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1 Next generation planar waveguide detection of
2 microcystins in freshwater and cyanobacterial
3 extracts, utilising a novel lysis method for portable
4 sample preparation and analysis

5
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18

19 Highlights

20 Sensitive assay for the detection of the most common and toxic microcystin variants

21 Detection of free and cell bound microcystin for a true reflection of toxin content

22 Novel, highly effective lysis method enabling fast and portable disruption of cells

23 Validated to measure microcystins below 1 and 0.1 ng ml⁻¹; free and intracellular

24 Next generation planar waveguide biosensor combining quantification and ease of use

25

26

27 Abstract

28 The study details the development of a fully validated, rapid and portable sensor based

29 method for the on-site analysis of microcystins in freshwater samples. The process

30 employs a novel lysis method for the mechanical lysis of cyanobacterial cells, with

31 glass beads and a handheld frother in only 10 min. The assay utilises an innovative

32 planar waveguide device that, via an evanescent wave excites fluorescent probes, for

33 amplification of signal in a competitive immunoassay, using an anti-microcystin

34 monoclonal with cross-reactivity against the most common, and toxic variants.

35 Validation of the assay showed the Limit of Detection (LOD) to be 0.78 ng ml⁻¹ and the36 CC β to be 1 ng ml⁻¹. Robustness of the assay was demonstrated by intra- and inter-

37 assay testing. Intra-assay analysis had % C.V.s between 8 and 26% and recoveries

38 between 73 and 101%, with inter-assay analysis demonstrating % C.V.s between 5 and

39 14% and recoveries between 78 and 91%. Comparison with LC-MS/MS showed a high

40 correlation ($R^2 = 0.9954$) between the calculated concentrations of 5 different41 *Microcystis aeruginosa* cultures for total microcystin content. Total microcystin

42 content was ascertained by the individual measurement of free and cell-bound

43 microcystins. Free microcystins can be measured to 1 ng ml⁻¹, and with a 10-fold

44 concentration step in the intracellular microcystin protocol (which brings the sample

45 within the range of the calibration curve), intracellular pools may be determined to 0.1

46 ng ml⁻¹. This allows the determination of microcystins at and below the World Health
 47 Organisation (WHO) guideline value of 1 µg l⁻¹. This sensor represents a major
 48 advancement in portable analysis capabilities and has the potential for numerous other
 49 applications.

50

51 **Keywords:** Microcystin, planar waveguide, portable biosensor, novel lysis, blue-green
 52 algae and cyanobacteria

53

54 1. Introduction

55

56 Cyanobacteria (Domain Bacteria) originate from the Precambrian era, 3.4 billion years
 57 ago and were the first prokaryotes to use water in the fixation of carbon dioxide [1].
 58 They are widespread throughout global waters, both marine and freshwater,
 59 encompassing, not only hot tropical to temperate waters, but even the chilly waters of
 60 the Antarctic ice shelves [2]. Of the many types of cyanobacteria, *Microcystis*
 61 *aeruginosa* (predominantly freshwater) is the most common species, which produces
 62 microcystins. In addition to *Microcystis*, microcystins are also produced by the
 63 following genera: *Anabaena*, *Nostoc*, *Planktothrix*, *Anabaenopsis* and
 64 *Hapalosiphon* [2]. Microcystins are cyclic heptapeptides with the structure; cyclo-(D-
 65 Alanine-X-D-MeAsp-Y-Adda-D-Glutamate-Mdha), where X and Y are variable L-
 66 amino acids, MeAsp and Mdha are Methylaspartic acid and Methyldehydroalanine,
 67 respectively and Adda is (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-
 68 phenyldeca-4(E),6(E)-dienoic acid [3]. There are over 90 variants of microcystins with
 69 the most common being Microcystin-LR (MC-LR), which has Leucine at position 2

