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Citation for published version:

Bridle, H, Miller, B & Desmulliez, MPY 2014, 'Application of microfluidics in waterborne pathogen monitoring: A review', *Water Research*, vol. 55, pp. 256-271. <https://doi.org/10.1016/j.watres.2014.01.061>

Digital Object Identifier (DOI):

[10.1016/j.watres.2014.01.061](https://doi.org/10.1016/j.watres.2014.01.061)

Link:

[Link to publication record in Heriot-Watt Research Portal](#)

Published In:

Water Research

Publisher Rights Statement:

Published in *Water Research*, 55 (2014), doi: 10.1016/j.watres.2014.01.061

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Accepted Manuscript

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PII: S0043-1354(14)00101-8

DOI: [10.1016/j.watres.2014.01.061](https://doi.org/10.1016/j.watres.2014.01.061)

Reference: WR 10464

To appear in: *Water Research*

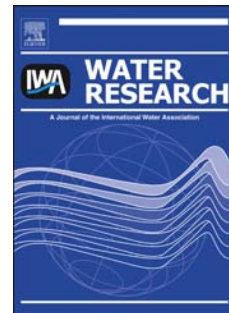
Received Date: 31 October 2013

Revised Date: 23 January 2014

Accepted Date: 24 January 2014

Please cite this article as: Bridle, H., Miller, B., Desmulliez, M.P.Y., Application of Microfluidics in Waterborne Pathogen Monitoring: A Review, *Water Research* (2014), doi: 10.1016/j.watres.2014.01.061.

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Highlights:

- First review of the application of microfluidics to waterborne pathogen monitoring
- Sample processing is divided into immunological, electrical and filtration methods
- Optical methods have had most attention but molecular methods maybe best
- Ultimate aim is multiplexed systems with integrated parts for automated operation
- Many challenges in applying microfluidics to this application: great future potential

Application of Microfluidics in Waterborne Pathogen Monitoring: A Review

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Abstract:

A review of the recent advances in microfluidics based systems for the monitoring of waterborne pathogens is provided in this article. Emphasis has been made on existing, commercial and state-of-the-art systems and research activities in laboratories worldwide. The review separates sample processing systems and monitoring systems, highlighting the slow progress made in automated sample processing for monitoring of pathogens in waterworks and in the field. Future potential directions of research are also highlighted in the conclusions.

Keywords: microfluidics, waterborne pathogens, monitoring techniques, sample processing, detection methods

1. Introduction

Inadequate access to clean water is hugely detrimental both to economic development and human health. In the developing world 2,200 children die daily from diseases transmitted through unsafe water and, despite the Millennium Development Goal of halving the proportion of people without access to an improved water source being met, 780 million people still lack access to safe water (www.cdc.gov/healthywater/global/wash_statistics.html). Far from being a solely developing world problem, waterborne disease is a threat to citizens in the developed world. For example, one of the largest recent outbreaks affected Milwaukee in 1993 in which approximately 400,000 people were infected by cryptosporidiosis (Corso et al.

35 2003). Moreover, it has been estimated that 10% of total hospital patients in the USA
36 contract diseases due to poor water sanitation, significantly increasing morbidity,
37 mortality and financial burden. Overall, lost productivity in the USA due to
38 waterborne diseases is estimated at \$20 billion per year (Straub and Chandler 2003).
39 According to the World Health Organisation (WHO), microbial hazards remain the
40 primary concern in both developing and developed countries (WHO 2011).

41
42 Waterborne pathogens include viruses, bacteria and parasites, several of which are
43 highly infectious, robust and long-lived in the environment as well as being resistant
44 to standard methods of water treatment. Viruses are the smallest of these pathogens,
45 typically around 20 – 300nm in diameter, which makes them difficult both to detect
46 and to remove. Additionally, viruses are highly infectious and often long-lived in the
47 aqueous environment, with norovirus for example being shown to remain infectious
48 after over 2 months in groundwater (Seitz et al. 2011). Furthermore, many viruses are
49 resistant to disinfection, particularly norovirus, which has demonstrated resistance to
50 chlorination, and adenovirus, which has remained viable even after UV treatment
51 (WHO 2011). Bacteria, with sizes on the order of a few micrometers, are often less
52 infectious, with some notable exceptions (e.g. *E. coli O157:H7* and *Shigella*). Bacteria
53 are more susceptible to chlorine disinfection. Parasites are the largest of the
54 waterborne pathogens, of around 5µm and larger, and comprise protozoa and
55 helminths. Helminth infections have decreased significantly over recent years as the
56 causative agents (e.g. the host within the water environment) are easily removed by
57 filtration. Protozoa however, remain a problem due to a low infectious dose, longevity
58 in the environment and resistance to water treatment methods (Baldersson and
59 Karanis 2011, Chen et al. 2007).

60
61 In recent years there have been numerous research advances in methods for
62 monitoring waterborne pathogens (Bridle 2013). Monitoring plays several key roles in
63 the design and implementation of water safety plans and can be applied for
64 surveillance, operational or investigative means (WHO 2011). This review focuses on
65 the role that miniaturisation, in particular using microfluidic systems, can play in the
66 delivery of “lab-on-a-chip” devices to perform monitoring procedures.

67

68 Microfluidic systems, *i.e.* fluid handling systems with channel dimensions on the
69 micrometer scale, have developed rapidly during the past decade and have found
70 many applications, especially within chemical analysis and biological assays. This is
71 unsurprising considering their numerous advantages which include reduced sample
72 consumption, increased speed of analysis, improved efficiency and process
73 parallelisation as well as access to phenomena and mechanisms that are not accessible
74 on the macroscopic scale (Beebe et al. 2002). For example, there have been
75 developments in using microfluidics to obtain better environmental control over cells
76 or bacteria during culture, even now to the level of creating organs on-chip (Huh et al.
77 2011), which could lead to improvement in traditional culture based pathogen
78 monitoring approaches. Microfluidics might also allow the design of environments to
79 promote culture of microorganisms, which have not yet been cultured in the lab. Yoon
80 and Kim, however, are not positive about this approach for foodborne (or waterborne)
81 pathogens with the justification that lab-on-a-chip has focussed on rapid methods of
82 detection while culturing is time-consuming (Yoon and Kim 2012). Their review from
83 2012 is an excellent overview of microfluidic detection methods and the latest
84 application to foodborne pathogens (Yoon and Kim 2012), many of which are
85 identical to waterborne pathogens. Another review of microfluidics for pathogens in
86 general is that by Mairhofer in 2009 (Mairhofer et al. 2009). The Nature review by
87 Yager discusses application of microfluidics for developing world settings (Yager et
88 al. 2006).

89
90 This review differs from the above by focussing specifically on waterborne
91 pathogens. To the best of the authors' knowledge, no previous review exists, which
92 concentrates specifically upon microfluidic approaches to waterborne pathogen
93 monitoring systems, despite many developments in this field in recent years.
94 Particular areas of focus in this article have been on sample processing applications, a
95 key part of any waterborne pathogen monitoring strategy, as well as on significant
96 developments in optical detection technologies in the last few years. This paper is
97 organised into two main sections. The first will discuss how microfluidics has been
98 applied to the challenge of sample processing within waterborne pathogen monitoring.
99 The second will provide an overview of research advances in the use of microfluidics
100 for waterborne pathogen detection. Finally, the paper concludes by summarising the
101 state-of-the-art approaches, looking forward to analysing the potential of

102 microfluidics within this application area and making recommendations for future
103 research.

104

105 **2. Existing Methods of Monitoring**

106

107 Existing methods of monitoring for waterborne pathogens mainly rely upon the
108 detection of faecal indicators (Gleeson and Gray 1996). This strategy assumes that
109 faecal indicators have contaminated water and will therefore also indicate the
110 presence of any pathogens. The advantage of this approach is that the tests are cheap
111 and easy to perform (2006). However, studies have indicated poor correlation between
112 the detection of indicators and presence of microbial pathogens (Savichtcheva and
113 Okabe 2006, Hunter 1997) and there are also concerns that this approach does not
114 allow a valid identification of the pathogen (Brettar and Höfle 2008).

115

116 The alternative to indicator monitoring is the implementation of tests to directly
117 identify particular pathogens. This is extremely challenging due to the low
118 concentrations of pathogens in large water volumes. Furthermore, it is often necessary
119 to determine the species, viability and infectivity of a microorganism to determine its
120 pathogenic potential. Indeed, for some pathogens, identification beyond the species
121 level is required, as in the case of the *Vibrio* species (Robinson et al. 2010).

122

123 Various methods of direct detection exist, including cell culture based methods,
124 immunological methods, microscopy and nucleic acid amplification approaches. Each
125 has advantages and disadvantages and the most suitable choice of monitoring
126 approach is likely to depend upon why monitoring is being undertaken (Bridle 2013).

127

128

129 **3. Sample Processing**

130

131 In some areas, e.g. medical applications such as detection of biomarkers (Mohammed
132 and Desmulliez 2011) or analysis of rare cells (Thomson et al. 2011), the capability of
133 microfluidics to enable analysis of small sample volumes is an attractive feature.

