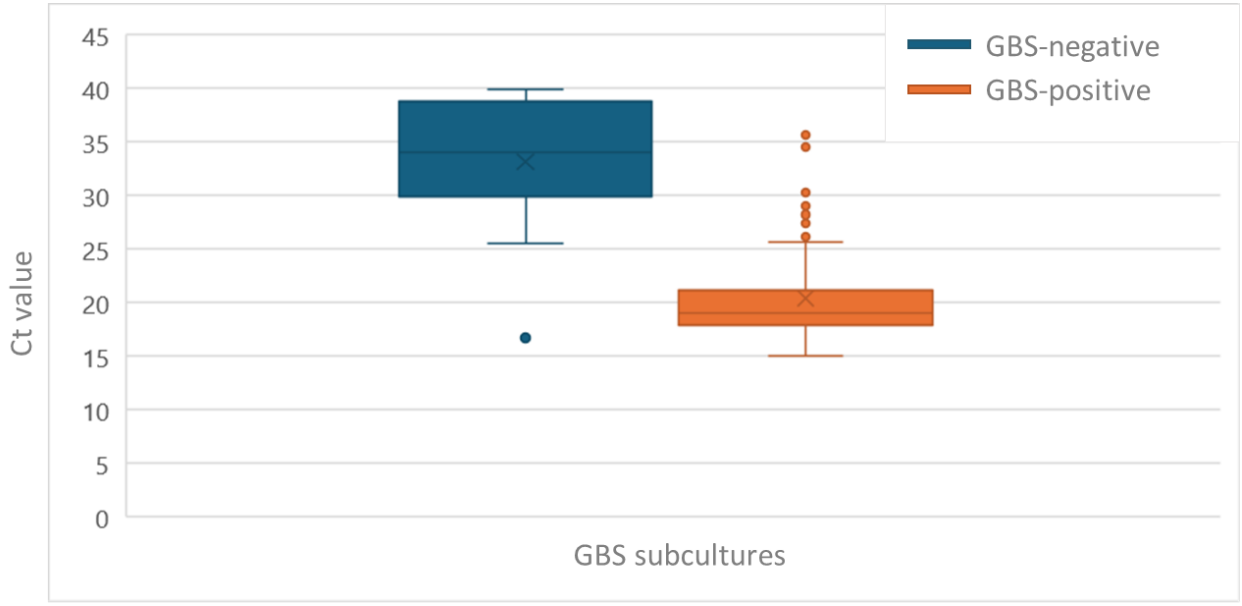

Figure 2: *Distribution of Ct values of GBS-positive broth-enriched PCR with respect to their broth-enriched subcultures (orange: positive subcultures, blue: negative subcultures).*

Table 5: *Overview of discrepant cases with a GBS-positive PCR and GBS-negative culture result in ante- or intrapartum samples. Antibiotic treatment, GBS bacteriuria during pregnancy, IAP and time interval between ante- and intrapartum screening are listed for all cases.*

	ANTEPARTUM					**Time interval** between antepartum screening and delivery	**INTRAPARTUM**			
CASES	Non-enriched PCR	Broth-enriched PCR	Culture	GBS bacteriuria	Antibiotic treatment (molecule, dose and duration)		Non-enriched PCR	Broth-enriched PCR	Culture	IAP
Case 1	NEG	POS (Ct 33.6)	NEG	No	Amoxicillin 3*500mg po, 5d (36 weeks of gestation)	6 weeks	Not available			No
Case 2	NEG	POS (Ct 32.8)	NEG	No	No	2 weeks and 2 days	NEG	POS (Ct 24.5)	POS	Penicillin
Case 3	NEG	POS (Ct 33.9)	NEG	No	No	2 weeks and 4 days	Not available			Penicillin
Case 4	NEG	POS (Ct 39.9)*	NEG	No	No	2 weeks	NEG	NEG	NEG	Penicillin
Case 5	NEG	POS (Ct 39.2)	NEG	No	No	4 weeks and 2 days	POS (Ct 29)	POS (Ct 17.7)	POS	Penicillin
Case 6	POS (Ct 23.4)	POS (Ct 16.3)	POS	Yes (12 6/7 and 36 weeks of gestation)	Fosfomycin (15 weeks and 2 days of gestation)	1 week and 1 day	POS (Ct 31.1)	POS (Ct 29.0)	NEG	Penicillin
Case 7	POS (Ct 34.6)	POS (Ct 17.3)	POS	Yes (17 weeks of gestation)	Fosfomycin and amoxicillin/clavulanic acid 3*875 mg po, 5d (17 weeks of gestation)	2 weeks and 4 days	POS (Ct 34.9)	POS (Ct 37.4)	NEG	Penicillin
Case 8	Not available			Yes (16 weeks of gestation)	Fosfomycin (21 weeks of gestation)	22 weeks	POS (Ct 31.6)	POS (Ct 16.7)	NEG	Penicillin
Case 9	NEG	POS (Ct 25.5)	NEG	No	Clindamycin 3*300mg po, 5d (12 weeks and 4 days of gestation)	NA				

POS: GBS-positive, NEG: GBS-negative, NA: not available, po: per os. Case 4 (*): GBS-positive PCR result not confirmed by the NRC.

