

Lab on a Chip

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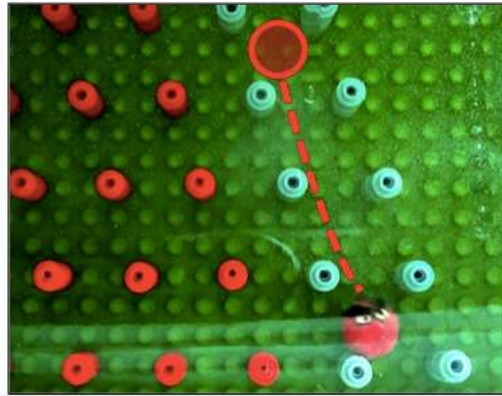
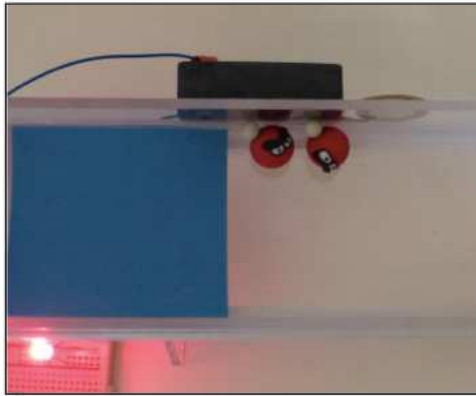
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I am really small...
I am present in water...
I can make you sick...



Would you be able to get rid of
me?



Microfluidic-based hands-on activities for introducing the notion of waterborne pathogen separation to children.

27

28 **Abstract**

29 The purpose of this paper is to present a new approach for introducing to a non-scientific
30 audience a major public health issue: access to safe drinking water. Access to safe drinking
31 water is a privilege in developed countries and an urgent need in the third world, which
32 implies always more efficient and reliable engineering tools to be developed. As a major
33 global challenge it is important to make children aware of this problem for understanding (i)
34 what safe drinking water is, (ii) how ingenious techniques are developed for this purpose and
35 (iii) the role of microfluidics in this area. This paper focuses on different microfluidic-based
36 techniques to separate and detect pathogens in drinking water that have been adapted to be
37 performed by a young audience in a simplified, recreational and interactive way.

38

39 **I. Introduction**

40 Diarrhoea is often considered in developed countries as a classical gastrointestinal symptom,
41 while not enjoyable nor usually too serious. However, this illness results in 1.5 million deaths
42 each year, most of which involving children, and is mainly due to the ingestion of pathogens
43 through water, food or unclean hands. The observation in (Prüss-Üstün *et al.*, 2008) [1]
44 highlights the high privilege in developed countries to have access to specific water
45 treatments, resulting in the delivery of safe drinking water. However, and despite these
46 treatments, several outbreaks are reported every month. The Drinking Water Inspectorate [2]
47 reports around 60 significant events caused by pathogens in water supplies in England and
48 Wales in 2012 whose sources are not always clearly identified. The main difficulties when
49 dealing with pathogens are first to deal with the large variety of existing harmful pathogens
50 (viruses, bacteria and protozoa) and second to detect their presence as they are flowing at
51 extremely small concentrations in large volumes of water. Their separation and detection are

52 thus time-consuming tasks (days are typically needed) that require an experienced staff [3].
53 As a consequence, only three microbiological parameters are set by the European regulation
54 to reflect the water quality: *E. coli*, *Enterococci* and *Pseudomonas aeruginosa*, all set to 0
55 bacteria per 100 mL of sample (per 250 mL for bottle water) [4]. Current limitations are thus
56 the correlation between these parameters and the concentration of all waterborne pathogens
57 and the delay of detection of a pathogen than can be long enough to affect a significant part
58 of the population. One could easily imagine how serious the situation could be in the
59 presence of dangerous pathogens resistant to treatment. *Cryptosporidium* for instance has
60 already been detected in water despite the absence of these indicators [3,5], and is routinely
61 tested for in UK waters. The development of new approaches is thus a growing and necessary
62 research area leading to several new national, European and international projects. For
63 instance, Aquavalens (<http://aquavalens.org/>) is a European project launched in April 2013
64 that “is centred on the concept of developing suitable platforms that harness the advances in
65 new molecular techniques to permit the routine detection of waterborne pathogens and
66 improve the provision of hygienically safe water for drinking and food production that is
67 appropriate for large and small systems throughout Europe”. Some of the techniques adapted
68 in this paper for the comprehension of children are funded by this project, which highlights
69 how the proposed public engagement is close to current laboratory techniques under
70 investigation. Both within this project and other research initiatives, many different detection
71 schemes have been proposed [3] and sample processing research is also developing.
72 Microfluidics has recently been applied to both sample processing and detection within
73 waterborne pathogen monitoring [6;7] with promising results. This paper focuses on how to
74 introduce the existing approach and microfluidic alternatives to children in an interactive and
75 recreational way.

76

77 II. Teaching objectives and workflow

78 There are a lot of different techniques that can be used for pathogen separation and detection.
79 Detection can be based on growing cultures or highly specific biosensors for instance [3].
80 Presenting all the existing techniques would be a tedious task beyond the scope of this
81 activity, and we here focus on emerging microfluidic approaches.

