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Loop-Mediated Isothermal Amplification (LAMP) assay for Rapid detection of *Streptococcus agalactiae* (Group B Streptococcus - GBS) in vaginal swabs – A Proof of Concept Study

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Abstract

Neonatal infection with *Streptococcus agalactiae* (Group B streptococcus – GBS) is a life threatening condition which is preventable if colonised mothers are identified and given antibiotic prophylaxis during labour. Conventional culture is time consuming and unreliable, and many available non-culture diagnostics are too complex to implement routinely at point of care. Loop-mediated isothermal amplification (LAMP) is a method which enables the rapid and specific detection of target nucleic acid sequences in clinical material without any requirements for extensive sample preparation. A prototype LAMP assay targeting the GBS *sip* gene is described. The assay was 100% specific for GBS with a Limit of Detection of 14 genome copies per reaction. The clinical utility of the prototype LAMP assay for rapid direct molecular detection of GBS was determined by testing a total of 157 vaginal swabs with

minimal sample processing using a rapid lysis solution. Compared to a reference qPCR assay the direct LAMP protocol had a sensitivity and specificity of 95.4% and 100% with a Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of 100% and 98.3%. Positive (LR+) and negative (LR-) likelihood ratios were infinity and 0.05 respectively. The direct LAMP method took a mean of 45 minutes from receipt of a swab to generation of confirmed result compared to 2 hours 30 minutes for the reference qPCR test. The direct LAMP protocol described is easy to perform facilitating rapid and accurate detection of GBS in vaginal swabs. This test has potential for use at point of care.

Introduction

Streptococcus agalactiae (Group B streptococcus - GBS) is the leading cause of early onset neonatal sepsis [1-5]. Asymptomatic GBS colonisation of the vagina and lower gastrointestinal tract occurs in 10-30% of pregnant women and is the most significant risk factor for neonatal GBS disease as transmission can occur from the recto/vaginal area during childbirth [4,6-9]. The implementation of culture based antepartum screening programmes for all pregnant women at 34-38 weeks and initiating intrapartum antibiotic prophylaxis has helped to reduce but not eliminate neonatal early onset disease (EOD) in many industrialised countries [4,10,11]. Despite technological advances the primary method for detection of GBS colonisation remains selective laboratory based culture of vaginal and/or rectal swab specimens followed by ancillary confirmation tests. This approach demonstrates variable sensitivity [12-14] and is associated with long turnaround times of 18-72 hours rendering culture unfeasible as an intrapartum test. Thus, women who give birth prematurely are not screened, and women who become colonised after screening are not identified [7,8]. A sensitive screening assay which could rapidly determine GBS status during labour would be

beneficial as it would facilitate targeted therapy whilst reducing the risks associated with antibiotic use in non-colonised women [15].

Quantitative Polymerase Chain Reaction (qPCR) assays can provide test results in a few hours [15-20]. However, requirements for expensive equipment, intricate sample preparation, skilled staff and/or support precludes the use of PCR at point of care (POC) [21-23].

Loop-mediated isothermal amplification (LAMP) is an isothermal molecular amplification technique which offers simple qualitative detection combined with exceptional sensitivity and specificity [24-26]. The single reaction temperature (60 – 65^0C) employed by LAMP means there are no requirements for thermocycling resulting in a need for less complex equipment making it amenable to use at POC [22,27]. Furthermore compared to PCR, LAMP is also demonstrably more resistant to inhibitors found in clinical material indicating potential for direct application to clinical specimens and/or crude sample preparations [21,28,29]. This is advantageous as one of the issues frequently hampering application of Nucleic acid amplification tests (NAAT) at POC are requirements to perform dedicated multistep nucleic acid extraction steps which add to test complexity, cost and turnaround time [21-23]. Whilst a number of LAMP assays for GBS detection have been developed previously, they are not amenable for POC use having requirements for an 18-24 hr pre-enrichment culture step [20], require multiple time consuming manipulations prone to contamination [30] or have unproven clinical efficacy [31,32].