134 However, for environmental applications, such as waterborne pathogen monitoring,
135 the need to process large sample volumes and complex matrices represents a major

136 challenge for such a technology. System parallelisation is one option to deliver
137 increased device throughput. Avoiding device clogging with complex samples is
138 another potential problem although lab-on-a-chip systems have been proven to handle
139 complex samples, like blood. Many different techniques have been utilised for cell
140 sorting within microfluidics and this review will present their applications to
141 waterborne pathogens, along with other approaches, which have been trialled for
142 waterborne pathogen sample processing on-chip.

143

144 Cell sorting in microfluidics is a well-studied and well-reviewed area (Chen et al.
145 2008, Kersaudy-Kerhoas et al. 2008). Methods can be sorted into those, which either
146 exploit intrinsic properties of the cell or resort to extrinsic 'labels' for cellular
147 differentiation. Additionally, separation can be performed by a variety of means
148 including optical, electric, magnetic or hydrodynamic forces. Examples of such
149 techniques are shown in Figure 1.

150

151 Only a small section of the vast literature of microfluidics methods used for cell
152 sorting has concentrated upon sample processing of waterborne pathogens, although
153 research studies in this topic have significantly increased in numbers recently (Table
154 1). Despite this recent focus, the water sample volumes studied remain small (μL - mL)
155 in relation to the volumes required for environmental applications (L - 1000L).
156 Furthermore, the performance of many of the developed devices has only been
157 demonstrated using purified water, a significantly simpler system than real water
158 samples. Centrifugation, which is often employed in traditional methods of
159 waterborne pathogen sample processing (Polaczyk 2008, Lindquist et al. 2007, Stine
160 2005, EPA 2005), has also been demonstrated on-chip (Madou 2006), though this has
161 not yet been applied to waterborne pathogens. Several on-chip approaches combine
162 some form of microfluidic enrichment with detection. One example of an existing
163 technique used for sample processing and detection that has been miniaturised into an
164 on-chip format, is flow cytometry. This technique is covered later under the optical
165 detection section.

166

167 **3.1 On-chip Immunological Concentration Methods**

168

169 Immunological methods of separation, the most common being immunomagnetic
170 separation (IMS) (EPA 2005, Hamza et al. 2012), are routinely applied in traditional
171 methods of waterborne pathogen detection. IMS works by mixing and incubating the
172 immunomagnetic beads and sample, allowing time for binding of the pathogen of
173 interest to the beads. Subsequently, the beads are pulled to one side of the container
174 using a magnet, isolating this pathogen from the rest of the sample, which is removed
175 and discarded. The bead-microorganism complex can be used for direct plating on
176 selective media, for enrichment of a bacterium or for nucleic acid isolation. However,
177 many protocols include dissolution of the bead-pathogen complex, often
178 accomplished by adding acid. The dissociated beads are then removed from the
179 pathogen sample by using the magnet to once again concentrate them at the side of the
180 container, and removing the solution which now contains the isolated pathogens. Both
181 the use of antibodies for selective capture and the use of immunomagnetic beads have
182 been applied within microfluidic systems.

183

184 The first demonstration of this approach was by Taguchi and co-workers who
185 investigated the microfluidic trapping of protozoa, for subsequent fluorescence
186 analysis (Taguchi et al. 2005). They developed a micro-well array strategy, consisting
187 of 32x32 microfabricated wells with a 10 or 30 μ m diameter and a 10 μ m depth, for
188 oocyst capture. After microfabrication, the micro-wells were selectively coated with
189 streptavidin and anti-*C. parvum* antibodies. For capture experiments, 10 mL of a
190 sample mixture of *C. parvum* oocysts (10^7 oocysts/mL) suspended in PBS was simply
191 deposited onto the array and the whole chip rotated horizontally for 1 h. While the
192 authors claimed this microarray capture process facilitated faster microscopic analysis
193 no data was provided to evidence this, and neither were recovery rates reported.

194

195 An alternative strategy employed IMS, using high aspect ratio curved microchannels,
196 and was demonstrated in 2010 by Dharmasiri et al (Dharmasiri et al. 2010). In this
197 case, *E. coli* was isolated from pre-filtered lake water with a 71% recovery rate. A 100
198 concentration factor was achieved and the device was capable of processing 1mL at a
199 flow rate of around 5 μ L/min. Another study published in 2010 by Guan et al used
200 antibody-coated microbeads inside the microchannels for the immunoseparation of *E.*
201 *coli* (Guan et al. 2012). The use of beads was employed to overcome the potential
202 limitations of planar microchannels, by increasing the surface area available for

203 antibody-pathogen binding. This was illustrated by the high recovery rate obtained, of
204 around 92 to 96%.

205

206 Automation and miniaturisation of IMS for *Cryptosporidium* and *Giardia* was
207 developed by Ramadan and colleagues in 2010 (Ramadan et al. 2010). Incubation of
208 the protozoan pathogens with the IMS beads occurs as in standard protocols and then
209 the bead-pathogen complexes are introduced into the flow-through system. The
210 system employs an external permanent magnet, the rotation of which repeatedly
211 captures and releases the magnetic particulate matter as it passes through the channel.
212 Performing multiple capture-release stages away from a wall avoids the problems of
213 aggregation, in which impurities might be trapped, as sometimes observed in IMS. At
214 the end of the flow channel the concentrated sample is captured by another magnet
215 (Figure 2). This system was reported to concentrate samples of 50mL down to 1mL,
216 with efficiencies comparable to the existing methods, for both tap and secondary
217 effluent water. Two different systems were trialled, fabricated from different
218 materials. When comparing channels made of disposable plastic with those made
219 from glass, the latter were found to perform better in tap water. One potential
220 explanation for this finding is that the rougher walls in the plastic cartridge offers sites
221 where pathogens could become trapped, thus reducing the recovery rate. The authors
222 state their work is a step towards creating automated sample processing for protozoa,
223 reducing the number of steps and human intervention required.

224

225 One final example of on-chip IMS is the system developed by Agrawal et al in 2012.
226 The 3D circular microfluidic system was fabricated in PDMS with imbedded
227 permanent magnets. The system was designed for multiplex pathogen detection, with
228 the first stage being capture of the bacteria with immunomagnetic nanoparticles
229 (Agrawal et al. 2012). The second stage involved pathogen detection with fluorescent
230 quantum dots. The recovery rate of the concentration stage was not reported.

231

232

233 **3.2 Electrical Based Concentration in Microfluidics**

234

235 The use of dielectrophoresis (DEP) to concentrate pathogen samples on a microfluidic
236 chip was first performed by Goater et al. in 1997, as part of an electrorotation

237 detection strategy for *Cryptosporidium* oocysts (Goater et al. 1997). Subsequently, in
238 2005, Gomez–Sjoberg reported DEP based concentration of *E. coli* in a 400pL
239 chamber, achieving concentration factors of 10^4 - 10^5 (Gomez-Sjoberg et al. 2005). In
240 2011, Chow and co-workers reported a DEP system for trapping and concentrating *E.*
241 *coli* (Chow and Du 2011). The system operated at a flow rate of 1 μ L/min and claimed
242 a 100% trapping efficiency if an appropriate DEP force was chosen. Additionally,
243 different voltages could be applied to distinguish between different species, and viable
244 and non-viable microorganisms. This example illustrates one of the key advantages of
245 electrical separation methods, e.g. the ability to distinguish between pathogens
246 according to their species and viability. For a general review of DEP in microfluidics
247 see the review by Cetin and Li (Cetin and Li 2011), and Jesus-Perez and Lapizco-
248 Encinas (Jesus-Perez and Lapizco-Encinas 2011) for the application of DEP to
249 environmental microorganisms.

250

251 An alternative approach to DEP was developed by Cabrera and Yager who utilised an
252 electrokinetic separation approach, at a flow rate of 83nL/s, to isolate bacteria
253 (Cabrera and Yager 2001). This technique could potentially be adapted to waterborne
254 pathogens. The advantages include continuous flow separations with a low power
255 requirement.

256

257 Lapizco-Encinas et al developed in 2005 an insulator-based DEP (iDEP) for
258 concentration of *E. coli* (Lapizco-Encinas et al. 2005). iDEP employs insulating
259 structures to generate non-uniformity in an electric field generated by remote
260 electrodes. Lapizco-Encinas and colleagues reported concentration factors on the
261 order of 3000, with recovery efficiencies nearing 100%. The technique was also
262 applied to viruses. In 2009, Cho et al applied iDEP in a plastic chip for the capture of
263 *E.coli* (Cho et al. 2009). The authors reported that a maximal capture efficiency of
264 66% was achieved at a flow rate 100 μ L/min. This seems relatively low, especially in
265 comparison to traditional IMS approaches, although it is comparable to recovery rates
266 obtained after membrane filtration and elution.