82 Laursen *et al.*[8] evaluated the impact of scientists in a classroom and features that enhance
83 positive student outcome regarding a specific activity. These features include: (i) equipment
84 and materials that enable science learning experiences, (ii) interesting science topics and (iii)
85 style of presentation with hands-on and inquiry approaches. The proposed activity tries thus
86 to encompass these parameters by selecting some specific separation and detection
87 techniques that can be reproduced easily and handled by children in a recreational but
88 educational approach.

89 As presented in Figure 1, this paper focuses on the introduction to waterborne pathogen
90 detection through a set of different modules dedicated to the standardized Immuno-Magnetic-
91 Separation (IMS), two microfluidic based separation techniques (IMS and Deterministic
92 Lateral Displacement) and then to pathogen detection by fluorescent labelling. All or a
93 selection of modules could be delivered according to the age of participants, learning
94 objectives, time available, cost, *etc.* in either schools or as an outreach activity at science
95 festivals. Indicative costs are given for each module independently, however some materials
96 are common to multiple modules, thus reducing total costs. Each module employs familiar
97 and widely available materials. To highlight the feasibility of these modules, each activity
98 has been performed “in-house” without laboratory facilities. Cartoons are also proposed
99 throughout the paper to help understand of the different topics introduced here and to broaden
100 the spectrum of the audience to a non-scientific arena.

101

	MODULE	TEACHING OBJECTIVES	MATERIALS	COST
1	Introduction to safe drinking water	<ul style="list-style-type: none"> • What is a safe drinking water? • What are waterborne (or harmful) pathogens ? 	<ul style="list-style-type: none"> • DinoLite® (microscope camera) and/or • Poster 	~£ 0 (excl. camera)
2	Pathogen separation by Immuno-Magnetic-Separation (IMS)	<ul style="list-style-type: none"> • What is IMS? • How to use this technique to remove pathogens from drinking water? 	<ul style="list-style-type: none"> • Particles in polymer clay • Magnetic antibodies in polymer clay • Strong magnets • Cup 	~ £40
3	Introduction to microfluidics	<ul style="list-style-type: none"> • What is a microfluidics ? • How manufacture microfluidic devices (or micro channels) ? 	<ul style="list-style-type: none"> • Polymer clay • Toothpicks • Plexiglas layer • Needle-tip bottles and squash 	~ £20
4	Pathogen separation by IMS using microfluidics	<ul style="list-style-type: none"> • How to couple microfluidics with IMS? • How to use microfluidics to detect pathogens using IMS? • Advantages of microfluidics? 	<ul style="list-style-type: none"> • Particles in polymer clay • Magnetic antibodies in polymer clay • Strong magnets • Y-channel • Piezoelectric sensor 	~ £80
5	Pathogen separation by Deterministic Lateral Displacement (DLD)	<ul style="list-style-type: none"> • What is the DLD? • How to use microfluidics and DLD to separate pathogens? 	<ul style="list-style-type: none"> • Particles in polymer clay • LEGO® DLD • Vase with shower gel 	~ £60
6	Pathogen detection by fluorescent microscopy	<ul style="list-style-type: none"> • What is fluorescence? • How this technique can be used for detecting pathogens? 	<ul style="list-style-type: none"> • Particles in polymer clay • Fluorescent antibodies in polymer clay • Magnifier in a black box 	~ £30

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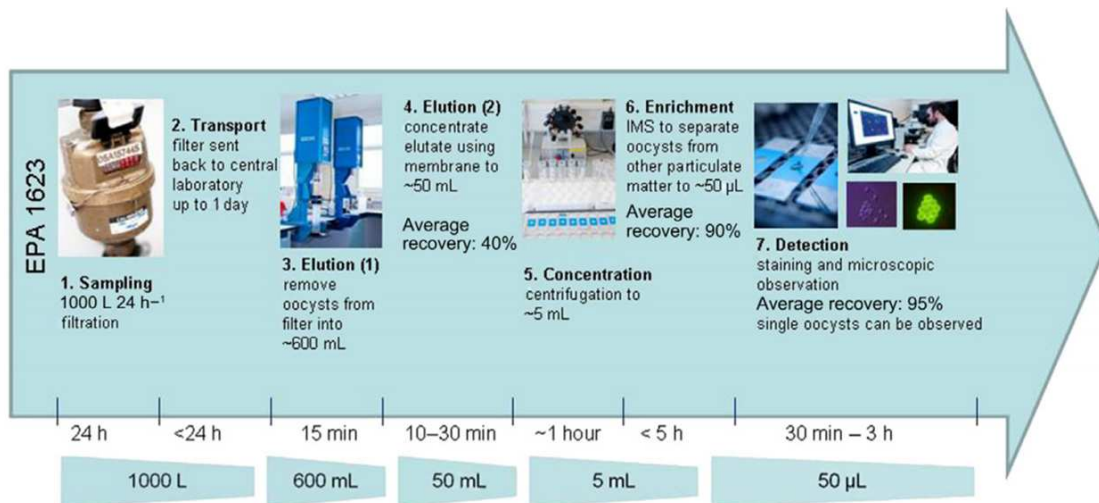
105 On one hand, the immuno-magnetic-separation is a well-known and efficient technique to
 106 separate and concentrate specific biological matters. This technique is part of the standard
 107 protocol (USEPA Method 1623) developed for recovery and detection of protozoa. To the
 108 best of the authors' knowledge, there is no activity relating this technique for public
 109 engagement. On the other hand, microfluidics is an increasingly growing research area whose
 110 applications for drinking water are quite scarce, though increasing in recent years [7]. Due to
 111 its success in research laboratories, literature for introducing microfluidics to students is
 112 flourishing as well [9-13]. However, most of these papers are targeting middle school, high
 113 school or undergraduate students. The audience of the proposed activity is young children, to
 114 enhance their interest in science, technology, engineering and mathematics (STEM) and to

115 promote the next workforce generation. By coupling microfluidics to waterborne pathogen
 116 detection, an interesting approach is proposed to children for understanding what
 117 microfluidics is and how relevant it could be for a concrete application.