Here we describe a newly designed GBS LAMP assay targeting *S. agalactiae sip* gene. As a proof of concept the LAMP assay was applied to residual vaginal swabs which had undergone minimal sample processing (Direct detection – no extraction protocol) with a rapid lysis solution containing the bacteriolytic enzyme achromopeptidase (ACH). ACH demonstrates high lytic activity against a range of gram positive cocci including GBS [23,33-35].

Materials and Methods

Clinical Specimens: The study was approved by the Office for Research Ethics committee Northern Ireland (reference 09/NIR02/43). A total of 157 residual vaginal swabs from pregnant women submitted to Department of Microbiology, Belfast Health and Social Care Trust (BHSCT) for bacterial investigations between March – July 2013 were opportunistically collected and utilised for the clinical validation study. The sample set consisted of 153 High Vaginal swabs (HVS) and 4 Low Vaginal Swabs (LVS). All vaginal swabs were collected on plastic rayon tipped swabs (Sterilin Ltd., Newport, UK) and transported to laboratory in AMIES transport medium. Culture of all swab specimens was carried out according to BHSCT Standard Operating Procedure by laboratory staff unaware that the samples were to be subsequently included in a study. Briefly, each swab was plated onto Columbia Blood agar (CBA) and differential Granada™ agar (bioMérieux, Basingstoke, UK). CBA plates were incubated in 10% CO_2 atmosphere with Granada™ plates incubated anaerobically at 37^0C. Plates were examined at 18-24 hours and again at 48 hours with presumptive positive GBS colonies on Granada™ agar identified by production of orange pigment. Presumptive GBS colonies on CBA were identified on the basis of haemolysis. All suspect colonies were appropriately identified by a combination of standard tests including Gram stain, catalase activity and latex agglutination using SLIDEX® Strepto Plus kit (bioMérieux, Basingstoke, UK) to identify the presence of Group B antigen. Following routine bacteriological culture all residual swabs were stored at $+4^0$C for a maximum of one week until required for processing and molecular analysis.

Rapid Sample preparation method for direct LAMP: Lysis solution consisted of 10 U/L of achromopeptidase (ACH) (Sigma-Aldrich, Dorset, UK) in 10 mmol/L Tris-HCL pH8.0, 1

mmol/L EDTA (Sigma-Aldrich, Dorset, UK). Residual swab specimens were removed from AMIES transport medium tubes with ~2 cm end physically broken off into 400 µl pre aliquoted lysis solution [ACH] by firmly pressing the swab against the side of a microfuge tube (Sarstedt, Leicester, UK). Tubes were capped and swabs incubated at ambient 'room temperature' (22 - 24^{0}C) for 5 minutes before boiling at 95^{0}C for 5 minutes in a dedicated heat block to simultaneously deactivate ACH and denature any target DNA for LAMP analysis. Samples were immediately cooled by placing directly on ice. A 5µl aliquot of lysis supernatant was used for GBS direct LAMP testing as outlined below. Remaining lysis supernatant was stored at 4^{0}C.

Nucleic acid (NA) extraction for qPCR and indirect LAMP: GBS reference and clinical isolates were pre-treated with ACH before undergoing total nucleic acid extraction using QIAamp blood DNA mini extraction kit (Qiagen, Crawley, UK). A 200 µl saline suspension of each GBS isolate was prepared and added to an equal volume of ACH 20 U/L for a final working concentration of ACH of 10 U/L. The suspension was incubated at room temperature for 5 minutes before being boiled at 95^{0}C/5 minutes. 200 µl aliquot of each lysate was then added to AL lysis buffer (Qiagen, Crawley, UK) and processed as per manufacturer's instructions to produce 100 µl of genomic extract.

Bacterial and fungal genomic DNA for specificity evaluation was obtained using QIAamp blood DNA mini extraction kit (Qiagen, Crawley, UK). 200 µl saline suspensions of each target organism were added to AL lysis buffer and processed as per manufacturer's instructions to produce 100 µl of genomic extract.