267

268 In 2010, a US patent was granted to Simmons et al. for the use of an iiDEP
269 microfluidic chip to capture *Cryptosporidium* (Simmons et al. 2010). The patent
270 claims that the device could process 1-10mL of water concentrating the sample to

271 25 μ L for further study such as immunofluorescence. Potential clogging problems
272 were addressed by the utilisation of an ultrafiltration membrane prior to sample entry
273 into the dielectrophoretic segment. A 2011 thesis reported the design of 3D iDEP
274 systems for trapping of bacteria, reporting stronger trapping forces at lower
275 temperatures, reducing the risk of thermal damage to the trapped cells, thus
276 potentially enabling further downstream analysis (Braff 2011). This work represented
277 the first observation of intra-species differences in membrane surface properties using
278 iDEP. One potential problem with DEP or iDEP as a concentration and isolation
279 technique is their operation in batch mode, which could complicate their integration
280 into continuous flow systems.

281

282 Two studies have utilised electrical concentration methods for viruses.

283 Balasubramanian presented a microfluidic system based on electrophoretic transport
284 and electrostatic trapping to study MS-2 virus and Echovirus 11 found in potable
285 water (Balasubramanian et al. 2007). MS-2 virus was captured with 88–99%
286 efficiency and Echovirus showed capture efficiency above 70%. Grom reported the
287 combination of electrohydrodynamic flow and dielectrophoretic forces to trap
288 Hepatitis A virus (Grom et al. 2006). They showed that the microsystem can be useful
289 to accumulate viral particles from relatively large sample volumes.

290

291 **3.3 On-chip Filtration**

292

293 Work carried out by Liu and colleagues employed sieves or filters as a means of
294 trapping *Cryptosporidium* oocysts (Zhu et al. 2004, Lay et al. 2008). In one example,
295 a weir was created by interfacing a deep channel (50 μ m) with a very shallow channel
296 (1 or 2 μ m) (Zhu et al. 2004). Using positive pressure, a mixture of protozoa in water
297 was injected into the channel, trapping the cells against the wall of the deep channel.
298 The common disadvantage of sieves or filters systems is their rapid clogging, perhaps
299 due to the weir system. However, in further work employing a so-called rain drop
300 bypass filter, Lay et al. significantly reduced this issue (Lay et al. 2008). The design
301 consists of 3 prefilters and a wide composite filter structure, which allows alternative
302 fluidic paths and therefore significantly reduces the pressure and the clogging on the
303 filter. The filters are made of fine arrays of rain drop like shaped pillars, arranged in
304 gaps ranging from 0.2 to 1mm, in the trapping zones and coarse arrays in the bypass

305 zones. The device was used for bacterial (and protozoal) capture and detection with a
306 limit of detection (LOD) of 10^5 colony forming units per millilitre (cfu/mL).
307 Application of such systems to viral capture would be challenging due to the small
308 size of structures that would be required.

309

310 Taguchi and co-workers refined their micro-well array method to increase the capture
311 efficiency. The laser-machined stainless steel micromesh consists of a 10x10 array of
312 2.7mm diameter cavities to capture single oocysts incorporated into a PDMS
313 microfluidic device (Taguchi et al. 2007). The maximum flow rate tested was
314 $350\mu\text{L}/\text{min}$, such that 5mL could be processed in under 15 min. When loading a
315 0.5mL test sample (spiked oocysts in PBS) at a concentration of 36 oocysts/mL a
316 recovery rate of 93% from the mesh was reported, comparable to that achieved by
317 IMS. Batch processing of the sample occurs in the current design; thus while
318 integration into automated systems would be possible, real-time continuous
319 monitoring is not.

320

321

322 **4. Use of Microfluidics in Waterborne Pathogen Detection**

323

324 The use of microfluidics for the detection of waterborne pathogens allows a variety of
325 detection technologies to be implemented. These include optical, electrical and
326 molecular means as well as biosensors. This section reviews the state of the art of
327 these techniques with emphasis on waterborne pathogens, and the data is summarised
328 in Table 2.

329

330 **4.1 Optical Methods**

331

332 Some optical detection techniques for waterborne pathogens have already benefited
333 from the advantages of lab-on-a-chip systems. These include optical microscopy of
334 stained oocysts and fluorescence techniques. Other potential systems, such as SERS
335 on chip reviewed by Chen and Choo in 2008 (Chen and Choo 2008), have not yet
336 been applied to waterborne pathogens detection.

337

338 In some of the examples of (oo)cyst and bacteria capture given in the previous
339 section, the sample processing module of the device was also utilised for detection,
340 through the addition of fluorescent stains. The systems by Taguchi and Liu (described
341 in section 3.3) represent a microfluidic alternative to the existing IMS and
342 fluorescence detection protocols for (oo)cysts. The method of Taguchi et al has the
343 advantage to include the pre-defined location of the binding of the oocysts and their
344 good adhesion to the substrate during the washing and staining steps (Taguchi et al.
345 2005). Their second design also allowed the detection to be done in 60 mins compared
346 to 2 to 3 hours for the IMS method (including staining) with automated FITC
347 labelling. Imaging was used for detection. A detection limit of 36 oocysts/mL was
348 reported, which is above the desired ability to detect single oocysts (Taguchi et al.
349 2007). Zhu et al confirmed that the use of microfluidics enhanced the signal detection,
350 reducing both the time and also claiming that reagent use could be 10-100x more
351 dilute for signals comparable to the traditional glass slide approach for (oo)cyst
352 detection (Zhu et al. 2004). However, fluorescence based detection of bacteria in
353 similar systems appears to be more challenging with Lay et al finding a limit of
354 detection of 1×10^5 cfu/mL (Lay et al. 2008).

355
356 Sandwich immunoassays on-chip have been applied to *E. coli* detection, in either a
357 0.4mL reaction chamber on-chip (Stokes et al. 2001) or in the case of Li and Su
358 processing a 1mL sample in 2 hours. It was claimed that a microfluidic approach
359 allowed for an improvement of detection sensitivity (to an LOD of 10-100cfu/mL)
360 due to reduced reagent consumption and increased immunoassay kinetics (Li and Su
361 2006).

362
363 A microfluidic flow cytometer with cheap, compact and low-power PIN photodiodes
364 was developed in 2004 (Tung et al. 2004). This device, made from PDMS,
365 hydrodynamically focuses the sample where it is excited and detected using optical
366 fibres and also uses lock-in amplification. While this system has not been applied to
367 waterborne pathogen detection, it did demonstrate the first single-cell fluorescence
368 detection using this type of photodiode. Device performance was characterised with
369 beads and yeast cells, with a sample volume of 5mL/hr. In 2005, Sakamoto et al
370 described a microfluidic flow cytometry set-up for *E. coli* detection capable of
371 analysing six 10 μ L samples in just 30 mins (Sakamoto et al. 2005). The system was

372 reported to deliver good agreement with traditional counts in shorter times, and was
373 tested with river water.

374

375 Flow cytometry on-chip for *E. coli* was also reported by Yamaguchi in 2011
376 achieving a good comparison to traditional counting methods, even in pond water
377 (Yamaguchi et al. 2011). The device integrated on-chip mixing of sample and
378 fluorescent stain to avoid pre-treatment steps off-chip and utilised automated image
379 analysis, delivering results in 1 hour. The authors note that using LED light sources
380 could allow for development of a portable system and that integration with sample
381 concentration microfluidics would be an option to reduce the LOD from the reported
382 10^4 cells/mL.

383

384 High throughput flow cytometry on-chip was demonstrated by Oakey et al
385 demonstrated (Oakey et al. 2010) delivering a 1ml/min volumetric throughput.
386 Though the study did not directly target pathogens it was able to discern individual
387 particles of around 10 μ m diameter, roughly similar in size to *Giardia lamblia*. The
388 system takes advantage of a hydrodynamic inertial focussing mechanism and could
389 characterise around 25,000 particles per second in a relatively low concentration of
390 sample (0.1% w/v). It was not tested with complex media but indicates the potential
391 of a miniaturised detection system, which could perhaps be coupled to a sample
392 processing mechanism to create a fully automated label-free monitoring solution.

393

394 Further optical methods include UV/visible spectroscopy and chemiluminescence In
395 2006, Li and Su produced a microfluidic chip to capture *E. coli* with antibodies for
396 detection by UV/visible spectroscopy (Li and Su 2006). A microfluidic approach
397 using IMS and chemiluminescence detected *E. coli* O157:H7 down to 34 cells in 90
398 mins, with a sample of 100 μ L (Varshney et al. 2006). *E. coli* O157:H7 activity has
399 also been monitored in real time, following immunoseparation by microbeads in
400 microchips, with bioluminescence technology (Guan et al. 2012). In 2008, Karsunke
401 and colleagues reported the detection of *Salmonella*, *E. coli* and *Legionella* using
402 chemiluminescence on-chip, with detection limits of 3×10^6 , 1×10^5 and 3×10^3
403 cells/mL, respectively, for an assay which took just 13 mins (Wolter et al. 2008). A
404 system redesign, incorporating six parallel flow through channels enabled easier

405 calibration and measurement on-chip improving the detection limits for *Salmonella*
406 and *E. coli* though not for *Legionella* (Karsunke et al. 2009). These later experiments
407 were also performed in water, making the results more relevant to water monitoring
408 than the previous PBS experiments.