118

119 III. How it works in research labs...

120 Figure 2 shows a standardised method for separating and detecting *Cryptosporidium*, a well-
 121 known and highly resistant pathogen encountered in water systems. This method incorporates
 122 five concentration steps with two stages of filtration and elution followed by centrifugation,
 123 to minimize the volume of liquid and thus concentrate particles.



124

125 Figure 2. Overview of the USEPA 1623 method for the detection of *Cryptosporidium*. Extracted from

126

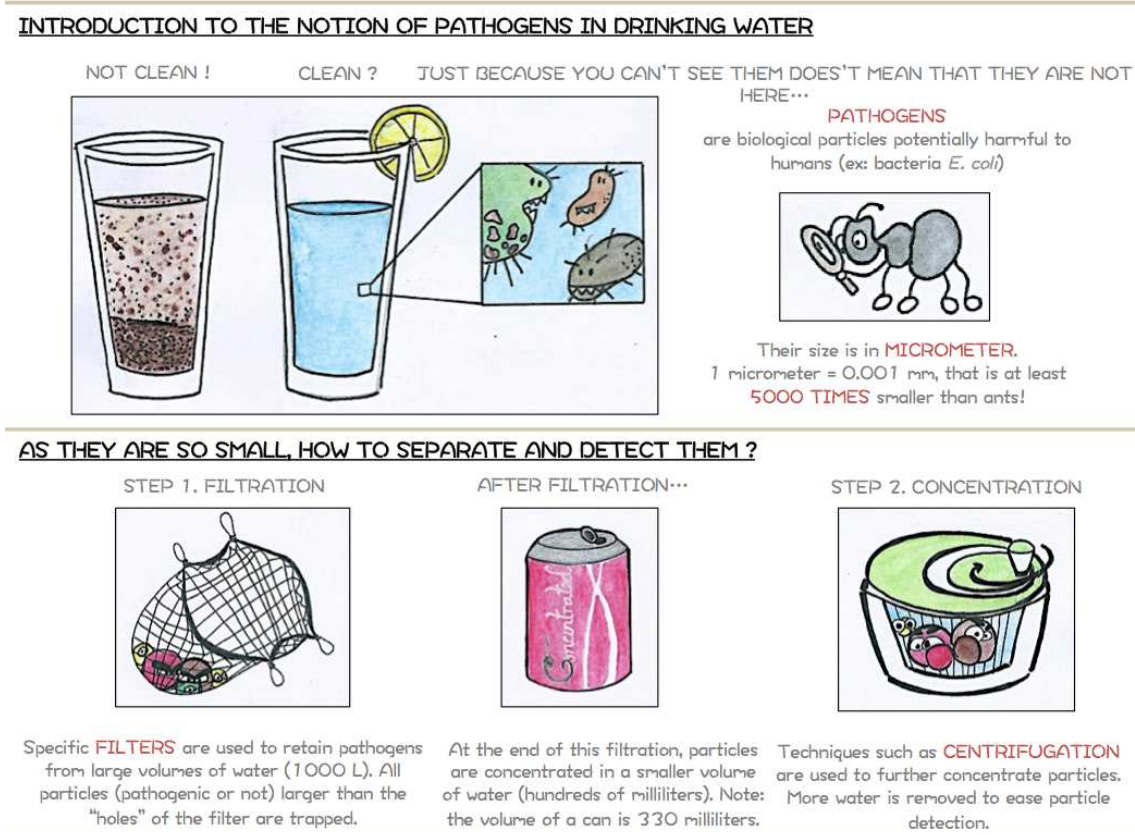
Bridle et al. [6].

127

128 The filtration steps rely on the size of particles to remove them from the water sample. All
 129 particles larger than the pore size of the filter will be trapped while the smaller particles will
 130 remain in water. As a consequence, a mix of different particles can be present after the
 131 concentration steps as long as they present a diameter larger than the filter pore size, only
 132 some of which will be pathogenic. Specific techniques are therefore needed to identify which
 133 particles are present to evaluate the water quality and if consumers can safely use this water.

134 The next paragraph presents one of them, namely the Immuno-Magnetic Separation. The
 135 accompanying support poster proposed for introducing in a simplified manner notions of
 136 waterborne pathogens and their separation is shown in Figure 3.

