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S. P. Tongeren, J. E. Degener, H. J. M. Harmsen

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1 **Comparison of three rapid and easy bacterial DNA extraction methods for use with**
2 **quantitative real-time PCR**

3

4 **S.P. van Tongeren*, J.E. Degener and H.J.M. Harmsen.**

5

6 Department of Medical Microbiology, University Medical Center Groningen, University of

7 Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands

8

*Corresponding author. Mailing address: Department of Medical Microbiology, University Medical Center Groningen, University of Groningen, PO Box 30001, 9700 RB Groningen, The Netherlands

Phone: +31 50 3613480

Fax: +31 50 3619105

Email: s.p.van.tongeren@med.umcg.nl

9 **Abstract**

10

11 The development of fast and easy on-site molecular detection and quantification methods for
12 hazardous microbes at solid surfaces is desirable for several applications where specialised
13 laboratory facilities are absent. Quantification of bacterial contamination necessitates the
14 assessment of the efficiency of the used methodology as a whole, including the preceding steps
15 of sampling and sample processing. We used quantitative real-time PCR (qrtPCR) for
16 *Escherichia coli* and *Staphylococcus aureus* to measure the recovery of DNA from defined
17 numbers of bacterial cells that were subjected to three different DNA extraction methods: the
18 QIAamp[®] DNA Mini Kit, Reischl et al. method and FTA[®] Elute. FTA[®] Elute significantly showed
19 the highest median DNA extraction efficiency of 76.9 percent for *E. coli* and 108.9 percent for *S.*
20 *aureus*. The Reischl et al. method and QIAamp[®] DNA Mini Kit inhibited the *E. coli* qrtPCR assay
21 with a 10-fold decrease of detectable DNA. None of the methods inhibited the *S. aureus* qrtPCR
22 assay. The FTA[®] Elute applicability was demonstrated with swab samples taken from the
23 International Space Station (ISS) interior. Overall the FTA[®] Elute method was found most suitable
24 to selected criteria in terms of rapidity, easiness of use, DNA extraction efficiency, toxicity and
25 transport and storage conditions.

26

27

28 Key words: Rapid, easy, DNA extraction, FTA, qrtPCR, *Escherichia coli*, *Staphylococcus aureus*

29

30 **Introduction**

31

32 The detection and quantification of microbes present on solid surfaces plays an important role in
33 clinical settings, the food industry, drinking water distribution systems, air-conditioning systems in
34 modern buildings or advanced life-support systems, such as manned spacecraft. Detection and
35 quantification by conventional culturing techniques however is laborious, lengthy, requires
36 specialist expertise, has poor diagnostic sensitivity and in addition, many bacteria such as
37 *Faecalibacterium prausnitzii* are difficult to culture or uncultivable which hampers their detection
38 [1]. Moreover, the culturing of hazardous microbes such as pathogens and so-called
39 “technophiles” may often be undesirable [2]. Especially in remote locations such as field
40 hospitals, disaster areas or manned spacecraft where specialised laboratory facilities and
41 technical personnel are not available, this results in the need for fast and easy methodologies that
42 can be used on-site by non-specialists. Accordingly, molecular methods have increasingly been
43 used for the detection and quantification of hazardous microbes overcoming many of the
44 disadvantages encountered by culturing [3].

45 Quantitative real-time PCR (qPCR) is a cultivation-independent method for the rapid
46 molecular detection and quantification of nucleic acid sequences [3]. The method has a high
47 specificity with a detection sensitivity of a few molecules per reaction and the potential to be
48 automated and miniaturized for use on-site by non-specialists. Moreover, for a reliable
49 quantification of bacterial contamination it is important to assess the efficiency of the used
50 methods as a whole, including both the molecular detection method as well as the preceding
51 steps of sampling and sample processing [3-5]. In addition, for increased sensitivity, methods with
52 the highest possible efficiency should be utilized and the dilution factor in all steps of sampling
53 and sample processing kept to a minimum.

54 Sample processing for use with qPCR involves cell lysis and subsequent recovery of DNA
55 free of amplification inhibitors. Factors that play a role in the efficiency of bacterial cell lysis may
56 be physiological characteristics of the species such as the constitution of the cell wall, the
57 physiological state the cell is in or cell concentration [6-8]. As a consequence, most DNA

58 extraction methods are optimal for just one or a group of bacterial species [6]. Ideally, for
59 applications where it is essential to measure the levels of a variety of hazardous species such as
60 in a natural environment, a universal DNA extraction method would be preferred that is optimal
61 for all bacterial species.

62 The aim of this study was to select and validate a suitable DNA extraction method for the
63 recovery of DNA from the potential pathogens *Escherichia coli* and *Staphylococcus aureus* to be
64 used in qrtPCR. These species were used as model organisms as the gram-negative bacterium
65 *E. coli* lyses differently than the gram-positive bacterium *S. aureus*, due to a different constitution
66 of its cell wall. In particular for use on-site with samples taken from solid surfaces, we sought to
67 select a fast and easy to use DNA extraction method that has a high efficiency, is universally
68 effective for bacterial species, makes use of non-toxic chemicals and has little or no transport or
69 storage requirements.

70

71

72 **Materials and methods**

73

74 Cultivation of bacterial cells

75

76 *S. aureus* subsp. *aureus* ATCC 25923 and *E. coli* ATCC 11775^T cells were each inoculated into 9
77 ml Brain Heart Infusion (BHI) (Mediaproducts BV, Groningen, The Netherlands) and grown
78 overnight at 37°C. From each culture, cells were washed twice by centrifugation of 1 ml cell
79 suspension for 10 min at 16100 × g, removal of the supernatant and resuspension of the pellet in
80 1 ml sterile physiological salt solution (0.85% NaCl, Mediaproducts BV, Groningen, The
81 Netherlands). The suspensions were kept on ice during the remainder of the procedure to
82 minimize cell growth and lysis.

83

84 DNA extraction methods description

85

86 With the commonly used QIAamp[®] DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) total DNA
87 can be purified from a variety of biological, clinical and forensic specimens. The method uses
88 chemical lysis by undisclosed lysis buffers containing chaotropic salt, enzymatic digestion by
89 treatment with proteinase K, and thermal lysis. The method uses a separate protocol for Gram-
90 positive bacteria, with the additional use of enzymatic digestion by lysozyme or lysostaphin with
91 Triton. DNA is bound to a silica-gel membrane in a spin-column, whilst PCR inhibitors are
92 supposedly not retained. The bound DNA is then washed, eluted in buffer and can then be stored
93 at -20°C.