70 and Arginine at position 4 [4, 3]. They are hepatotoxic, due to their uptake via the bile
71 acid transport system delivering them into hepatocytes, where they inhibit
72 Serine/Threonine Proteases via Adda, causing over phosphorylation of proteins. Acute
73 toxicosis results in the disruption of the cytoskeleton, loss of cell structure and
74 adhesion, loss of tissue structure and the collapse of sinusoidal capillaries resulting in
75 hepatic haemorrhaging and ultimately death [5–8]. The deaths of 60 patients in a
76 dialysis unit in Brazil, in 1996, have been attributed to the use of contaminated water
77 during dialysis [9]. Lower levels of microcystins, although not acutely lethal, have
78 been shown to promote cancer (Group 2B carcinogen; possibly carcinogenic to
79 humans) and have immunotoxic and genotoxic effects [10–12]. The World Health
80 Organisation (WHO) have set the recommended limit at $1 \mu\text{g l}^{-1}$ for drinking water and
81 $20 \mu\text{g l}^{-1}$ for recreational waters [13–15]. Microcystins are small (MC-LR being 995.2
82 Da) and stable compounds, withstanding harsh conditions, such as high temperatures
83 and extreme pH. Removal is usually achieved by activated carbon filtration, ozonation
84 or chlorination; all being commonly utilised in water treatment [16–18].

85
86 The ability to detect microcystins is important and many tests exist. The vast majority
87 are laboratory based, with immunoassays enabling high throughput screening for total
88 microcystin concentrations and analytical methods (such as HPLC, Mass
89 Spectrometry[19–21]), although slower, allowing the quantification and identification
90 of individual variants within a sample [22, 3]. Recent attention has turned towards the
91 on-site detection of microcystins. Portable tests have long since focused on the lateral
92 flow format, a major drawback of which is that quantification usually relies on the
93 user's determination. However, the first lateral flow device (LFD) for microcystins,
94 devised by Kim et al (2003) [23], employed a custom made portable-laser fluorescence

95 scanner, eliminating the subjective nature of results interpretation. For this they used
 96 Alexa Fluor 647®, a Cy5 labelled fluorescent probe, as the secondary, detection,
 97 antibody, in a competitive assay, whereby a MC-LR conjugate was coated to the
 98 surface and an anti-MC-LR monoclonal was used as the primary antibody. This assay
 99 format works well for most biosensors, including planar waveguide, a technology, that
 100 has been around for decades. Two portable assays, have been developed recently by
 101 Long et al (2005) and Herranz et al (2012) [24, 25]. Both utilise modified versions of
 102 the aforementioned assay format; Long et al use a Cy5 labelled monoclonal, and
 103 Herranz coat the sensor surface with MC-LR as opposed to a MC-LR-conjugate. The
 104 biosensors used are the Trace Organic Pollutant Analyser (TOPA) and a
 105 commercialised version of the US Naval Research Laboratory (NRL) Array Biosensor
 106 prototype, by Long et al and Herranz et al respectively. For the TOPA assay, one
 107 analyte was measured at a time, with a cycle time of 20 min, 150 cycles per surface and
 108 an LOD of 30 ng l⁻¹. The NRL Biosensor, can detect up to 6 analytes at a time, with
 109 15 cycles per surface, a cycle time of 60 min and an LOD of 16 ng l⁻¹. The present
 110 study utilises the next generation of evanescent wave/ planar waveguide detection. This
 111 platform has all the advantages related to single use LFDs and portable biosensors yet
 112 has the ability to perform the accurate quantification only associated with laboratory
 113 based, methods. The unique MBio SnapEsi® LS sensor [26] employed in the present
 114 study uses, a patented design, whereby the lens is integrated on a disposable cartridge; a
 115 cartridge into which the sample and reagents are added and held. The cartridges are
 116 custom made, spotted with (in-house prepared) toxin-protein conjugates, chosen and
 117 optimised for the sensitive and selective binding of anti-microcystin monoclonal
 118 antibody (also produced in-house). These cartridges eliminate the need for
 119 cumbersome and expensive microfluidics often associated with advanced portable

120 sensors, thus there is no need for pumps, valves, tubing or buffer/waste reservoirs. In
121 the SnapEsi® LS assay there are no concerns about tubing becoming blocked; either by
122 lines drying out due to warm temperatures, air bubbles, or sample particulate blockages.
123 The cartridge can simply be used, read and disposed of. Thus a simple to use, low cost
124 and accurate means of detecting microcystins will be presented.