137



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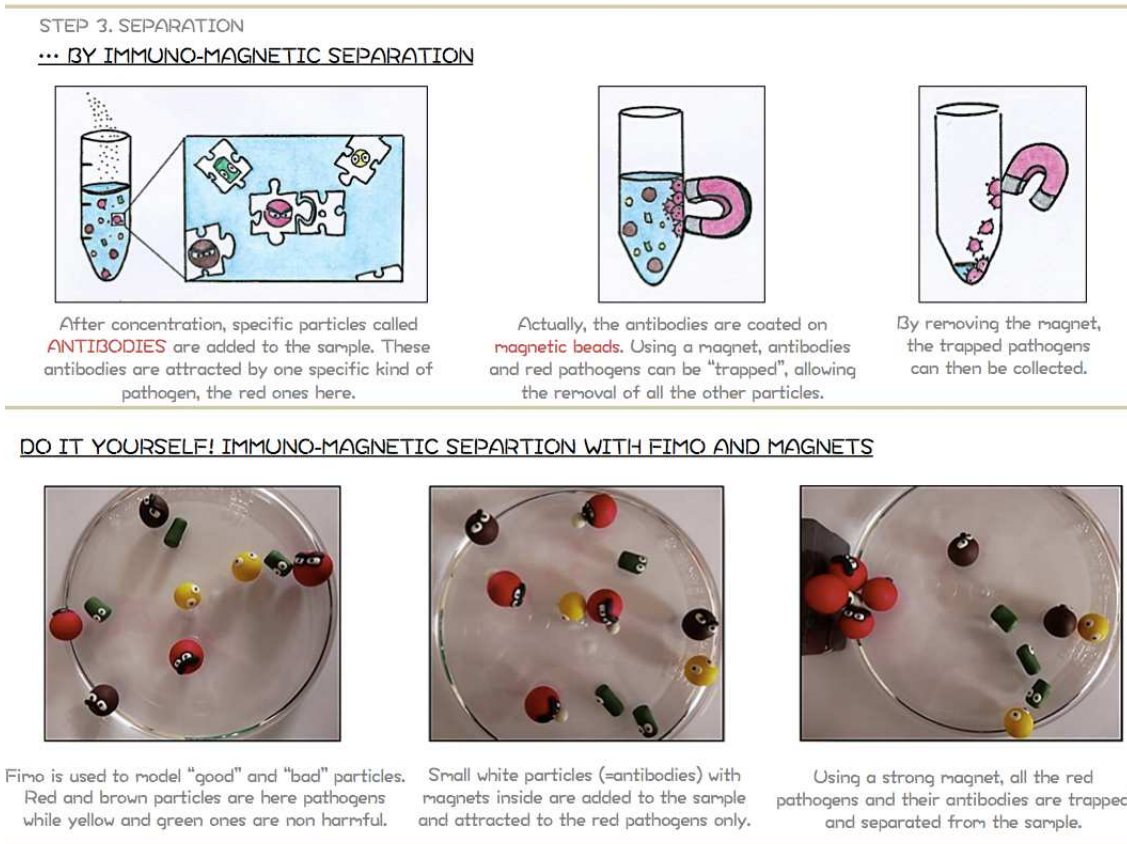
139 *Figure 3. Module 1 support poster or how to introduce notion of safe drinking and pathogens to a*
 140 *young audience.*

141

142 *A. Standardized Immuno-Magnetic Separation (IMS)*

143 The principle of the Immuno-Magnetic-Separation (IMS) is schematically represented in
 144 Figure 4. It relies on the addition of specific magnetic beads coated with antibodies [14] (e.g.
 145 anti-*Cryptosporidium* if the presence of *Cryptosporidium* needs to be confirmed). Particles in
 146 the sample are only captured if they correspond to the specific anti-bodies coated on the
 147 magnetic beads and can then easily be removed using a strong magnet. Although IMS is a

148 powerful technique for separating specific biological particles such as pathogens, the standard
 149 protocol is usually limited to small volumes of samples and requires the intervention of
 150 experienced staff. Microfluidic-based techniques are a growing topic for proposing smart
 151 alternatives to water issues, and one approach that has been taken is to perform on-chip IMS
 152 [15-18].
 153



154

155 *Figure 4. Module 2 support poster. Top: Schematic representation of separation by Immuno-*

156 *Magnetic-Separation (IMS). Bottom: hands-on activity to reproduce standard IMS.*

157

158 *B. Microfluidic based separation techniques*

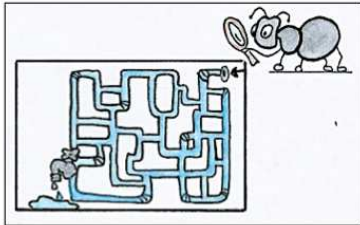
159 Microfluidics is defined as "the science and technology of systems that process or manipulate
 160 small (10^{-9} to 10^{-18} litres) amounts of fluids, using channels with dimensions of tens to
 161 hundreds of micrometres" [19]. Figure 5 proposes a cartoon for introducing the notion of

162 microfluidics and the manufacturing procedure of microchannels. The module related to
 163 microfluidics (Module 3) is of prime importance for allowing children to have a better
 164 representation of systems that are presented in the following modules (Module 4 and 5).

165

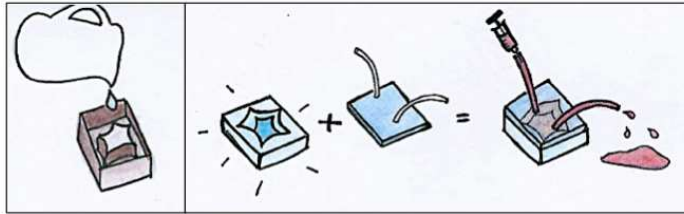
HOW MICROFLUIDICS CAN BE USED FOR PATHOGENS SEPARATION?

