# Optimizing Exact Diagnostics Group B Streptococcus Quality Control in Lim Broth Processed on the Cepheid GBS LB Assay: Importance of Swabbing and Matrix Influence

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## Abstract

**Background:** Group B Streptococcus (GBS) colonization in expectant mothers poses a risk of infection and transmission to newborns, potentially leading to severe illness or even death. Screening for GBS in expectant mothers is typically performed using culture or real-time PCR around week 37 of gestation. While real-time PCR offers greater sensitivity, it requires GBS enrichment in Lim broth prior to testing.

Adequately designed Quality Controls (QCs) play a crucial role in ensuring accurate and reliable GBS screening results. These controls should simulate patient specimens by containing whole organisms in clinically relevant matrices, allowing comprehensive monitoring of the PCR process and exposure to similar challenges. However, GBS screening involves an additional step of enrichment in Lim broth and, in some instances, subsequent swab dipping, as is the case with the Cepheid GBS LB assay.

In this study, we evaluate the effects of swab immersion timing and matrix type on the detection of GBS.

**Methods:** We formulated two GBS QCs that simulate enriched low positive specimens: one in an adapted Lim broth and the other in a synthetic matrix (both at 1000 copies/mL by Bio-Rad ddPCR™). We dipped clean swabs in 250 µL of the Lim broth QC or the synthetic matrix QC for either <1, 3, or 30 seconds. Samples were immediately run on the Cepheid GBS LB assays according to the assay's instructions for use. For comparison against the differential swab adsorption volume, we also transferred 100 µL of Lim broth QC directly into the Cepheid cartridge.

**Results:** Our data revealed that dipping and immersing a swab in a GBS QC formulated in Lim broth for <1 second not only caused an average delay of 2.1 Ct values but also resulted in a false negative compared to the 3- and 30-seconds immersion. This highlights the variability introduced by immersion timing during the dipping step. Furthermore, we observed that rapid dipping in Lim broth led to a 1.7 Ct delay compared to the synthetic matrix, suggesting differential efficiency of GBS absorption on the swab between Lim broth and the synthetic matrix.

**Conclusion:** This study highlights the critical importance of (i) formulating GBS QC in Lim broth and (ii) controlling the swab-dipping step in the laboratory workflow. By incorporating these measures, laboratory users can ensure reliable QC GBS results. These findings emphasize the need for careful attention to these factors during GBS QC testing, ultimately contributing to improved outcomes for newborns.

## Background

*Streptococcus agalactiae*, also known as Group B Streptococcus (GBS), is a gram-positive bacterium that commonly colonizes healthy adults - including 20-30% of pregnant women - without causing noticeable symptoms. More importantly, 50-70% of neonates become infected with GBS when born from colonized mothers[1]. This poses a severe risk of life-threatening infections and potentially harmful complications to these newborns if GBS goes undetected[2]. Therefore, it is vital for expecting mothers to be screened for GBS, which typically occurs at weeks 35-37 of gestation[3].

Identifying GBS may involve laborious culture-based techniques with long turnaround times and low sensitivity. These traditional approaches often lead to diagnostic delays and the risk of yielding false negative outcomes. As a result, the gold standard bacterial culture is being replaced more and more frequently with molecular testing, which more generally represents a significant advancement in the realm of infectious disease diagnostics[4].

Adequately designed Quality Controls (QCs) are a cornerstone in ensuring the reliability of test results, which is particularly critical for GBS testing. Whether for assay verification/validation or routine run controls, QCs should simulate patient specimens. This includes containing whole organisms within matrices that replicate clinical conditions. QCs designed this way successfully mirror the same challenges and workflow intrinsic to clinical specimens, which are expected attributes of QC materials. Hence, such QCs allow for comprehensive monitoring of steps related to the pre-analytical and analytical processes seen in patient testing. GBS molecular testing, in particular, introduces an additional layer of complexity by involving Lim broth enrichment and the subsequent swab dipping within this enriched Lim broth, a characteristic seen in assays like the Cepheid GBS LB and GBS LB XC. The pre-analytical swab dipping step should also be evaluated by the QC materials because the extent of GBS bacteria adsorption from the Lim broth onto the swab will influence the results.