Q3.3 FINANCIAL IMPACT OF THE PANTHER FUSION® GBS ASSAY COMPARED TO CULTURE

To estimate the **financial impact** of the use of the Panther Fusion® GBS assay for antenatal screening in HETUMO, laboratory costs of culture and PCR were compared. Costs related to HOT, identification using MALDI-TOF, the CHROMID Strepto B agar plates (bioMérieux, France) and the Panther Fusion® GBS tests (based on list price) were taken into account. Costs for rectovaginal swabs and AST account for both PCR and culture and were therefore excluded. All costs were calculated in euros (€) and based on an average of 2819 GBS screenings per year in HETUMO (data from 2023). Data are shown in Table 6. The Panther Fusion® GBS assay is approximately **ten times more expensive** than culture, i.e. 43215.27€ for PCR *versus* 4510.40€ for culture per year. The Belgian reimbursement authority "Rijksinstituut voor ziekte- en invaliditeitsverzekering - Institut national d'assurance maladie-invalidité" (RIZIV-INAMI) provides 2.73€ per GBS culture-based screening, note only once per pregnancy (54). It is not surprising that PCR costs exceed those of culture. This was also observed by Berg *et al,* who compared the cost of 5 antepartum culture- and PCR-based screening approaches, estimating a 13-fold higher cost for broth-enriched PCR compared to culture (AST cost not included) (55).

***Table 6**: Cost (in euros excl. VAT) of the Panther Fusion® GBS assay compared to culture.*

Cost per sample	Cost for culture (€)	Cost for the Panther Fusion® GBS assay (€)
Labour (HOT)	0.51	0.33
Reagents	1.09	15.00
Total cost	1.60	15.33

HOT: hands-on time

When calculating the **AST cost** separately, the expenses for a Mueller Hinton agar containing 5% sheep blood, antibiotic disks for disk diffusion, and HOT were considered. All costs were calculated in euros and were based on an average of 459 ASTs per year for GBS. GBS positivity rate in screening samples was 17% in HETUMO in 2022 and note that AST is performed for each GBS-positive screening sample in our centres, regardless of penicillin allergy status. This results in an additional cost of 1806.39€ for PCR-based screening and 1059.14€ for culture-based screening, respectively (see Table 7). The higher AST cost associated with PCR is due to the need for reflex culture. RIZIV-INAMI provides 3.12€ per AST that is performed (56). It is important to realise that the increased sensitivity of PCR could result in an increased detection of GBS, resulting in more AST. Based on our data (see Figure 2), one could suggest a threshold Ct of 36 to perform reflex culture for AST.

***Table 7**: Cost (in euros excl. VAT) of antimicrobial susceptibility testing when using the Panther Fusion® GBS assay or culture.*

Cost of AST	Cost in case of screening with culture (€)	Cost in case of PCR screening (€)
Per sample	2.31	3.94
AST for all GBS-carrying women (per year)	1059.14	1806.39

AST: antimicrobial susceptibility testing

In the present cost analysis, the potential time savings of broth-enriched PCR compared to culture methods were not taken into account. Broth-enriched PCR results are typically available within 18-24h, whereas culture methods typically take up to 42-72h. To address this better, as well as to assess the clinical benefits and relate these to the additional costs, cost-effectiveness studies are needed.

Q4: WHAT IS THE PREDICTIVE VALUE OF ANTENATAL SCREENING AT 35-37 WEEKS OF GESTATION FOR GBS CARRIAGE AT DELIVERY?

Q4.1 BACKGROUND ON THE PREDICTIVE VALUE OF ANTENATAL SCREENING

Guidelines suggest a **predictive window** of maximum **5 weeks** for GBS screening, a time interval that we also apply in HETUMO (19,23). The final aim of this CAT was to assess whether screening at 35-37 weeks of gestation provides an accurate estimation of GBS colonisation in pregnant women at delivery.

To our knowledge, **Boyer *et al* (1983)** were the first to study the predictive window of antenatal GBS screening by comparing ante- and intrapartum GBS carriage using culture-based methods (23). The GBS prevalence in their study population was 23%. These authors defined the predictive value of a positive antepartum culture as the proportion of patients with positive rectovaginal prenatal cultures who were confirmed to carry GBS at time of labour. Similarly, the predictive value of a negative antepartum culture was defined as the proportion of patients with negative prenatal rectovaginal cultures who were confirmed GBS-negative at time of labour (23). The authors determined an overall positive and negative predictive value of antepartum culture of 67.2% and 91.5%, respectively (23). All GBS-carrying pregnant women who gave birth within 6 weeks after the antepartum screening (n = 16) remained GBS-positive. The mean rate of colonisation loss was 1.8% per extra week of gestation (23). On the other hand, all GBS-negative pregnant women who gave birth within 10 weeks after the antepartum screening remained GBS-negative. When the interval between ante- and intrapartum exceeded 10 weeks, a mean rate of colonisation acquisition of 0.4% per extra week of gestation was observed. These data showed an inverse relationship between the predictive value of antepartum screening and the interval between antepartum screening and time of delivery (23). The estimated overall sensitivity and specificity of culture-based antepartum screening were 70.0% and 90.4%, respectively. The authors suggested that screening at 36-38 weeks would be optimal, though this approach would not cover women who deliver prematurely (23).