94 The simple boiling procedure developed by Reischl et al. involves chemical lysis in
95 combination with thermal lysis and has been used for the extraction of *S. aureus* total DNA [9].
96 The concentrated lysis buffer (10 ×) contains 10% Triton X-100, 5% Tween 20, 100 mmol l⁻¹ Tris-
97 HCl (pH 8.0) and 10 mmol l⁻¹ EDTA.

98 The FTA[®] Elute method (Whatman plc., Maidstone, UK) was developed for room temperature
99 collection, transport, storage and isolation of nucleic acids from a wide variety of biological,
100 clinical and forensic sample types. The method uses chemical lysis by a cellulose paper matrix
101 treated with proprietary reagents that lyses cells and denatures proteins upon contact causing the
102 release and subsequent entrapment of nucleic acids in the fibres of the matrix. The paper rapidly
103 inactivates organisms, decreasing the risk of contamination for the individuals handling the
104 sample. Sample volume requirements are minimal, of approximately 12-40 µl per collection area.
105 After drying, the samples can be stored for many years. DNA is recovered from the FTA[®] Elute
106 matrix through a simple hot water elution procedure. Inhibitory components are supposedly
107 retained on the FTA[®] Elute matrix or disposed of during a short washing step.

108

109 DNA extraction method comparison

110

111 The experiments were performed using DNA-free laboratory techniques. The cells in both *E. coli*
112 and *S. aureus* suspensions were enumerated by 4',6-diamidino-2-phenylindole (DAPI) staining
113 and subsequent visual cell counting with an Olympus BH2 epifluorescence microscope by the

114 method as described previously [10], with the exception that the cells were not fixed by
115 paraformaldehyde. The enumerations were performed in duplicate and averaged. Subsequently,
116 the cell suspensions were serially diluted in sterile physiological salt solution and divided into
117 aliquots of 5.3×10^5 , 5.3×10^4 , 5.3×10^3 , 5.3×10^2 and 5.3×10^1 cells.

118 Volumes of the aliquots were adjusted with sterile physiological salt solution to suit the
119 recommended input volume for each DNA extraction method after which DNA was extracted in
120 quadruplicate according to the instructions of the authors and manufacturers and subjected to
121 qrtPCR as described below.

122 To monitor contamination during the procedure, negative (extraction) controls were prepared
123 consisting of physiological salt solution processed through each DNA extraction method. PCR
124 inhibition was determined by adding a template of 1 ng of the qrtPCR standard DNA, prepared as
125 described below, together with either 2 μ l of an extraction control or 2 μ l Molecular Biology Water
126 to triplicate reactions. PCR inhibition will cause a shift of the threshold cycle (Ct) to a higher cycle
127 number compared to the standard (Online Resources 1 and 2). Positive controls were performed
128 by adding a template of 100 fg of the standard together with 2 μ l Molecular Biology Water to
129 reactions in triplicate.

130

131 DNA extraction of swab samples taken from the International Space Station interior using the
132 FTA[®] Elute method

133

134 To demonstrate the applicability of the FTA[®] Elute method for the analysis of surface samples,
135 DNA was extracted with this method from triplicate swab samples taken from the interior of the
136 International Space Station (ISS) as part of the "SAMPLE" experiments which were previously
137 described in van Tongeren et al. 2007 [2]. The DNA contents were subsequently quantified with
138 the qrtPCR assays of *S. aureus* and *E. coli* as described below.

139

140 Quantitative real-time PCR methods

141

142 Two TaqMan PCR assays were used to amplify specific regions of the genome of *S. aureus* and
143 *E. coli*. Oligonucleotide primers and double-dye probes were obtained from Eurogentec S. A.
144 (Seraing, Belgium). Primers SauV58 (5'-GCTGTGATGGGGAGAAGACAT-3') and SauR54b (5'-
145 CGGTACGGGCACCTATTTTC-3') were used to amplify a 90 bp fragment of the 23S rRNA gene
146 of *S. aureus* [7]. Primers UIDA-F (5'-TGGTGATTACCGACGAAAAC-3') and UIDA-R (5'-
147 GCGTGGTTACAGTCTTGC-3') were used to amplify a 145 bp fragment of the *uidA* gene of *E.*
148 *coli* [11]. Probes Sta59bTQ (5'-AGAGGCTTTTCTCGGCAGTGTGAAATCAACGA-3') and UIDA-
149 Pr (5'-GCCGGGATCCATCGCAGCGTAATGCTC-3') were hybridized in real-time with the PCR
150 product of *S. aureus* and *E. coli*, respectively [7, 11]. The probes were labelled with
151 carboxyfluorescein (FAM) on the 5'-end and a 4-([4-(dimethylamino)phenyl]azo)benzoic acid
152 (DABCYL) quencher on the 3'-end. All qrtPCR reactions were performed in a total volume of 30 μ l
153 consisting of 3 μ l of target DNA and 27 μ l of amplification mixture containing PCR Reaction Buffer
154 (Smart™ Kit No ROX, Eurogentec S. A.), primers and probe and Molecular Biology Water
155 (AccuGENE®, Lonza Group Ltd, Basel, Switzerland). The Smart™ Kit PCR reagents contained
156 HotGoldStar DNA polymerase and Uracil-N-Glycosylase to prevent carry-over contamination. The
157 end concentration of each primer was 150 nmol l⁻¹, of the Sta59bTQ probe 80 nmol l⁻¹ and of the
158 UIDA-Pr probe 300 nmol l⁻¹. Amplification and detection were carried out on a Smart Cycler®
159 System (Cepheid, Sunnyvale, USA) with a profile of 50°C for 2 min to activate Uracil-N-
160 Glycosylase, 95°C for 10 min to activate HotGoldStar DNA polymerase, followed by 45 cycles of
161 95°C for 15 s and 60°C for 60 s.

162 Negative controls for each qrtPCR assay were performed by running reactions without the
163 addition of template DNA.

164

165 Generation of qrtPCR standards with bacterial genomic DNA

166

167 Cells were cultivated as described above. To obtain a sufficient amount of relatively good quality
168 DNA, the commonly used QIAamp® DNA Mini Kit was used to extract DNA from the cells
169 according to the instructions of the manufacturer. The procedure was followed by ethanol

170 precipitation to purify the DNA solution from contaminants such as PCR inhibiting substances.
171 The DNA purity and concentration was determined with a NanoDrop® ND-1000
172 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). A standard curve of mean
173 qrtPCR Ct values was prepared with triplicate replicates of serial dilutions of bacterial genomic
174 DNA (three series of 10 ng, 1 ng, 100 pg, 10 pg, 1 pg, 100 fg and 10 fg for *S. aureus* subsp.
175 *aureus* ATCC 25923 and two series of 30 ng, 3 ng, 300 pg, 30 pg, 3 pg, 300 fg and 30 fg for *E.*
176 *coli* ATCC 11775^T, respectively). The amount of bacterial DNA in samples that were subjected to
177 the various DNA extraction methods was estimated by qrtPCR with this curve under identical
178 PCR conditions.