409

410 In 2007, Ymeti et al developed a sensor utilising a Young's interferometer that utilises
411 an off- chip light source and CCD to detect the presence of Herpes Simplex
412 Virus type 1 (HSV-1)(Ymeti et al. 2006). While HSV-1 is not primarily transmitted
413 through water, cutaneous manifestations of infection occur due to its presence in
414 water (Elko et al. 2003). Furthermore, this type of detection mechanism can easily be
415 adapted for the detection of other viruses and other microbial agents. Measurements
416 were taken after flowing buffer through the chip for 30 mins to establish a baseline
417 signal and virus detection occurred over approximately 2.5 hours. The flow rates and
418 sample volume are not reported though the author stipulates that it is suitable for
419 volume of sample measured in micro-litres. Most interesting is the assertion that the
420 LOD is sensitive enough to detect a single binding instance, though only detection of
421 ~700 virus particles is firmly established; this still represents a highly sensitive and
422 easily miniaturised platform. It is important to note however that generally, the higher
423 sensitivity of detection, the more specificity to the target analyte or purity of the
424 sample is necessary to reduce interference from non-target particles in the sample
425 matrix.

426

427 Single-cell level detection was reported using a latex immunoagglutination for real-
428 time detection of *E. coli* on a microfluidic chip, reading out the light scattering results
429 using a proximity (i.e. one not integrated on-chip) optical fibre (Han et al. 2008).

430 Single cell detection per microdevice was reported which increased to 4 cfu upon the
431 addition of an extra washing step to determine only viable bacteria. While the volume
432 processed by each device was 100 μ L, later work by the authors connected the
433 microfluidic system to a larger piped network, sampling a fraction of the water supply
434 and achieving a 10 cfu/mL LOD in less than 5 minutes (Kwon et al. 2010). The same
435 group also applied this technology for the detection of *Cryptosporidium*, via isolation
436 of the *Cryptosporidium* oocyst wall protein (COWP), reporting single oocyst
437 sensitivity, analysing a small volume of sample subsequent to the traditional
438 filtration/concentration processes (Angus et al. 2012). For faster detection, on the

439 order of 10 mins, filtration and concentration could be skipped though the detection
440 limit in this case was raised to 1-10 oocysts/mL.

441

442 In 2011 *E. coli* from lettuce wash was detected using a particle immunoagglutination
443 assay to an LOD of 10 cfu/mL in just 6 mins (You et al. 2011). The drawback of this
444 system was that it only tested 60µL of sample. However, the microfluidic chip was
445 integrated into a handheld portable unit. The same year another approach for *E. coli*
446 detection involved incorporation of single microorganisms into droplets, offering the
447 advantages of fast fluorescent tracer dye accumulation within droplets as well as
448 digital counting (Marcoux et al. 2011). Detection was based on metabolism, showing
449 good agreement with traditional colony counts, achievable within 2 hours for some
450 bacteria, with full quantification possible in less than 10 hours. Although this method
451 was aimed at foodborne pathogens the detection was performed in water, and thus
452 could be directly applied to waterborne pathogen detection.

453

454 In 2012, Connelly and co-workers developed a microfluidic system for virus
455 detection, combining pre-concentration and signal amplification via liposomes with
456 different detection methods (Connelly et al. 2012). Liposomes and the virus sample
457 are incubated and then pre-concentrated through electrokinesis towards a nanoporous
458 membrane. The sample is then eluted and captured downstream, where the liposomes
459 are lysed releasing fluorescent signal. The authors note that the LOD of 1×10^5 pfu/mL
460 is currently quite high, and suggest several improvements, as well as highlighting the
461 suitability of the device for easy-to-use, rapid and portable systems.

462

463 Also, in 2012, Agrawal and colleagues used secondary fluorescently labelled
464 antibodies for the fluorescent detection of immunomagnetically captured pathogens,
465 within a circular microfluidic system (Agrawal et al. 2012). The authors stated that the
466 use of the magnetic nanoparticles facilitated the capture of the antigen in a confined
467 space thus enhancing the subsequent fluorescence signal. Cd-Te QDs with different
468 emission wavelengths were conjugated to capture *E. coli* and *S. typhimurium*,
469 respectively. Simultaneous detection was possible in the range of 10^3 - 10^7 cfu/mL for a
470 20µL sample.

471

472 **4.2. Electrical Methods**

473

474 Despite the relative ease of electrode integration on-chip, there appears to be fewer
475 studies demonstrating electrical methods of waterborne pathogen detection with
476 microfluidics. Existing examples are given in this section.

477

478 The Connelly system mentioned in section 4.1 could also be operated using an
479 electrical method for virus detection, with electrochemical compounds encapsulated
480 in liposomes, which can be lysed releasing the compounds for detection. Detection of
481 whole viruses using carbon nanotube thin film field effect devices has been reported
482 by Mandal (Mandal et al. 2012).

483

484 Houssin and colleagues reported the detection of *Cryptosporidium* using EIS and
485 could distinguish between viable and non-viable oocysts in 2010 (Houssin et al.
486 2010). The detection took place on a PDMS chip and was due to ion release in hypo-
487 osmotic conditions, thus requiring oocysts to be resuspended in purified water. A chip
488 consisting of an arrangement of four sensors with 4 μ m wide interdigitated electrodes
489 was manufactured by optical lithography and metal deposition on a Pyrex substrate.
490 The limit of detection of the device was measured to be 10⁴ oocysts/mL in the buffer.

491

492 Dielectrophoresis has been applied to the study of both *Cryptosporidium* and *Giardia*
493 (oo)cysts. It has been shown that viable and non-viable oocysts electrorotate at
494 different rates and in opposite directions, depending upon the field strength (Goater et
495 al. 1997, Dalton et al. 2001). Goater et al. designed a system in which travelling wave
496 dielectrophoresis was used to collect oocysts in the centre of a spiral electrode where
497 electrorotation was applied for detection. It was observed that, in the frequency
498 window of 20-600 kHz, viable oocysts rotated faster than non-viable ones, at rates
499 discernible to the human eye or an automated image recognition system. Recently, it
500 has been shown that *Plasmodium falciparum* trophozoites modify the zeta potential of
501 red blood cells, potentially opening the way to for characterization of infectivity by
502 measurement of zeta potential changes (Tokumasu et al. 2012).

503

504 4.3. Biosensors

505

506 A biosensor is a device that convert biological target recognition into a detectable
507 response; this signal transduction can be performed using optical, electrical or mass-
508 sensitive means. This definition specifically refers to systems where the action of
509 biological recognition triggers the signal transduction (and therefore excludes the
510 previous examples given under the optical and electrical sections).

511

512 From a review of the literature on biosensor development for waterborne pathogens it
513 was clear that electrochemical methods of biosensing have been the least applied to
514 waterborne pathogens (Bridle 2013). The same conclusion is true about the
515 integration of this type of sensor into microfluidics for waterborne pathogens.

516 However, impedance based biosensors have been applied to waterborne pathogens
517 within microfluidics. While there are several examples of the use of optical or mass-
518 sensitive transduction for waterborne pathogen detection, this strategy has rarely been
519 integrated on-chip.

520

521 A system, developed by Gomez and colleagues, consists of a flow-through system to
522 measure the impedance of pathogenic bacteria, testing for metabolic activity as an
523 indicator of viability (G. They claimed to process low numbers of cells (1-5000)
524 though the sample volume was also very small, being just 30nL. Impedance biosensor
525 chips were also developed for detection of *E. coli* O157:H7 based on the surface
526 immobilization of affinity-purified antibodies onto indium tin oxide (ITO) electrode
527 chips (Ruan et al. 2002). The biosensor can detect the target bacteria with a detection
528 limit of 6×10^3 cells/mL. In 2008, Yang employed electrochemical impedance
529 spectroscopy (EIS) for the detection of *Salmonella*, which were captured, and
530 concentrated, using IMS (Yang 2008).

531

532 In 2010, Mannoor et al described a microfluidic impedance system for bacterial
533 detection (Mannoor et al. 2010). In the flow-through set-up the LOD was 10^4
534 cells/mL, as opposed to 1 bacterium/ μ L for static operationas shown in Figure 3. In
535 the static set-up the sample was incubated on top of the electrode array for 10-12 mins
536 whereas in continuous flow the sample was passed over the array at a flow rate of 5
537 μ L /min. The authors suggest the difference in LOD between these two approaches, is
538 due to the influence of flow (opposing effects of shear and mixing forces) on the
539 binding kinetics, resulting in reduced binding, as has been reported in other similar

540 flow through assays. Appropriate system design that take into consideration binding
541 kinetics and flow rates is very important (Squires et al. 2008).