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## Materials and Methods

This study aims to understand the adequacy and robustness of Exact Diagnostics GBS Quality Control using both the Cepheid GBS LB assay and the newer Cepheid GBS LB XC assay by thoroughly evaluating (i) three swab dipping conditions; and (ii) two different matrices.

The first component of our study involved dipping a clean single-use disposable swab (the type recommended in both Cepheid's instructions for use) in Exact Diagnostics GBS QC formulated in adapted Lim broth (consisting of Lim broth with a preservative). The Exact Diagnostics GBS QC materials used for this study were prepared using absolute quantification as determined by a validated in-house laboratory developed test (LDT) on Bio-Rad's droplet digital PCR (ddPCR™) platform. Comparative analysis of Ct values was performed after rapidly (<1 second) dipping a swab versus introducing a deliberate 3-second delay and 30-second delay (data not shown) during that dipping step using both the aforementioned Cepheid assays. To gain deeper insights into the impact of the swabbing technique on QC performance, we tested various GBS concentrations (100, 1000, and 3000 copies/mL) on the Cepheid GBS LB XC assay. Additionally, we compared the performance of the QC when formulated in a synthetic matrix (resembling universal transport media - UTM) versus in adapted Lim broth on the Cepheid GBS LB assay. Lastly, in order to have a more complete perspective alongside the analysis of GBS adsorption onto the swab, we took an additional approach consisting of directly introducing 100 µL of GBS QC in Lim broth into Cepheid cartridges.

## Results

**Table 1:** Results with the GBS QC in Lim broth using the Cepheid GBS LB assay

| Technique: Rapid Dip (<1sec) | Technique: Slow Dip (3 sec) |
|---|---|
| Titers assessed | Titers assessed |
| 1000 copies/mL | 1000 copies/mL |
| 37.0 | 37.4 |
| 40.4 | 36.7 |
| 38.6 | 37.5 |
| 43.1 | 37.8 |
| 39.7 | 37.8 |
| 39.2 | 36.6 |
| 40.1 | 36.6 |
| 38.6 | 39.2 |
| 39.1 | 37.1 |
| 38.7 | 37.4 |
| Avg Ct 39.5 | Avg Ct 37.4 |
| Std Dev 1.6 | Std Dev 0.8 |

**Assay:** **Cepheid GBS LB assay**
Fill Volume: 250µL
Matrix: Adapted LIM Broth
Legend
Detected
Not Detected

**Table 2:** Results with the GBS QC in Lim broth using the newer Cepheid GBS LB XC assay

| Technique: Rapid Dip (<1sec) | | | | Technique: Slow Dip (3sec) | | |
|---|---|---|---|---|---|---|
| Titers assessed | | | | Titers assessed | | |
| | 3000 copies/mL | 1000 copies/mL | 100 copies/mL | 3000 copies/mL | 1000 copies/mL | 100 copies/mL |
| | 32.4 | 37.1 | ND | 31.8 | 33.6 | 38.7 |
| | 32.0 | 34.7 | 37.4 | 32.0 | 32.4 | 39.1 |
| | 32.8 | 35.6 | 38.2 | 32.2 | 32.7 | 37.7 |
| | 34.0 | 37.6 | 40.1 | 32.0 | 32.3 | ND |
| | 32.2 | 35.5 | 39.1 | 31.7 | 32.8 | 38.5 |
| | 33.2 | 34.2 | 39.7 | 32.2 | 34.1 | 40.5 |
| | 33.3 | 33.6 | ND | 32.2 | 34.1 | 36.6 |
| | 33.2 | 34.6 | ND | 32.0 | 33.2 | 36.3 |
| | 34.2 | 35.3 | 40.7 | 31.6 | 32.6 | 36.6 |
| | 34.3 | 37.1 | 37.1 | 32.6 | 35.8 | 36.0 |
| | 34.0 | 34.2 | 37.0 | 31.3 | 32.6 | 37.8 |
| | 33.5 | 36.6 | ND | 31.9 | 32.5 | 37.2 |
| | 32.4 | 35.5 | 40.0 | 32.0 | 33.2 | 36.9 |
| | 34.8 | 37.3 | 39.7 | 31.9 | 34.3 | 37.0 |
| Avg Ct | 33.3 | 35.7 | 38.9 | Avg Ct 32.0 | 33.3 | 37.6 |
| Std Dev | 0.9 | 1.3 | 1.4 | Std Dev 0.2 | 1.0 | 1.3 |