YOU SAID MICROFLUIDICS???



MICROFLUIDICS is like plumbing adapted to the micrometric world, with channels the width of one human hair.

HOW TO MANUFACTURE SUCH SMALL PLUMBING??



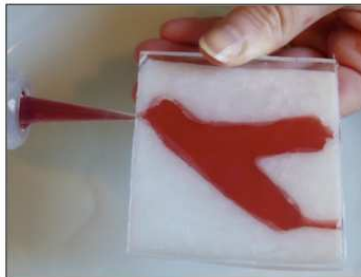
A specific liquid (ex: PDMS) is poured in a mold. After being heated, the liquid is hardened.

A transparent layer (ex: glass) is then used to "close" the channel. Holes and tubes are added for the inlet/outlet of the liquid.

DO IT YOURSELF! A MICROCHANNEL WITH FIMO AND PLEXIGLAS



Fimo molded with a "Y" wooden letter. Inlet and outlets made with toothpick.



After baking the bottom of the channel (Y-shaped Fimo), silicon is used to bond it with a Plexiglas layer.



Squash is injected through one hole with a needle-tip bottle. You can then play with the shape of your channel !

166

167 *Figure 5. Module 3 support poster. Top: Schematic representation of a microfluidic system and the*
 168 *manufacturing procedure. Bottom: hands-on activity to produce a "micro" channel.*

169

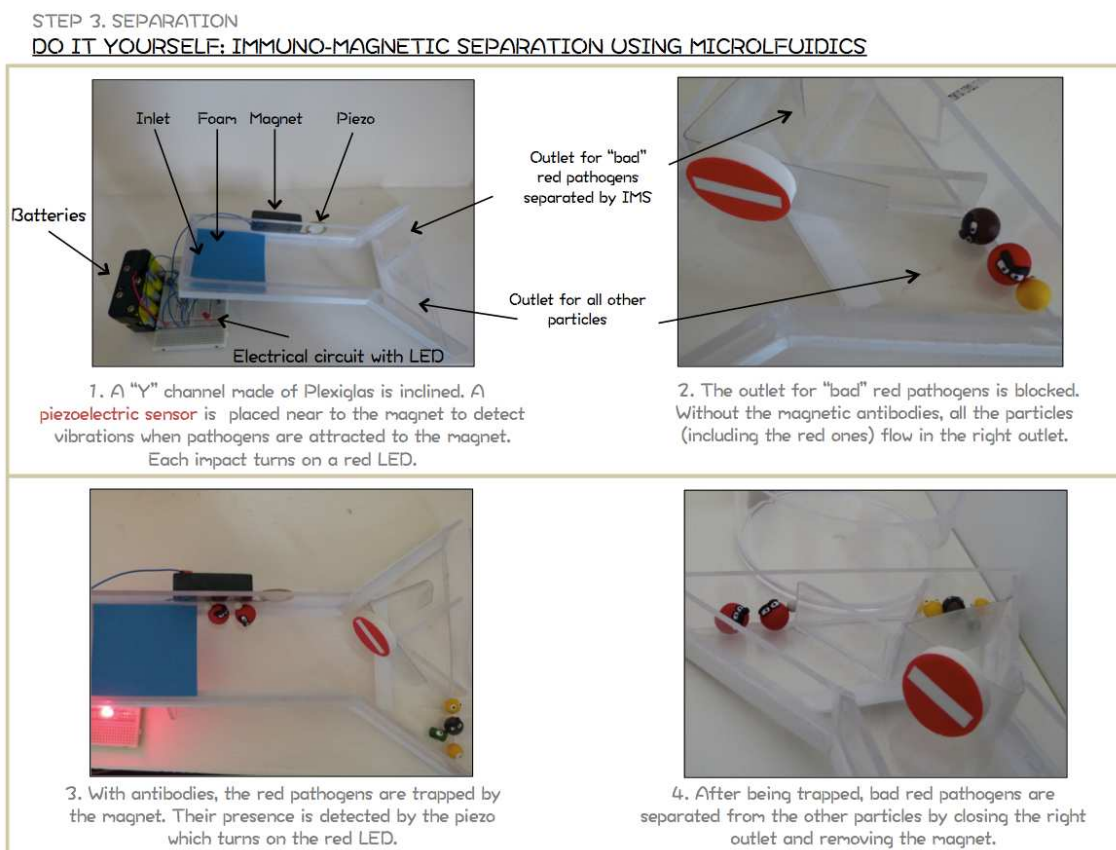
170 *B.1. Microfluidic based Immuno-Magnetic Separation*

171 Microfluidics can offer several advantages to the standardized IMS, which explains the wide
 172 range of publications related to this topic [15-18]. A microfluidic-based IMS is more
 173 automated, can deal with larger volumes of sample than standard IMS and miniaturize the
 174 procedure into one on-chip unit. One other main advantage is the possibility to integrate
 175 several other procedures within the same device such as the detection of trapped particles.

176 Techniques based on fluorescence detection are for instance proposed in the literature for
 177 identifying on-chip the presence of pathogens [18].