179

180 Statistical analysis

181

182 The quantification limit was determined for each qrtPCR assay as the lowest concentration on the
183 linear part of the standard curve at which 7 out of 9 reactions or more gave a positive result. The
184 quantification limit was used as the cut-off level or, instead of this, the value of a negative control
185 in case it had a positive result. In case a Ct of zero (negative result) or above the mean Ct (+ 1 s.
186 d.) of the quantification limit was measured, a Ct equal to the mean Ct (+ 1 s. d.) of the
187 quantification limit was assumed for data analysis.

188 DNA extractions were performed in quadruplicate. The efficiency of DNA extraction was
189 determined by the measured amount of bacterial DNA in each sample as compared to the
190 estimated amount of bacterial DNA before DNA extraction as a reference (100%). The reference
191 was estimated by microscopic cell counting and assuming a chromosomal weight calculated from
192 the chromosome length and GC content of approximately 3.0 fg for *S. aureus* and 5.7 fg for *E.*
193 *coli*, respectively. Within each DNA extraction method, efficiencies of extraction amongst different
194 cell amounts were compared by Kruskal-Wallis analysis. Friedman analysis with additional
195 Bonferroni-protected contrasts was performed to compare DNA extraction methods amongst
196 each species. Differences were considered statistically significant when P values were less than
197 0.05 (two-sided), resulting in 0.05/(number of contrasts performed) after Bonferroni correction.

198 Statistical and graphical analyses were performed by using SPSS (version 16.0 for Windows,
199 SPSS Inc. [<http://www.spss.com/>]) and Microsoft® Office Excel 2003 (Microsoft Corporation
200 [<http://office.microsoft.com/>]) software, respectively.

201

202

203 **Results**

204

205 The processing time for 42 samples was 40 minutes for the Reischl et al. method, 110 minutes
206 for FTA® Elute and 5 hours 30 minutes for the QIAamp® DNA Mini Kit. These processing times
207 did not include preparation of the solutions.

208 The sample input volumes were 90 µl for the Reischl et al. method, 40 µl for the FTA® Elute
209 paper and 180 µl for the QIAamp® DNA Mini Kit. Output volumes for the samples were 100 µl for
210 the Reischl et al. method, approximately 13.4 aliquots of 30 µl for the FTA® Elute paper and 100
211 µl for the QIAamp® DNA Mini Kit.

212 The concentration of the *E. coli* cell suspension was determined in duplicate at 1.09×10^7 cells
213 0.01ml^{-1} (cumulating variation coefficient (CVC)=10.52) and 9.51×10^6 cells 0.01ml^{-1} (CVC=
214 9.76), with a mean of 1.02×10^9 cells ml^{-1} . In addition, the concentration of the *S. aureus* cell
215 suspension was determined in duplicate at 3.47×10^7 cells 0.01ml^{-1} (CVC=9.43) and 3.23×10^7
216 cells 0.01ml^{-1} (CVC=10.72), with a mean of 3.35×10^9 cells ml^{-1} .

217 QrtPCR standard curves with E and R² values that were determined for *E. coli* and *S. aureus*
218 are presented in Online Resources 1 and 2. The qrtPCR quantification limit was determined at 30
219 fg of the standard for *E. coli* and at 10 fg of the standard for *S. aureus*, respectively.

220 Subsequently, when assuming a Ct equal to the mean Ct (+ 1 s. d.) of the qrtPCR quantification
221 limit, extrapolated limits of quantification per extraction could be determined for *E. coli* to be 552
222 fg DNA for FTA® Elute and 137 fg DNA for the Reischl et al. method and QIAamp® DNA Mini Kit.
223 For *S. aureus* extrapolated limits of quantification per extraction were determined at 961 fg DNA
224 for FTA® Elute and 238 fg DNA for the Reischl et al. method and QIAamp® DNA Mini Kit.

225 Figs. 1A and 1B show the median DNA levels measured per cell amount after extraction, their
226 corresponding reference and the extrapolated limits of quantification for each DNA extraction
227 method.

228 The median efficiencies of DNA extraction of different cell amounts calculated for each DNA
229 extraction method are presented in Table 1. Within each method, no significant differences were
230 observed by Kruskal-Wallis analysis amongst efficiencies of DNA extraction of different cell
231 amounts. Figs. 2A and 2B give an overview of the median efficiency of DNA extraction of the 5.3
232 $\times 10^5$, 5.3×10^4 and 5.3×10^3 cell amounts for each method. P values of $P \leq 0.017$ were
233 considered statistically significant for the performed three contrasts after Bonferroni correction.
234 The experiments show that for *E. coli* ATCC 11775^T the FTA[®] Elute paper had the highest
235 median DNA extraction efficiency of 76.9 percent, followed by the Reischl et al. method with 43.7
236 percent. The QIAamp[®] DNA Mini Kit had the significantly lowest median DNA extraction efficiency
237 of 7.7 percent as compared to FTA[®] Elute ($P=0.001$) and the Reischl et al. method ($P=0.004$). For
238 *S. aureus* subsp. *aureus* ATCC 25923, FTA[®] Elute paper and QIAamp[®] DNA Mini Kit performed
239 with similar efficiencies of 108.9 and 97.7 percent, respectively. The efficiency of the Reischl et al.
240 protocol was significantly lower with 9.0 percent as compared to the QIAamp[®] DNA Mini Kit
241 ($P=0.001$) and FTA[®] Elute paper ($P=0.001$).

242 The level of PCR inhibition of the DNA extraction methods on the *E. coli* qrtPCR assay is
243 presented in Fig. 3. The results show that compared to added water to 1 ng of the qrtPCR
244 standard DNA, both the added extraction controls of the Reischl et al. method and QIAamp[®] DNA
245 Mini Kit caused a shift towards a higher Ct, indicating an inhibitory effect, in contrast to the
246 extraction control of the FTA[®] Elute method. No inhibitory effects were noticeable on the *S.*
247 *aureus* qrtPCR assay (data not shown).

