**QUEEN'S
UNIVERSITY
BELFAST**

A comparison of different pre-lysis methods and extraction kits for recovery of *Streptococcus agalacticae* (Lancefield group B *Streptococcus*) DNA from whole blood

Burke, R. M., McKenna, J. P., Cox, C., Coyle, P. V., Shields, M. D., & Fairley, D. J. (2016). A comparison of different pre-lysis methods and extraction kits for recovery of *Streptococcus agalacticae* (Lancefield group B *Streptococcus*) DNA from whole blood. *Journal of Microbiological Methods*, 129, 103-108.
<https://doi.org/10.1016/j.mimet.2016.08.016>

Published in:
Journal of Microbiological Methods

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights

© 2016 Elsevier Ltd. This manuscript version is made available under the CC-BY-NC-ND 4.0 license <http://creativecommons.org/licenses/by-nc-nd/4.0/> which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

A comparison of different pre-lysis methods and extraction kits for recovery of *Streptococcus agalacticae* (Lancefield group B Streptococcus) DNA from whole blood.

Rachael M. Burke,^{a,b} James P. McKenna,^b Ciara Cox,^b Peter V. Coyle,^b Michael D. Shields,^{a,c} Derek J. Fairley^{a,b} #

Centre for Infection and Immunity, Queens University Belfast, Northern Ireland^a; Regional Virus Laboratory / Department of Microbiology, Belfast Health and Social Care Trust, Northern Ireland^b; Royal Belfast Hospital for Sick Children, Northern Ireland^c

Running head: *S. agalactiae* DNA recovery from blood

Address correspondence to Derek J. Fairley, derek.fairley@belfasttrust.hscni.net

REVISION

ABSTRACT

Sub-optimal recovery of bacterial DNA from whole blood samples can limit the sensitivity of molecular assays to detect pathogenic bacteria. We compared 3 different pre-lysis protocols (none, mechanical pre-lysis and achromopeptidase pre-lysis) and 5 commercially available DNA extraction platforms for direct detection of Group B Streptococcus (GBS) in spiked whole blood samples, without enrichment culture. DNA was extracted using the QIAamp Blood Mini kit (Qiagen), UCP Pathogen Mini kit (Qiagen), QuickGene DNA Whole Blood kit S (Fuji), Speed Xtract Nucleic Acid Kit 200 (Qiagen) and MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics Corp). Mechanical pre-lysis increased yields of bacterial genomic DNA by 51.3 fold (95% confidence interval; 31.6 - 85.1, $p < 0.001$) and pre-lysis with achromopeptidase by 6.1 fold (95% CI; 4.2 - 8.9, $p < 0.001$), compared with no pre-lysis. Differences in yield due to pre-lysis were 2-3 fold larger than differences in yield between extraction methods. Including a pre-lysis step can improve the limits of detection of GBS using PCR or other molecular methods without need for culture.

INTRODUCTION

Streptococcus agalactiae (Lancefield Group B streptococcus [GBS]) is the most common cause of serious bacterial illness in neonates. Incidence of GBS disease in neonates less than 90 days old is 0.43 per 1000 births, with a case fatality of 12% (1). Conventional detection of GBS from patient samples using culture is both time-consuming and unreliable, particularly if samples are taken after antibiotics are administered (2). Laboratory diagnosis involves culture of recto-vaginal swab samples (when screening for antepartum or intrapartum carriage) and culture of blood and/or cerebrospinal fluid (CSF) samples from unwell neonates or neonates known to have been exposed to maternal GBS (3). Effective molecular tests for GBS would be valuable to clinicians by obviating the need for

55 several days of culture. Several quantitative real-time polymerase chain reaction (PCR) assays for
56 GBS have been developed and validated, most for use on vaginal swab samples rather than whole
57 blood (4-7). One recent study showed a real-time PCR assay to detect the *cylB* gene in blood and/or
58 CSF was significantly more sensitive than culture for diagnosis of GBS infection in neonates (2).

59

60 Effective DNA extraction from clinical specimens is critical for molecular pathogen detection. This is
61 particularly the case for whole blood, where the complex matrix and presence of PCR inhibitors can
62 make DNA extraction difficult (8, 9). It is well established that different methods of sample
63 preparation and DNA extraction have a significant impact on overall assay sensitivity (10, 11). GBS is
64 a Gram-positive organism with a robust cell wall, and accordingly it can be difficult to lyse bacterial
65 cells to release genomic DNA (9). This makes molecular detection of GBS much more challenging
66 than, for example, *Neisseria meningitidis* (an easily-lysed Gram-negative organism) where PCR
67 testing is widely recognised as a gold standard method (12, 13).