125

126 Further to this, we present a highly novel sample preparation method for the portable
127 lysis of cyanobacterial, *Microcystis aeruginosa*, cells, for the quantification of intra-
128 cellular microcystin levels. Microcystins are inherently endotoxins, therefore, the
129 measurement of freshwater only, fails to detect the majority of toxin present, as has
130 been highlighted by Codd et al (2005) [2] who state that guideline values “should
131 therefore apply to the sum of the intracellular and extracellular microcystin pools”; yet
132 the focus has remained on water testing only. This may be in part due to the difficulties
133 encountered in dealing with cyanobacterial samples in current analytical methods.
134 Cheap, quick and portable lysis usually relies on chemical disruption of cells, using
135 harsh reagents which may interfere with downstream assays, causing matrix
136 interference and thus skewing results. The best cellular disruption occurs via
137 mechanical lysis of the cells, such as that of glass bead beating, which has been
138 demonstrated to achieve full lysis of algal cells, in a quick time of only 10 min [27].
139 This laboratory based method required a paint shaker to mix the sample/glass bead
140 combination and a centrifuge to separate cells from freshwater samples. To overcome
141 this, centrifugation steps were switched to filtration and the paint shaker was substituted
142 for a, low cost, hand-held, battery operated frother. Other non mechanical methods
143 require filtration of the sample (glass fibre filters, GF/C) followed by slow toxin
144 extraction using solvents which are not compatible with immunoassays.

145
146 Presented here is an assay, proven to be capable of detecting microcystins in both free
147 and intracellular states, to a level of 1 ng ml⁻¹ for free and 0.1 ng ml⁻¹ for intracellular
148 microcystins. This rapid, semi-quantitative test, has an assay time of only 15 min for
149 free microcystins, and under 30 min for intracellular microcystins, inclusive of sample
150 preparation.

151

152

153

154

155 2. Materials and Methods

156

157 2.1. Reagents and Chemicals

158 Microcystin-LR was purchased from Enzo Life Sciences, through Alpha Technologies
159 Ltd, Larne Northern Ireland. Bovine Serum Albumin (BSA), Phosphate buffered saline
160 (PBS) tablets, Tween-20 and apo-Transferrin (bovine) were purchased from Sigma-
161 Aldrich, Gillingham, UK. Cyanobacterial cultures were obtained from the Culture
162 Collection of Algae and Protozoa (CCAP), Oban, Scotland and the Laboratory of
163 Ecotoxicology, Genomics and Evolution (LEGE) at the Centre of Marine and
164 Environmental Research (CIIMAR), Porto, Portugal. From CCAP *M. aeruginosa* strains
165 1450/3 (non-toxic) and 1450/6 (toxic) were purchased and from CIIMAR, strains
166 LEGE 91093, LEGE 91094, LEGE 91095 and LEGE 91096 were received. Jaworski's
167 and BG11 media were purchased from CCAP, Oban, Scotland. Alexa Fluor 647 goat
168 anti—rabbit IgG and Alexa Fluor 647 goat anti—mouse IgG antibodies were purchased

169 from Invitrogen Ltd, Paisley, Scotland. The anti-microcystin, 5C4, monoclonal was
 170 prepared in-house and details will be published elsewhere.

171

172 2.2. Apparatus

173 For cell lysis a hand held frother was purchased from Argos Direct, Stafford, UK, while
 174 the paint shaker, a Minimix standard shaker, was purchased from Merris Engineering,
 175 Berkshire, UK. Swinnex, 25 mm filter holders, 25mm gaskets and 25 mm MF-
 176 Millipore (mixed cellulose esters, hydrophilic, 0.45 μm and black gridded) membranes,
 177 together with Millex-HA (0.45 μm , mixed cellulose) filters were purchased through
 178 Premier Scientific Ltd, Belfast, Northern Ireland. The SnapEsi® LS System [28] was
 179 supplied by MBio Diagnostics Inc, Boulder, Colorado, USA, as were the microarray
 180 cartridges.