248 The applicability of the FTA[®] Elute method was examined by extracting DNA with this method
249 from swab samples taken from the interior of the ISS. For *S. aureus*, a potential pathogen
250 commonly found on the human skin, a location associated with human contact was positive with a
251 mean of 4.50 ± 0.21 log fg DNA ($\sim 3.16 \times 10^4$ fg). Calculated from the chromosomal weight, this
252 amount of DNA would correspond with $\sim 1.05 \times 10^4$ *S. aureus* cells per sample. Sampling in the

253 toilet area was positive for *E. coli* with a mean of 4.50 ± 0.33 log fg DNA ($\sim 3.18 \times 10^4$ fg), as can
254 be expected since the potential pathogen *E. coli* is a common inhabitant of the human gut. This
255 amount of DNA would correspond with $\sim 5.58 \times 10^3$ *E. coli* cells per sample. In contrast, the mean
256 *S. aureus* and *E. coli* DNA content from a location between wall panels was below the cut-off
257 level.

258

259

260 **Discussion**

261

262 Molecular methods have increasingly been used for the detection and quantification of hazardous
263 microbes. Moreover, the need for a rapid and easy to use on-site molecular detection and
264 quantification method is imperative. An equally important step in nucleic-acid based quantification
265 of bacteria is the part of sample processing preceding the actual measurement, as has previously
266 been mentioned [3-5]. The results of this study confirm that DNA extraction is a key step in this
267 process.

268 Several strategies can be used for the extraction of DNA from microbial samples, such as
269 enzymatic, chemical or thermal lysis, mechanical disruption of the cell wall by beads or sonication
270 or a combination of the above [6, 9]. A disadvantage of enzymatic lysis is that commercially
271 available enzymes can be contaminated with microbial DNA. Highly sensitive and specific nucleic
272 acid-based methods for the detection of bacteria necessitate the use of DNA extraction reagents
273 that are free of contaminating bacterial nucleic acids. In addition, the use of enzymes often
274 requires special conditions and preservation requirements such as refrigeration and buffer
275 storage. Chemical lysis often involves the usage of aggressive and toxic chemicals which is less
276 desirable for on-site detection where laboratory safety conditions are absent. A disadvantage for
277 application on-site to thermal lysis, mechanical disruption by beads, or methods that use
278 centrifugation is that the required equipment generally takes up a lot of weight and volume.

279 We selected to study three different DNA extraction methods to meet criteria for use on-site
280 with qrtPCR on samples taken from solid surfaces such as processing time, easiness of use,

281 efficiency, range of bacterial species that it is effective for, chemical toxicity and transport and
282 storage requirements, which will be discussed below.

283 In our study, both the Reischl et al. protocol and FTA[®] Elute were indeed fast and easy to use
284 by single step isolation procedures and processing times of 40 and 110 minutes for 42 samples,
285 respectively. DNA extraction methods with comparable processing times were reported before [3,
286 12-14].

287 In all, FTA[®] Elute paper significantly had the highest efficiency to recover DNA from *E. coli*
288 ATCC11755 and *S. aureus* subsp. *aureus* ATCC 25923 compared to the other two methods. As
289 shown by the results, the measured amount of DNA in some cases exceeded that of the
290 reference which was estimated by the 'gold standard' of microscopic cell counting, resulting in
291 percentages above 100 percent. An underestimation of the amount of chromosomal DNA by
292 microscopic cell counting can easily occur due to factors such as cell clumping, cell division or
293 cell lysis.

294 DNA extraction efficiency is limited by several factors. An important factor is cell concentration,
295 which can be a delimiting factor for quantification of natural samples where cell concentrations
296 will vary. At low concentrations, method sensitivity will play a role and at high concentrations,
297 overloading of the method can reduce DNA extraction efficiency. For FTA[®] Elute problems can
298 arise for certain harder to lyse species at concentrations above 10^7 cells ml⁻¹ and overloading of
299 the column of the QIAamp[®] DNA Mini Kit may lead to significantly lower yields than expected,
300 according to the manufacturers instructions. A second factor is the type of bacterial species that
301 is being subjected to the extraction. For instance, the constitution of the bacterial cell wall may
302 impede cell lysis and the liberation of DNA from the cell. A third factor may be the physiological
303 state of the cells that are subjected to cell lysis [7], in which context it can be noted that the cells
304 used in the present study were of liquid cultures in stationary phase. Another factor that may
305 influence the outcome of quantification is the size of the DNA fragments generated by a specific
306 method [6]. Long fragment sizes generated by the DNA extraction method may result in
307 incomplete denaturation during the thermal cycling of qrtPCR, whilst on the other hand short
308 fragment sizes may result in reduced amplification efficiency.

309 The QIAamp[®] DNA Mini Kit was developed and commonly used for a variety of bacterial
310 species [15-24]. The FTA technology concept, including FTA[®] Elute, was also developed for use
311 with a broad spectrum of bacterial species [25-27]. To our knowledge, the use of FTA[®] Elute for
312 quantitative bacterial applications has not been assessed to date. The protocol by Reischl et al.
313 was previously used for *S. aureus*, however the main constituents and boiling procedure of the
314 method have also been used for other species [9, 19, 28-33]. However, as confirmed by our
315 results, it must be taken into account that inter-species and even intra-species differences in DNA
316 extraction efficiencies will exist, influencing the outcome of microbial quantification [6, 8, 34].
317 Furthermore, in contrast to FTA[®] Elute, both the Reischl et al. and QIAamp[®] DNA Mini Kit method
318 showed inhibitory effects on the *E. coli* qrtPCR assay but not on the *S. aureus* qrtPCR assay.
319 Whilst the final volume of the FTA[®] Elute extract is four times more dilute than that of the other
320 methods, the concentration of any inhibitory compounds it may possibly contain is too low to
321 cause an inhibitory effect when used according to the manufacturer's instructions. As such,
322 inhibitory effects of a DNA extraction method can differ per PCR assay as shown by comparison
323 of our results and those of other authors [8, 16]. PCR inhibition reduces sensitivity, thereby
324 negatively influencing the outcome of quantitative measurements. As a result, different rates of
325 PCR inhibition on separate PCR assays will complicate quantification of microbial samples with a
326 complex composition. Moreover, these observations should also be carefully considered in the
327 choice of inhibition controls [3]. In addition to PCR inhibition generated by the DNA extraction
328 method itself as described above, PCR inhibition needs to be assessed depending on the
329 specimen type. In this study, we sought to select a DNA extraction method suitable for the
330 processing of samples taken from solid surfaces in various surroundings. However, a wide variety
331 of possible PCR inhibiting compounds or, most often, none at all can be emphasized to be
332 present on such surfaces, leaving any prediction on PCR inhibition generated by this particular
333 specimen type speculative.

334 The least toxic method appears to be FTA[®] Elute which, apart from the proprietary chemicals
335 on the paper, only requires the use of water. The protocol developed by Reischl et al. utilizes the

336 detergents Tween 20 and Triton X-100, whilst the QIAamp[®] DNA Mini Kit involves the use of
337 irritating substances.