Vaginal swab lysates were extracted as follows; A 200 µl aliquot of ACH lysis supernatant (produced as indicated in previous section) was removed from each clinical swab specimen and extracted using MagNA Pure 96 viral NA small volume kit and MagNA Pure instrument

(Roche Diagnostics, Mannheim, Germany) as per manufacturer's instructions. Resultant 100 µl genomic extract aliquots were stored at -80^{0}C until required for qPCR and LAMP (indirect) analysis.

LAMP primers and reaction: LAMP primers targeting a highly conserved region of *S. agalactiae sip* gene (HiberGene Diagnostics, Ireland) were employed throughout. The LAMP primer set consisted of forward (sip_FIP) and reverse (sip_BIP) 'inner' primers, forward (sip_F3) and reverse (sip_B3) 'outer' primers and forward (sip_FL) and reverse (sip_BL) 'loop' primers. Optimised GBS LAMP consisted of 5 µl of template DNA, 12.5 µl of LAMP isothermal Master Mix ISO 001 (OptiGene Ltd., Horsham, UK) containing novel strand displacing DNA polymerase (*GspSSD*), propriety thermostable inorganic pyrophosphatase, reaction buffer, MgCl, dNTP's, DNA intercalating dye, 2 µM each of FIP and BIP, 0.2 µM each of F3 and B3, 1 µM each of FL and BL and Nuclease Free Water (Promega, Southampton, UK) for a final reaction volume of 25 µl. The GENIE® II (OptiGene Ltd, Horsham, UK) real time fluorometer was utilised for concurrent target isothermal amplification and detection. LAMP reactions were performed at a previously identified optimal reaction temperature of 62^{0}C for 30 minutes followed by a heating and cooling step to 98–80 ^{0}C (0.05 ^{0}C/s) which allows re-annealing of amplified DNA and display of a specific annealing curve. Positive and negative controls were included for each run and stringent precautions undertaken to prevent cross contamination. Positive LAMP reactions were identified by GENIE® II (OptiGene Ltd, Horsham, UK) instrument software. In the results section each positive reaction had a Time to positivity (T_T) value in minutes and seconds and a specific melting point temperature (Tm) in degrees centigrade (^{0}C).

Real Time PCR (qPCR): qPCR was employed as the reference molecular detection method. Primer and hybridisation probe sequences targeting *S. agalactiae sip* gene were as previously described [17]. Primer sequences as follows: Forward primer 5'–ATCCTGAGACAACACTGACA–3', and reverse primer 5'-TTGCTGGTGTTTCTATTTTCA-3'. The hybridisation probe 5'-ATCAGAAGAGTCATACTGCCACTTC3' contained a FAM label on 5' end with a Black Hole Quencher 1 (BHQ1) on 3' end. Primers and hybridisation probe were synthesised by Eurogentec (Eurogentec, Liège Belgium). The final qPCR reaction mix contained 1X Platinum® UDG Mastermix (Life Sciences, Paisley, UK), 0.2 µM Bovine serum albumin (Sigma, Dorset, UK), total of 4 mmol/L $MgCl_2$, 0.4 µM forward and reverse primers, 0.2 µM hybridisation probe, Nuclease Free Water (Promega, Southampton, UK) and 3 µl of target template for a final reaction volume of 12 µl. qPCR was performed using a Light Cycler 480 (LC480) instrument (Roche Diagnostics, Mannheim, Germany). A No Template Control (NTC) consisting of Nuclease Free Water (Promega, Southampton, UK) and a plasmid reference standard containing 3.50E06 *sip* genome copies per ml were included for all qPCR runs. The qPCR run parameters consisted of an initial step of 95°C for 10 minutes followed by an amplification program for 45 cycles of 10 seconds at 95°C, 30 seconds at 60°C, 1 second at 72°C with fluorescence acquisition at the end of each extension. Data was analysed using LC480 software and GBS genome copy number for positive specimens determined from crossing point threshold (Cp) relative to an external positive control curve.