178 In order to introduce simply the notion of “multitask” chip, a microfluidic-based IMS is
 179 coupled with a piezoelectric sensor (Figure 6). For pathogen detection, antibodies are usually
 180 immobilized onto the surface of a piezoelectric sensor. When pathogens are trapped, a shift in
 181 the resonance frequency of the sensor is detected and correlated to the mass of pathogens
 182 blocked at the surface [20-21]. This approach is here extended to the detection of antibody-
 183 coated magnetic beads and pathogens onto the magnet. For a simple realisation, the
 184 piezoelectric sensor will detect the vibration due to the impact of particles onto the magnet
 185 that will turn on a red LED.

186



187

188 *Figure 6. Module 4 support poster. Hands -on activity to mimic a microfluidic based IMS with*
 189 *detection of trapped pathogens.*

190

191 Immuno-magnetic separation provides excellent recovery rates but remains specific to one
192 particle/antibody combination. This procedure has to be iterated if different particles have to
193 be detected and requires the corresponding specific antibodies, which are not always readily
194 available and can be very expensive. When applied to drinking water purposes, this iterative
195 procedure is a limiting step to the fast detection of all the potential harmful pathogens.
196 Moreover, smaller pathogens such as viruses are not concentrated by the centrifugation step
197 (Step 5 in Figure 2), they will remain in the supernatant and require further specific and
198 expensive steps to be separated such as ultracentrifugation.

199

200

201 *B.2. Deterministic Lateral Displacement (DLD)*

202 Different techniques have been developed in the literature for sorting particles using
203 microfluidic devices [7;22]. This paper only focuses on one of these techniques, referred to as
204 Deterministic Lateral Displacement (DLD), initiated by the work of Huang *et al.* in 2004 [23]
205 and easily reproducible at a macroscopic scale with LEGO® [24-26], thus highly suitable for
206 manipulation/visualisation by children. The basic idea of DLD is to separate particles by
207 changing their trajectory within a channel depending on their size. “Large” particles (*i.e.*,
208 particles larger than a critical diameter defined below) are deviated from their initial position
209 due to the presence of posts placed in the microchannel.

210 These posts are designed within a specific geometry and periodicity in order to separate
211 particles above a desired critical diameter D_c [27]:

212

$$213 \quad D_c = 1.4G\varepsilon^{0.48}, \quad (1)$$

214 with G the distance between two posts (see Figure 5) and ε defined as

215

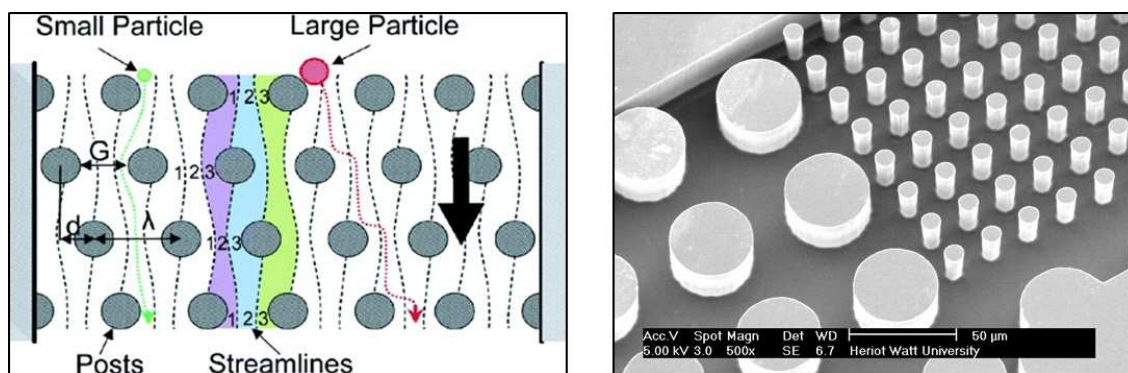
216

$$\varepsilon = \frac{d}{\lambda} = \frac{1}{N} = \tan \theta, \quad (2)$$

217

218 where d is the shift between two successive vertical posts, λ is the centre-to-centre distance219 between two successive horizontal posts (see Figure 7), N is the periodicity of the post array220 and θ is the angle of deviation of the posts.

221



222

223 *Figure 7. Example of microfluidic channel for particle separation based on DLD. Left: Schematic*224 *representation of DLD principle extracted from Davis et al.[28]. Right: Magnified view of a DLD*225 *device used for separating bacteria from blood – IB3 and MISEC group at Heriot Watt University.*

226

227 Due to the specific fluid motion present in devices containing posts, particles above the

228 critical diameter are deviated while small particles follow an ultimately straight path. This

229 technique is relevant for introducing the safe drinking water challenge since pathogens

230 present different characteristic sizes, depending on their kingdom (nanometres for viruses,