68

69 Molecular testing of neonatal blood and/or CSF specimens may improve the diagnosis of early-onset
70 sepsis caused by GBS, as detection of GBS DNA in a sterile site specimen would confirm the
71 diagnosis. However, failure to lyse GBS cells will adversely affect detection limits and clinical
72 sensitivity of molecular GBS tests. This study aimed to optimise extraction of GBS DNA from whole
73 blood, and to improve detection limits for molecular GBS detection. We report data from three
74 different pre-lysis methods and five different DNA extraction kits on the yield of GBS DNA from
75 spiked samples of saline and whole blood. Significant differences in yield were observed using
76 different extraction methods, with exceptionally low yields seen when commonly used extraction kits
77 were used without pre-lysis.

78

79 **METHODS**

80

81 **Preparation of GBS-spiked samples**

82

83 *Streptococcus agalactiae* (strain ATCC 12386) was cultured overnight on Columbia Blood Agar
84 (CBA) at 37°C in a 10% CO₂ atmosphere and resuspended in sterile phosphate buffered saline (PBS) to
85 an optical density of ~1.5. Tenfold serial dilutions were prepared with the number of colony forming
86 units per ml (CFU.ml⁻¹) ascertained by the spread plate method (14). Triplicate aliquots (100µl) of
87 duplicate serial dilutions were plated onto CBA plates. Following overnight incubation at 37°C in 10%
88 CO₂ atmosphere individual colonies were counted and the mean CFU.ml⁻¹ count determined. Aliquots
89 (200µl) of the GBS suspension were stored at -20°C until required. Each aliquot of GBS cells
90 underwent one freeze-thaw cycle only. Aliquots (2.8ml) of sterile PBS or whole EDTA-treated human
91 blood were inoculated with cell suspension (200µl) containing either 6.3×10^4 cfu.µl⁻¹ (“high spike”
92 sample) or 63 cfu.µl⁻¹ (“low spike” sample) prior to DNA extraction.

93

94 **Sample pre-lysis protocols**

95

96 Two protocols for sample pre-lysis prior to DNA extraction were compared: enzymatic lysis using
97 achromopeptidase (ACH, lysyl endopeptidase, EC 3.4.21.50) and mechanical lysis using bead-beating.
98 Controls were extracted without any pre-lysis.

99

100 For enzymatic lysis, ACH (100kU; Sigma-Aldrich, Gillingham, U.K.) was dissolved in 5.2ml of Tris-
101 EDTA buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). Sample aliquots (200µl) were mixed with an
102 equal volume of ACH stock (200µl, containing 3.85kU ACH) and incubated at room temperature
103 (22°C) for 5 minutes. For spiked saline samples, ACH was inactivated prior to extraction by heating to
104 95°C for 5 minutes. Preliminary experiments found the efficiency of ACH lysis in EDTA-blood was
105 significantly reduced compared to lysis in saline (data not shown). Dilution (1:4) with Tris-HCl buffer

106 (10mM, pH 8.0) prior to ACH treatment resolved this, so blood samples were diluted before addition
107 of ACH and incubation. For blood samples, instead of heating (which caused blood to clot), ACH was
108 inactivated prior to extraction by addition of lysis buffer from the extraction kit being evaluated.

109

110 For mechanical lysis, samples were processed by bead-beating using Pathogen Lysis Tubes S (Qiagen,
111 Manchester, UK). Saline or blood (400µl) spiked with GBS cells were mixed with lysis buffer (100µL)
112 containing anti-foam Reagent DX (0.67% v/v) in a Lysis Tube. The recommended lysis buffer for each
113 extraction protocol being evaluated was used (Qiagen Buffer ATL, Fuji Buffer LDB or Roche Lysis
114 Buffer). Bead beating was done using a Mini-BeadBeater-1 (Biospec Products Inc., Bartlesville, USA)
115 on full speed for 90 seconds.