Specificity & sensitivity: Specificity of GBS LAMP was evaluated using total genomic extracts (containing approximately 10E07 genome copies) derived from a range of bacteria (n=55) and fungi (n=1). These included a number of GBS reference, clinical and veterinary isolates (n=17). Reaction mix without DNA template was included as a negative control.

The following organisms were tested; *Streptococcus agalactiae* (NCTC 11360, NCTC 8187, NCTC 11078, NCTC 11079, NCTC 11080, NCTC 11930, NCTC 9829, ATCC 12386, ID: 603532, ID: 603535, ID: 605872, RVH2419/12, RVH2543/12, RVH60/13, RVH524/13, VSD04988, VSD04946), *Streptococcus pneumoniae* (ATCC 49619, RVH59/07), *Streptococcus parasanquis* (RVH74/07), *Streptococcus intermedius (*RVH305/07), *Streptococcus pyogenes* (ATCC 19615), *Streptococcus salivarius* (RVH557/13), *Streptococcus vestibularis* (RVH527/13), *Streptococcus mitis/oralis* (RVH508/13), *Streptococcus dysglactiae* (RVH497/13), *Streptococcus anginosus* (RVH452/13), *Staphylococcus aureus* (ATCC 25923), MRSA (NCTC 10442), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus lugdunensis* (RVH isolate), *Staphylococcus capitis* (RVH isolate), *Enterococcus faecalis* (ATCC 29212, RVH009/07), *Micrococcus luteus* (RVH290/07), *Brevibacillus brevi* (RVH381/13), *Bacillus cereus* (RVH299/13), *Neisseria meningitidis* (NCTC 10791 serogroup Y, RVH531/07 serogroup B), *Neisseria sicca* (RVH920/06), *Klebsiella pneumoniae* (RVH45/07), *Klebsiella oxytoca* (RVH135/07), *Enterobacter aerogenes* (NCTC 10006, RVH001/07), *Enterobacter cloacae* (NCTC 9394), *Serratia marcescens* (RVH287/07), *Acinetobacter baumanii* (RVH36/07), *Acinetobacter lwoffii* (RVH98/07), *Escherichia coli* (NCTC 9001, ATCC 25922), *Citrobacter freundii* (NCTC 9750), *Haemophilius influenzae* (NCTC 4560), *Sphingobacterium spiritivorum* (ATCC 33861), *Stenotrophomonas maltophilia* (ATCC 17666), *Pseudomonas aeruginosa* (ATCC 27853), *Gardnerella vaginalis* (ATCC 49145), *Candida albicans* (ATCC 10028).

LAMP analytical sensitivity (limit of detection - LOD) was determined using tenfold serial dilutions of *S. agalactiae* ATCC 12386 nucleic acid extracts analysed in duplicate containing 2.8E07 to 2.8E02 genome copies per ml respectively. A No Template Control (NTC) consisting of Nuclease Free Water (Promega, Southampton, UK) was included with all LAMP runs.

Statistical analysis: For purposes of validation the reference molecular method was defined as achromopeptidase (ACH) pre-treatment of a clinical swab specimen followed by Nucleic Acid (NA) extraction and qPCR testing. A specimen was considered a true positive if: (i) GBS was obtained following culture; or (ii) Specimen was identified as GBS qPCR positive. All qPCR and LAMP results were compared with routine culture results. Sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV), positive (LR+) and negative (LR-) likelihood ratios for each test method were calculated. The level of agreement between LAMP and qPCR assays was assessed using Kappa (κ) coefficient with 95% confidence levels. κ was calculated using the formula Pr(*o*) - Pr(*e*)/1 - Pr(*e*), where Pr(*o*) is the observed agreement and Pr(*e*) is the hypothetical probability of chance agreement.