**Assay:** **Cepheid GBS LB XC assay**
Fill Volume: 250µL
Matrix: Adapted LIM Broth
Detected
Not Detected

## Results

**Table 3:** Results when comparing GBS QC in Lim broth Vs in a synthetic matrix using the Cepheid GBS LB assay

| Matrix: LIM Broth | Matrix: Synthetic |
|---|---|
| Titers assessed | Titers assessed |
| 1000 copies/mL | 1000 copies/mL |
| 37.0 | 37.4 |
| 40.4 | 36.7 |
| 38.6 | 37.5 |
| 43.1 | 37.8 |
| 39.7 | 37.8 |
| 39.2 | 37.6 |
| 40.1 | 39.5 |
| 38.6 | 39.2 |
| 39.1 | 37.1 |
| 38.7 | 37.4 |
| Avg Ct 39.5 | Avg Ct 37.8 |
| Std Dev 1.6 | Std Dev 0.9 |

Technique: Rapid Dip (<1sec)
**Assay:** **Cepheid GBS LB assay**
Fill Volume: 250µL
Detected
Not Detected

## Discussion

Our data indicates that when a clean swab is rapidly (<1 second) dipped into a GBS QC solution formulated in Lim broth , it not only introduces an average delay of 2.1 and 2.4 Ct values at a working concentration of 1000 copies/mL with the Cepheid GBS LB and Cepheid GBS LB XC assays, respectively (tables 1 and 2), but also has the potential to generate false negative QC results when compared to a longer (3-second) dipping duration (table 1). A much longer (and not workflow friendly) 30-second dipping duration was also tested and was not significantly different from dipping for 3 seconds on the GBS LB assay (Mean 37.6 Ct, n=2). Moreover, loading 100 µL of 5E3 cp/mL GBS QC in Lim broth directly into the GBS LB XC cartridge results in a mean Ct of 33.2 (n=2). Taken together, these observations emphasize the criticality of controlling for the pre-analytical swab dipping step (if required by the assay such as the Cepheid GBS LB and GBS LB XC assays) in addition to PCR processing.

Furthermore, that rapid (<1 second) swabbing technique was also performed using the same QC but formulated in a synthetic matrix. At a working concentration of 1000 copies/mL, the results show an average 1.7-Ct delay in Lim broth compared to the synthetic matrix (T able 3), suggesting that the efficiency of GBS adsorption onto the swab surface differs between Lim broth and the synthetic matrix. In essence, Lim broth represents a more challenging and realistic matrix when compared to the synthetic matrix tested. This highlights the importance of the matrix effect and for QCs to fully simulate patient specimens.

## Conclusion

It is important for laboratories to rely on Quality Controls appropriately designed to fully simulate patient specimens and to undergo the same workflow as those clinical specimens, whether those QCs are used for assay verification/validation or as run controls. For routine screening purposes, GBS testing requires that the swabs collected from patients are enriched in Lim broth and, in the case of assays like the Cepheid GBS LB and GBS LB XC ones used here, for a new swab to then be dipped into that Lim broth before being loaded into the testing platform. This study specifically emphasizes the importance for GBS QCs to be formulated in Lim broth and that these QCs also provide the ability to control for the swab dipping step when it is part of the patient specimen workflow. Hence, the importance of the matrix effect and to control the GBS adsorption onto a swab (when applicable) were highlighted here.

By incorporating these measures, laboratory staff can establish a solid foundation for reliable GBS testing results. These findings highlight key factors that must be considered when selecting GBS QC to assess and control assays, ultimately contributing to improved outcomes for newborns.

## References

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