338 The protocol according to Reischl et al. and the FTA[®] Elute method both occupy little volume
339 and weight. The Reischl et al. protocol only requires one lysis buffer, thermal heating and
340 centrifugation. FTA[®] Elute consists of small paper sample cards and in addition requires water, a
341 small puncher, thermal heating and centrifugation. Additional transport and storage advantages
342 for the FTA[®] Elute method are room temperature sample storage, protection of DNA against
343 radiation, UV damage and enzymatic breakdown and the immediate deactivation of potentially
344 hazardous microbes, according to the manufacturer. The QIAamp[®] DNA Mini Kit makes use of
345 several solutions, spin-columns, centrifugation and thermal heating steps and as such occupies
346 more volume and weight than the other methods.

347 Finally, the applicability of the FTA[®] Elute method for the analysis of environmental samples
348 was demonstrated with swab samples taken from the interior of the ISS. The bacterial levels that
349 correspond with the amount of DNA measured in these samples are also in agreement with
350 hygiene measures taken aboard the ISS aimed to keep bacterial levels below the bacterial
351 acceptability limit of 10^4 colony forming units per 100 cm^2 [35].

352 The importance of the DNA extraction step in nucleic acid based molecular detection and
353 quantification methodology for bacteria and the importance of the assessment of its efficiency is
354 evident, as is the case in fungal diagnostics [36]. Following the results of this study, FTA[®] Elute
355 paper would be our method of choice for use on-site with samples taken from solid surfaces,
356 based on processing time per sample, easiness of use, DNA extraction efficiency, broad-range
357 species effectiveness, chemical toxicity and transport and storage advantages.

358

359

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361

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367

368

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506 **Table 1** Efficiencies of DNA extraction of different amounts of cells of *E. coli* ATCC 11775^T and
 507 *S. aureus* subsp. *aureus* ATCC 25923 by three different methods: FTA[®] Elute, the Reischl et al.
 508 method and QIAamp[®] DNA Mini Kit.

Number of cells		5.3 × 10 ⁵	5.3 × 10 ⁴	5.3 × 10 ³	5.3 × 10 ²	5.3 × 10 ¹
		Median % (n=4)				
<i>E. coli</i> ATCC 11775 ^T	FTA [®] Elute	40.5	84.8	96.3	BC ^d	BC ^d
	Reischl et al.	48.9	59.6	24.5	BC ^b	ND
	QIAamp [®] DNA Mini Kit	4.6	32.3	13.5	BC ^b	BC ^c
<i>S. aureus</i> subsp. <i>aureus</i> ATCC 25923	FTA [®] Elute	112.6	94.9	119.4	BC ^b	BC ^c
	Reischl et al.	10.2	8.8	8.5	BC ^b	BC ^a
	QIAamp [®] DNA Mini Kit	69.4	117.0	97.7	149.9	BC ^a

509 ND, not detected; BC, Below Cut-off level, i.e. not all of the reactions were positive and
 510 quantifiable.

511 ^aFour, ^bthree, ^ctwo, ^done of four reactions produced a positive result.

512

513 **Fig 1** DNA levels extracted by three methods with varying amounts of cells. Median DNA levels
514 as determined by qrtPCR resulting from quadruplicate extractions (n=4) of different amounts of
515 cells of (A) *E. coli* ATCC 11775^T and (B) *S. aureus* subsp. *aureus* ATCC 25923. Extractions were
516 performed by three different DNA extraction methods: FTA[®] Elute, the Reischl et al. method and
517 QIAamp[®] DNA Mini Kit. Error bars indicate minimum and maximum values. Extrapolated
518 quantification limits of the methods are represented by dotted lines: ---, FTA[®] Elute, — — —,
519 Reischl et al. and QIAamp[®] DNA Mini Kit.

520

521 **Fig 2** DNA extraction efficiencies of three methods. Percentage of DNA extraction efficiency
522 (n=12) of FTA[®] Elute, the Reischl et al. method and QIAamp[®] DNA Mini Kit for (A) *E. coli* ATCC
523 11775^T and (B) *S. aureus* subsp. *aureus* ATCC 25923. Horizontal bars show the median and
524 quartiles.

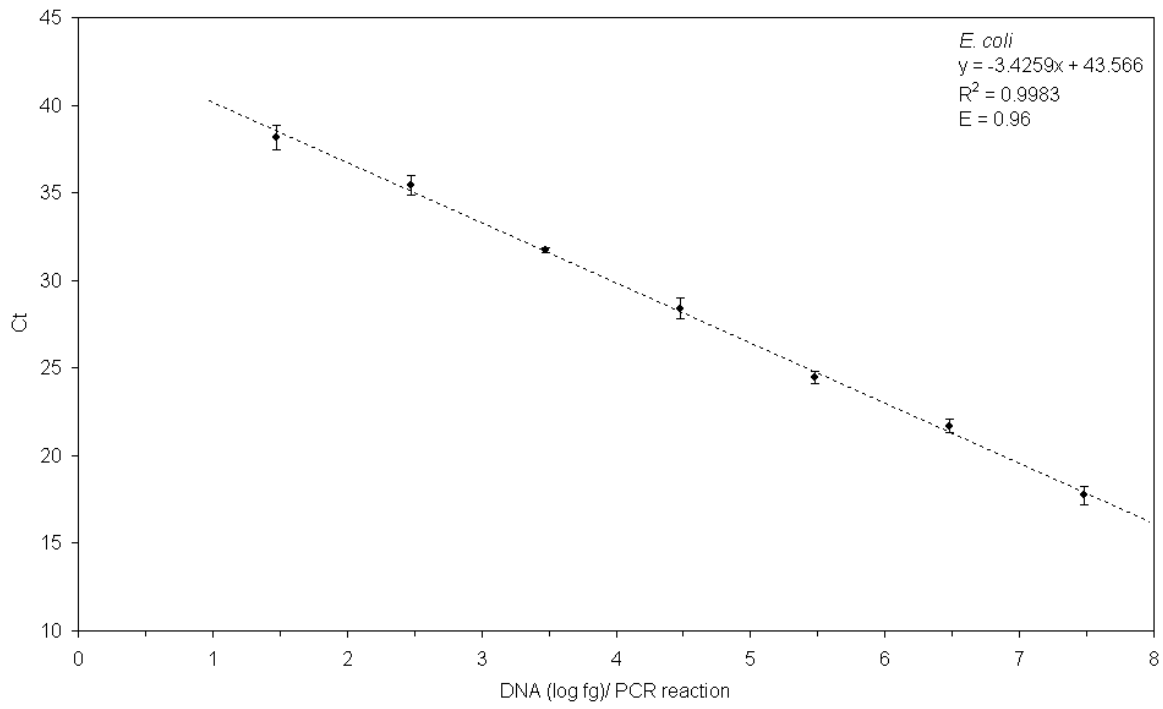
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526 **Fig 3** Inhibition of the *E. coli uidA* qrtPCR assay. Mean levels of DNA were determined with
527 qrtPCR by adding a template of 1 ng of the standard together with either water or an extraction
528 control of one of the three following DNA extraction methods to triplicate reactions (n=3): FTA[®]
529 Elute, the Reischl et al. method and QIAamp[®] DNA Mini Kit. The dotted line represents part of the
530 standard curve. (O) FTA[®] Elute, (□) QIAamp[®] DNA Mini Kit, (◇) Reischl, (Δ) Water.