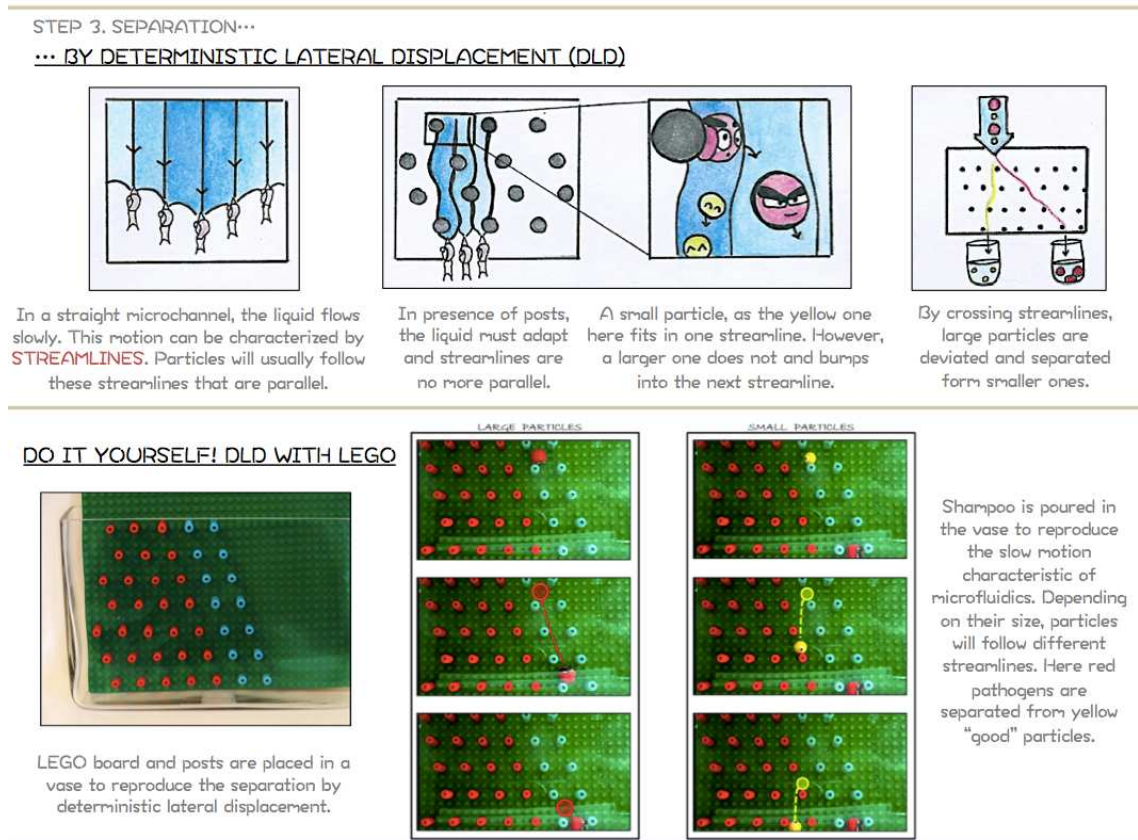
231 around a micrometre for bacteria and several micrometres for protozoa). Although studies

232 focusing on the separation of non-spherical biological particles are limited and need further

233 investigation before this method can be fully applied to waterborne pathogens, DLD devices

234 can be produced at a macroscopic scale with LEGO[®]. This offers an excellent interactive

235 approach to introduce current research aims to children and is easy to implement in schools
 236 or during outreach activities for example. The support poster proposed for introducing the
 237 notion Deterministic Lateral Displacement is proposed in Figure 8.
 238



239
 240 *Figure 8. Module 5 support poster. Top: Schematic representation of the DLD principle. Bottom:*
 241 *hands-on activity to mimic a size-based separation using DLD.*

242

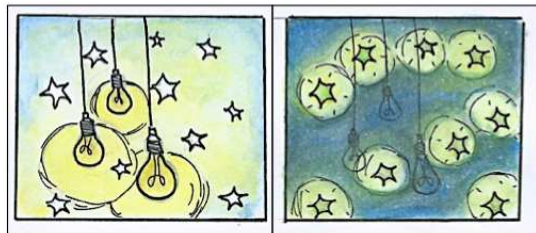
243 *C. Detection*

244 The last step of the process to be introduced to children is the detection of the separated
 245 pathogens. This process usually relies on the labelling of pathogens with specific fluorescent
 246 antibodies. Using a fluorescent microscope, pathogens conjugated with fluorescent antibodies
 247 can then easily be detected and counted (Figure 9).

248

STEP 4. DETECTION

After separating the pathogens, specific techniques are used to identify them. Antibodies are used again (see IMS). But this time, they are **fluorescent!**



Fluorescent means that they can emit light if excited with an appropriate light source.

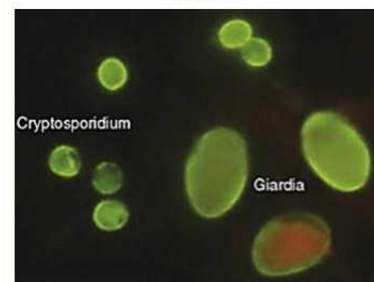


Because pathogens are really small, a **MICROSCOPE** is used to see this fluorescence.

DO IT YOURSELF! A FLUORESCENT MICROSCOPE

An insect magnifier is placed in a cardboard box painted in black. Red pathogens (with magnets inside) separated by STEP 3 are mixed with antibodies (also with magnet inside) made of a fluorescent FIMO. When placed in the dark, the fluorescent beads can be observed.

IT LOOKS ACTUALLY LIKE THIS...



Real pathogens observed using a microscope and fluorescent antibodies.

249

250 *Figure 9. Module 6 support poster. Top: Schematic representation of the fluorescence phenomenon.*

251 *Bottom: hands-on activity to visualize fluorescent particles using a low-cost microscope. Image with*
 252 *real pathogens reproduced from Bouzid et al.[29].*

253

254 **IV. How it works with children...**

255 Now that the challenge of pathogen separation and detection has been introduced, this paper
 256 presents an easy and interactive way to reproduce and illustrate these different techniques
 257 with children. Detailed explanations to reproduce all the experiments are proposed in the
 258 supplementary information.

