



Comparison of Real-time PCR Methods and Membrane Elisa for Rapid Detection of Group B Streptococcus



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INTRODUCTION

Group B Streptococcal (GBS) disease in neonates is preventable with screening recommended at 28-32 weeks. Antibiotic prophylaxis is provided if maternal carriage is detected. However it is recognised that colonisation at 28-32 weeks does not correlate with carriage at partum. Thus mothers with high risk factors are admitted prophylactically placing considerable pressure on beds.

PCR and EIA methods are available as an alternative to culture, and offer more rapid turn-around-times. The aim of this study was to compare various PCR and EIA methods for the detection of GBS to our culture based method.

METHODS

The "gold standard" was considered to be routine culture where swabs are incubated in GBS broth Gray *et al* J Clin Microbiol 1979 Apr; 9(4):466-70 for 18-24 hours and then sub-cultured onto GBS agar at 24 hours.

BD GeneOhm Strep assay

854 duplicate vaginal/rectal swabs were collected, one for routine culture and the other for the BD GeneOhm Strep assay. Assays were performed 24/7 as urgent tests by Microbiology staff, following manufacturer's protocols.

Multiplexed-tandem (MT-) PCR

MT-PCR (Stanley, KK and Szewczuk, E. Nucleic Acids Res. 2005 Nov 24;33(20):e180) was used to detect GBS from various specimens (Table 2) using Easy-Plex assay 3201.01 <http://www.ausdiagnostics.com/>. The Gene-Plex automated robot performed the 1st step PCR and prepared the 72-well disc ready for 2nd step amplification in the Rotor-Gene 6000 (Corbett Life Science). Computer-generated analysis generated results of "present" or "absent" within 3 hours of test commencement. An internal spike control and Listeria-specific primers were incorporated into the assay. Assay sensitivity was determined using serial dilutions of wild-type GBS diluted 10 fold to 10⁻⁸ as template. Plate counts were performed to determine actual CFU of starting inocula.

Biostar OIA Strep immunoassay

PBS immunoassay single test was used to test 30 PBS vortexed samples that were culture and MT-PCR concordant using the urine protocol and 9 discordant samples.

RESULTS

BD GeneOhm Strep assay

Table 1. BD GeneOhm Strep assay compared with culture

N=854	PCR	Culture	
		POSITIVE	NEGATIVE
	POSITIVE	165	12 ^a
	NEGATIVE	19 ^b	658

Sensitivity 89.7 %; Specificity 98.2%

^a After Plate review 17 adjusted to 12

^b After plate review 21 adjusted to 19

Mean time to detection – 2.5 hrs from collection

MT-PCR GBS assay

Initial validation of dilutions of GBS prepared and tested in MT-PCR for LOD found MT-PCR detected 8 CFU starting template .

Table 2 MT-PCR template results compared to broth culture

Template used for MT-PCR Easy-Plex assay	Nos of samples tested	Culture pos/MT-PCR pos	Culture Neg/MT-PCR Neg	Culture Pos/PCR MT-Neg	Culture Neg/MT-PCR Pos
GBS broth (diluted 1:10,000)	92	28	59	0	5
GeneOhm Sample Buffer tubes	46	17	23	6	0
GeneOhm Lysis tubes	46	22	22	1	1
Swab vortexed in 1mL PBS	147	25	113	6	3

Table 3 Sensitivity and Specificity

Template used for MT-PCR Easy-Plex assay	Sens %	Spec %
GBS broth (diluted 1:10,000)	100	92.2
GeneOhm Sample Buffer tubes	73.9	100
GeneOhm Lysis tubes	95.7	95.7
Swab vortexed in 1mL PBS	80.6	97.4

Biostar OIA Strep B

Results of swabs vortexed in PBS using urine protocol

15 culture neg/MT-PCR neg All Negative
18 culture Pos/ MT-PCR pos All Positive

Table 4 Results of 9 culture/MT PCR discrepant samples tested with Biostar OIA StrepB

PBS samples tested	Number discrepant	Biostar POS	Biostar NEG
Culture Neg/MT-PCR Pos	3	1	2
Culture Pos/MT-PCR Neg	6	2	4

DISCUSSION

The BD GeneOhm GBS Assay uses 1.5 µL inocula and may be prone to operator sampling error. Repeat testing of Culture Pos/PCR Neg samples using either original or new lysate found 9/19 were now concordant.

The Easy-Plex GBS assay had very good specificity overall but its sensitivity appeared to be affected by the concentration of organisms present or quality of template. Using broth enrichment or DNA extracts as template, sensitivity was high (>97%). In direct specimen sampling, MT-PCR was less sensitive than culture unless using template from enrichment or extraction. The use of the Gene-Plex Robot made sample preparation and loading of discs simple and required minimal operator time. Cost is slightly cheaper than culture but it is batch dependent and if prior enrichment is used this will increase overall cost, add to result time and processing workload.

Although more testing is needed with the Biostar OIA Strep B Kit, the results are promising although sensitivity issues are evident when compared to broth culture for GBS detection. Broth culture can give 5-15% increase in yield of GBS compared with direct plating. The clinical significance of detecting low carriage numbers at birth needs to be assessed. The kit was relatively easy to use although slightly more expensive than culture. It requires subjective interpretation which may create interpretation errors.

CONCLUSION

Although expensive, the BD GeneOhm assay is ideally suited for random access and sampling at partum.

MT-PCR is promising but extraction may be needed to increase sensitivity of assay.

Biostar OIA Strep represents an alternative to instrument based real-time PCR methods but is more suited to single specimen processing.

These methods allow practical alternatives for differently sized laboratories, workloads and indications for detection of GBS in antenatal patients either as part of a routine antenatal screening strategy or for rapid detection during labor.