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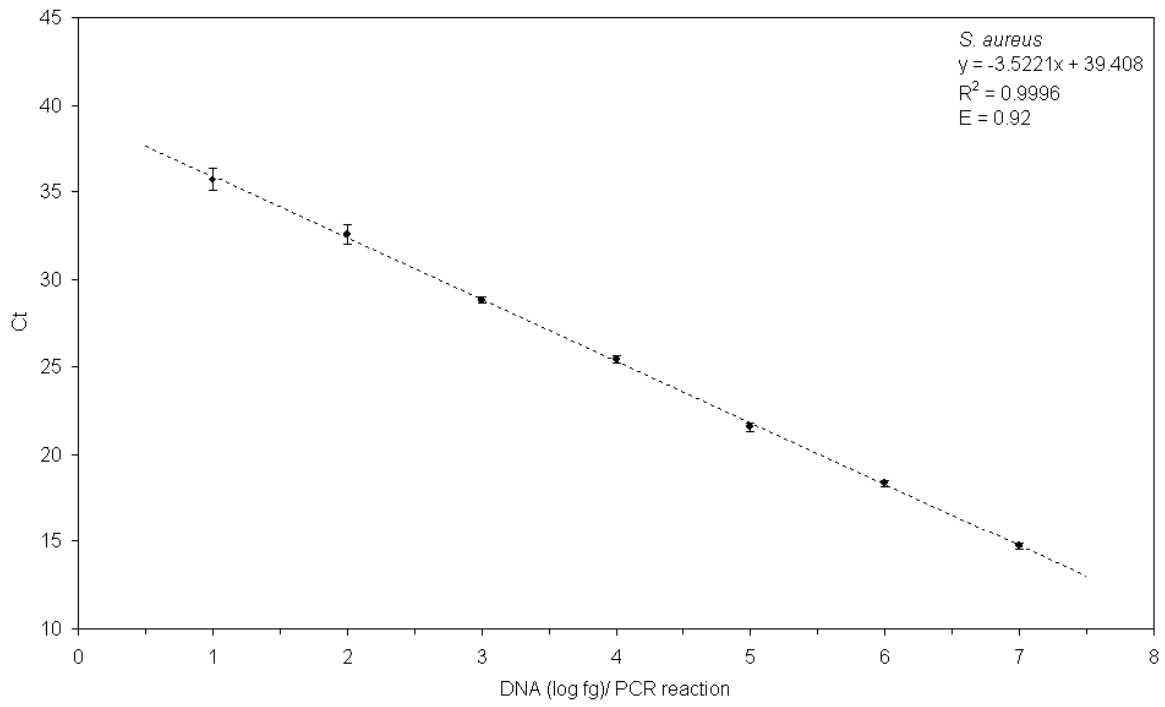
532 **Online Resources 1 and 2** QrtPCR standard curves with E and R² values of *E. coli* (n=6) and *S.*
533 *aureus* (n=9).