Regan *et al* (1996) concluded that cervicovaginal GBS colonisation at 23-26 weeks was a poor predictor for GBS EOD, though the predictive accuracy could be increased when also sampling rectally and at 31-36 weeks of gestation. The GBS prevalence in their study population was 21% (57). Similar findings were reported by **Goodman *et al* (1996)**, though they did not observe any improvement in the predictive value of antepartum screening at 37 weeks. The GBS prevalence in this study population was 14.0% (17). **Yancey *et al* (1996)** studied the predictive value of late antepartum culture-based screening at 33-39 weeks of gestation using ante- and intrapartum specimens (58). In contrast to Goodman *et al*, they demonstrated a higher sensitivity and specificity of antepartum screening, at 87% and 96%, respectively. The predictive value improved when women gave birth within 5 weeks after antenatal screening, also suggesting that screening at 35-37 weeks of gestation would be optimal (58). The GBS prevalence in this study population was 26.5% and these authors mentioned that high GBS prevalence results in a more accurate prediction of GBS carriage at delivery (58). In **2010**, **Valkenburg-van den Berg *et al*** conducted a systematic review on the timing of GBS screening during pregnancy (59). These authors underlined the current recommendations to screen at 35-37 weeks of gestation, but also emphasised the limitations of culture-based antepartum screening. The positive and negative predictive values of culture-based antenatal screening at 35-37 weeks of gestation were 69% and 94%, respectively, indicating that approximately 6% of GBS-carrying women at delivery had a GBS-negative antepartum screening (59). Previous research has shown that approximately 50% of GBS-carrying women transmit GBS during labour to their neonates and, in the absence of IAP, approximately 1-3% of these neonates develop GBS EOD (4,12,21). The authors also noted the variability in sampling locations (cervicovaginal, vaginal, rectovaginal, rectal or a combination of those sites) and culture conditions (with or without enrichment, use of different agar media and incubation protocols), as well as gaps in follow-up data, complicating comparisons between these studies. A more recent study by **Hussain *et al* (2019)** evaluated the predictive value of rectovaginal culture-based antepartum screening at 35-37 weeks of gestation and found a relatively weak agreement (Cohen's $\kappa < 0.60$) between antepartum cultures at 35-37 weeks and intrapartum results (60)**.**

Q4.2 A MULTICENTRE STUDY TO EVALUATE THE PREDICTIVE VALUE OF ANTEPARTUM GBS SCREENING

To evaluate the predictive value of antepartum GBS screening and to gain more insight in the optimal timing of antenatal GBS screening, a prospective study was conducted. For this, we compared ante- to intrapartum results obtained with culture and broth-enriched PCR. To our knowledge this is the first study to evaluate the use of antenatal PCR screening to estimate GBS colonisation in pregnant women at delivery.

Q4.2.1 Methods

Patient selection, sample collection, sample processing, sample analyses and interpretation of PCR results are described in Q3.2.1. The rate of agreement between ante- and intrapartum GBS results was assessed by calculating the OPA, PPA, NPA and Cohen's κ. To evaluate the predictive value of antenatal GBS screening at 35-37 weeks of gestation in our centres, PPV and NPV were determined for each weekly interval (from ≤1 to ≤7 weeks) between antepartum screening and delivery. Overall acquisition and loss rates of rectovaginal GBS colonisation were determined. A Kaplan-Meier curve was generated using R (V.4.4.0). Antepartum GBS carriage was determined by either GBS screening or presence of GBS bacteriuria at 35-37 weeks during the current pregnancy. Physicians were notified about women with a GBS-negative antepartum screening who tested GBS-positive at labour. Although IAP could not be administered, physicians were able to monitor both mother and child more closely after delivery. Note that due to unavailability of PCR tests for 12 days, 28 samples were analysed with a delay of 8 to 19 days after collection. This is conflicting with the IFU of the Panther Fusion® GBS assay which instructs analysis of the Aptima Lysis Tubes after a maximum of 7 days of storage. However, we have found that delayed PCR analyses up to 19 days did not result in qualitatively different results (data not shown).

Q4.2.2 Results & Discussion

A total of 188 ante- and intrapartum results were matched. Note that for 1 woman GBS carriage was determined based on GBS bacteriuria at 35 weeks of pregnancy. The mean interval between ante- and intrapartum screening was 3-4 weeks (minimum 1 day, maximum 6 weeks and 6 days). The mean gestational age at delivery was 39-40 weeks (minimum 35 weeks and 3 days, maximum 41 weeks and 4 days). **OPA, PPA and NPA between ante- and intrapartum results** were 94.7%, 87.5% and 97.1%, respectively (see Table 8). A Cohen's κ of 0.86 was calculated, indicating a near-perfect agreement between ante- and intrapartum screening. Hussain *et al* reported a lower Cohen's κ of 0.45, but their study only used culture to determine GBS carriage (60).

