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Original article

Evaluation of hibergene loop-mediated isothermal amplification assay for detection of group B streptococcus in recto-vaginal swabs: a prospective diagnostic accuracy study

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ABSTRACT

Objectives: To prospectively evaluate HiberGene's loop-mediated isothermal amplification (LAMP) assay for detection of group B *streptococcus* (GBS) in maternal recto-vaginal swabs and compare it with enrichment culture.

Methods: Following ethical approval and informed written consent, two low vaginal and rectal swabs were obtained from 400 pregnant women. One swab was tested for GBS using the rapid LAMP assay (index test), the second swab was tested using enrichment culture (reference standard). Antimicrobial susceptibility testing was performed according to EUCAST guidelines.

Results: There were 376 concordant results, 20 discordant and four invalid LAMP results. Among discordant results, six were LAMP negative/culture positive and 14 were LAMP positive/culture negative. The sensitivity was 92.2%, specificity 95.6%, positive predictive value 83.5% and negative predictive value 98.1%. The prevalence of GBS carriage was 19.25% (77/400). Forty-eight of 77 GBS-positive women were colonized vaginally (62.3%) and 70 were colonized rectally (90.9%). Erythromycin resistance was 22.4% (17/76) and clindamycin resistance was 17.1% (13/76).

Conclusions: The LAMP assay is a rapid and simple test with results available in approximately 1 h compared with 48 h for culture. The test has good sensitivity and specificity compared with enrichment culture. This test can be used for rapid antenatal GBS screening. **A. Curry, Clin Microbiol Infect 2018;▪:1**

Introduction

Group B streptococcus (GBS) is an important cause of maternal and neonatal infection. It is the most common cause of severe infection in newborns in the first week of life in many developed countries, and is associated with bloodstream infection, pneumonia and meningitis. Approximately 15%–25% of women are colonized with GBS [1,2]. Various international guidelines have been published recommending use of intrapartum antibiotic prophylaxis in pregnant women at risk of transmitting GBS to their neonate, using either a risk-based or screening approach [3,4]. If performed, microbiological screening is recommended at 35–37 weeks of gestation, using enrichment culture [3,5]. Culture results are less reliable the longer the time between screening and delivery, especially if more than 5 weeks have elapsed due to the transient carriage of the organism [6,7]. Furthermore, as culture takes up to 48 h to become positive, it is not useful during the intrapartum period.

It is recognized that a rapid, sensitive and specific GBS test could be of benefit during the intrapartum period or following rupture of membranes. Nucleic acid amplification tests are suitable, although they are not in widespread use for GBS screening. These tests require equipment and reagents which may not be available in all microbiology laboratories and are more expensive than culture. Loop-mediated isothermal amplification (LAMP) takes place at a single temperature, so there is no need for thermal cycling. This reduces the cost compared with PCR.

The study objective was to prospectively evaluate HiberGene's rapid LAMP assay (index test) for detection of GBS in maternal

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recto-vaginal swabs and compare it with enrichment culture (reference standard).

Materials and methods

This was a prospective diagnostic accuracy study evaluating HiberGene's LAMP assay by comparing it with enriched culture, the reference standard currently in use in our laboratory. Women were eligible to participate if they were pregnant and attending the National Maternity Hospital, Dublin. The National Maternity Hospital is a large tertiary referral maternity hospital delivering approximately 9000 infants per annum or one in every eight infants born in the Republic of Ireland. A research midwife attended antenatal clinics between June and October 2016 and informed pregnant women of the study. Following informed written consent, two separate low vaginal and two separate rectal swabs were obtained from 400 pregnant women who were between 30 and 40 weeks of gestation.

One set of swabs (low vaginal and rectal) was pooled and tested for GBS using the rapid HiberGene LAMP assay (index test), (HiberGene Diagnostics, Dublin, Ireland).

Bacterial cells are first eluted from the swab, and the eluate is subjected to enzymatic lysis before undergoing a heat denaturation step. Then, 25 μL of the denatured lysate is added to the HiberGene reaction strip, which contains primers both for a highly conserved gene region of GBS and an exogenous bacteriophage sequence used as the assay Extraction Control, together with an intercalating dye. Primers, polymerase, intercalating dye for detection of amplified product, and all other reactants, are provided in freeze-dried format in the HiberGene LAMP assay reaction tubes.

The LAMP technology has been described previously [6,7]. Briefly, six primers targeting eight gene regions are designed to generate a self-priming dumbbell-shaped template upon isothermal incubation with strand-displacing polymerase. Large quantities of complex amplicon are rapidly generated; in HiberGene's embodiment of the technology, this amplification is visualized in real-time on the HiberGene Swift instrument by means of an intercalating fluorescent dye.