116

117 **DNA extraction protocols**

118

119 Following sample pre-lysis, five different DNA extraction kits were compared (Table 1): QIAamp
120 Blood Mini kit (Qiagen); QIAamp UCP Pathogen Mini kit (Qiagen); QuickGene DNA Whole Blood
121 kit S (Fuji); MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics Corp.,
122 Indianaopolis, US); and SpeedXtract Nucleic Acid Kit 200 (Qiagen). Each kit was used according to
123 the manufacturers' instructions, with one exception. The SpeedXtract kit uses two rounds of binding
124 onto magnetic beads, leaving the target DNA in solution after the second magnetic separation. The
125 protocol was modified to include ACH pre-lysis between the two magnetic separations, prior to
126 removal of the magnetic beads. Buffer EN (400µl) was added to spiked EDTA whole blood (200µl)
127 and incubated with SpeedXtract Suspension A magnetic beads, according to the manufacturer's
128 protocol for liquid samples. Following magnetic separation, removal of supernatant and a wash step
129 using Buffer EN, the Suspension A beads were resuspended in ACH (100µl) and incubated at room
130 temperature for 5 minutes followed by heating to 95°C for 5 minutes. After ACH treatment, Buffer SL
131 (100µl) was added and samples were heated again to 95°C for 5 minutes before completing the
132 manufacturer's protocol. Controls were extracted without ACH by resuspending the Suspension A

133 magnetic beads in Buffer SL (200µl) and heating to 95°C for 10 minutes. It was not feasible to
134 incorporate bead-beating into the SpeedXtract protocol without major deviation from the
135 manufacturer's protocol. Bead-beating would either lyse bacterial cells in blood prior to the first
136 magnetic separation (with loss of the bacterial DNA) or would require bead-beating of the magnetic
137 particles prior to the second magnetic separation (with possible mechanical breakdown of the
138 particles).

139

140 The QIAamp UCP Pathogen kit was not evaluated without pre-lysis or using ACH pre-lysis, as the
141 manufacturer's protocol specifies bead-beating. Conversely, bead-beating was not evaluated for the
142 QIAamp Blood Mini kit because the extraction chemistry is identical to the QIAamp UCP Pathogen
143 kit. Data from these two kits were therefore combined as a single "Qiagen column" method for
144 comparison with other methods. For both QIAamp kits, extraction columns were processed by
145 centrifugation, according to the manufacturer's instructions. For Fuji and Roche methods, the
146 QuickGene Mini-80 and MagNA Pure Compact systems were used respectively. Following extraction,
147 purified DNA was eluted using the manufacturers' elution buffer for each kit. Elution volume was
148 100µl for all methods, except SpeedXtract where 200µl elution was used. Combinations of different
149 sample pre-lysis and extraction methods introduced different overall dilution factors (Table S1), so
150 appropriate corrections were required for calculation of yield.

151

152 **Reference DNA extraction and quantification**

153

154 Genomic DNA was extracted from a suspension of *S. agalactiae* ATCC 12386 in PBS, using the
155 Roche MagNA Pure method with pre-lysis using both ACH and bead-beating. DNA concentration was
156 determined using a NanoDrop™ 2000 UV spectrophotometer (ThermoFisher Scientific Inc., Waltham,
157 USA) and genome copy number per µl calculated assuming a genome size of 2.13×10^6 bp (*S.*
158 *agalactiae* A909 whole genome; RefSeq NC_007432). Calibrators for qPCR were prepared by dilution
159 of the reference DNA stock in Tris-HCl buffer (1mM, pH 8.0) containing $0.1 \mu\text{g} \cdot \mu\text{l}^{-1}$ yeast tRNA

160 (Sigma-Aldrich, Gillingham, U.K.). Ten-fold serial dilutions over a 6 log range were prepared for
161 qPCR calibration and to evaluate limits of detection.

162

163 **Real-time quantitative PCR (qPCR)**

164

165 A previously published Taqman® qPCR assay targeting the *sip* gene was used to detect and quantify
166 GBS DNA (7). Primers and probe were synthesised by Eurogentec (Eurogentec, Liège Belgium). The
167 probe was labelled with 5'-FAM and 3'-Black Hole Quencher 1. The final qPCR reaction mix
168 contained 1X Platinum® UDG Mastermix (Thermo Scientific, Manchester, UK), 0.2µM Bovine serum
169 albumin (Sigma, Dorset, UK), 4 mmol.L⁻¹ MgCl₂, 0.4µM forward and reverse primers, 0.2µM probe,
170 Nuclease Free Water (Promega, Southampton, UK) and 3µl of target template for a final reaction
171 volume of 12µl. qPCR was performed using a Light Cycler 480 (LC480) instrument (Roche
172 Diagnostics, Mannheim, Germany) using the following thermal cycling program: 95°C (10 minutes)
173 followed by 45 cycles of 95°C (10 seconds) / 60°C (1 minute), with fluorescence acquisition at the end
174 of each extension cycle. Data were analysed using LC480 software and GBS genome copy number for
175 positive specimens determined from crossing point threshold (Cp) relative to an external calibration
176 curve, prepared as described above, with triplicate assays run on duplicate dilution series. Calibrators
177 and no-template (water) controls were also run with each batch of qPCR samples. PCR efficiency (%
178 efficiency = $[10^{(-1/\text{slope})}-1] \times 100$) and linearity were evaluated by linear regression of log-transformed
179 calibration data. Data were analysed in Excel 2011 (Microsoft Corp., Seattle, USA) and Stata 11 (Stata
180 Corp., Texas, USA).