Results

Analytical reactivity, specificity and sensitivity: The LAMP assay successfully amplified DNA from all GBS reference, clinical and veterinary strains analysed with Time to positivity (T_T) values of less than 20 minutes and specific melt point temperature values (Tm) ranging from 85.5 – 86.21^{0}C obtained. No amplification was recorded for any other bacterial or fungal species tested. This confirmed 100% specificity of the GBS LAMP assay.

Employing tenfold serial dilutions of a known concentration (2.8E07 to 2.8E02 copies per ml) of GBS ATCC 12386 nucleic acid extracts in duplicate the LAMP assay consistently detected 2.80E03 *sip* genome copies per ml corresponding to an analytical Limit of Detection (LOD) of 14 genome copies per reaction (for 5µl input volume and final reaction volume of 25µl). The mean GBS LAMP T_T and Tm for 2.80E03 *sip* genome copies per ml were determined as 11 minutes and 85.98^{0}C respectively. The LAMP LOD was slightly higher than that achieved for qPCR which in our laboratory had a previously determined LOD of 1.00E03 genome copies per ml equating to 2 genome copies per reaction.

Clinical Validation: Routine bacteriological culture was compared to the reference qPCR molecular method, direct LAMP and indirect LAMP methods. Summary of findings are outlined in Tables 1 and 2. A total of 36/157 (22.9%) specimens analysed were confirmed as GBS culture positive. All 36 culture positive GBS specimens were correctly identified as positive by qPCR and by the direct and indirect LAMP methods. An additional 7 specimens were identified as GBS positive by qPCR giving a total of 43/157 (27.4%) 'true' positive specimens. Comparison of difference between qPCR and culture results was statistically significant (*p=0.0005)*. The loads of GBS determined by qPCR (genome copies per ml) for positive clinical specimens were as follows: Mean = 1.23E+08; Max= 2.50E+09; Min=

1.90E+03; Median= 2.40E+06. Compared to culture qPCR had a sensitivity and specificity of 100% and 94.2% with a PPV and NPV of 83.7% and 100%. Positive (LR+) and negative (LR-) likelihood ratios were infinity and 0.16 respectively. The mean genome copies per ml of the seven identified specimens missed by culture but positive by qPCR was 1.09E06 (Range 1.90E03 – 7.60E06; Median 5.50E03) compared to 1.47E08 (Range 4.60E03 - 2.50E09; Median 3.65E06) for specimens identified as both culture and qPCR positive. This difference in mean genome copies per ml was statistically significant (*p=<0.0001*).

The direct LAMP method took a mean of 45 minutes from receipt of a swab to generation of confirmed result compared to 1 hour 45 minutes for indirect LAMP (extraction protocol) and 2 hours 30 minutes for reference qPCR test.

There was a very high level of agreement between LAMP (Indirect and direct) methods and reference qPCR assay. Compared to qPCR agreement ranged from 100% for indirect LAMP (Kappa = 1.0; Strength of agreement = perfect) to a level of agreement of 98.7% for direct LAMP (Kappa = 0.968; Strength of agreement = very good). Indirect LAMP method identified 43/157 (27.4%) specimen extracts as GBS positive with the direct LAMP method identifying a total of 41/157 (26.1%) of crude specimen ACH lysates as GBS positive. Compared to qPCR the indirect LAMP method had a sensitivity and specificity of 100% and a PPV and NPV of 100%. Positive (LR+) and negative (LR-) likelihood ratios were infinity and 0.00 respectively. Compared to qPCR the direct LAMP had a sensitivity and specificity of 95.4% and 100% with a PPV and NPV of 100% and 98.3%. Positive (LR+) and negative (LR-) likelihood ratios were infinity and 0.05 respectively. Two specimens (405 & 453 see Table 2) were identified as false negative after analysis by direct LAMP method. Following nucleic acid extraction and analysis by qPCR these specimens tested GBS positive with genome copy loads of 1.90E+03 per ml (9.5 copies per 5µl addition) and 1.91+E03 per ml (9.6 copies per 5µl addition) respectively. Additionally following nucleic acid extraction both

of the specimens tested LAMP positive returning T_T values of 12.15 and 29.00 minutes respectively. Overall the mean time to achieve positivity (T_T) for direct LAMP method was higher compared to indirect LAMP method; 14.83 minutes (range 8.15 – 26.00 minutes) compared to 9.60 minutes (range 5.00 – 29.00 minutes). Testing performance comparison is displayed in Table 1 with all specimens identified as positive by either culture, qPCR and/or LAMP is presented in Table 2.