The LAMP assay run time is 66 min from the time when analysis begins, compared with enrichment culture, which takes 40–48 h. As the HiberGene Swift assigns positive status in real-time as soon as amplification is detected, a positive result can be available in a shorter time than the 66-min run time.

The second set of swabs were tested separately for GBS using enrichment culture (reference standard) to determine site of carriage. However, the results were treated as per patient for the purposes of comparison with the LAMP assay. An enrichment culture method is recommended by many GBS guidelines because it is more sensitive than direct culture [3,5]. The swab was inoculated onto blood agar (Fannin L.I.P., Galway, Ireland), chromogenic Brilliance GBS agar (Fannin L.I.P.) and Todd–Hewitt broth (Fannin L.I.P.) and incubated at 35°C aerobically for 16–20 h. After overnight incubation the direct agar plates were read and the Todd–Hewitt broth was subcultured onto blood agar and chromogenic Brilliance GBS agar and incubated overnight at 35°C anaerobically (blood agar) and aerobically (Brilliance GBS agar). All suspect colonies underwent Lancefield streptococcal grouping (Oxoid) to confirm GBS.

The rapid LAMP assay was performed by a HiberGene scientist and GBS culture was performed by the microbiology department at the National Maternity Hospital. The culture results were not available to the scientist performing the index test. When the rapid LAMP assay and culture results were completed, results of the LAMP assay (index test) were matched with the culture results (reference standard) using a common laboratory number, by the corresponding author. The enriched culture result was that reported to the patient's chart as the accredited test to ISO 15189 standards; HiberGene's LAMP assay was a research test only during this study.

Antimicrobial disc susceptibility testing was performed on GBS culture isolates according to EUCAST guidelines [8]. The following antibiotics were tested: penicillin, erythromycin, clindamycin and vancomycin.

Statistical analysis was carried out using Epi-Info™ version: 7.2.0.1. Frequencies and percentages are used to describe the data and 95% CI are presented where relevant. Statistical significance testing was carried out using chi-squared test and two-tailed Fisher's Exact test where appropriate.

Institutional ethical board approval was granted for the study on 27 June 2016 (EC 22.2016).

Results

All patients who took part in the study were pregnant women. Fig. 1 shows women undergoing GBS recto-vaginal screening and the results of the LAMP test and enrichment culture.

There were 376 concordant, 20 discordant and four invalid LAMP assay results. Among discordant results, six were LAMP GBS negative and culture positive and these were considered false-negative LAMP results. One GBS was detected by direct culture and five only following enrichment culture, indicating a lower quantity of organism present. A further 14 were LAMP GBS positive and culture negative and we considered these results inconclusive. It is possible that these were false-positive LAMP results or false-negative culture results due to quantity of microorganism/DNA present, improved sensitivity with a molecular method, patient on recent antibiotic therapy, laboratory error or contamination.

In direct comparison with the reference standard and after the exclusion of the four invalid LAMP results, the LAMP assay had a sensitivity of 92.2% (95% CI 83.8%–97.1%) and specificity 95.6% (95% CI 92.75%–97.6%). The positive predictive value was 83.5% (95% CI 75.2%–89.5%) and negative predictive value was 98.1% (95% CI 95.9%–99.1%). These statistical data assume that the LAMP-positive and culture-negative GBS results were all false-positive LAMP results, which may not be the case.

The prevalence of recto-vaginal GBS carriage was 19.25% (77/396) using enrichment culture. 53.2% (41/77) of GBS culture-positive patients had GBS detected in both rectal and low vaginal swabs; 37.7% (29/77) had GBS detected in the rectal swab alone and 9.1% (7/77) had GBS detected in the low vaginal swab only. Seventy-six GBS isolates underwent susceptibility testing. All isolates were susceptible to penicillin and vancomycin, as expected. Seventeen isolates were resistant to erythromycin (22.4%) and 13 were resistant to clindamycin (17.1%).

Discussion

The main findings from this diagnostic accuracy study is that HiberGene's GBS LAMP assay has good sensitivity, specificity, positive predictive value and negative predictive value compared with the reference enrichment culture. The turnaround time is superior to enrichment culture, providing rapid results with a run time of up to 66 min from the time when testing commences, compared with 40–48 h for enrichment culture. It is also comparable to other commercial nucleic acid amplification tests including PCR [3]. Furthermore, the test is straightforward to perform and could be used as an on-call test, thereby facilitating intrapartum testing. The opportunity for intrapartum testing compares favourably with late antenatal culture at 35–37 weeks of gestation due to the concerns regarding the accuracy of antenatal screening for an organism that may be carried intermittently [9,10].

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