542

543 Among optical approaches, surface plasmon resonance (SPR) within microfluidics
544 has been demonstrated, though there is little work with waterborne pathogens. Cell-
545 based SPR is less common than small molecule detection and so SPR lab-on-a-chip
546 devices perhaps hold most promise for detection of the outputs of molecular methods.
547 SPR based biosensors are currently implemented in field-deployable devices sensing
548 of small molecules, proteins, viruses and whole microbes using a 24-channel
549 SPREETA (Sensata) sensor unit (Mairhofer et al. 2009).

550

551

552 **4.4. Molecular Methods**

553

554 The first microfluidic PCR was demonstrated in a 50 μ L silicon chip in 1993
555 (Northrup et al. 1993). Since then numerous designs and components for performing
556 molecular methods on-chip have been developed, which are now starting to be
557 incorporated into integrated systems. In addition, many authors have adopted
558 polymeric materials to reduce the turnaround time in device design refinement and to
559 reduce cost. The main advantage of microfluidic PCR is the rapid temperature
560 cycling, which can be obtained due to the reduced thermal mass; in commercial PCR
561 systems this step accounts for over 90% of the operation time. Single cell detection is
562 possible (Whitea et al. 2010).

563

564 For PCR there have been two main approaches to on-chip systems, although methods
565 such as oscillatory PCR, convective PCR and continuous flow thermal gradient PCR
566 have been developed (Ahmad and Hashsham 2012). The first is static PCR where the
567 sample volume is held in a chamber and the temperature cycling is performed by
568 means of some kind of heating system. The second is flow-through PCR where the
569 device is designed such that sample travelling in a serpentine channel passes through
570 different temperature zones. The advantage of this approach is faster delivery time of
571 the results (90s to 10mins) at the cost of lower detection sensitivity. The high surface
572 area in the serpentine channel, compared to static reactor volume, could be a

573 significant adsorption factor of reagents. There are several commercially available
574 static PCR systems but as yet no flow-through devices.

575

576 . A pocket-sized convective PCR system developed by Agrawal et al in 2007 was the
577 cheapest prototype molecular method lab-on-a-chip, costing just \$10 for the hardware
578 and a few cents per reaction (Agrawal et al. 2007). The continuous flow thermal
579 gradient is the first demonstration of a real-time flow-through system (Pjescic et al.
580 2010). Microfluidic systems for isothermal molecular methods have also been
581 developed such as on-chip NASBA (Dimov et al. 2008, Gulliksen et al. 2004,
582 Gulliksen et al. 2005) and LAMP (Ahmad and Hashsham 2012).

583

584 Although significant progress has been made in the design and operation of the
585 amplification and detection stages of lab-on-chip systems for molecular methods, only
586 a few systems have presented full-integration with sample processing also performed
587 on-chip but this is an area requiring more development. For more details about the
588 general state-of-the-art regarding microfluidic molecular methods we recommend the
589 2009 review article by Zhang and Ozdemir (Zhang and Ozdemir 2009) and the more
590 recent 2012 review article by Ahmad and Hashsham (Ahmad and Hashsham 2012).
591 See also Figure 4 for an overview of stages in molecular detection and microfluidic
592 components capable of performing these tasks.

593

594 While there have been considerable progress developing microfluidic systems that
595 enable on-chip molecular detection, the number of studies applying these types of
596 technologies to waterborne pathogens is very low. In terms of waterborne viruses we
597 found that RT-PCR on a microfluidic device with integrated amplification and
598 fluorescence detection, which could be performed within 1 hour, was demonstrated for
599 rotavirus in 2011 (Li et al. 2011). Work developing a multiplexed PCR system for
600 bacteria was announced in 2006, with promising early results (Weigl et al. 2006). In
601 2010, Ramalingam and colleagues presented the simultaneous detection of four
602 waterborne bacteria in a PDMS PCR array, which utilised capillary flow for sample
603 loading (Ramalingam et al. 2010).

604

605 With regard to protozoa, the two publications that relate to molecular sensing of
606 *Cryptosporidium* in microfluidic systems describe the performance of NASBA off-

607 chip (Nugen et al. 2009) with only the detection of the mRNAs amplicons performed
608 on-chip. Esch et al. developed a fluorescence based detection assay chip, relying on a
609 sandwich hybridization of the NASBA product between capture probes and reporter
610 probes (Esch et al. 2001). The microfluidic device consists of one channel in a PDMS
611 block bonded to a glass slide with a gold pad at its centre to immobilise the capture
612 probe. The reporter probes were tagged with carboxyfluorescein-filled liposomes
613 giving out better fluorescent intensities than usual fluorophores. This technique gave a
614 LOD of 5 femtomoles of amplicon per test (12.5 μ L). The overall time for the full
615 analysis was 1-2 hours, including the heat shock and implementation of the NASBA
616 procedure (Esch et al. 2001).

617

618 **4.5 Commercial Systems**

619

620 *CryptoDetect CARD*TM is a platform, shown in Figure 5, with on-chip integrated
621 sample preparation features, developed by Rheonix and reportedly capable of
622 detecting *Cryptosporidium* in raw water samples (Rheonix 2011). The technology
623 involves integrated IMS and washing of the oocysts, heat shock, lysis, extraction,
624 purification and detection of RNA amplicons, using fluorescent liposomes. However,
625 the technology is at an early stage and no LOD or recovery rate was communicated.

626

627 Early Warning Inc. have combined sample processing and detection into an
628 automated platform for on-line monitoring of pathogens including viruses, bacteria
629 and protozoa (Early Warning Inc 2011). Unlike previous examples, the platform
630 includes a concentrator capable of sampling 10L of water and filtering it to a 10mL
631 sample. The concentrator unit also features hydrodynamic cavitation to disaggregate
632 clumps. The inclusion of this unit also makes the system relatively large (182x139x76
633 cm) and heavy (around 200kg), which would make it difficult for portable usage in
634 the field. After concentration and filtration, analytes of interest in the sample are
635 subsequently separated using IMS, lysed and pathogen RNA is amplified by NASBA.
636 The detection happens when the hybridization of target RNA amplicons on specific
637 biomolecular probes generates a current via a guanine oxidation process. The
638 company website reports the limit of detection of this device after concentration,
639 filtration and detection on the biosensor chip as 1 cell per 100mL equivalent,
640 sampling a total volume of 10L. Additionally the total load quantification and

641 viability testing of up to 25 species can be performed on a single chip with a total
642 processing time of less than 3 hours from sampling to results.

643

644 **5. Conclusions**

645

646 This paper has reviewed the applications of microfluidics for waterborne pathogen
647 monitoring. Detection to the single-cell level has been demonstrated on-chip, though
648 the range of pathogens studied needs to be expanded. Work with *E. coli* is most
649 common and *Cryptosporidium* is also relatively well studied. Research activities
650 involving other pathogens are significantly more limited. For almost all types of
651 detection approaches demonstrated for waterborne pathogens, there are microfluidic
652 systems for performing these tests. Amongst the optical, electrical and biosensor
653 systems presented, the optical detection option seems to have received the most
654 attention from the lab-on-a-chip community.

655

656 The advantages of performing fluorescent detection on-chip are: (1) the reduced
657 sample volume resulting in a lower background noise signal and therefore improved
658 sensitivity and signal to noise ratio; (2) the small sample volume needed and control
659 of flow enhancing binding kinetics and increasing sensitivity; and (3) the reduced
660 consumption of reagents (Rivet et al. 2011). However, there are challenges, especially
661 with the relatively short shelf life of reagents for field devices, and particularly in the
662 production of low-cost, sensitive optics. There has been some progress towards this
663 latter goal with the development of on-chip microscopes and photodiode detectors.
664 Despite this, some authors believe label-free technologies seem more promising for
665 ease of integration with microfluidics (Rivet et al. 2011). However, field devices at
666 fixed locations will require regular operations, such as filter changes, sterile packing
667 and samples transport, to maintain their functionality and integrity as monitoring
668 systems and hence the reagent shelf-life might not be such a problem.

669

670 Performing molecular methods on a microfluidic chip exploits many of the
671 advantages of lab-on-a-chip miniaturisation and is likely to become one of the
672 promising directions for waterborne pathogen detection in microfluidic systems.
673 However, lab-on-a-chip systems have not yet been applied too extensively to the
674 challenge of waterborne pathogen detection. The technology is reaching the stage of

675 integration although more work needs to be done on incorporating the sample
676 processing module. This challenge is greater for “real” environmental water samples
677 where both the initial sample volumes and the potential number of
678 interferents/inhibitors are high. In general, the smaller the sample volumes or flows
679 used in detection, the greater the burden is on the sample processing for concentration
680 and enrichment. The different detection methods, described above for whole-cell
681 microorganisms can mostly also be applied to molecular detection and so there are
682 many options to perform the on-chip detection. An EU project, entitled
683 “AQUAVALENS: Protecting the health of Europeans by improving methods for the
684 detection of pathogens in drinking water and water used in food preparation”, which
685 started in the spring of 2013 aims to further the use of molecular methods for
686 monitoring the microbial quality of drinking water with one important element
687 focussing on the delivery of technological platforms, including microfluidics, to
688 perform the testing.