181

182 2.3. Preparation of Toxin Protein Conjugate (TPC); 183 MC-LR-Transferrin

184 MC-LR, 0.25 mg, was reconstituted in 50 μl Dimethyl sulfoxide (DMSO). To this was
 185 added 50 μl *N*-Hydroxysuccinimide (NHS) (130 mM) and 100 μl *N*-(3-
 186 Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) (39 mM), both
 187 dissolved in 50 mM 2-(*N*-Morpholino) ethanesulfonic acid (MES), pH 4.7. This was
 188 stirred for 30 min, protected from light, at room temperature. The active ester solution
 189 was then added, dropwise to a vial containing 1 mg of Transferrin dissolved in 1 ml
 190 PBS pH 7.4. This was stirred at room temperature, for 2 hours, again protected from
 191 light. The TPC was dialysed against PBS pH 7.4 and tested by ELISA. The TPC was
 192 sent to mBio Diagnostics for microarray printing, at 100 $\mu\text{g ml}^{-1}$.

193

194 **2.4. Microarray Printing**

195 Microarrays were printed using a Bio-Dot AD3200 robotic arrayer. Briefly, spots were
196 produced, with a diameter of 0.5 mm, using a Bio-Jet print head that dispensed 20 nl.
197 Four replicates of the TPC were printed onto a grid with 1 mm centres. Microarrays
198 were then blocked with a protein-based blocking agent (0.5% casein in PBS with
199 Proclin300 antimicrobial) prior to spin-drying. Microarrays were then assembled into
200 an injection moulded cartridge, which contained a 5 mm wide fluidic channel (max
201 volume 30 μ l) with a single inlet port for the addition of sample and reagents. Assays
202 were carried out on a rack, angled for optimum flow rate, enhancing passive fluid flow,
203 which is aided by capillarity due to the narrow fluidic channel.

204

205 **2.5. Culturing**

206 CCAP cultures were maintained in Jaworski's Medium, while CIIMAR LEGE cultures
207 were maintained in BG11 Medium. Culturing was done in glass Erlenmeyer flasks
208 (wide necked) with cotton wool plugs and foil lids and incubated at 20 °C with a light
209 intensity of 116 μ mol m⁻² s⁻¹. All consumables were sterilised by autoclaving at 121 °C
210 for 15 min. Culturing work was carried out in a pre-sterilised (via 30 min UV
211 exposure) UV3 HEPA PCR cabinet. Cultures were maintained in log phase, with fresh
212 cultures being seeded from denser cultures (nearing stationary phase); 40 ml dense
213 culture added to 160 ml fresh media. Culture growth was monitored by cell counting
214 using Lugol's stain and a haemocytometer under x200 magnification.

215

216 2.6. Sample Lysis

217 A 25 mm swinnex filter (in a 25 mm Swinnex Filter Holder) was used to filter 50 ml of
 218 sample/culture. The filter membrane was then transferred to a 7 ml bijou and the
 219 captured cells resuspended with 5 ml PBS pH 7.4. To 5 ml of sample/culture, 1 g of
 220 0.1 mm glass beads was added to a small glass beaker (15 ml), whose diameter was just
 221 large enough to accommodate the frother whisk, whilst limiting the space for
 222 beads/cells to accumulate in. The frother was then switched on and allowed to 'whisk'
 223 the sample/bead mixture for 10 min. Once finished the sample was twice filtered (0.45
 224 μm Millipore), to ensure complete removal of glass beads/fragments; once should be
 225 enough, but a second filtration was included in case the first filter was perforated. A
 226 negative toxin producer (CCAP 1450/3) was treated in the same way to produce the
 227 matrix in which calibrants are prepared.

228

229 2.7. Assay

230 Reagent/Sample Preparation

231 Assay Buffer

232 The buffer was PBS pH 7.4 containing 0.05% Tween-20, 0.45 μm filtered.

233

234 Antibody Solutions

235 The monoclonal antibody, 5C4 was diluted (v/v), 1 in 100, from the stock solution of
 236 approximately 0.88 mg ml^{-1} . The diluent was Assay Buffer containing 1% BSA, the
 237 BSA acting as a stabiliser. The working stock was maintained at 4 °C and diluted
 238 again, with Assay Buffer (without the added BSA) to a final concentration of 1 in
 239 10000, for use in the assay. The 5C4 monoclonal antibody was shown to have cross-

240 reactivity for the most common microcystin variants, as follows: MC-RR, 108%; MC-
241 YR, 68%; MC-LA, 69%; MC-LW, 71%; MC-LF, 68%; and Nodularin, 94%.