***Table 8**: Rate of agreement between ante- and intrapartum GBS screening results (n = 188).*

	Intrapartum screening		Overall Percent Agreement: 94.7% (95%CI, 0.905-0.971); Positive Percent Agreement: 87.5% (95%CI, 0.753-0.941); Negative Percent Agreement: 97.1% (95%CI, 0.929-0.989); Cohen's κ: 0.86
Antepartum screening	**+**	**-**	
+	42	4	
-	6	136	

Table 9 provides an overview of the ten cases with a discrepant result between ante- and intrapartum screening. In total, 6 pregnant women carrying GBS at delivery were missed by antepartum screening, whereas 4 pregnant women received unnecessary IAP due to loss of GBS carriage between antepartum screening and labour. These results confirm previous literature about the dynamic colonisation of GBS in pregnant women (7,15–18). For case 1, a very weak GBS-positive broth-enriched PCR result (Ct 39.9) was found with antepartum screening, while both non-enriched PCR and culture were negative for GBS. Note that this very weak positive PCR result obtained with the Panther Fusion® GBS assay was not confirmed by the NRC (for more information see Q3.2.2.). Due to lack of evidence on correlation between Ct values and viability of PCR-detected GBS, this woman was considered a true GBS carrier antepartum and therefore IAP was advised. Two GBS-carrying women antepartum (cases 2 and 6) received antibiotics after 35 weeks of pregnancy, which may have contributed to the eradication of GBS. However, it is more likely that the negative intrapartum results of these women reflect the dynamic nature of GBS colonisation during pregnancy. All positive intrapartum samples from antepartum GBS-negative women showed a positive culture result and/or a significant decrease in Ct values after broth enrichment. These data indicate the presence of viable GBS in the rectovaginal area of those women at labour. Although none of these GBS-carrying women received IAP, no neonatal GBS EOD or maternal complications related to GBS carriage were observed. These findings emphasize that GBS colonisation at delivery does not necessarily lead to invasive GBS disease (4,21). On the other hand, even low-level GBS colonisation could still result in vertical transmission when IAP is not administered (17,23). Future studies are needed to investigate the impact of viable GBS load and PCR Ct values on the risk of vertical transmission and GBS EOD.

***Table 9**: Overview of cases with discrepant ante- and intrapartum GBS screening results determined by PCR (with and without broth enrichment), broth-enriched culture and/or GBS bacteriuria at 35-37 weeks of gestation (the latter only for antenatal GBS carriage). Antenatal antibiotic treatment, intrapartum antibiotic prophylaxis and time interval between ante- and intrapartum screening are also listed.*

	ANTEPARTUM					**Time interval** between ante- and intrapartum screening	**INTRAPARTUM**			
Cases	Non-enriched PCR	Broth-enriched PCR	Broth-enriched culture	GBS bacteriuria	Antibiotic treatment (molecule, dose and duration)		Non-enriched PCR	Broth-enriched PCR	Broth-enriched culture	**IAP**
Case 1	NEG	POS (Ct 39.9)*	NEG	No	No	2 weeks	NEG	NEG	NEG	Penicillin
Case 2	NA		POS	No	Clindamycin 3*300 mg po (1 day before delivery)	5 weeks and 3 days	NEG	NEG	NEG	Clindamycin
Case 3	NEG	NEG	NEG	39 weeks**	No	3 weeks and 5 days	POS (30.7)	Not available	POS	No
Case 4	NEG	NEG	NEG	No	No	2 weeks and 3 days	POS (Ct 33.7)	POS (Ct 17.7)	POS	No
Case 5	NEG	NEG	NEG	No	No	4 weeks and 2 days	POS (Ct 22.2)	POS (Ct 18.4)	POS	No
Case 6	NA			35 weeks	Amoxicillin 3*1g po and fosfomycin (at 35-36 weeks)	3 weeks and 6 days	NEG	NEG	NEG	Penicillin
Case 7	NEG	NEG	NEG	No	No	2 weeks and 2 days	POS (Ct 35.6)	POS (Ct 20.1)	POS	No
Case 8	NEG	NEG	NEG	No	No	4 weeks and 4 days	Not available	POS (Ct 16.9)	POS	No
Case 9	NEG	NEG	NEG	No	No	5 weeks and 1 day	POS (Ct 20.1)	POS (Ct 18.5)	POS	No
Case 10	NEG	POS (Ct 30.4)	POS	No	No	5 weeks	NEG	NEG	NEG	Penicillin

POS: GBS-positive, NEG: GBS-negative, IAP: intrapartum antibiotic prophylaxis, NA: not available, po: per os. Case 1 (*): GBS-positive PCR result not confirmed by the NRC (for more information see Q3.2.2.). Case 3 (**): considered as GBS-negative antepartum.