689

690 Microfluidics has even been applied to sample processing, though dealing with real
691 water samples is challenging, due to potential problems with device clogging as well
692 as obtaining sufficient throughput. In order to overcome this latter limitation high
693 levels of parallelisation would be required which may complicate the possibility of
694 any portable sized instruments based around these technologies. However, online
695 monitoring of waste water using a microfluidic system has been demonstrated, albeit
696 for phosphate sensing, which is simpler than microorganism analysis (Cleary et al.
697 2008).

698

699 Foodborne pathogen processing and detection in microfluidics seem to have received
700 more attention than waterborne pathogens, though often the pathogens are the same
701 and identical detection procedures are possible. The main difference between two lies
702 in the sample processing, and it is easier to test wash from food samples as opposed to
703 process large volumes of complex environmental water, which might be the
704 explanation for the focus. Additionally, there may be differences in the food and water
705 testing markets, influencing the drive towards, and perhaps also the funding for, the
706 different target areas.

707

708 In general for wider exploitation of microfluidic devices integration of optical and
709 electrical detection components would be essential to realise a fully automated
710 system. Either these components need to be included on-chip at low-cost or systems
711 need to be designed where low-cost optical and electrical hardware integrates with a
712 microfluidic chip. Additionally, multiplexed detection systems capable of classifying
713 a range of pathogens from the different kingdoms (virus, bacteria, and protozoa),
714 ideally to beyond the species level would be highly desirable and is perhaps the
715 ultimate goal of any detection system for waterborne pathogens. Some microarray
716 work, e.g. that delivered by the EU project Healthy Water, suggests this is a
717 possibility on-chip. Determination of pathogen viability and nocivity still remains an
718 elusive goal, and off-chip optimisation of these approaches will be required before
719 microfluidics systems can be produced.

720

721 Quilliam et al report that increases in the sensitivity and specificity of detection
722 methods for waterborne pathogens are currently being achieved by combining
723 advances in microfluidics technology and analytical chemistry with molecular and
724 immunological methods (Quilliam et al. 2011). Mairhofer, Roppert and Ertl believe
725 the next generation of pathogen sensing developments will be facilitated by advances
726 in lab-on-a-chip devices (Mairhofer et al. 2009). The global market for microfluidic
727 technology is growing at a great pace and was estimated to be worth US\$6.2 billion in
728 2011 (Zhang and Ozdemir 2009). Given their many advantages, and providing some
729 of the above challenges can be met, it can be expected that in the future microfluidics
730 for waterborne pathogens will have an increasing share in that growing market.

731

732

733 **Acknowledgements**

734 HB would like to acknowledge The Royal Academy of Engineering/EPSRC for her
735 research fellowship. BM would like to acknowledge his BBSRC Industrial Case
736 Studentship, supported by Scottish Water. Both HB and MD would like to
737 acknowledge EU funding for the project "AQUAVALENS: Protecting the health of
738 Europeans by improving methods for the detection of pathogens in drinking water and
739 water used in food preparation".

740

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1005 Figure Captions

1006
1007
1008
1009 Figure 1:

1010
1011 Schematic illustrating different methods of cellular separation in microfluidic
1012 systems. Reproduced with permission from Kersaudy-Kerhoas (2008).

1013
1014
1015 Figure 2

1016
1017 Schematic view of the magnet array system. The microfluidic channel sits on top of
1018 the magnet array and as sample passes through the channel several trapping and
1019 releasing events occur before the sample is finally captured at the end of the channel.
1020 In contrast to the existing procedure, where the sample is pulled to the side of a tube
1021 with one magnet, the several steps involved here avoids the problem of aggregation
1022 and impurity capture. Figure 2 from Ramadan. Reproduced with permission.

1023
1024
1025 Figure 3

1026
1027 Impedance measurements with a microfluidic device. Figure 5 from [38]. Real-time
1028 binding of bacteria to AMP sensors.

1029 (A) Digital photograph of the microfluidic flow cell, indicating the small size of this
1030 system, the device set-up and operation.

1031 (B) Optical micrograph of the microfluidic channel with an embedded interdigitated
1032 microelectrode array chip, showing the design of the electrodes.

1033 (C) Optical image of the embedded microelectrode array after exposure to 10^7 cfu/mL
1034 bacterial cells for 30 min. The cells have been captured on the electrodes.

1035 (D) Real-time monitoring of the interaction of the AMP-functionalized sensor (and an
1036 unlabeled control chip) with various concentrations of *E. coli* cells, showing how this
1037 device can successfully discriminate between different concentrations of bacteria.

1038
1039

1040 Figure 4

1041

1042 Schematic of stages in molecular detection and the appropriate microfluidic
1043 technology. Table 1 from Liu (2009)

1044

1045 Figure 5

1046 Top view: schematic of the Rheonix system, which performs on-chip detection of
1047 *Cryptosporidium* from a 5mL starting sample. The sample firstly enters a large
1048 chamber where IMS takes places. Next the sample moves to a chamber for heat-shock
1049 to induce viable oocysts to activate the heat shock protein gene that is subsequently
1050 used for detection. From there the sample passes into a chamber where controlled
1051 interactions with the chemicals required for lysis and purification occurs before the
1052 RNA is isolated. Finally the sample flows along the channel at the bottom left of the
1053 chip before entering the lateral flow assay component where detection takes place.

1054 Bottom view: optical image of the microfluidic indicating the various stages and
1055 showing the size of the device relative to a US dollar coin.

1056

1057

Microorganism		Approach	Example	Volume Processed	Time Required	Water Sample	Concentration Achieved	Recovery Rate	Reference
Viruses	<i>Tobacco Mosaic Virus</i>	Electrical	iDEP	20 μ L	2 min	DI water	Unreported	Unreported	Lapizco-Encinas (2005)
	<i>Hepatitis A</i>	Electrical	DEP	Unreported	9 mins	1:5 PBS/Water	10^7	Accumulated, Unrecovered	Grom (2006)
	<i>MS-2</i>	Electrical	DEP	2 mL	2 mL/hr	Potable bottled water	14.2 x * *maximum reported with salmonella	90-99%	Balasubramanian (2007)
	<i>Echovirus 11</i>	Electrical	DEP	2 mL	2 mL/hr	Potable bottled water	14.2 x * *maximum reported with salmonella	70%	Balasubramanian (2007)
Bacteria	<i>Lysteria</i>	Electrical	DEP	40 μ L	2 μ L /min	HLB, LB and DI Water	10^{4-5}	at least 10%	Gomez-Sjoberg (2005)
	<i>E. coli</i>	Electrical	iDEP	20 μ L	2 min	DI water	3200x	Nearing 100%	Lapizco-Encinas (2005)
	<i>E. coli</i>	Filtration	Raindrop bypass microfilter	100 μ L	10 μ L/min	PBS	Unreported	Unreported	Lay (2008)
	<i>E. coli</i>	Electrical	iDEP	Unknown	100 μ L/min	PBS + Wash buffer	Unreported	66%	Cho (2009)
	<i>E. coli</i>	IMS	High aspect ratio curved channels coated in antibody	1 mL	5 μ L/min	Pre-filtered lake water	10^2	71%	Dharmasiri (2010)
	<i>E. coli</i>	IMS	Antibody coated	1 μ L	5 μ L/min	PBS	Unreported	92-96%	Guan (2010)

			microbeads in channels						
	<i>E. coli</i> and <i>E. faecalis</i>	Electrical	DEP	60 μ L	1 μ L/min	Nanopure water	Unreported	>90%	Chow (2011)
	<i>E. Coli</i> and <i>Salmonella Typhimurium</i>	IMS	3D circular system with immunomagnetic nanoparticles	20 μ L	10 μ L /min	PBS	10 ⁴	Not reported (for sample processing stage)	Agrawal (2012)
Protozoa	<i>Cryptosporidium</i>	Electrical	DEP/travelling wave	300 μ L	Mins	PBS	Unreported	90%	Goater (1997)
	<i>Cryptosporidium</i> and <i>Giardia</i>	Filtration	Weir filters	1-5 μ L	20 μ L/min	PBS	Unreported	Up to 100%, depending on weir size	Zhu (2004)
	<i>Cryptosporidium</i>	IMS	Antibody capture in microfabricated wells	10 mL	1hr	PBS	Unreported	Unreported	Taguchi (2005)
	<i>Cryptosporidium</i>	Filtration	SUS micromesh	500 μ L	60 mins	Tap Water	Unreported	93%	Taguchi (2007)
	<i>Cryptosporidium</i> and <i>Giardia</i>	Filtration	Raindrop bypass microfilter	Unreported	Unreported	PBS	Unreported	Unreported	Lay (2008)
	<i>Cryptosporidium</i> ; <i>Giardia</i>	IMS	Use of an in-channel micromesh for oocyst capture	5 mL	350 μ L/min 5mL in 15mins	Tap, effluent and DI water	5x (extrapolated)	93%	Ramandan (2010)
	<i>Cryptosporidium</i>	Electrical	iDEP	1-10mL	Unreported	Water	To 25 μ L	Unreported	Simmons (2010)