181

182 Data from the “high spike” samples are presented for numerical comparisons of yield, with 95%
183 confidence intervals (95% CI), whereas data from the “low spike” samples served to delineate the
184 lower limits of detection. Numerical comparison of yield for the “low spike” samples was not feasible
185 because the yield for many of the methods was so low that many (or all) replicates were negative for
186 GBS DNA when tested. Genome copy number data are shown for all PCR-positive samples.

187 **RESULTS**

188

189 **Performance of qPCR assay**

190

191 Efficiency of the *sip* gene qPCR, calculated from calibrator dilutions over a 6 log range (from 35
192 genome copies to 3.5×10^6 genome copies per reaction) was 100.3% with very high linearity ($R^2 =$
193 0.999), indicating the assay had excellent dynamic performance. The lower limit of detection for the
194 assay was found to be 7 genome copies per reaction, as 5/5 replicate assays were positive at that level
195 of dilution. Copy numbers below 7 per reaction were not evaluated.

196

197 **Reference DNA extraction method**

198

199 The highest yields of genomic DNA were obtained by using combined ACH and mechanical pre-lysis
200 of stock GBS suspensions in PBS, followed by MagNA Pure extraction. Genome copy numbers
201 recovered from PBS using this method were 5.5× higher than expected copy numbers calculated from
202 viable count (cfu/ml) data for the GBS spike. This was presumably due to the presence of non-viable
203 and non-culturable cells and chains of GBS cells in the spiked samples, making culture-based
204 estimates of copy number inaccurate. The analytical performance of the qPCR assay meant reliable
205 estimates of genome copy number could be obtained, but only post-extraction. These estimates
206 probably still underestimate the actual genome copy number in the spike, as the yield of the reference
207 extraction method is unlikely to be 100%. The yields of genomic DNA using other extraction methods
208 (each quantified using qPCR, as absolute genome copy numbers) were calculated relative to this
209 reference extraction method, and presented as “expected genome copies per ml”.

210

211 As determined by reference extraction of aliquots of GBS cells suspension in PBS (using combined
212 bead-beating, ACH treatment and MagNA Pure extraction) there were 1.84×10^7 (95% CI $4.4 \times 10^6 -$
213 2.3×10^7) genome copies per ml of high spike EDTA blood and 1.68×10^4 (95% CI $9.8 \times 10^3 - 2.1 \times$

10⁴) genome copies per mL of low spike blood. Expected copies per PCR reaction (3µl aliquot from a 100µl or 200µl DNA extract) varied from 9.4×10^3 to 1.7×10^5 copies in the case of high spike samples, and 9 to 152 copies in the case of low spike samples, depending on the dilution factor involved in pre-lysis and extraction (Table S1).

Comparison of different pre-lysis and extraction protocols.

Figure 1 summarises the differences in absolute yield of gDNA in the high-spike experiments, according to extraction protocol. Table 2 shows percentage yield of each extraction, relative to the reference method, after taking into account the different dilution factors involved.

The overall effect of mechanical pre-lysis in the high spike samples was to increase yields of DNA by 51.3 fold (95% CI; 31.6 – 85.1 fold, $p < 0.001$) compared with no pre-treatment. Pre-lysis with ACH increased yields by 6.1 fold (95% CI; 4.2 – 8.9 fold, $p < 0.001$). In preliminary experiments we found that ACH treatment is ineffective in undiluted whole blood, and that a 1 in 4 dilution prior to ACH treatment is optimal for maximal yield (data not shown). This introduces an unavoidable dilution step in the ACH pre-lysis protocol for whole blood, compared with mechanical lysis (Table S1). Treatment with ACH was slightly superior to mechanical lysis in terms of increased % recovery of DNA (1.8 fold greater percentage recovery of DNA, $p = 0.020$) but because the ACH pre-lysis protocol required more sample dilution, the mechanical pre-lysis protocol gave higher overall higher yields of DNA.

There was no significant difference in DNA yield between the Qiagen and Fuji column-based extraction methods ($p = 0.238$). The MagNA Pure extraction method gave the highest absolute yield of DNA, giving 1.96 fold greater yield than the column-based extraction methods (95% CI; 1.26 to 3.07 fold, $p = 0.004$). The SpeedXtract system performed slightly less well. The spin-column based methods gave a yield 2.41 fold greater than the SpeedXtract kit (95% CI; 0.74 to 4.31 fold, $p = 0.004$), although the SpeedXtract protocol was the simplest, requiring only a magnetic rack.