In total, 188 intrapartum swabs were analysed to **estimate the predictive window of antenatal GBS screening**. Of these, 46 antepartum samples were GBS-positive and 142 were GBS-negative. The PPV of antepartum screening was defined as the number of women with a positive antepartum screening who remained GBS-positive at labour. Similarly, the NPV of antepartum screening was defined as the number of women with a negative antepartum screening who remained GBS-negative at labour. Table 10 provides an overview of the results. With a 5-week interval between antepartum screening and delivery, PPV and NPV of antepartum screening were 92.9% and 96.1%, respectively. The overall observed GBS acquisition and loss rates were 4.2% and 8.7%, respectively. In other words, **8.7**% of antepartum GBS-carrying women received **unnecessary IAP** due to loss of GBS colonisation. Conversely, **12.5%** of GBS-carrying women at labour would have been **missed** if only antepartum screening was used. Figure 3 displays a Kaplan-Meier curve which graphically represents the retention of antenatal GBS carriage status at labour.

According to our data, a 3-week interval between antepartum screening and delivery appears to be optimal, yielding the highest PPV and NPV (see Table 10). Boyer *et al* observed an NPV of 100% for antepartum screening with an optimal interval of up to 10 weeks between screening and delivery, while we observed the first acquisition of GBS carriage at labour after a 2-week interval (23). Also, we observed the first loss of GBS carriage at labour within 2 weeks, while Boyer *et al* observed this within 6 weeks. Boyer *et al* included a significantly higher number of pregnant women, but they only used culture for the detection of GBS (23). It is also important to note that our first two observed losses of GBS colonisation were based on an episode of GBS bacteriuria at 35-36 weeks of gestation (case 6) and on a very weak GBS-positive antepartum result (case 1). The increased sensitivity of PCR used in our study may have contributed to these different findings.

Table 10: *Overview of intrapartum GBS-positive and GBS-negative women in relation to their antenatal GBS carriage status. Positive (PPV) and negative (NPV) predictive values are listed for increasing weekly time intervals between intra- and antepartum screening (n = 188).*

	GBS-positive women antepartum (n = 46)			GBS-negative women antepartum (n = 142)		
Time interval (weeks)	No. of persistent GBS colonisations	No. of GBS losses	PPV (%)	No. of persistent GBS-negative cases	No. of GBS acquisitions	NPV (%)
≤1	2	0	100.0	5	0	100.0
≤2	5	1*	83.3	29	0	100.0
≤3	18	1	94.7	65	2	97.0
≤4	26	2**	92.9	102	3	97.1
≤5	39	3	92.9	124	5	96.1
≤6	42	4	91.3	135	6	95.7
≤7	42	4	91.3	136	6	95.8

*: broth-enriched antepartum PCR showing a Ct value of 39.9 (very weak positive) (case 1, see Table 9); **: antepartum GBS carriage based on bacteriuria at 35 weeks of gestation (case 6, see Table 9).

As demonstrated by El Helali *et al*, the PPV and NPV of intrapartum screening are superior to those of antepartum screening (16). Interestingly, Boyer *et al* found that women with persistent GBS colonisation are at high risk for vertical transmission of GBS (23). Our data showed a persistent GBS colonisation rate of 91.3%, indicating that while antenatal screening is not perfect, it successfully identifies the majority of GBS carriers at labour. Additionally, antenatal screening allows for AST to be performed. As long as the detection of clindamycin resistance in GBS strains is not possible using molecular-based tests, reflex cultures for AST remain essential, particularly for women with (severe) penicillin allergy. Development of PCR assays that also detect clindamycin resistance genes, either as commercial tests or self-developed tests with an open-access system like the Panther Fusion, would be usefull. Ideally, we would prefer to perform both ante- and intrapartum GBS screening. This approach would enable us to identify GBS-carrying women at 35-37 weeks of gestation, allowing to determine clindamycin susceptibility. In addition, intrapartum screening with fast PCR tests would allow us to identify all GBS carriers at labour, including women who acquire GBS between antenatal screening and labour, as well as women with an unknown GBS carriage status at labour. However, given the costs of fast PCR, practical challenges and the lack of reimbursement, these tests are currently not considered in our hospitals.