242

243 The detection antibody, Alexa Fluor 647 goat anti-mouse IgG, was used to prepare a
244 detection antibody working stock solution, also stored at 4 °C (without added BSA),
245 protected from light. The stock solution was prepared by adding 100 µl of each
246 antibody to 2 ml of Assay Buffer. For use in assay conditions, a further 1 in 10 dilution
247 (with Assay Buffer) was required to get to the final 10 µg ml⁻¹ working solution.

248

249 Calibrants

250 Calibrants were prepared from a 1 µg ml⁻¹ solution of MC-LR, to yield concentrations
251 of 200, 50, 20 and 0 ng ml⁻¹, with dilutions made using Assay Buffer. These were then
252 used to spike 950 µl of matrix, using 50 µl per spike, to get final solutions with the
253 following concentrations: 10, 2.5, 1 and 0 ng ml⁻¹.

254

255 Assay Conditions

256 Equal volumes of 5C4 antibody and sample/calibrant were mixed and 150 µl
257 immediately applied to the cartridge. After 7 ½ min, 150 µl of detection antibody was
258 added. After 7 ½ min (15 min total time) the cartridge was read using the SnapEsi
259 reader.

260

2.8. Liquid Chromatography Tandem- Mass Spectrometry (LC-MS/MS) Validation

Freeze-drying and solid phase extraction (SPE) were used to prepare and extract microcystins from *Microcystis aeruginosa* samples for LC-MS/MS detection, as reported elsewhere; using modified methods from Kim et al. (2009), Lawton et al. (1994), Msagati et al. (2006) and Mooney et al. (2011) [29–31, 20]. Briefly, 50 ml samples were freeze-dried and resuspended in, 5 ml, 75% methanol before enrichment and purification with OASIS HLB cartridges, after the methanol content was diluted to 15%. Microcystins were eluted with 6 ml methanol, containing 0.1% Trifluoroacetic acid, dried under nitrogen, resuspended in 80% methanol and analysed by LC-MS/MS; using a Waters Acquity UPLC and a Quattro Premier XE Mass Spectrometer, run in electrospray positive mode (ESI).

2.9. Surface Plasmon Resonance (SPR) Assay

CM5 research grade chips were used on a Biacore Q instrument. CM5 chips were coated with MC-LR using the method devised by Vinogradova et al [32]. The flow rate was 20 $\mu\text{l min}^{-1}$ for 4 min per cycle, with the 5C4 monoclonal antibody used at a 1 in 1000 dilution (v/v) using the 0.88 mg ml^{-1} stock solution. 5C4 was mixed with sample prior to injections using a blend of 30% antibody and 70% sample. The calibration curve consisted of six points; 10, 5, 2.5, 1, 0.5 and 0 ng ml^{-1} MC-LR. Calibrants were prepared in matrix (*M. aeruginosa* CCAP 1450/3 lysate) to normalise for matrix effects. Regeneration of the chip surface was achieved by injecting 75 mM Sodium hydroxide and 10% Acetonitrile (in deionised water) for 1 min at 20 $\mu\text{l min}^{-1}$.

286 3. Results and Discussion

287 3.1.Milk Frother Validation

288 Validation of the frother required testing samples lysed by both the laboratory based
 289 paint shaker [27] and portable frother by SPR. This was due to the observation that
 290 disintegration of the glass beads occurred upon mixing, which resulted in the
 291 appearance of small fragments, which could be mistaken for *M. aer* cells when viewed
 292 microscopically, See Fig 1 for images. To overcome this, identical samples were tested
 293 by SPR. For one sample, the paint shaker and 0.5 mm beads were employed and for
 294 the other the frother and 0.1 mm beads were used. The ratio of 1 g per 2 ml of sample
 295 was maintained, thus 2.5 g of 0.1 mm glass beads were added to the 5 ml sample. The
 296 same mixing time was used for both, 10 min. The final samples were then tested by
 297 SPR to determine the toxin concentrations present. The concentration as determined by
 298 the paint shaker method was 9.0 ng ml⁻¹, while that of the frother method was 9.25 ng
 299 ml⁻¹, resulting in a 103 % recovery of microcystin.. This confirmed that the lysis
 300 procedure was as effective as that of the paint shaker.

