Group B Streptococcus - Rapid Molecular Assay

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Technology description

CONTEXT

The Problem

Group B streptococcus (GBS) is one of the most important infectious causes of neonatal morbidity and mortality in developed countries, causing meningitis, pneumonia and septicaemia in newborn babies and their mothers. Vaginal colonisation by GBS is reported in up to 27% of women, and can lead to intrapartum infection of neonates. Early-onset disease (0-7 days) presents as pneumonia and/or bacteraemia, with case fatality rates of up to 29% reported. Late onset disease (7-90 days) presents as bacterial meningitis, with case fatality rates of up to 6%. Up to 30% of babies who survive late-onset disease suffer permanent disability.

In addition GBS is a prominant animal pathogen. GBS remains a leading cause of bovine mastitis leading to a decrease in the quality and quantity of milk production which has significant economic implications for the diary industry worldwide. Further, GBS has emerged as a significant fish pathogen causing outbreaks of disease and substantial economic loss especially for aquaculture industry based in warm water environments.

Some countries (notably the USA, Canada and Australia) have implemented routine culture-based universal screening and intrapartum antibiotic prophylaxis for pregnant women identified as colonised with GBS. This has been shown to prevent vertical transmission and to reduce the risk of early onset GBS infection. As maternal colonisation with GBS can be intermittent, transient or persistent it is crucial to screen pregnant woman as close to delivery as possible.

Conventional enriched culture of lower vaginal and rectal swabs remains the reference standard for the detection of GBS maternal colonization but this requires up to 72 hours for results to be known which precludes its use for intrapartum screening. In addition diagnosis of GBS sepsis in newborns by conventional culture methods is unreliable and time consuming. In the absence of an effective vaccine the rapid, sensitive and specific detection of GBS is crucial for patient mangement and reducing mortaility.

The Need

There is a clear requirement for improved methods to detect GBS which can be used immediately before or during labour, to identify women who should be offered prophylaxis and which could diagnose infection in newborns. Such methods also have widespread veterinary diagnostic applications reducing economic loss and maintaining animal welfare. The ideal method would be rapid, highly

sensitive and specific, and amenable to possible use as a near-patient or point-of-care (POC) or 'in the field' test. The only methods which can meet this specification are molecular "nucleic acid amplification" tests.

Existing solutions for GBS detection

Enriched culture of vaginal and anorectal swabs in LIM selective broth for 24 hours followed by subculture to blood agar for 40-48 hours is current HPA reference standard for processing swabs for GBS carriage. The turn around times associated with this testing algorithim effectively precludes its use as an intrapatrum screening assay.

Other solutions either use an expensive and automated system that is only applicable for laboratory based testing or require a long (18 hours +) enrichment step prior to amplification.

These characteristics make existing tests unsuitable for rapid near patient intrapartum use.

TECHNOLOGY

The Invention

The invention is a prototype in vitro diagnostic method for the rapid and specific detection ofStreptococcus agalactiae(Group B Streptococcus, GBS) nucleic acid (DNA) from clinical specimens. The designed assay utilises previously described Loop mediated isothermal amplification (LAMP) technology combined to detect a portion of the surface immunogenic protein (sip) gene unique to GBS.

LAMP is an isothermal molecular amplification technique which offers simple detection combined with high sensitivity and specificity. The robust chemistry, ease of operation, rapidity, low cost, and modest equipment requirements represent significant advantages over real-time PCR and potential to be deployed as a Point of Care (POC) test for the intrapartum detection of GBS.

Analytical Specificity

Analytical specificity of GBS LAMP was evaluated using a variety of nucleic acid preparations (extracts, boiled preps & Achromopeptidase lysates) derived from a wide range of bacterial and fungal strains. A total of 10 differentstreptococcusspecies were analysed. This included a number of GBS reference, clinical and veterinary strains. **The LAMP assay displays 100% specificity for GBS detection.**

Analytical sensitivity (Limit of Detection, LOD)

Analytical sensitivity was determined using total nucleic extracts containg a known quantity of GBS genome copies. LOD was determined as 14 genome copies per reaction (Equivalent to 2.80E03 genome copies per ml).

Clinical Validation (Initial)

Compared performance of LAMP assay with a qPCR assay and culture (direct plating only) for detection of GBS from screening swabs. Swabs undergo nucleic acid extraction with extracts tested by qPCR initially to ascertain GBS positivity and load status (genome copies/ml). A total of 121 swabs have been analysed.

LAMP	**qPCR**	**Culture**	
Positive	Negative	Positive	Negative
Positive	20	0	15	5

Negative	3	98	0	101
Sensitivity	87%	100%		
Specificity	100%	95.3%		
PPV	100%	75%		
NPV	97%	100%		

Three specimens identified as LAMP negative but qPCR positive (False Negatives) were determined to have genome copies per ml of 1.60E03, 1.20E03 & 2.50E03 respectively by qPCR.

Advantages

(i) Speed. The test offers a very low 'time-to-result', typically less than 30 minutes from receipt of specimen.
(ii) Sensitivity & specificity. The test offers very high sensitivity and specificity, comparable to the most sensitive laboratory tests available.
(iii) Simplicity. The test can be performed without specialised or expensive equipment and by staff with minimal training. Most importantly, it can be performed in a 'near-patient' setting (as well as a conventional laboratory setting), allowing immediate feedback of results to clinical staff.
The GBS LAMP assay consists of six LAMP primer sets termed Forward Inner Primer (FIP), Backward Inner Primer (BIP), Foward outer primer (F3), Backward outer primer (B3), Forward Loop Primer (FL) and Backward Loop Primer (BL).

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