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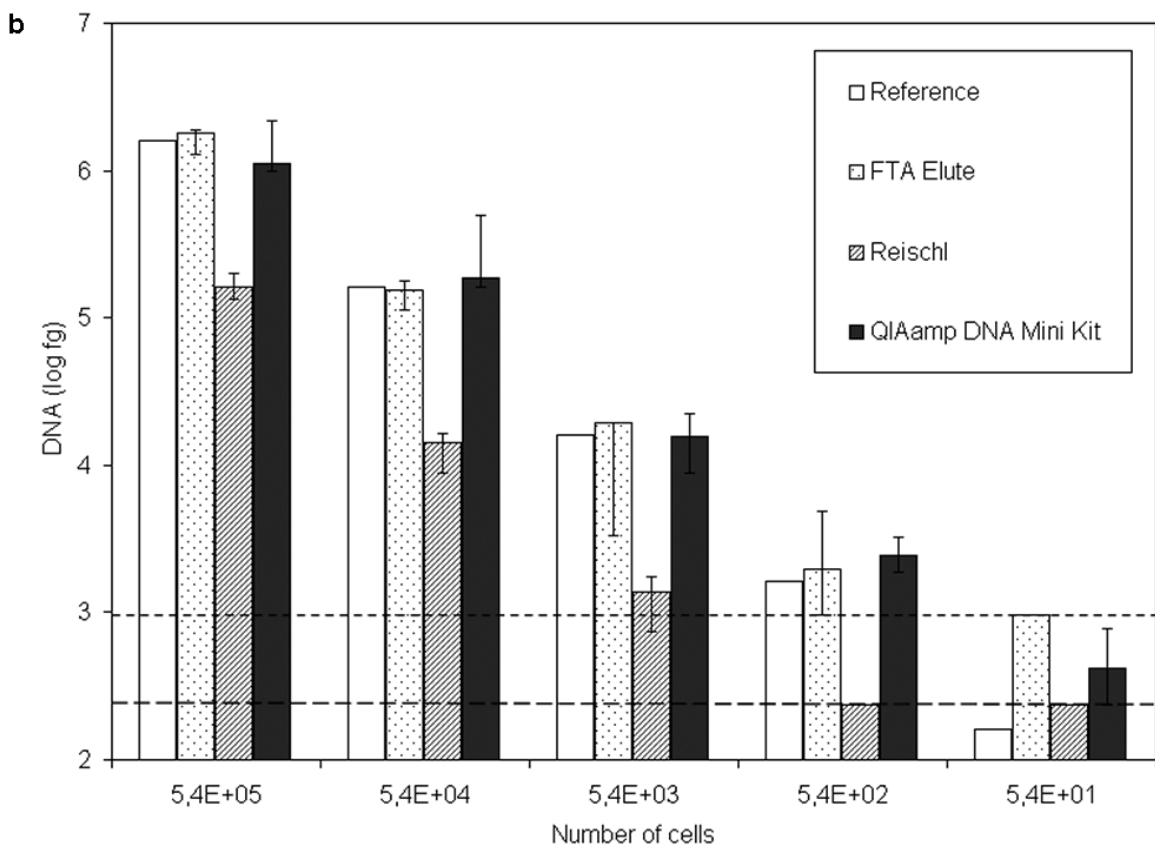
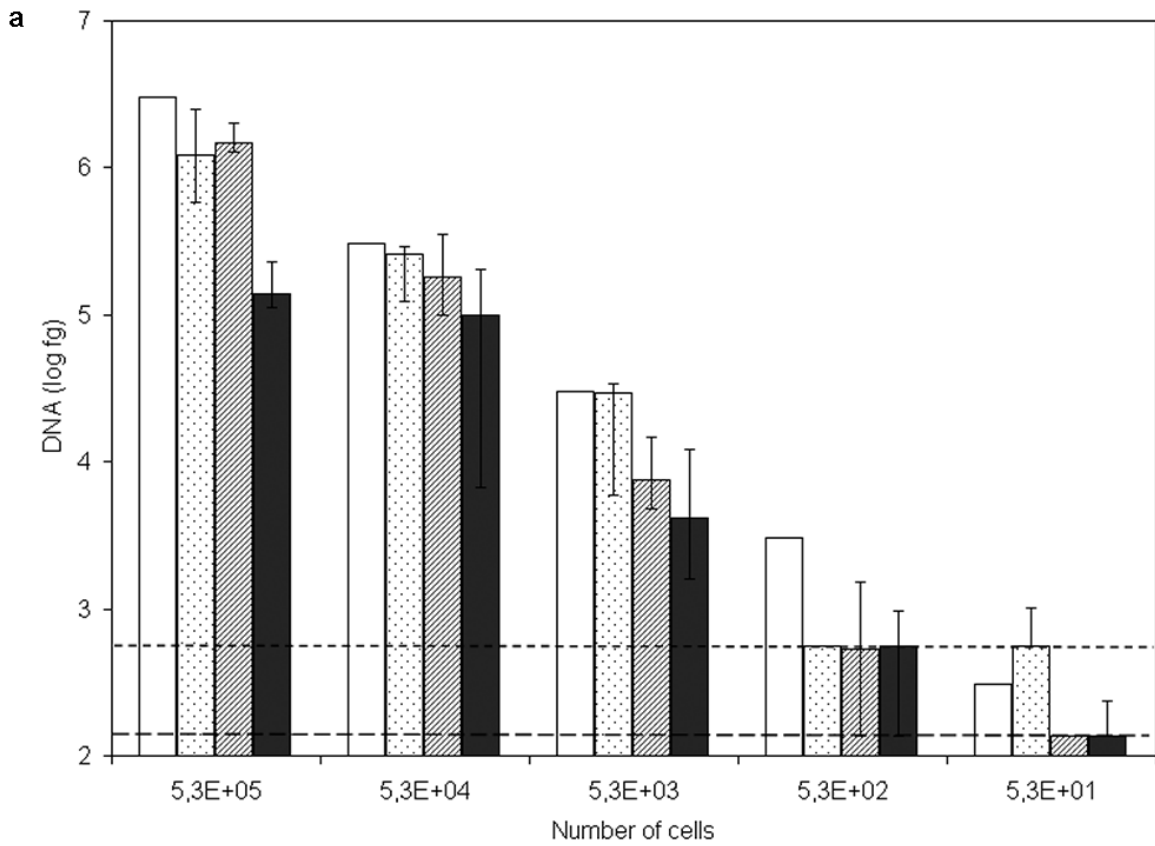
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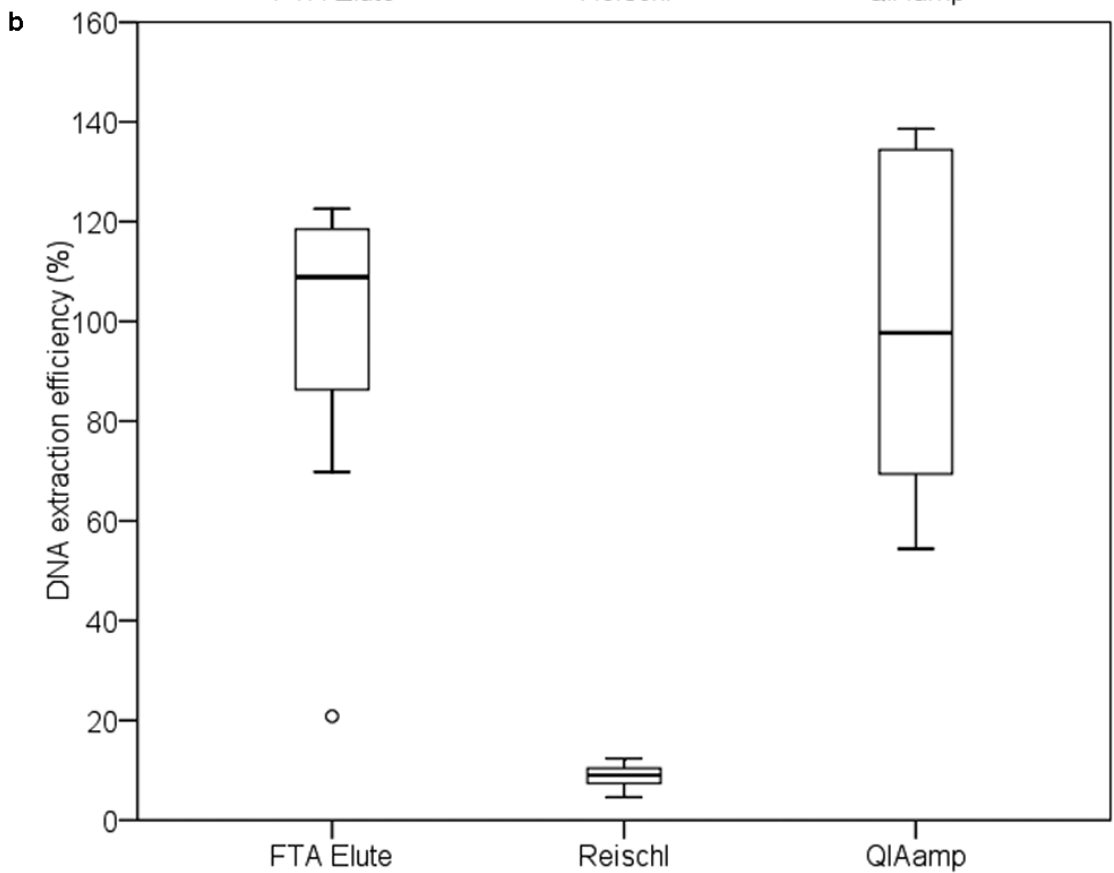