Discussion

Screening for maternal GBS colonisation at 35-37 weeks via selective culture is a time consuming and inadequate method which fails to identify all cases of GBS [12,13,20]. There is a recognised clinical need for rapid, highly sensitive and specific methods to detect GBS which can be used at POC before or during labour to identify women for prophylactic antibiotic treatment. Currently qPCR offers the required specification to achieve these goals however technical obstacles including requirements for stringent sample preparation, complex instrumentation and skilled personnel have restricted their introduction at POC [21-23]. The robust chemistry, ease of operation, rapidity, low cost, and modest equipment requirements associated with LAMP represent significant advantages over existing molecular methods for detection of pathogens [22,27,29,36].

The primary objective of this proof of concept study was to validate the effectiveness of a prototype LAMP assay (HiberGene diagnostics, Ireland) in combination with a simple rapid specimen lysis protocol (without extraction) for direct detection of GBS in residual vaginal swabs. Pre-lysis with ACH at a concentration of 10 U/L for 5 minutes at room temperature has been previously shown in our laboratory to be a highly effective method to significantly increase the available yield of GBS nucleic acid for molecular analysis [35]. Eliminating the

299 requirement for nucleic acid extraction not only simplified the test procedure but also resulted
300 in significant time savings with direct LAMP taking 45 minutes from receipt of swab to
301 definitive GBS result compared to 60 minutes and >2 hrs for indirect LAMP and qPCR
302 respectively. The GBS LAMP assay had an analytical LOD of 2.80E03 genome copies per ml
303 which compared favourably with the determined reference qPCR analytical LOD of 1.00E03
304 genome copies per ml. Crucially when applied to clinical specimens, qPCR and both direct
305 and indirect LAMP methods resulted in identification of additional GBS positives (n=7)
306 missed by culture. These findings were not unexpected and are consistent with previous
307 studies demonstrating an increase in GBS positivity rate when Nucleic acid amplification
308 tests (NAAT's) are directly compared to culture [13,16,18,19,20]. Higher detection rates of
309 NAAT's have been claimed to be due to the inability of culture to detect low numbers of
310 GBS bacteria but may also be attributed to the presence of antagonistic flora and/or the
311 detection of non-culturable cells [19,37].
312 Compared to the reference qPCR method the direct LAMP had a sensitivity of 95.4% and
313 specificity of 100%. High specificity is an intrinsic feature of LAMP and reflects the
314 presence of six specific LAMP primers recognising eight target regions on *sip* gene. The
315 direct LAMP assay returned higher mean T_T values for identified positive specimens
316 compared to the indirect LAMP assay applied to the same samples which had undergone
317 nucleic acid extraction (14.69 minutes versus 9.61 minutes respectively - difference of 5.08
318 minutes). Additionally the direct LAMP assay failed to detect GBS in two specimens
319 subsequently shown to contain low genomic loads by qPCR. These findings indicate that the
320 presence of component(s) in crude lysate matrices can impede LAMP and in conjunction
321 with low initial GBS loads may have contributed to the two false negative results. Solution(s)
322 to combat this could include prior centrifugation and/or a dilution step(s) although this would
323 complicate the assay protocol and make application less attractive at POC. At present the

clinical significance of specimens containing a low GBS burden remains to be determined [16]. The excellent performance of qPCR and LAMP in this study must also be taken in context as 'second hand' residual vaginal swabs post bacteriological culture as described would certainly benefit from further field evaluation employing dedicated recto/vaginal swabs in order to fully establish diagnostic and analytical characteristics. Efforts are currently underway to address this.