241

242 **Lower limits of detection**

243

244 In the “low spike” experiments we investigated the lower limits of detection for GBS in whole blood
245 samples. Based on preliminary experiments the GBS load in these blood samples (1.68×10^4 genome
246 copies.mL⁻¹) was expected to be at or below the limits of detection using some extraction methods
247 (data not shown). Due to the very low copy numbers expected, additional replicates were included in
248 these experiments.

249

250 Firstly, results were compared based on pre-lysis method. In low spike samples without any pre-lysis,
251 8/25 (32%) were positive for GBS DNA, with a mean genome copy number of 3.8 (95% CI; 3.0 – 4.5)
252 copies per reaction in positive samples. In samples with mechanical pre-lysis, 18/18 (100%) were
253 positive for GBS DNA, with a mean copy number of 58.1 (95% CI; 42.2 – 74.0) copies per reaction.
254 In samples with ACH pre-lysis, 16/34 (47%) were positive for GBS, with a mean copy number of 19.6
255 (95% CI; 5.3 – 33.8) copies per reaction. Of note, in the low-spike samples using the ACH pre-lysis
256 protocol, 6/6 (100%) replicates extracted using the SpeedXtract system were positive for GBS DNA.
257 This compares to 5/10 (50%) using Qiagen Blood Amp Mini kit, 2/10 (20%) using the Fuji QuickGene
258 and 3/8 (37.5%) using MagNA Pure. As previously stated, ACH pre-lysis required a 1 in 4 dilution of
259 whole blood. The chemistry of the Speed Xtract kit is different from the other kits; the supernatant
260 containing most of the whole blood components is discarded early in the process following magnetic
261 separation. It was not possible to incorporate mechanical pre-lysis into this protocol, but ACH could be
262 used without an additional dilution step which may explain the superior performance.

263

264 Secondly, we compared the effects of extraction platform using samples without any pre-lysis (i.e.
265 exactly according to manufacturer’s protocol). GBS DNA was detected in: 3/6 (50%) replicates
266 extracted using the Qiagen Blood Mini Kit with a mean of 3.6 (95% CI; 0.9 – 6.3) genome copies per
267 reaction; 3/6 (50%) replicates extracted with the Speed Xtract kit with a mean of 3.7 (95% CI; 0.7 –

268 6.7) genome copies per reaction; and 0/6 (0%) replicates extracted with the Fuji Quickgene extraction
269 platform. GBS DNA was detected in 2/7 (28.6%) replicates where samples were extracted using the
270 MagNA Pure system, with a mean of 4.1 (95% CI; 3.3 – 4.9) genome copies per reaction.

271

272 **Discussion**

273

274 In this study, we found yields of *S. agalactiae* genomic DNA from blood, using several different
275 commercial DNA extraction kits, were extremely low. Unsurprisingly, much higher yields were seen
276 when kits were modified to include mechanical lysis, although the improvements using a very simple
277 and rapid enzyme treatment were also impressive. To our knowledge, no previous studies have
278 demonstrated use of ACH to improve lysis of *S. agalactiae*, or use of this enzyme at room
279 temperature. We also used higher ACH unit activities than reported for other Gram-positive bacteria.
280 Previous studies used arbitrary and varying amounts of ACH: 1000U/ml (18), 1500U/ml (15),
281 2000U/ml (16) or 4000U/ml (19). Using 4000U/ml, Niwa et al (19) reported complete lysis of a range
282 of Gram-positive bacteria in 10-15 minutes at 37°C. Our method further increased the quantity of ACH
283 (to 9625U/ml) in a simple, fast (5 minute) room temperature protocol. There may be scope to further
284 improve yields by extending the incubation time, increasing the incubation temperature, or both.

285

286 Large differences in yield from the high-spike samples were seen between different extraction methods
287 using the same pre-lysis protocol. With no pre-lysis, or using bead-beating, both the Quickgene and
288 MagNA Pure methods consistently gave better performance than the Qiagen method. Using ACH pre-
289 lysis, the yield for the Quickgene method was significantly reduced. The reasons for this are unclear.
290 Comparing all 3 pre-lysis protocols, the MagNA Pure method was the most effective overall. This
291 justified use of this method, with combined pre-lysis using both ACH and bead-beating, as the
292 reference method for estimation of genome copy number in the spiked samples, and for yield
293 calculations. The yields were lowest for extraction of the high-spike samples using the SpeedXtract
294 method. However, the strong performance of this method for extraction of low-spike samples (due to

the smaller dilution factors involved) should be emphasised. This method is attractive in practical terms, and ACH pre-lysis did increase yield, so additional work to optimise this approach for GBS testing may be worthwhile.