Table 1: Microfluidics Applications in Sample Processing for Waterborne Pathogens

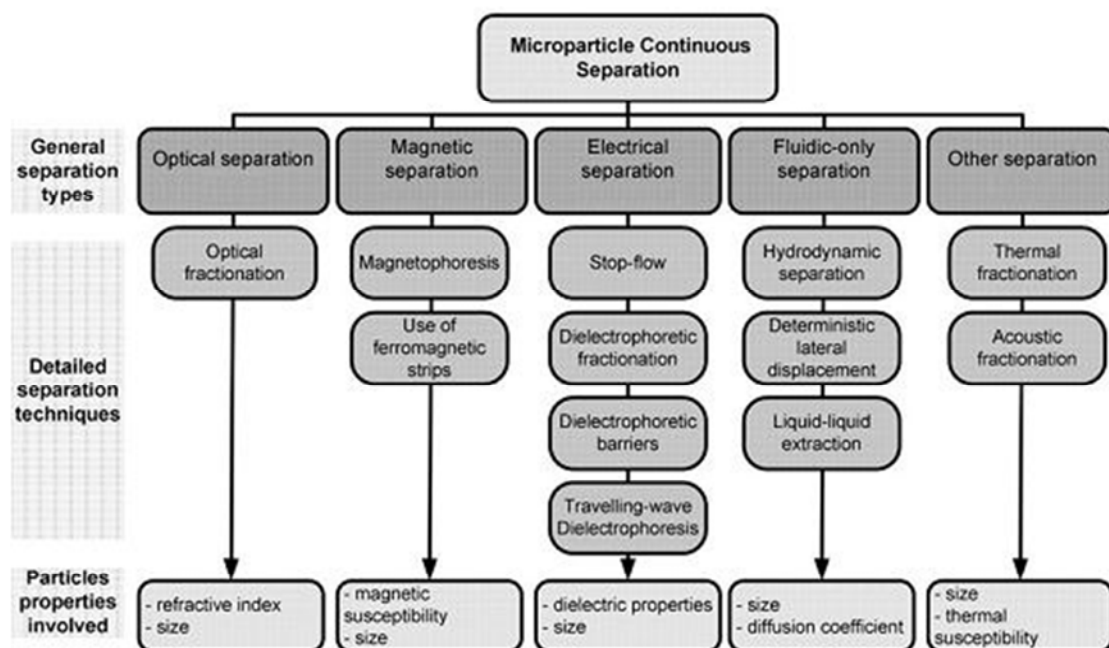
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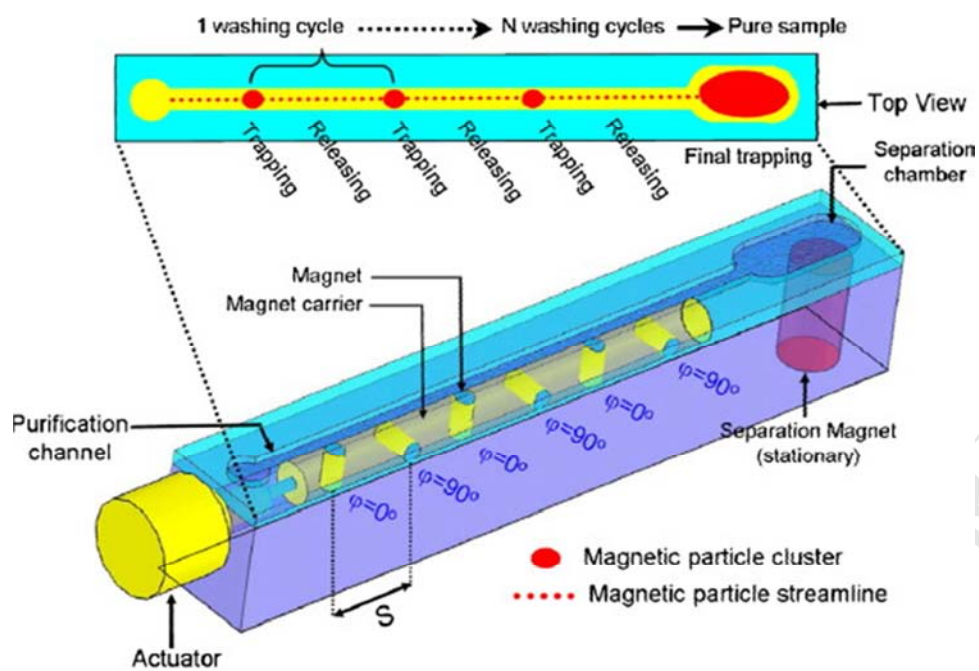
	Microorganism	Approach	Example	Volume Processed	Time Required	Water Sample	Limit of detection	Reference
Viruses	<i>Herpes Simplex Virus- Type 1</i>	Optical	Youngs Interferometer	~ μL	2.5 hrs	PBS	700 pfu	Ymeti (2006)
	<i>Rotavirus</i>	Molecular	RT-PCR (fluorescence detection)		1 hr			Li (2011)
	<i>Feline Calicivirus</i>	Optical or electrical detection	With liposome amplification	60 μL	2.5 hrs	PBS	1×10^5 pfu/mL	Connelly (2012)
	<i>M13 Phage</i>	Electrical	Impedance	~ nL	6 hrs	PBS	550 pfu	Mandal (2012)
Bacteria	<i>E coli</i>	Optical	Sandwich immunoassay	0.4 mL	1 hr	PBS +/- milk +/- BSA	20 cfu	Stokes (2001)
	<i>E coli</i>	Optical	Sandwich immunoassay	1 mL	2 hrs	PBS	10-100 cfu/mL	Li (2005)
	<i>E coli</i>	Optical	Flow cytometry	60 μL	30 mins	River water	Comparable to fluorescence microscopy ~1	Sakamoto (2005)
	<i>E coli</i>	Optical	UV-vis	1 mL	2hrs	PBS	10-100 cfu/mL	Li (2006)
	<i>E coli</i> O157:H7	Optical	Chemiluminescence	100 μL	90 mins	Food sample	34 cells	Varshney (2006)
	<i>E.coli</i>	Optical	Fluorescence	100 μL	20 mins	PBS	1×10^5 cfu/mL	Lay (2008)
	<i>E coli, Salmonella and Legionella</i>	Optical	Chemiluminescence	1.2 mL	13 mins	PBS	1×10^5 , 3×10^6 , 3×10^3 cells/mL	Wolter (2008)