301

302 3.2.Assay Format

303 The format of the assay was competitive inhibition, whereby the more microcystins that
 304 were present in a sample/calibrant, the less anti-microcystin, 5C4, monoclonal there
 305 was available to bind to the surface bound TPC. Thus the more microcystins present in
 306 a given sample, the lower the signal would be. This is shown in Fig 2, showing the
 307 fluorescence recorded for the four identical microcystin spots, on cartridges with
 308 calibrants added.

309
 310

3.3. Assay Validation

The limit of detection (LOD) was determined by taking the mean response for 20 negative (toxin free) samples and subtracting 3x standard deviations (S.D.) as is standard for inhibition assays [33]. The response was then converted to concentration via the calibration curve and derived as 0.78 ng ml^{-1} (see Table 1). This level was used as a guide to estimate the detection capability ($\text{CC}\beta$), which was 1 ng ml^{-1} , equivalent of the $1 \mu\text{g l}^{-1}$ WHO recommended level. Taking into consideration the 10-fold concentration step in the sample preparation method for intra-cellular measurement, this brings samples containing 0.1 ng ml^{-1} into the measureable range of the calibration curve. No sample preparation method, other than filtration ($0.45 \mu\text{m}$) was included for free microcystin measurement, as this would only serve to reduce the speed of analysis. As can be seen from Fig 3, there was no overlap between the $20 \times 1 \text{ ng ml}^{-1}$ spiked samples and the 20 negative samples from the LOD calculation (to $1 \times \text{S.D.}$). Populations were also shown to be significantly different with a p value < 0.0001 ; 2-tailed, unpaired t-test. The assay had a dynamic range (IC_{10} to IC_{90}) of 0.22 to 5.12 ng ml^{-1} .

Repeatability and reproducibility (intra- and inter-run robustness) were demonstrated by spiking at 3 different toxin levels, with 4 replicates per spiking level, across 2 batches of cartridges and repeating this over 3 days. The data was then analysed per day to determine repeatability of the assay and then across the 3 days to determine the reproducibility of the assay. The 3 spiking levels chosen were $\text{CC}\beta$, the midpoint (IC_{50}) and the level at which 75% inhibition of signal was achieved (IC_{75}), which were 1, 1.27 and 2.78 ng ml^{-1} respectively. Inter-run analysis showed that spiking level recoveries all lay between 78 and 91% with % C.V.s lower than 15% (see Table 2). For intra-run

analysis, recoveries were between 73 and 96 % with % C.V.s below 22% (see Table 2). This demonstrated a high degree of repeatability for what is a semi-quantitative assay, and providing values that could be expected with a fully quantitative, laboratory based assay,

Final validation of the assay was performed by testing samples by the SnapEsi method and comparing them to values determined by LC-MS/MS analysis. Table 3 shows the calculated concentrations of the 5 *M aeruginosa* strains tested. The two microcystin pools were combined to give a total microcystin content of each sample. These were then compared to the concentrations derived by LC-MS/MS analysis and as can be seen from the data in Table 4 the difference between the calculated concentrations is between 0.8 to 1.3-fold, with an average of a 1.04-fold difference. Differing sample preparation methods in addition to the differing detection methods generally result in much greater variation in data generated. The R^2 value, when the calculated concentrations were plotted against each other, Fig 4, was 0.9954, demonstrating the reliability and accuracy of the method.