In terms of cost, the requirement for an automated extraction system (i.e. a MagNA Pure Compact or MagNA Pure 96 instrument) makes the MagNA Pure method significantly more expensive overall than the other methods. The reagent cost for MagNA Pure extractions was also the highest (£5.46/sample), while the SpeedXtract method was the least expensive (£1.57/sample). The reagents for the Quickgene (£2.78/sample) and the Qiagen Blood Mini (£2.68/sample) and Qiagen UCP (£3.34/sample) were intermediate in cost. The additional costs for pre-lysis were similar, at £2.58/sample for bead-beating and £2.46/sample for ACH. The overall extraction cost for the tested combinations with pre-lysis ranged from £4.03 to £10.50 per sample, excluding instrument costs. The optimum method in terms of both cost and performance was bead-beating with Quickgene extraction (£5.36/sample).

Conventional culture methods for detection of Group B streptococcus are time consuming, and can be unreliable. It has previously been shown that molecular methods can be used to detect GBS in culture-negative EDTA-blood samples, although at a low rate; 2/35 culture-negative blood samples of babies with probable sepsis were positive by PCR (2). As Qiagen Blood Mini kits were used to extract DNA from samples without any pre-lysis in that study, our results suggest that poor DNA recovery may have limited the sensitivity of PCR.

Recovering Gram-positive bacterial DNA from whole blood samples without a culture enrichment step remains a challenge. The utility of direct PCR in addition to culture to detect septicaemia and meningitis is well established for meningococcal septicaemia (12) , and PCR may become the gold standard method for many other invasive bacterial infections, provided that optimised extraction methods are used.

322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348

We found that processing samples with ACH or mechanical pre-lysis significantly increases the yield of GBS DNA with a mean increase of 6.1 fold and 51.3 fold respectively, after allowing for different dilution factors for different protocols. The ACH pre-lysis method is straightforward, amenable to high-throughput or routine use, and the enzyme retains lytic activity for GBS for 30 days when stored at 4°C (data not shown). Although more effective overall, and requiring no dilution step, the mechanical pre-lysis protocol requires a bead beating instrument and involves more hands-on sample preparation time.

There is growing clinical interest in the use of rapid molecular tests to detect GBS in late pregnancy, especially during labour, and in near-patient settings. Intrapartum screening of all pregnant women for GBS using rapid molecular methods was recommended following a European consensus conference in 2015 (20). However, sub-optimal recovery of GBS DNA from clinical specimens is a potentially important confounding factor that could affect the outcome of clinical trials in this area. A recent study in France (21) concluded that intrapartum PCR testing could improve diagnosis and prevention of GBS disease, compared to culture-based screening earlier in pregnancy. The study did not directly compare the analytical performance of PCR to intrapartum culture, although a separate French study in a different hospital (22) reported the sensitivity of intrapartum GBS PCR, compared to broth enrichment culture, was 94.4%. A Japanese study (23) concluded that intrapartum PCR testing for GBS was effective, although sensitivity was only 83.3% compared to broth enrichment culture on specimens collected at the same time.

Our data suggest that problems with DNA extraction efficiency could adversely affect the performance of molecular tests to detect GBS in clinical specimens, leading to underestimation of both analytical and clinical sensitivity, and systematic bias in clinical trials. Unless this issue is properly evaluated and addressed, these problems might limit the clinical utility of these potentially very important testing methods for rapid detection of GBS in intrapartum screening and diagnosis of neonatal infections.

349

350 **Acknowledgements**

351

352 RB was funded by an Academic Research Fellowship (Queen’s University Belfast). This work was
353 supported by charitable funding from the McClay Foundation, Northern Ireland, U.K.

354

355

356 **Legends**

357

358 Table1.

359 Sample pre-lysis and extraction methods evaluated in this study.

360

361 Table 2.

362 Yield of GBS genomic DNA from “high spike” saline and blood samples (1.84×10^7 genome
363 copies.ml⁻¹).

364

365 Figure 1.

366 Box plot showing recovery of GBS genomic DNA from “high spike” saline and blood samples ($1.84 \times$
367 10^7 genome copies. ml⁻¹). A: reference extractions from PBS; B: blood extraction, no pre-lysis; C:
368 blood extraction, bead-beating pre-lysis; D: blood extraction, ACH pre-lysis. Abbreviations: nil = no
369 pre-lysis; ACH = pre-lysis with achromopeptidase; BB = bead-beating pre-lysis.

