

Rapid oligochromatographic assay for the detection of GBS (group B streptococcus) in vaginal samples

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Introduction and Purpose

Antenatal screening of pregnant woman to detect colonization by group B streptococci (GBS) has led to a reduction of GBS infections in newborns, traditionally one of the main causes of mortality in this group. Culture methods have been widely used for this purpose. Yet, the long time required to get results (between 24 and 48 hours) has promoted the search of alternative diagnostic methods. Antigen detection and amplification of DNA have been proposed. While the former seems to lack the sensitivity and specificity demanded for this type of test, the PCR technique has proved to be a suitable alternative for this purpose. Yet, detection of amplicons from end-point PCR assays has traditionally been time-consuming, technically demanding and associated to the use of hazardous substances, while the newer real-time PCR (qPCR) assays imply the use of expensive reagents and equipment, not always available in all laboratories.

More recently, oligochromatography (a technique based in the well-known lateral-flow immunochromatographic method widely used for immunological assays) has been used for an easy, rapid and cheaper detection of PCR products.

The aim of this study was to evaluate a rapid and simple new commercial assay (Speed-oligo GBS, Vircell) for the detection of GBS based on a PCR plus a rapid test device that provides results in less than one hour.

Methods

• **Clinical samples:** vaginal and recto-vaginal swabs submitted to the Microbiology Service of NT Hospital in Jaén for routine GBS detection were included in the study. Double swabs collected in Amies transport medium were used for the study. One was used for culture, while the second one was used for molecular assays. DNA was extracted from the swab by simple heating at 95°C for 5 minutes.

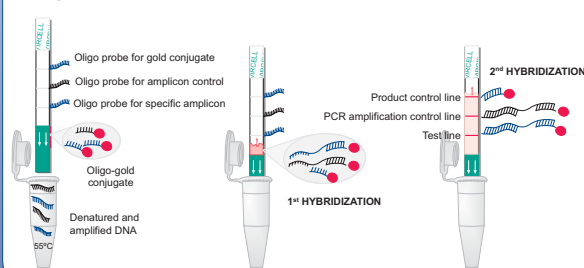
• **Culture:** isolation of GBS was carried out in Granada medium. Blood agar plates were also used: a commercial agglutination was used to confirm the identification of b-haemolytic colonies.

• **Speed-oligo GBS:** this new assay includes two steps. A PCR technique targets the *cfb* gene of GBS. Amplicons are detected in a second step by means of a dipstick comprising a probe conjugated to gold particles able to hybridize with the amplified DNA, and a test line containing a second complementary probe that captures the complexes formed between the amplicons and the gold conjugate to produce a pink colored line in positive samples. The oligochromatographic device also includes a PCR control line (reacting with a PCR product that is amplified from a spurious plasmid and its corresponding primers included in the PCR mix) to discard inhibitory effects and a control line to verify the correct flow through the dipstick. Figure 1 schematically describes this process. 5 µl of denatured PCR products are mixed with 35 µl of running buffer preheated at 55°C in a heating block. A dipstick is inserted into the reaction tube and the reaction mixture is allowed to run through it for 5 min while the reaction tubes are kept in the heating block. The reaction is visually read.

• **Analytical sensitivity and specificity:** dilutions of a quantified DNA from *S. agalactiae* were used to establish analytical sensitivity of the test. Swabs artificially inoculated with 75 µl of quantified bacterial suspensions were also used; replicas of these swabs were seeded onto blood agar plates for counting. DNA extracted from other microorganisms were tested to check the specificity of the assay.

• **qPCR:** Real Time PCR on a different target gene (*sip*) was carried out as described in the literature (Bergseng et al. 2007. J Med Microbiol 56: 223) to help solve possible discrepancies with the culture technique. Additional sequencing was carried out in those samples repeatedly discrepant by the Speed-oligo GBS assay.

Figure 1



Results

• Analytical sensitivity of Speed-oligo GBS was established in 8 genome copies per reaction as calculated from DNA dilutions.

• When swabs artificially inoculated with *S. agalactiae* were tested, the oligochromatographic assay was able to detect 100% of the swabs inoculated with 250 CFU per swab. An average of 5 CFU grew when replicas of these swabs were seeded onto agar plates.

• No microorganism other than GBS (see table 1) reacted in the Speed-oligo test.

• Taking culture as the gold standard, the new oligochromatography-PCR assay showed a 93% sensitivity, 93% specificity, 93% overall agreement (table 2).

• False negative results in Speed-oligo GBS corresponded to samples with a low bacterial load (<5 CFU per plate). PCR from colonies gave positive Speed-oligo GBS results, excluding genotype variations as the cause of these false negative results.

• When a second PCR was performed, 9 out of the 11 false positive results of Speed-oligo GBS were also positive by the qPCR, which targeted a completely different gene.