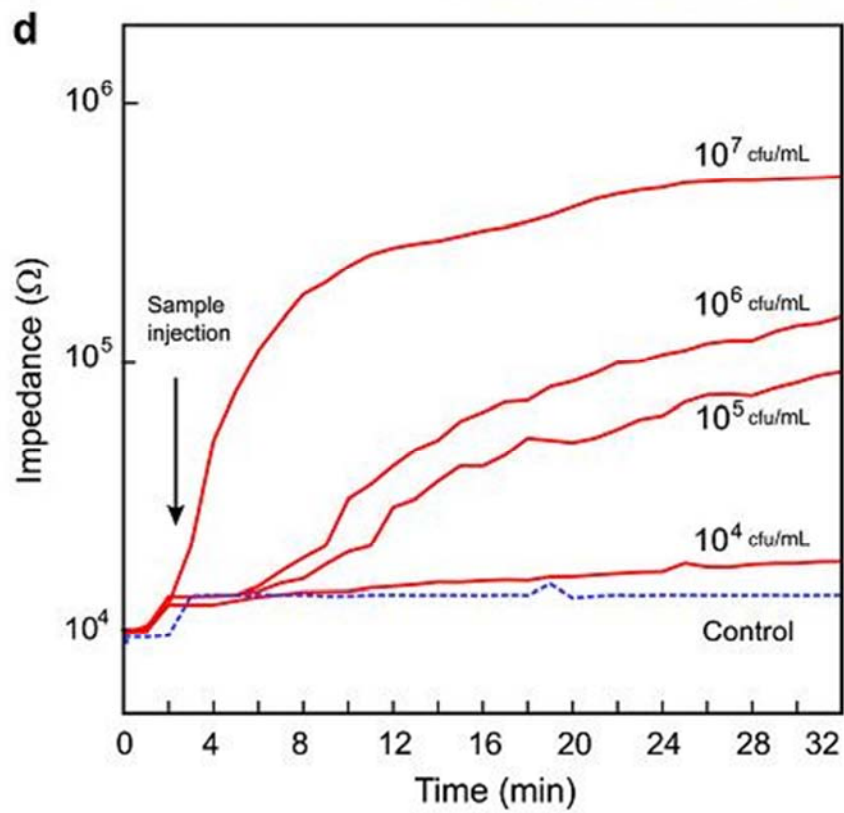
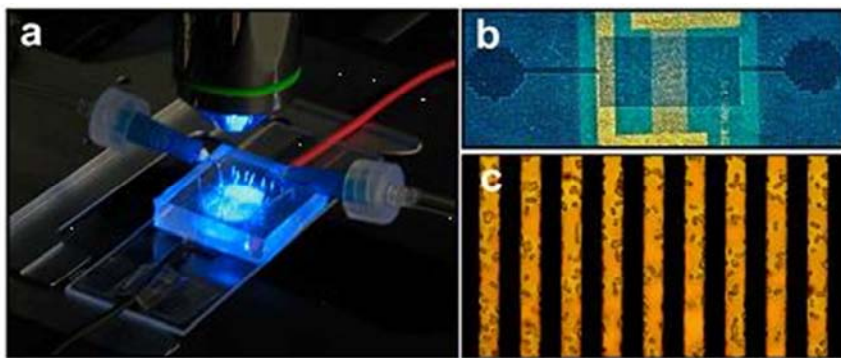
							respectively	
<i>E coli</i>	Optical	Immunoagglutination, light scattering	100 μ L	5 mins	PBS	Single cells or 4 cfu/mL (if viable only)		Han (2008)
<i>E coli, Salmonella and Legionella</i>	Optical	Chemiluminescence	1.2 mL	13 mins	PBS	1×10^5 , 3×10^6 , 3×10^3 cells/mL respectively		Wolter (2008)
<i>E coli, Salmonella and Legionella</i>	Optical	Chemiluminescence	0.64 mL	18 mins	Millipore water	1.8×10^4 , 7.9×10^4 and 2.0×10^7 respectively		Karsunke (2009)
<i>E coli</i>	Optical	Immunoagglutination, light scattering	100 μ L	5 mins	Sampling piped network	10 cfu/mL connected to sample pipe		Kwon (2010)
<i>E coli</i>	Optical	Flow cytometry	35 μ L	~45mins	Pond water	10^4 cells/mL		Yamaguchi (2011)
<i>E coli</i>	Optical	Particle immunoagglutination	60 μ L	6 mins	Lettuce wash	10 cfu/mL		You (2011)
<i>E coli</i>	Optical	Droplet counting, metabolism	<1 μ L	2 hrs	Water (pL droplets in oil)	Single cells		Marcoux (2011)
<i>E coli</i> O157:H7	Optical	Bioluminescence	1 μ L	20 mins	PBS	3.2 cfu/ μ L		Guan (2012)
<i>E coli and S typhimurium</i>	Optical	Fluorescence	50 μ L	Not reported	PBS	8.8 and 9.3 10^5 cfu/mL, respectively, simultaneous detection		Agrawal (2012)

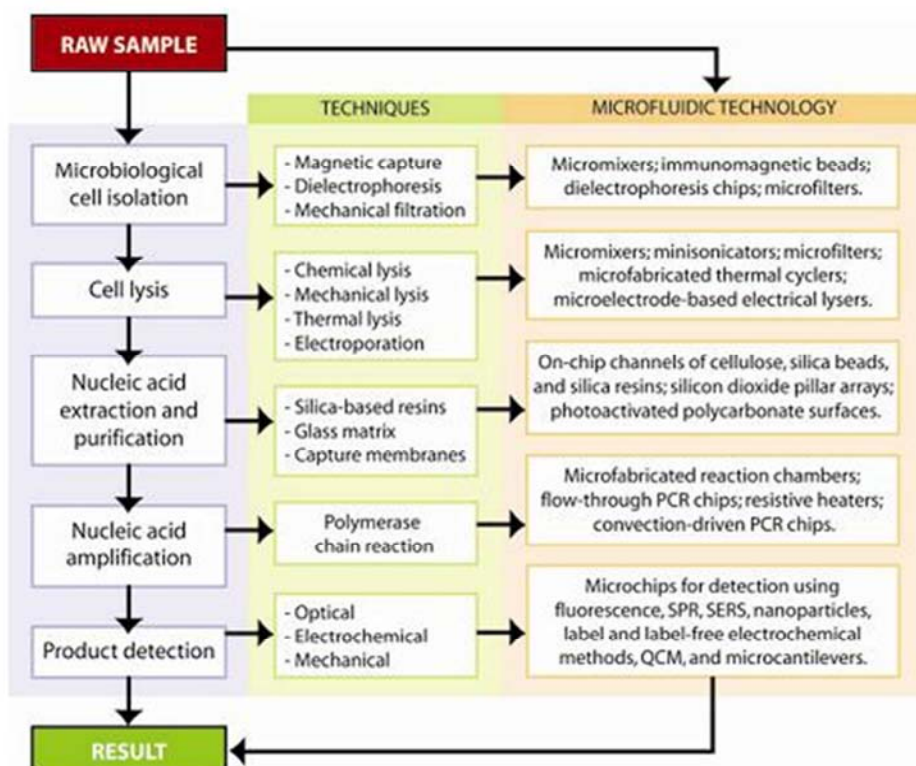
	<i>L. innocua</i>	Electrical	Impedance	30 nL	15 mins	Tris-Gly Buffer	10 cells	Gomez (2001)
	<i>E.coli</i>	Electrical	Impedance	100 μ L	>1hr	Modified PBS	6×10^3 cfu/mL	Ruan (2002)
	<i>Salmonella</i>	Electrical	Impedance	20 μ L	25 mins	DI Water	3.45×10^6 cfu/mL	Yang (2007)
	<i>E coli</i>	Electrical	Impedance	5 μ L/min	32 mins	PBS	10^4 cells/mL	Manoor (2010)
	<i>Shigella dysenteriae serotype 1, Shigella toxin-producing Escherichia coli, E. coli 0157, Campylobacter jejuni, and Salmonella and Shigella species</i>	Molecular	Immunocapture of pathogens plus on-chip nucleic extraction and detection	Unknown	Unknown	Unknown	Unknown	Weigl (2006)
	<i>A. hydrophilia, K. pneumoniae, S. aureus and P. aeruginosa and E. coli</i>	Molecular	On-chip PCR	Not reported	Not reported	Not reported	51 cfu/mL	Ramalingam (2010)
Protozoa	<i>Cryptosporidium</i> and <i>Giardia</i>	Optical	Fluorescence	5 μ L	Max 12 mins	PBS	Not reported; images show single (oo)cyst	Zhu (2004)
	<i>Cryptosporidium</i>	Optical	Fluorescence	10 μ L	Not reported	PBS	Not reported, images show	Taguchi (2005)

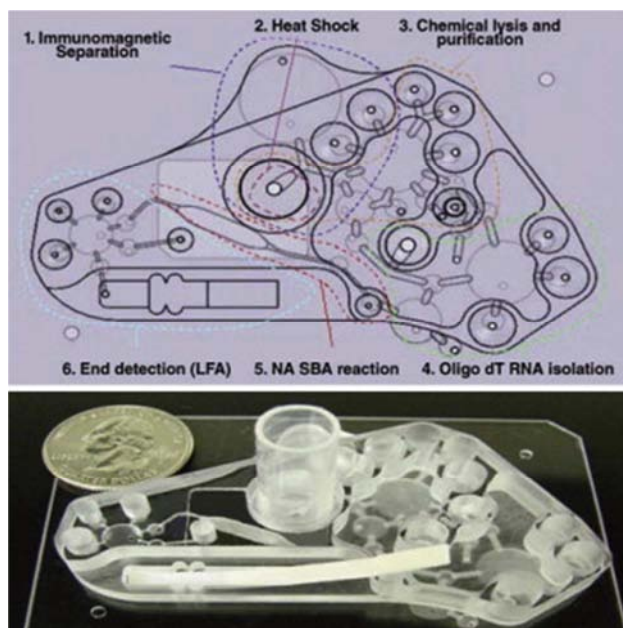
							single (oo)cysts	
<i>Cryptosporidium</i>	Optical	Fluorescence	100-500 μ L	1 hr	Tap water and Milli-Q water	36 oocysts/mL		Taguchi (2007)
<i>Cryptosporidium</i>	Optical	Immunoagglutination	15 μ L of 1000L concentrate	10 mins (not including concentration)	Tap water	Single oocyst with pre-concentration; 1-10 oocysts/mL without		Angus (2012)
<i>Cryptosporidium</i>	Electrical	Impedance	110 μ L	~22 mins	WFI	$<1 \times 10^4$ oocysts/mL		Houssin (2010)
<i>Cryptosporidium</i>	Molecular	NASBA (off-chip) and on-chip fluorescence detection	12.5 μ L of NASBA amplicon	1-2 hrs	Not reported	5 fmol of amplicon/ 0.4 fmol/ μ L		Esch (2001)
<i>Cryptosporidium</i>	Molecular	NASBA (off-chip) and on-chip electrochemical detection	1 μ L of NASBA amplicon	~30 mins (not including lysis time etc)	Nuclease free water	1 oocyst		Nugen (2009)











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