References

1. **Edmond KM, Kortsalioudaki C, Scott S, Schrag SJ, Zaidi AKM, Cousens S, Heath PT.** 2012. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. *Lancet* **379**:547–556.
2. **de Zoysa A, Edwards K, Gharbia S, Underwood A, Charlett A, Efstratiou A.** 2012. Non-culture detection of *Streptococcus agalactiae* (Lancefield group B Streptococcus) in clinical samples by real-time PCR. *Journal of Medical Microbiology* **61**:1086–1090.
3. **van den Brand M, Peters RPH, Catsburg A, Rubenjan A, Broeke FJ, van den Dungen FAM, van Weissenbruch MM, van Furth AM, Kõressaar T, Remm M, Savelkoul PHM, Bos MP.** 2014. Development of a multiplex real-time PCR assay for the rapid diagnosis of neonatal late onset sepsis. *J. Microbiol. Methods* **106**:8–15.
4. **Ke D, Ménard C, Picard FJ, Boissinot M, Ouellette M, Roy PH, Bergeron MG.** 2000. Development of conventional and real-time PCR assays for the rapid detection of group B streptococci. *Clinical Chemistry* **46**:324–331.
5. **Wernecke M, Mullen C, Sharma V, Morrison J, Barry T, Maher M, Smith T.** 2009. Evaluation of a novel real-time PCR test based on the *ssrA* gene for the identification of group B streptococci in vaginal swabs. *BMC Infect. Dis.* **9**:148.
6. **Morozumi M, Chiba N, Igarashi Y, Mitsuhashi N, Wajima T, Iwata S, Ubukata K.** 2015. Direct identification of *Streptococcus agalactiae* and capsular type by real-time PCR in vaginal swabs from pregnant women. *J. Infect. Chemother.* **21**:34–38.

- 397 7. **Bergseng H, Bevanger L, Rygg M, Bergh K. 2007.** Real-time PCR targeting the sip gene for
398 detection of group B Streptococcus colonization in pregnant women at delivery. *Journal of*
399 *Medical Microbiology* **56**:223–228.
- 400
- 401 8. **Al-Soud WA, Rådström P. 2001.** Purification and characterization of PCR-inhibitory
402 components in blood cells. *J. Clin. Microbiol.* **39**:485–493.
- 403
- 404 9. **Mahalanabis M, Al-Muayad H, Kulinski MD, Altman D, Klapperich CM. 2009.** Cell lysis
405 and DNA extraction of gram-positive and gram-negative bacteria from whole blood in a
406 disposable microfluidic chip. *Lab Chip* **9**:2811.
- 407
- 408 10. **Metwally L, Fairley DJ, Coyle PV, Hay RJ, Hedderwick S, McCloskey B, O'Neill HJ,**
409 **Webb CH, Elbaz W, McMullan R. 2008.** Improving molecular detection of *Candida* DNA in
410 whole blood: comparison of seven fungal DNA extraction protocols using real-time PCR.
411 *Journal of Medical Microbiology* **57**:296–303.
- 412
- 413 11. **van Tongeren SP, Degener JE, Harmsen HJM. 2011.** Comparison of three rapid and easy
414 bacterial DNA extraction methods for use with quantitative real-time PCR. *Eur. J. Clin.*
415 *Microbiol. Infect. Dis.* **30**:1053–1061.
- 416
- 417 12. **Bourke TW, McKenna JP, Coyle PV, Shields MD, Fairley DJ. 2015.** Diagnostic accuracy of
418 loop-mediated isothermal amplification as a near-patient test for meningococcal disease in
419 children: an observational cohort study. *The Lancet Infectious Diseases* **15**:552–558.
- 420
- 421 13. **Bryant PA, Li HY, Zaia A, Griffith J, Hogg G, Curtis N, Carapetis JR. 2004.** Prospective
422 study of a real-time PCR that is highly sensitive, specific, and clinically useful for diagnosis of
423 meningococcal disease in children. *J. Clin. Microbiol.* **42**:2919–2925.

424

425 14. **Cruickshank R.** Medical Microbiology: a guide to the laboratory diagnosis and control of
426 infection, 11th ed. Livingstone, London.

427

428 15. **Ezaki T, Suzuki S.** 1982. Achromopeptidase for lysis of anaerobic gram-positive cocci. J. Clin.
429 Microbiol. **16**:844–846.

430

431 16. **Slifkin M, Cumbie R.** 1987. Serogrouping single colonies of beta-hemolytic streptococci with
432 achromopeptidase extraction. J. Clin. Microbiol. **25**:1555–1556.