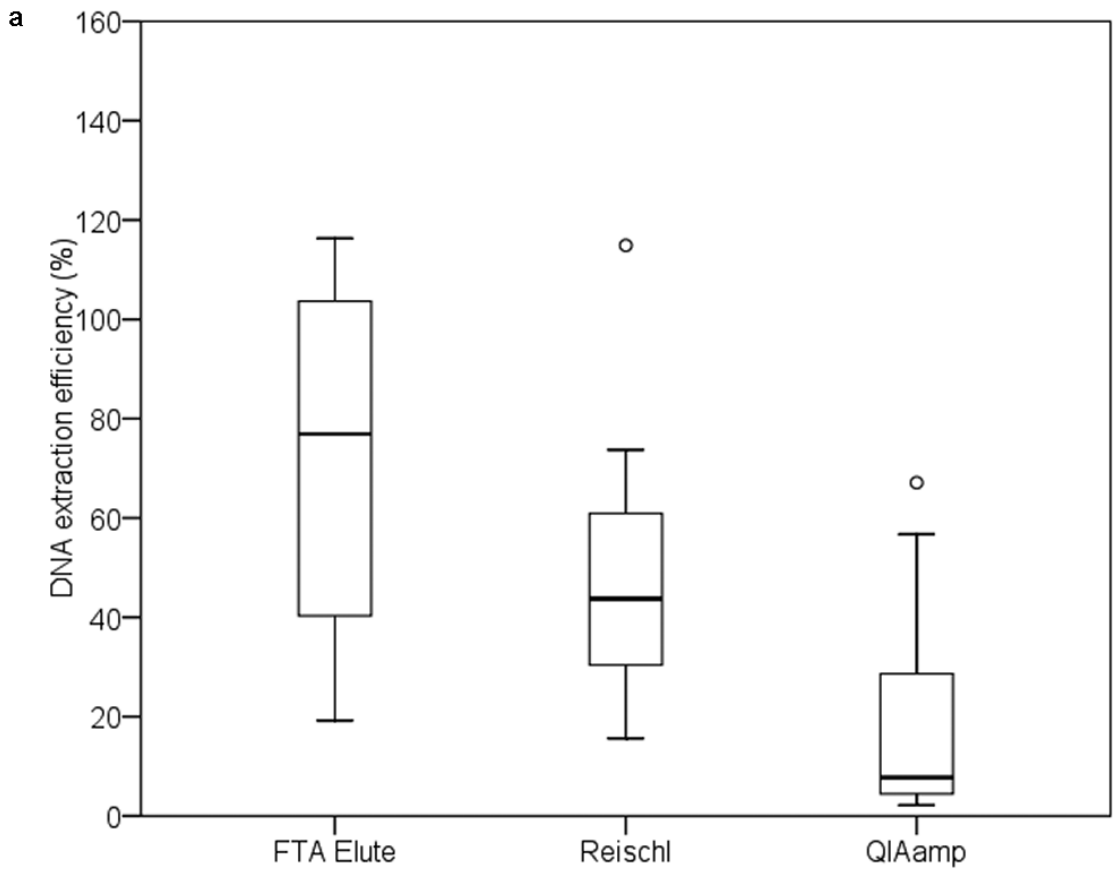
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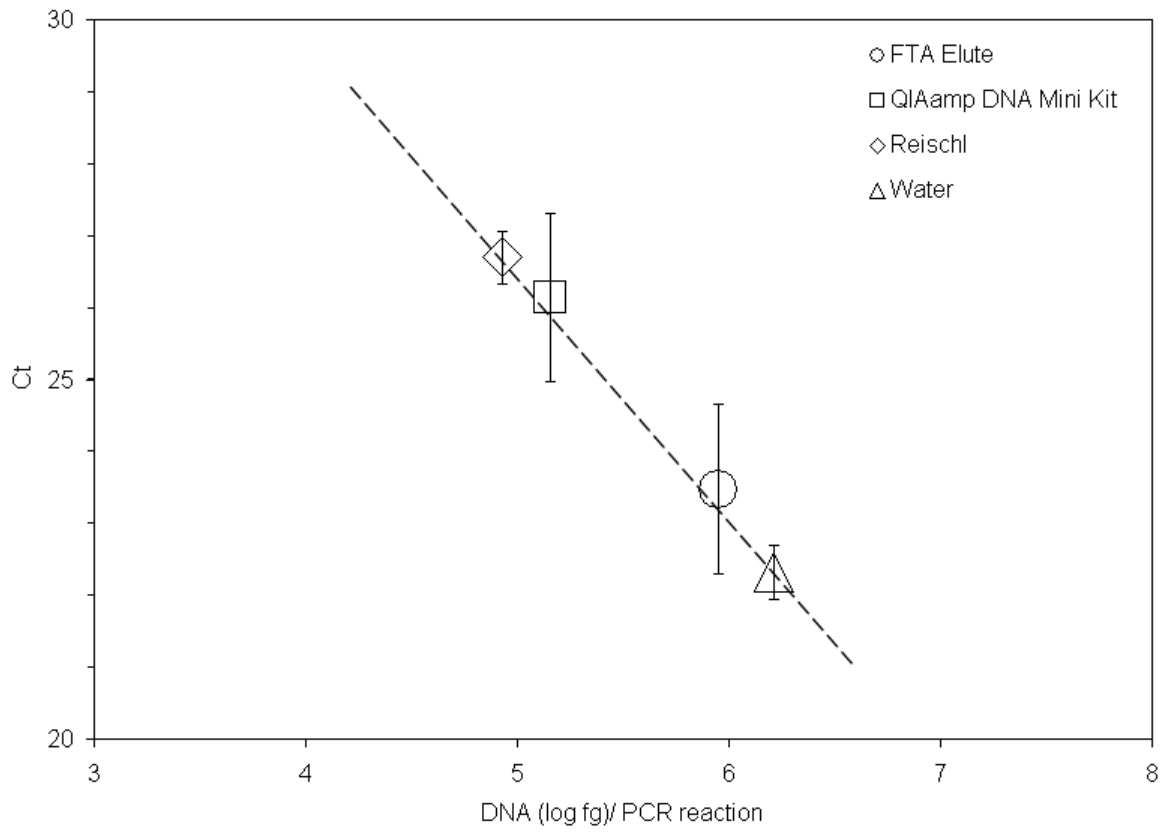
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