Comparison of culture with two different qPCR assays for detection of rectovaginal carriage of *Streptococcus agalactiae* (Group B streptococci) in pregnant women

Nabil Abdullah El Aila,1 Inge Tency,2 Geert Claeys,1 Hans Verstraelen,2 Pieter Deschagt,1 Ellen Decat,1 Guido Lopes dos Santos Santiago,1 Piet Cools,1 Marleen Temmerman,2 Mario Vaneechoutte1

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1 Department Clinical Chemistry, Microbiology and Immunology, Ghent university, Belgium, 2 Department of Obstetrics & Gynaecology, Ghent University Hospital, Belgium

Introduction:

Group B streptococcal (GBS) infection remains a leading cause of neonatal sepsis. In Belgium, 13-25% of pregnant women are colonized with GBS and GBS is responsible for 30% of the early neonatal infections. Guidelines from the Centers for Disease Control and Prevention (CDC) recommend that all women should be screened at 35-37 weeks of gestation and that those women found to be colonized with group B streptococci should receive intrapartum intravenous antibiotic prophylaxis either with penicillin G or ampicillin, effective in reducing the incidence of early-onset neonatal GBS infections. The standard method for the diagnosis of group B streptococcal colonization comprises culture of combined vaginal and rectal samples in a selective broth medium, with subculture onto solid media, which is time consuming, requiring 48-72 h (4,5). New rapid, sensitive and specific methods for detection of GBS in pregnant women are needed in order to provide timely treatment of neonates. This study compared three different culture media and two qPCR methods, to detect GBS colonization in pregnant women, starting directly from vaginorectal swabs or after Lim broth enrichment.

Methods

A total of 100 pregnant women at 35-37 weeks of gestation were included. For each subject, one vaginorectal ESwab was collected. Plating onto Columbia CNA agar (CNA), group B Streptococcus differential agar (Granada Agar) and chromID™ Strepto B agar (ChromAgar), directly from the swab or after Lim broth enrichment, was carried out. For qPCR evaluation, we extracted DNA with the easyMAG (BioMérieux) platform, directly from the vaginorectal swab and after Lim broth enrichment. Two different qPCR formats were compared, i.e. the hydrolysis probe (Taqman, Roche) format targeting the sip gene (1.3) and the hybridization probe (Hyprobe, Roche) format, targeting the cfb gene (2.6).

Conclusions

> When detecting GBS directly from vaginorectal swabs, the application of qPCR, irrespective of the target (sip or cfb) or of the chemistry (hybridization probes or hydrolysis probe), increased the number of GBS positive women (27%) compared to culture (22%)(p < 0.05; McNemar’s test).

> The sensitivity of qPCR after Lim broth enrichment (33%) was again significantly higher than qPCR after DNA extraction directly from the vaginorectal swabs (27%) (p < 0.05; McNemar’s test).

Results

> Of a total of 100 pregnant women tested, 66 were negative by both culture and qPCR, 22 were positive by culture, 27 by direct qPCR and 33 by qPCR after Lim broth enrichment (Table 1).

> The one sample that was positive by culture and negative by qPCR, was culture positive only after enrichment.

> For the 21 samples that were positive for both culture and qPCR, both the DNA directly from the sample and the DNA extracted after Lim broth enrichment were qPCR positive.

> Samples with the highest Cq-values, which can be assumed to be the samples with the lowest bacterial load, were also the ones that were culture negative, which is also indicative of a lower bacterial load, i.e. the mean Cq value for the 21 culture/qPCR positive samples was 21.5 (Cl: 18.7-24.3), whereas that for the 12 culture negative, PCR positive samples it was 26.5 (Cl: 22.3-30.8), strongly indicative for a lower bacterial load in the latter (p = 0.05).

> For the 12 samples that were positive only by qPCR, the mean Cq value for the six samples, that were positive also when the DNA was extracted directly from the samples, was 22.6 (Cl: 15.4-29.8), whereas the Cq-value of the six samples, that were only positive when DNA was extracted from the Lim broth after enrichment, was 30.3 (Cl: 27.7-32.9), strongly indicative for a lower bacterial load in the latter (p = 0.05).

Conclusions

Table 1: Sensitivity, specificity, and positive and negative predictive values of the culture method compared with qPCR for detection of group B Streptococcus (GBS)

<table>
<thead>
<tr>
<th>Culture method</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>qPCR method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Positive</strong></td>
<td>21</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>1</td>
<td>66</td>
<td>67</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>22</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>92.4%</td>
<td></td>
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<tr>
<td><strong>Specificity</strong></td>
<td></td>
<td>84.6%</td>
<td></td>
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<tr>
<td><strong>Positive predictive value</strong></td>
<td>62.5%</td>
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<td></td>
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<tr>
<td><strong>Negative predictive value</strong></td>
<td>98.5%</td>
<td></td>
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</tbody>
</table>

Legend: X-axis: C+: culture positive, C-: culture negative; D+: qPCR positive, directly from sample, D-: qPCR negative, directly from sample; Lim+: qPCR positive only after Lim broth enrichment. Y-axis: Cq-value of qPCR.

![Figure 1. Cq values of the GBS-qPCR positive rectovaginal samples using the hybridization probe assay (c/- cfb gene).](image-url)

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Literature