433

434 17. **Leonard RB, Carroll KC.** 1997. Rapid lysis of gram-positive cocci for pulsed-field gel
435 electrophoresis using achromopeptidase. Diagn. Mol. Pathol. **6**:288–291.

436

437 18. **Paule SM, Pasquariello AC, Hacek DM, Fisher AG, Thomson RB, Kaul KL, Peterson LR.**
438 2004. Direct detection of *Staphylococcus aureus* from adult and neonate nasal swab specimens
439 using real-time polymerase chain reaction. J Mol Diagn **6**:191–196.

440

441 19. **Niwa T, Kawamura Y, Katagiri Y, Ezaki T.** 2005. Lytic enzyme, labiase for a broad range
442 of Gram-positive bacteria and its application to analyze functional DNA/RNA. J Micro Methods
443 **61**:251– 260

444

445 20. **Di Renzo GC, Melin P, Berardi A, Blennow M, Carbonell-Estrany X, Donzelli GP,**
446 **Hakansson S, Hod M, Hughes R, Kurtzer M, Poyart C, Shinwell E, Stray-Pedersen B,**
447 **Wielgos M, El Helali N.** 2015. Intrapartum GBS screening and antibiotic prophylaxis: a
448 European consensus conference. J Matern Fetal Neonatal Med. 28(7):766-82

449

- 450 21. **Raignoux J, Benard M, Huo Yung Kai S, Dicky O, Berrebi A, Bibet L, Chetouani AS,**
451 **Marty N, Cavalie L, Casper C, Assouline-Azogui C.** 2016. Is rapid intrapartum vaginal
452 screening test of group B streptococci (GBS) during partum useful in identifying infants
453 developing early-onset GBS sepsis in postpartum period? [Article in French] Arch Pediatr.
454 **23:899–907**
- 456 22. **Defez M, Khizar F, Maurin M, Biot F, Pons JC, Sergent F.** 2016. Usefulness of a rapid
457 intrapartum real-time PCR assay in comparison with the group B Streptococcus culture
458 screening at the end of pregnancy in pregnant women. [Article in French] J Gynecol Obstet Biol
459 Reprod (Paris). S0368-2315(16)30057-6
- 461 23. **Tanaka K, Iwashita M, Matsushima M, Wachi Y, Izawa T, Sakai K, Kobayashi Y.** 2016.
462 Intrapartum group B Streptococcus screening using real-time polymerase chain reaction in
463 Japanese population. J Matern Fetal Neonatal Med. **29:130-4**
- 464
465
466

Table1. Sample pre-lysis and extraction methods evaluated in this study

	DNA extraction kit	Pre-lysis method¹		
		None	ACH	Bead-beating
Qiagen columns	QIAamp Blood Mini	+	+	–
	QIAamp UPC Pathogen Mini	–	–	+
	QuickGene DNA Whole Blood S	+	+	+
	MagNA Pure Compact	+	+	+
	SpeedXtract	+	+	–

¹ Some combinations could not be evaluated for technical reasons; see text for details.

Table 2. Yield of GBS genomic DNA from “high spike” saline and blood samples (1.84×10^7 genome copies.ml⁻¹).

Pre-lysis	Extraction platform	Expected copies per reaction	Mean copies detected per reaction ($\pm$ SE)	Yield (%)
Saline:				
ACH & bead-beating*	MagNApure	5.53×10^4	$5.53 \times 10^4 (\pm 9410)$	100%
None	MagNApure	1.10×10^5	2160 (± 929)	1.95%
EDTA-whole blood:				
None	QIAamp Blood Mini	1.10×10^5	503 (± 132)	0.46%
None	QuickGene Mini-80	1.10×10^5	480 (± 332)	0.43%
None	MagNApure	5.53×10^4	792 (± 336)	1.43%
None	SpeedXtract	5.53×10^4	247 (± 50)	0.45%
Bead-beating	UCP Pathogen Mini	1.66×10^5	$1.55 \times 10^4 (\pm 6410)$	9.34%
Bead-beating	QuickGene Mini-80	5.53×10^4	$4.35 \times 10^4 (\pm 2.79 \times 10^4)$	26.2%
Bead-beating	MagNApure	1.10×10^5	$3.54 \times 10^4 (\pm 9570)$	32.0%
ACH	QIAamp Blood Mini	1.38×10^4	5240 (± 1978)	37.9%
ACH	QuickGene Mini-80	1.38×10^4	1800 (± 1390)	13.1%
ACH	MagNApure	9400	7190 (± 3220)	76.4%
ACH	SpeedXtract	5.53×10^4	804 (± 211)	1.45%

* Reference extraction