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

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

3

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10

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

12

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## REVISION

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### 30 **ABSTRACT**

31

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

44

### 45 **INTRODUCTION**

46

47 *Streptococcus agalactiae* (Lancefield Group B streptococcus [GBS]) is the most common cause of  
48 serious bacterial illness in neonates. Incidence of GBS disease in neonates less than 90 days old is  
49 0.43 per 1000 births, with a case fatality of 12% (1). Conventional detection of GBS from patient  
50 samples using culture is both time-consuming and unreliable, particularly if samples are taken after  
51 antibiotics are administered (2). Laboratory diagnosis involves culture of recto-vaginal swab samples  
52 (when screening for antepartum or intrapartum carriage) and culture of blood and/or cerebrospinal  
53 fluid (CSF) samples from unwell neonates or neonates known to have been exposed to maternal GBS  
54 (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% CO<sub>2</sub> 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<sup>-1</sup>) 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 CO<sub>2</sub> atmosphere individual colonies were counted and the mean CFU.ml<sup>-1</sup> 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  $6.3 \times 10^4$  cfu.µl<sup>-1</sup> (“high spike”  
92 sample) or 63 cfu.µl<sup>-1</sup> (“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  $2.13 \times 10^6$  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<sup>-1</sup> MgCl<sub>2</sub>, 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  $3.5 \times 10^6$  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  $1.84 \times 10^7$  (95% CI  $4.4 \times 10^6 -$   
213  $2.3 \times 10^7$ ) genome copies per ml of high spike EDTA blood and  $1.68 \times 10^4$  (95% CI  $9.8 \times 10^3 - 2.1 \times$

214  $10^4$ ) genome copies per mL of low spike blood. Expected copies per PCR reaction (3 $\mu$ l aliquot from a  
215 100 $\mu$ l or 200 $\mu$ l DNA extract) varied from  $9.4 \times 10^3$  to  $1.7 \times 10^5$  copies in the case of high spike  
216 samples, and 9 to 152 copies in the case of low spike samples, depending on the dilution factor  
217 involved in pre-lysis and extraction (Table S1).

218

### 219 **Comparison of different pre-lysis and extraction protocols.**

220

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

224

225 The overall effect of mechanical pre-lysis in the high spike samples was to increase yields of DNA by  
226 51.3 fold (95% CI; 31.6 – 85.1 fold,  $p < 0.001$ ) compared with no pre-treatment. Pre-lysis with ACH  
227 increased yields by 6.1 fold (95% CI; 4.2 – 8.9 fold,  $p < 0.001$ ). In preliminary experiments we found  
228 that ACH treatment is ineffective in undiluted whole blood, and that a 1 in 4 dilution prior to ACH  
229 treatment is optimal for maximal yield (data not shown). This introduces an unavoidable dilution step  
230 in the ACH pre-lysis protocol for whole blood, compared with mechanical lysis (Table S1). Treatment  
231 with ACH was slightly superior to mechanical lysis in terms of increased % recovery of DNA (1.8 fold  
232 greater percentage recovery of DNA,  $p = 0.020$ ) but because the ACH pre-lysis protocol required more  
233 sample dilution, the mechanical pre-lysis protocol gave higher overall higher yields of DNA.

234

235 There was no significant difference in DNA yield between the Qiagen and Fuji column-based  
236 extraction methods ( $p = 0.238$ ). The MagNA Pure extraction method gave the highest absolute yield of  
237 DNA, giving 1.96 fold greater yield than the column-based extraction methods (95% CI; 1.26 to 3.07  
238 fold,  $p = 0.004$ ). The SpeedXtract system performed slightly less well. The spin-column based methods  
239 gave a yield 2.41 fold greater than the SpeedXtract kit (95% CI; 0.74 to 4.31 fold,  $p = 0.004$ ), although  
240 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 ( $1.68 \times 10^4$  genome  
246 copies.mL<sup>-1</sup>) 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

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

298

299 In terms of cost, the requirement for an automated extraction system (i.e. a MagNA Pure Compact or  
300 MagNA Pure 96 instrument) makes the MagNA Pure method significantly more expensive overall  
301 than the other methods. The reagent cost for MagNA Pure extractions was also the highest  
302 (£5.46/sample), while the SpeedXtract method was the least expensive (£1.57/sample). The reagents  
303 for the Quickgene (£2.78/sample) and the Qiagen Blood Mini (£2.68/sample) and Qiagen UCP  
304 (£3.34/sample) were intermediate in cost. The additional costs for pre-lysis were similar, at  
305 £2.58/sample for bead-beating and £2.46/sample for ACH. The overall extraction cost for the tested  
306 combinations with pre-lysis ranged from £4.03 to £10.50 per sample, excluding instrument costs. The  
307 optimum method in terms of both cost and performance was bead-beating with Quickgene extraction  
308 (£5.36/sample).