4. Conclusions

Due to the true and accurate level of microcystins in a water sample only being ascertained if both free and cell-bound levels are quantified, a method has been developed, and fully validated, to allow the calculation of the total microcystin content of a sample. To allow for the development of a rapid and portable assay, a novel method of cell lysis was also developed and validated, whereby a frother was used to vigorously agitate a sample containing, 0.1 mm glass beads, mechanically lysing the

360 cyanobacterial cells, without the need for harsh chemicals, in just 10 min. The planar
 361 waveguide assay, using a SnapEsi® LS sensor, is rapid, taking only 15 min and using
 362 an anti-microcystin monoclonal antibody (5C4), that detects the more common, and
 363 toxic, variants of microcystin; MC-LR, MC-RR, MC-YR, MC-LA, MC-LW and MC-
 364 LF. The assay can detect free microcystins to 1 ng ml⁻¹ and intracellular microcystins
 365 to 0.1 ng ml⁻¹. The difference being due to a 10x concentration step that was included
 366 in the intracellular sample preparation method; no sample preparation method was
 367 required in the measurement of the free microcystin fraction. In addition to this, a
 368 single, cheap, cartridge is used per sample that is simply discarded after reading, to give
 369 an assay that is not only rapid and portable, but has quantitative capabilities that many
 370 semi-quantitative laboratory based methods fail to reach.

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443 List of Figures:

444 Fig 1. Image a (x 200 magnification) shows 0.1 mm glass beads after 10 min mixing
445 with the milk frother. The majority of beads disintegrate forming small fragments that
446 may be mistaken for cyanobacterial cells. Image b (x 200 magnification) shows CCAP

447 1450/6 *M. aeruginosa* cells; which would be difficult to distinguish amongst the bead
448 fragments in image a.

449 Fig 2. Images of microcystin spots as treated with calibrants: a, 10 ng ml⁻¹; b,
450 2.5 ng ml⁻¹; c, 1 ng ml⁻¹; and d, 0 ng ml⁻¹.

451 Fig 3. Determination of CCβ as 1 ng ml⁻¹, as shown by the 0 ng ml⁻¹ and 1 ng ml⁻¹
452 populations not overlapping (to 1 S.D.)

453 Fig 4. Comparison of mBio and LC-MS/MS results, showing a good correlation,
454 whereby $R^2 = 0.9954$.

455

456 List of Tables:

457 Table 1. Determination of LOD as 0.78 ng ml⁻¹

458 Table 2. Measured concentrations of spiked samples, along with S.D., % C.V.s and
459 mean recoveries, for repeatability and reproducibility analysis of assay.

460 Table 3. Microcystin content of *M. aeruginosa* cultures as calculated by mBio

461 SnapEsi.

462 Table 4. Comparison of mBio and mass spec concentrations for toxin producing *M.*
463 *aeruginosa* samples.

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Response (Normalised Signal)					Calc Conc (ng ml ⁻¹)
Mean	S.D.	%C.V.	3x[S.D.]	LOD	
1060.9	133.8	12.6	401.4	659.5	0.78

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	Spiking Level (ng ml ⁻¹)	Measured Concentration (ng ml ⁻¹) ± S.D.	C.V. (%)	Mean Recovery (%)
Day 1	1	0.95 ± 0.13	14.0	95.1
	1.27	0.99 ± 0.28	21.9	78.2
	2.78	2.03 ± 0.39	19.3	73.1
Day 2	1	0.86 ± 0.13	15.5	85.9
	1.27	1.06 ± 0.28	26.3	83.3
	2.78	2.02 ± 0.36	17.6	72.6
Day 3	1	0.91 ± 0.10	10.5	90.9
	1.27	1.29 ± 0.11	8.4	101.3
	2.78	2.49 ± 0.44	17.8	89.4
Overall (Days 1 to 3)	1	0.91 ± 0.05	5.0	90.6
	1.27	1.11 ± 0.15	13.9	87.6
	2.78	2.18 ± 0.27	12.2	78.4

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Culture	Cell bound Concentration (ng ml ⁻¹)	Free Concentration (ng ml ⁻¹)	Total Concentration (ng ml ⁻¹)
1450/06	7.9	21.2	29.2
91093	63.6	16.5	80.1
91094	38.7	21.6	60.3
91095	128.2	461.5	589.7
91096	72.5	18.2	90.7

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Culture	Calculated Concentration (ng/ml)		Difference (x-fold)
	mBio	Mass Spec	
1450/06	29.2	23.7	0.8
91093	80.1	108.1	1.3
91094	60.3	64.5	1.1
91095	589.7	536.2	0.9
91096	90.7	98.9	1.1

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1. Sample Filtration

2. Resuspension of cells

3. Sample Lysis

6. Read Signal

5. Application of sample

4. Sample filtration