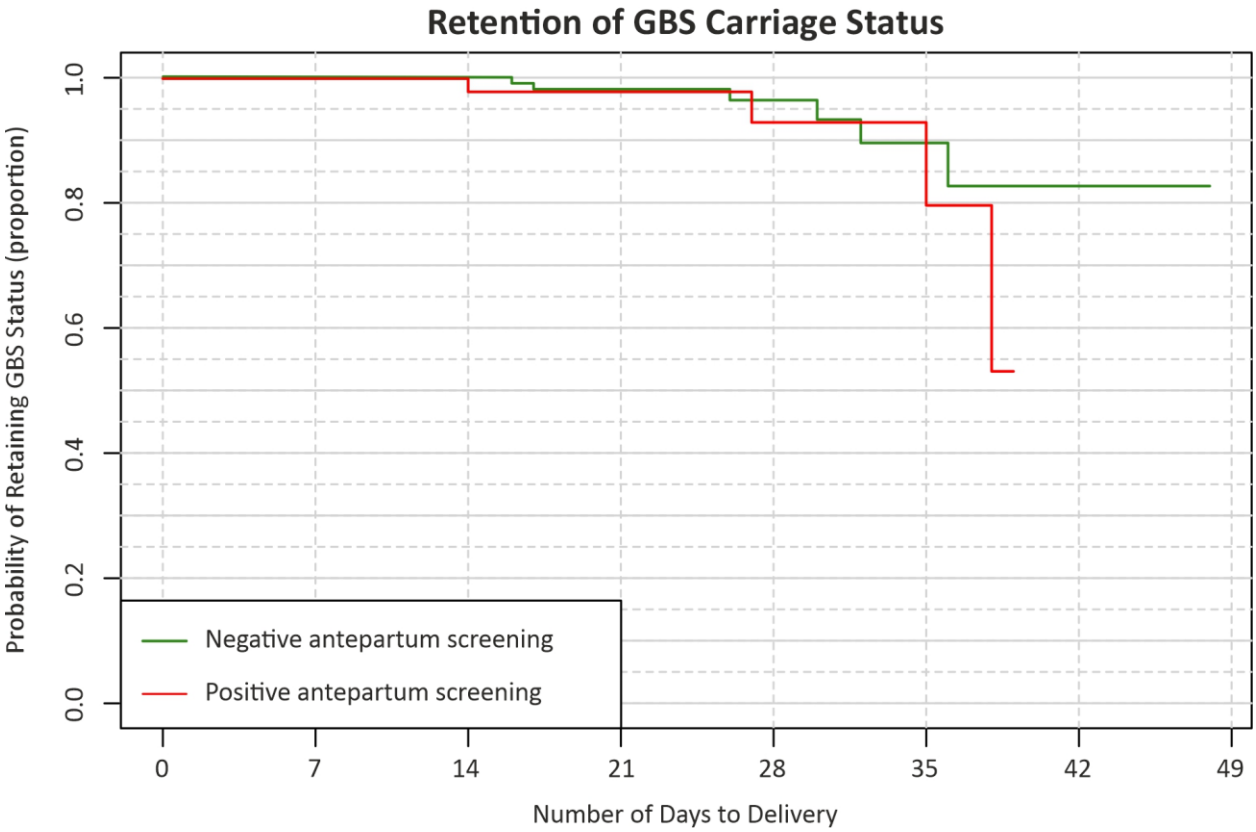

***Figure 3**: Kaplan-Meier curve displaying the probability of retention of antepartum GBS carriage.*

In summary, the 6 acquisitions and 4 losses of GBS colonisation we observed confirm the dynamic nature of GBS colonisation in pregnant women (7,15–18). When extrapolating our data, we estimate that annually approximately 90 pregnant women in HETUMO would be additionally identified as GBS carriers during labour that would have been missed with antepartum screening (based on 2819 deliveries annually in HETUMO). As previously mentioned, research has shown that approximately 50% of GBS-carrying women transmit GBS during labour to their neonates and, in the absence of IAP, approximately 1-3% of these neonates develop GBS EOD (4,12,21). Therefore, by using intrapartum testing instead of antepartum testing, we could potentially prevent an additional 0.9 GBS EOD per year. Conversely, approximately 60 women annually would show a loss of GBS colonisation by the time of delivery but would receive unnecessary IAP due to GBS-positive antepartum screening results. There are some limitations to our study. First, only 188 ante- and intrapartum matches were included to estimate the predictive window of antenatal GBS screening. No power analysis was performed before this study started, so the required study population size for statistical analysis was not determined. Further research is needed to investigate the correlation between Ct values and viability of PCR-detected GBS as well as on the risk of vertical transmission and GBS EOD. Finally, clinical studies are essential to explore the exact role of GBS density, as determined by PCR results, in the risk of vertical transmission and GBS EOD.

CONCLUSIONS

Vertical transmission of GBS during delivery can result in serious neonatal infections with high morbidity and mortality. Identifying GBS-carrying women during labour enables the administration of IAP to prevent vertical transmission. However, the optimal strategy for GBS screening remains a topic of ongoing debate. In this CAT, the current guidelines for GBS screening were reviewed, as well as their application in Belgian hospitals. Additionally, a multicentre study was conducted to assess the analytical performance of the Panther Fusion® GBS assay and to evaluate its financial implications compared to culture. Finally, we examined the predictive value of antepartum GBS screening in our centres.

First, we found that there is **no universal consensus** on which GBS screening strategy is most fitted to determine or estimate GBS colonisation in pregnant women at delivery. Although guidelines recognise the limitations of culture-based antenatal screening, most of them continue to recommend this strategy. At the same time, they acknowledge broth-enriched NAATs as an acceptable alternative to culture for antenatal screening. Molecular tests demonstrate strong analytical performance, particularly when used after broth enrichment, but they are associated with higher costs. The European consensus favours intrapartum GBS screening as the preferred strategy, although this is also associated with higher costs and requires trained staff as well as reliable molecular infrastructure at delivery wards. Further research is necessary to evaluate intrapartum PCR as a preventive strategy against GBS EOD. Our **national survey** on GBS screening revealed that most laboratories perform **culture-based antepartum screening**. However, a majority expressed interest in molecular-based tests, with increased costs, lack of reimbursement, and unavailability of AST cited as the major drawbacks.