Whilst this study utilised a GENIE® II fluorometer (cost £5000) to conduct isothermal amplification and specific detection of GBS in real time it would be entirely feasible to perform GBS LAMP testing using a simple heating block in conjunction with simple visual endpoint interpretation. This represents a commonly applied and valid approach for LAMP testing particularly in resource limited settings [27,38,39].

In conclusion the data presented demonstrates the efficacy of employing LAMP on minimally processed vaginal swabs for rapid direct molecular detection of GBS. The simplicity of the direct LAMP protocol combined with described sensitivity and specificity are desirable characteristics amenable to POC use in resource limited settings.

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Table 1 LAMP (Indirect & direct) and culture testing performance for clinical swabs (n=157) compared to reference GBS qPCR method. For LAMP the mean, maximum, minimum and median time to achieve LAMP positivity in minutes: seconds (T_T) and specific melt temperatures (Tm) in degrees centigrade are shown.

GBS qPCR	**LAMP (INDIRECT)**		**LAMP (DIRECT)**		**Culture**	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive	43	0	41	2	36	7
Negative	0	114	0	114	0	114
Sensitivity	**100%**		**95.4%**		**100%**	
Specificity	**100%**		**100%**		**94.2%**	
PPV	**100%**		**100%**		**83.7%**	
NPV	**100%**		**98.3%**		**100%**	
(LR+)	**Infinity**		**Infinity**		**Infinity**	
(LR-)	**0.00**		**0.05**		**0.16**	
Mean T_T (Tm)	09.61 mins (86.66^{0}C)		14.69 mins (86.68^{0}C)		NA	
Max T_T (Tm)	29.00 mins (87.60^{0}C)		26.00 mins (87.60^{0}C)		NA	
Min T_T (Tm)	05.00 mins (85.86^{0}C)		08.15 mins (85.75^{0}C)		NA	
Median T_T (Tm)	08.30 mins (86.39^{0}C)		14.00 mins (86.40^{0}C)		NA	

NA; Not applicable

Table 2 Individual vaginal swab specimens identified either as GBS positive by culture, qPCR, indirect and/or direct LAMP methods. For qPCR genome copies per ml detected are shown. For LAMP the time to achieve positivity in minutes T_T and specific melt temperatures (Tm) in degrees centigrade are shown

Specimen Code	**Culture Result**	**qPCR Result copies/ml**	**Indirect LAMP Result T_T mins (Tm)**	**Direct LAMP Result T_T mins (Tm)**
334	+	+ 2.4E06	+ 08.20 (87.20^{0}C)	+ 17.50 (87.60^{0}C)
337	**+**	+ 5.6E03	+ 11.50 (87.60^{0}C)	+ 22.20 (87.60^{0}C)