309

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

316

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

322

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

330

331 There is growing clinical interest in the use of rapid molecular tests to detect GBS in late pregnancy,  
332 especially during labour, and in near-patient settings. Intrapartum screening of all pregnant women for  
333 GBS using rapid molecular methods was recommended following a European consensus conference in  
334 2015 (20). However, sub-optimal recovery of GBS DNA from clinical specimens is a potentially  
335 important confounding factor that could affect the outcome of clinical trials in this area. A recent study  
336 in France (21) concluded that intrapartum PCR testing could improve diagnosis and prevention of  
337 GBS disease, compared to culture-based screening earlier in pregnancy. The study did not directly  
338 compare the analytical performance of PCR to intrapartum culture, although a separate French study in  
339 a different hospital (22) reported the sensitivity of intrapartum GBS PCR, compared to broth  
340 enrichment culture, was 94.4%. A Japanese study (23) concluded that intrapartum PCR testing for  
341 GBS was effective, although sensitivity was only 83.3% compared to broth enrichment culture on  
342 specimens collected at the same time.

343

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

349

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351

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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 ( $1.84 \times 10^7$  genome  
363 copies.ml<sup>-1</sup>).

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<sup>-1</sup>). 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.



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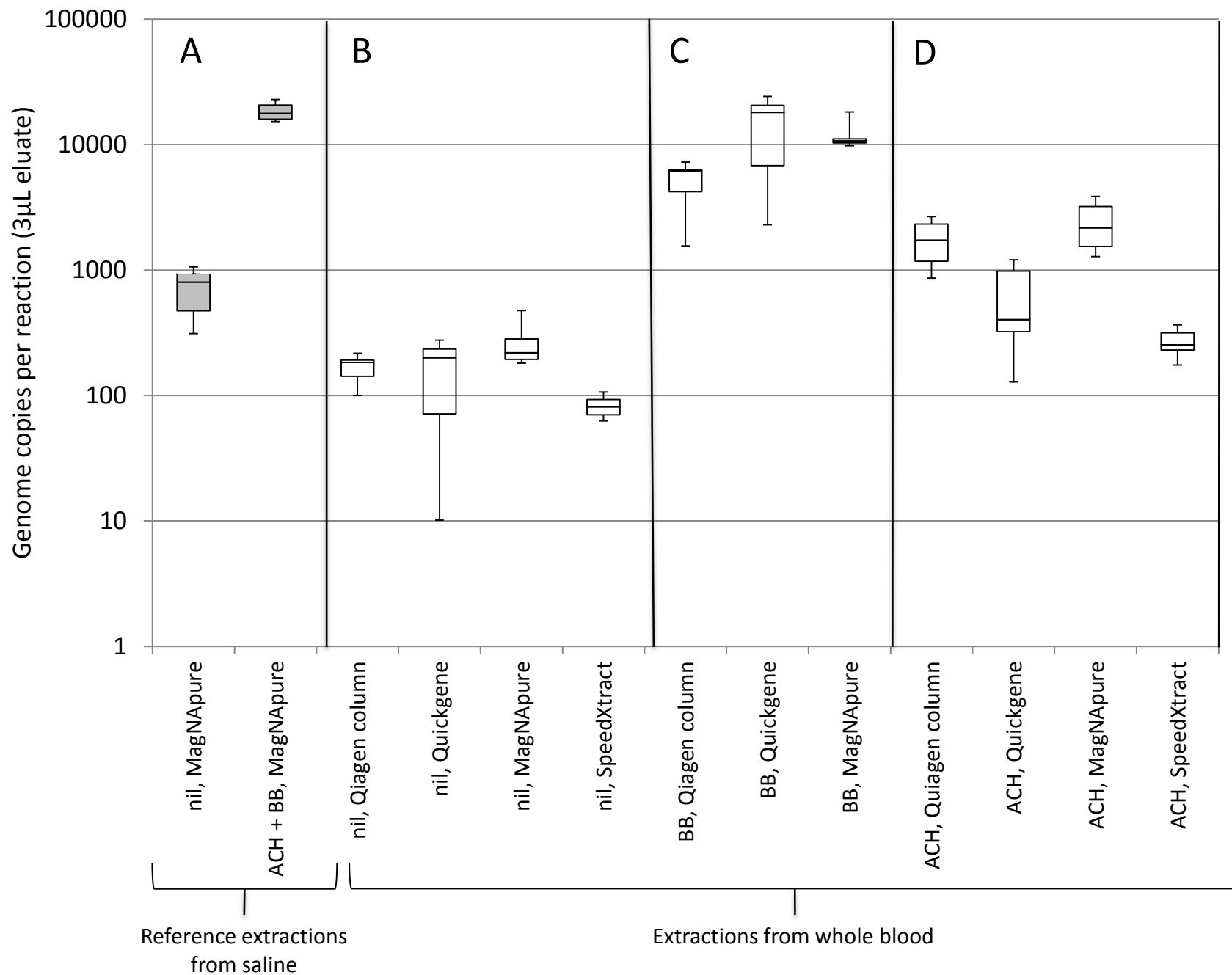


Table 1. Sample pre-lysis and extraction methods evaluated in this study

	<b>DNA extraction kit</b>	<b>Pre-lysis method<sup>1</sup></b>		
		None	ACH	Bead-beating
Qiagen columns	QIAamp Blood Mini	+	+	-
	QIAamp UPC Pathogen Mini	-	-	+
	QuickGene DNA Whole Blood S	+	+	+
	MagNA Pure Compact	+	+	+
	SpeedXtract	+	+	-

<sup>1</sup> 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 ( $1.84 \times 10^7$  genome copies.ml<sup>-1</sup>).

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

\* Reference extraction