• A new gold standard was defined in which all samples with a positive culture result and/or a positive PCR consensus result were considered positive. All tests were evaluated against this new gold standard (table 3) with sensitivities (Ss) and specificities (Sp) values of 89% and 100%, 90% and 100%, 94% and 99% respectively for culture, qPCR and Speed-oligo GBS.

• The sequences from the 2 samples that were only positive in the oligochromatographic assay only showed agreement with *S. agalactiae*. In one case, the sequence presented a mutation in one base pair against the canonic sequence of GBS in this region, decreasing the probability of cross-over contamination as the cause of this positive result.

Table 1

Microorganism	Strain	Source
<i>Aspergillus fumigatus</i>	MCV-CH10	ATCC
<i>Bacillus cereus</i>	Type strain	NCTC
<i>Bordetella pertussis</i>	F	ATCC
<i>Candida albicans</i>	3153	NCPF
<i>Chlamydia trachomatis</i>	434	ATCC
<i>Chlamydia pneumoniae</i>	CM-1	ATCC
<i>Chlamydia psittaci</i>	6BC	ATCC
<i>Coxiella burnetii</i>	Nine Miles	ATCC
<i>Haemophilus influenzae</i>	Clinical isolate	HNTJ
<i>Haemophilus ducreyi</i>	Type strain	NCTC
<i>Legionella pneumophila</i>	Philadelphia-1	ATCC
<i>Mycoplasma pneumoniae</i>	FH	ATCC
<i>Neisseria meningitidis</i> ser. A	Type strain	NCTC
<i>Neisseria meningitidis</i> ser. B	Type strain	NCTC
<i>Neisseria meningitidis</i> ser. C	Type strain	NCTC
<i>Rickettsia conorii</i>	Moroccan	ATCC
<i>Salmonella typhi</i>	Clinical isolate	HNTJ
<i>Streptococcus pneumoniae</i>	Clinical isolate	HNTJ
<i>Toxoplasma gondii</i>	RH	ATCC
<i>Brucella abortus</i>	S-99	Comm
HSV1	MacIntyre	ATCC
HSV2	MS	ATCC
Group C streptococcus	Clinical isolate	HNTJ
Group F streptococcus	Clinical isolate	HNTJ
<i>Streptococcus constellatus</i>	Clinical isolate	HNTJ
<i>Streptococcus viridans</i>	Clinical isolate	HNTJ
<i>Enterococcus faecalis</i>	Clinical isolate	HNTJ
<i>Streptococcus pyogenes</i>	Clinical isolate	HNTJ
<i>Gardnerella vaginalis</i>	Clinical isolate	HNTJ
<i>Ureaplasma urealyticum</i>	Clinical isolate	HNTJ
<i>Mycoplasma hominis</i>	Clinical isolate	HNTJ

Table 2

Speed-oligo GBS		CULTURE		
		POS	NEG	TOTAL
POS	69	11 [#]	80	
NEG	5*	150	155	
TOTAL	74	161	235	

* < 5 colonies/ plate- colony PCR positive

[#] 9 confirmed as positive in the qPCR assay based on a different target (*sip* gene)

Table 3

Speed-oligo GBS		Culture + PCR consensus			Ss= 94% Sp= 99%
		POS	NEG	TOTAL	
POS	78	2	80	Ss= 89% Sp= 100%	
NEG	5	150	155		
Culture	POS	74	0	74	Ss= 90% Sp= 100%
	NEG	9	152	161	
qPCR	POS	75	0	75	Ss= 90% Sp= 100%
	NEG	8	152	160	
TOTAL		83	152	235	

Conclusions

• Speed-oligo GBS proved to be a rapid, sensitive and specific assay for the detection of GBS colonization, based on nucleic acid amplification techniques coupled to a simple oligochromatographic method.

• The few false negative results in the oligochromatography-PCR assay were probably due to the low colonization level of those patients. The fact that different swabs were used for culture and PCR assays can account in part for this difference.

• When the test was carried out with GBS colonies isolated from these patients, positive results were obtained, discarding the possibility that the test was missing certain GBS genotypes present in those samples.

• Most culture negative samples scored as positive in the commercial assay, could also be detected with an alternative qPCR test, based on a different target gene, thus ruling out the possibility of cross-over contamination. The use of double swabs as pointed above, together with the fact that culture requires the presence of viable microorganisms, can explain these differences.

• Sequencing of the amplicons from the two samples that were only positive in the oligochromatographic assay also indicates a high degree of specificity for the test. This specificity is sustained on the double hybridization used for the detection of the amplified PCR products.

• No cross-reactivity was found with microorganisms other than GBS.

• Although Speed-oligo GBS and culture did not show significant differences (McNemar chi-square test) in classifying the samples, the oligochromatographic test was slightly more sensitive when compared with the "Culture + PCR consensus" gold standard.