354	+	+ 1.1E09	+ 05.30 (87.30^{0}C)	+ 09.51 (87.40^{0}C)
355	-	+ 4.2E03	+ 10.30 (87.11^{0}C)	+ 21.00 (87.10^{0}C)
370	+	+ 9.0E04	+ 09.30 (87.60^{0}C)	+ 19.00 (87.25^{0}C)
372	-	+ 6.3E03	+ 15.15 (87.41^{0}C)	+ 16.00 (86.00^{0}C)
373	+	+ 2.2E06	+ 08.00 (87.10^{0}C)	+ 14.50 (87.10^{0}C)
374	+	+ 2.5E09	+ 05.00 (87.30^{0}C)	+ 09.30 (87.41^{0}C)
376	+	+ 3.6E04	+ 09.30 (87.43^{0}C)	+ 20.15 (87.60^{0}C)
380	+	+ 2.8E04	+ 12.00 (87.09^{0}C)	+ 24.15 (87.20^{0}C)
382	+	+ 4.6E03	+ 17.15 (87.55^{0}C)	+ 21.00 (87.40^{0}C)
392	-	+ 5.5E03	+ 12.00 (87.40^{0}C)	+ 14.50 (86.61^{0}C)
395	+	+ 2.4E04	+ 11.45 (87.35^{0}C)	+ 20.30 (87.55^{0}C)
403	+	+ 1.1E04	+ 09.45 (86.04^{0}C)	+ 22.45 (87.38^{0}C)
405	-	+ 1.9E03	+ 12.15 (86.12^{0}C)	-
408	+	+ 1.7E05	+ 09.00 (86.09^{0}C)	+ 26.00 (87.44^{0}C)
425	+	+ 5.6E07	+ 07.00 (85.86^{0}C)	+ 08.45 (85.75^{0}C)
426	-	+ 3.7E04	+ 12.15 (86.14^{0}C)	+ 15.45 (86.12^{0}C)
427	+	+ 2.8E06	+ 08.30 (86.11^{0}C)	+ 11.30 (85.92^{0}C)
446	+	+ 1.5E08	+ 08.00 (87.00^{0}C)	+ 17.45 (87.11^{0}C)
448	-	+ 7.6E06	+ 09.45 (86.91^{0}C)	+ 20.00 (87.02^{0}C)
451	+	+ 1.3E08	+ 07.15 (87.00^{0}C)	+ 15.45 (87.21^{0}C)
453	-	+ 1.9E03	+ 29.00 (87.14^{0}C)	-
685	+	+ 2.2E08	+ 07.00 (86.30^{0}C)	+ 12.30 (86.50^{0}C)
687	+	+ 1.0E08	+ 07.20 (86.30^{0}C)	+ 11.00 (86.51^{0}C)
695	+	+ 9.0E04	+ 11.15 (86.49^{0}C)	+ 17.45 (86.39^{0}C)
711	+	+ 1.1E08	+ 06.50 (86.20^{0}C)	+ 10.30 (86.38^{0}C)
729	+	+ 5.6E07	+ 07.21 (86.20^{0}C)	+ 13.15 (86.43^{0}C)
732	+	+ 2.6E04	+ 11.50 (86.50^{0}C)	+ 18.15 (86.40^{0}C)
736	+	+ 9.4E05	+ 08.00 (86.30^{0}C)	+ 11.30 (86.30^{0}C)
755	+	+ 5.2E07	+ 07.00 (86.28^{0}C)	+ 10.45 (86.40^{0}C)
782	+	+ 2.4E06	+ 08.45 (86.49^{0}C)	+ 11.10 (86.30^{0}C)
789	+	+ 1.6E04	+ 17.00 (86.39^{0}C)	+ 19.00 (86.20^{0}C)
793	+	+ 2.3E07	+ 08.00 (86.49^{0}C)	+ 11.00 (86.40^{0}C)
825	+	+ 4.5E06	+ 08.15 (86.29^{0}C)	+ 10.45 (86.26^{0}C)
826	+	+ 1.1E07	+ 08.30 (86.29^{0}C)	+ 09.45 (86.30^{0}C)
831	+	+ 9.3E07	+ 06.45 (86.39^{0}C)	+ 09.00 (86.40^{0}C)

843	**+**	+ 4.2E08	+ 06.15 (86.29^{0}C)	+ 08.15 (86.36^{0}C)
864	**+**	+ 2.0E08	+ 06.30 (86.29^{0}C)	+ 08.15 (86.33^{0}C)
870	**+**	+ 7.0E05	+ 09.30 (86.39^{0}C)	+ 14.00 (85.80^{0}C)
872	**+**	+ 8.5E06	+ 08.00 (86.00^{0}C)	+ 10.15 (85.80^{0}C)
876	**+**	+ 5.2E07	+ 07.15 (86.29^{0}C)	+ 11.00 (86.42^{0}C)
891	**+**	+ 1.8E06	+ 08.30 (86.29^{0}C)	+ 11.00 (86.39^{0}C)
Totals	**36**	**43**	**43**	**41**

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