Next, we conducted a **multicentre study**, involving 1001 women who underwent rectovaginal screening at 35-37 weeks of gestation, with a subset of women (n = 238) also receiving intrapartum screening. Specimens were analysed using broth-enriched culture and (non-)broth-enriched PCR. This study demonstrated superior analytical performance of the Panther Fusion® GBS assay compared to culture when used on broth-enriched specimens. This method also enables a more optimised lab workflow with more high-throughput screening, but is also associated with a 10-fold higher cost compared to culture. It is important to note that the Panther Fusion® GBS assay would be better suited for ante- rather than intrapartum screening, due to its 2-hour and 20-minute time-to-result once samples are loaded and because of its superior performance with broth-enriched specimens compared to non-enriched ones. The lack of clindamycin susceptibility determination is a major pitfall of all currently available PCR assays, which restricts their use for intrapartum screening.

Finally, antepartum GBS carriage in pregnant women was compared to their GBS carriage at time of labour. Among 188 matched ante- and intrapartum screenings, 10 discrepancies were observed, indicating a near-perfect agreement between ante- and intrapartum screening (Cohen's $\kappa = 0.86$). Predictive values of antenatal screening decreased as the interval between antenatal screening and delivery increased. Overall, **12.5%** of GBS-carrying women did not receive IAP due to GBS-negative antepartum screening, whereas **8.7**% of antepartum GBS carriers received unnecessary IAP due to loss of GBS colonisation at labour. Based on our findings, a 3-week time interval between antepartum screening and delivery appears to be an optimal predictive window.

In conclusion, we prefer to maintain an antepartum GBS screening strategy in HETUMO, but consider using PCR instead of culture to increase its sensitivity. Ideally, we would additionally perform intrapartum PCR in women with an unknown GBS carriage status. However, given the limited recommendations for intrapartum PCR in local guidelines, the inability of NAATs to determine clindamycin resistance, the lack of reimbursement, the available evidence and our study results, intrapartum PCR is currently not considered in our hospitals.

COMMENTS

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TO DO/ACTIONS

1. The data obtained from this CAT will be incorporated into the Panther Fusion® GBS assay validation report.
2. The findings will be compiled into a poster format and presented at symposia and/or other scientific events.
3. The outcomes of this CAT will inform decisions regarding the potential use of antenatal PCR as an alternative to culture for GBS screening in HETUMO.
4. Demographic parameters from all pregnant women included in the estimation of the predictive window will be collected at a later stage to correlate with our data on the predictive window of antenatal screening (for more information see Supplementary Table 3).

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ATTACHMENT 2: SUPPLEMENTARY TABLES

***Supplementary Table 1**: Overview of guidelines on ante- and intrapartum GBS screening (10,15,19–21).*

	Superior Health Council, Belgium, 2003 (10)	European consensus, 2014 (15)	ASM/CDC/ACOG, USA, 2019-2020
Laboratory request form	Specification 'culture for GBS-screening'	Identify on the orders that the specimens are for GBS culture	-
Antepartum screening	Standard	Alternative	Standard
Who to screen?	All pregnant women, except • Previous child with invasive GBS disease • GBS bacteriuria during current pregnancy	Not specified.	All pregnant women, except • Previous child with GBS infection • GBS bacteriuria during current pregnancy
Timing of screening	35-37 weeks of gestation	35-37 weeks of gestation	36 0/7 – 37 6/7 weeks of gestation
Sample collection	Rectovaginal specimen, one or two swabs, no use of speculum.	• Rectovaginal specimen • Single flocked swab is recommended. No use of speculum. • (Self-collection is acceptable with proper explanation)	• Rectovaginal specimen • Single flocked swab, no use of a speculum
Transport conditions	• Non-nutritive liquid medium • Ideally within 24h and max 48h to the lab • In case of delay, to be stored at 2-8°C	Selective enrichment broth (e.g., LIM broth) • As soon as possible to the lab • In case of delay store at room temperature for a maximum of 4 days If not available: non-nutritive medium	• Liquid-based, non-nutritive transport medium • Within 24h to the lab (otherwise rejection) • In case of delay, to be stored in the fridge (2-8°C)
Enrichment in selective broth	LIM broth, overnight incubation at 35-37°C	Selective enrichment broth (LIM), 18-24h, 35-37°C, ambient air or CO_2	Selective enrichment broth (e.g.Trans-Vag or LIM broth), 18-24h incubation at 35-37°C, in ambient air or 5% CO_2
Screening agar and incubation conditions	Granada: anaerobic incubation at 35-37°C for 18-24h (aerobic if inoculated media is covered with a coverslip); or (sheep)blood agar: aerobic incubation.	Granada: anaerobically; or GBS chromogenic media (e.g. StrepBSelect® (BioRad), ChromID® Strepto B (bioMérieux), Brillance GBS® (ThermoScientific)). Incubate at 35-37°C for 48h in appropriate atmosphere.	Non-selective or selective media (e.g. Granada or chromogenic media). Incubate at for 48h in appropriate conditions (cfr specific IFU). Attention to detection of both (non)-haemolytic strains.
Culture examination	Granada: orange-red coloured colonies.	Granada: orange or red colonies; StrepBSelect: pale to dark turquoise; ChromID Strepto B: pale pink to red; Brillance GBS: bright dark pink to red; Blood agar: gray/translucent/small zone of beta-hemolysis.	Identify suspected colonies according to IFU for specific media.
Identification of GBS	Granada: based on orange-red coloured colonies: blood agar: based on latex-agglutination or analogue test.	Granada: based on orange-red coloured colonies; chromogenic agars: latex or co-agglutination test, GBS antigen detecting test and/or MALDI-TOF	Identification of candidate isolates with CAMP-test, latex agglutination, MALDI-TOF, DNA-probes or NAAT
Reporting of the results	Qualitative	Qualitative	Qualitative
Culture without broth enrichment	Possible, but only additionally to enriched cultures	Possible, but only additionally to enriched cultures	Possible, but only additionally to enriched cultures
Antimicrobial susceptibility testing	-	Orderd in case of penicillin allergy with high risk for anaphylaxis. Interpretation according to EUCAST procedure. D-zone test to determine clindamycin and erythromycin susceptibility. Penicillin-allergy should be mentioned on laboratory request form.	Perform AST for all pregnant women with severe penicillin allergy, routine AST is not required. D-zone test to determine clindamycin and erythromycin susceptibility, also report vancomycin if the GBS strain is clindamycin resistant.
Intrapartum screening	Not extensively mentioned	Recommended as new strategy	Not extensively mentioned
Fast screening	Fast PCR is not extensively mentioned. Strep B OIA can be used, but only in case of unknown GBS status at time of labour	Intrapartum PCR is highly recommended as first line screening	Intrapartum NAAT without broth enrichment is not recommended.

NAAT: nucleid acid amplification test.

Supplementary Table 2: *Analytical performances of different PCR assays and culture as demonstrated in several papers studying the Panther Fusion® GBS assay (32,36,45).*

Study	Comparison of assay's	Analytical performances
Berry et al 2019 (45)	Hologic Panther Fusion® GBS assay • Enriched rectovaginal specimens • Target: *cfb* and *sip* BD MAX® GBS assay (comparator) • Enriched rectovaginal specimens • Target: *cfb*	Agreement study: • Overall agreement: 99.0% • Positive percent agreement: 96.6% • Negative percent agreement: 99.7%
Shin and Pride, 2019 (32)	Enriched culture (comparator 1) • Enriched rectovaginal specimens • Trypticase soy agar containing 5% sheep blood • ID: MALDI-TOF for all beta-haemolytic colonies Consensus (comparator 2) • Based on NAAT results • True positive if positive by two or more assays	Culture vs consensus: • Sensitivity: 73.5% • Specificity: 100.0%
	Hologic Panther Fusion® GBS assay • Enriched rectovaginal specimens • Target: *cfb* and *sip*	PCR vs consensus: • Sensitivity: 95.9% • Specificity: 99.4%
	Luminex Aries® GBS assay • Enriched rectovaginal specimens • Target: *cfb*	PCR vs consensus: • Sensitivity: 96.6% • Specificity: 96.3%
	Cepheid Xpert® GBS LB assay • Enriched rectovaginal specimens • Target: *cfb*	PCR vs consensus: • Sensitivity: 95.9% • Specificity: 98.3%
Thwe, et al, 2022 (36)	Enriched culture (comparator 1) Culture: • Enriched rectovaginal specimens • Tryptic soy with sheep blood agar and Columbia nalidixic acid agar • ID: MALDI-TOF for all beta-hemolytic colonies Cepheid Xpert® GBS LB XC assay • Enriched rectovaginal specimens • Target: glucosyl transferase family protein and a region in the *LysR* family transcriptional regulator of GBS	PCR versus culture • Sensitivity: 99.1% • Specificity: 91.8% • PPV: 71.6% • NPV: 99.8%
	Hologic Panther Fusion® GBS assay and culture (comparator 2) Culture: cfr above Hologic Panther Fusion® GBS assay: • Enriched rectovaginal specimens • Target: *cfb* and *sip* Cepheid Xpert® GBS LB XC assay • Enriched rectovaginal specimens • Target: glucosyl transferase family protein and a region in the *LysR* family transcriptional regulator of GBS	PCR versus comparator method • Sensitivity: 99.3% • Specificity: 98.7% • PPV: 95.9% • NPV: 99.8%

CI: confidence interval, NPV: negative predictive value, PPV: positive predictive value

Supplementary Table 3: *Overview of demographic data that will be collected for future research in collaboration with the NRC for S. agalactiae.*

Demographics
• Maternal age • Ethnicity • Gestational age at birth • Parity/gravidity • Pregravidity weight (BMI) and weight at delivery • Birth weight infant • Acquired or previously present diabetes • Penicillin allergy status • GBS bacteriuria • Previous GBS-infected child • Mode of delivery • Premature rupture of membranes • Preterm premature rupture of membranes • Stillbirth • Preterm labour/delivery • Intrapartum antimicrobial prophylaxis Antibiotics at time of delivery