259

260 *A. Immuno-Magnetic Separation with FIMO®*

261 Pathogens and other biological particles are represented in a simplified and magnified manner
 262 using FIMO® clay (Figure 4- Bottom). FIMO® is a soft polymer clay, available in a large

263 range of colours, that can be easily shaped and then hardened after baking for 30 minutes in
264 an oven at 110°C.

265 In this paper and for ease of children's understanding, two kinds of particles have been
266 represented:

267 - "Bad" particles, red and brown particles in Figures 4, 6 and 8. "Bad" particles
268 represent waterborne pathogens, defined by the Environmental Agency as
269 microorganisms capable of causing disease that may be transmitted via water and
270 acquired through ingestion, bathing or by other means. The size of red and brown
271 particles is roughly the same (diameter around 1.6 cm). In order to let children
272 identify which are these "bad" particles, they are represented with angry faces. Note
273 that faces could be directly painted on baked polymer clay by children. In this paper,
274 angry faces are also made with the polymer clay. To reproduce the immuno-magnetic
275 separation, small magnetic beads are incorporated inside the red "bad" particles
276 (Figure 4-Bottom) before baking.

277

278 - "Good" particles defined as non-harmful for humans. These particles are the yellow
279 and green ones throughout the paper. These particles are smaller than the bad ones to
280 be then separated using DLD which, as mentioned previously, is a separation
281 technique considering the particle size as the sorting parameter.

282

283 Note that this representation of "good" and "bad" particles with different sizes is obviously
284 largely simplified in comparison to the reality. Even within a same "family" of pathogens,
285 some are harmful while some others are not. Challenges for researchers are still to define the
286 pathogenic characteristics of these particles, a problematic far too complex to be introduced
287 within minutes to children.

288

289 Assuming this simplification, the Immuno-Magnetic separation focuses here on the removal
290 of red particles. The magnetic antibodies are represented in Figure 4 by small fluorescent
291 beads made also with FIMO[®] (fluorescent FIMO[®] no. 04). Small magnetic beads are also
292 incorporated in these fluorescent beads before baking to be attracted toward the red particles.
293 Children only have to incorporate these fluorescent beads in the sample and observe that they
294 are directly attracted by the red “bad” particles. A strong magnet is then used to remove all
295 (and only) the red “bad” particles.

296

297 *B. Microfluidics*

298 In order to understand the notion of microfluidics and its relevance to waterborne pathogen
299 separation, a simple procedure based again on FIMO[®] is proposed. Using a block of FIMO[®]
300 that is flattened with a book or a rolling pin, a channel is created by using a mould, a Y
301 wooden letter for example here. A Y-channel is produced to complement the Y-channel
302 proposed for the microfluidic-based IMS (Module 4), although this approach allows an
303 infinite number of designs to be created (see angry pathogen device bottom right of Figure 4).
304 Three smaller channels are then produced using a toothpick to allow the liquid to enter and
305 exit the device. To close the channel, a piece of Plexiglas is used. After baking the FIMO[®]
306 block, transparent silicon for bathrooms is finally used to bond it to the Plexiglas layer. Using
307 a needle-tip bottle, red liquid (*e.g.* squash or food dye) is incorporated through one of the
308 hole.

309 Yang *et al.* [8] proposed in their paper an interactive and hands-on activity for manufacturing
310 magnified microfluidic devices with Jell-O[®] dessert. This fun and simplified approach, closer
311 to the actual procedure of manufacturing, can directly be related to the proposed activity if

312 time is available. However, the FIMO[®] approach allows children to easily touch and mould
313 their own device during an outreach activity for instance.

314

315 *B.1. Microfluidic based Immuno-Magnetic Separation*

316 For the microfluidic-based IMS, a Y-shaped channel (29 cm in length, 5 cm in width and 3.5
317 cm in height) made of Plexiglas is inclined. A similar device made with a plastic bottle is also
318 proposed in the supplementary information for reducing the costs of the activity. The outlets
319 of the channels (two branches of the Y-channel) can be let opened to allow the fluid and
320 particles to be collected in two different cups. A small support is fixed on the wall of the
321 channel to hold the magnet while being easily removable by children. A piezoelectric sensor
322 is then placed next to the magnet with transparent blue tack to detect the shock of trapped
323 particles against the magnet. For safety reasons, the magnet and piezoelectric sensor are
324 placed outside the channel to avoid any contact with water. A small piece of foam (see Figure
325 6) is placed at the bottom of the channel inlet to absorb the shock when particles are entering
326 the channel and to avoid false detections by the piezoelectric sensor. Each shock detected by
327 the sensor propagates a current through an electrical circuit (*cf.* supplementary information)
328 to finally here turn on a light (LED). Extensions of this system can easily be imagined by
329 placing a buzzer, several LEDs to know the force of the impact against the magnet, *etc.* At
330 the beginning of the experiment, a set of particles is poured in the device just above the foam
331 (Figure 6). Since the device is inclined, particles will roll down by gravity. The outlet on the
332 left of the channel is initially closed, here by a piece of flexible plastic from a plastic bottle.
333 All the particles will then flow in the right outlet of the device. A second experiment is
334 performed with, this time, magnetic beads incorporated inside the red bad pathogens and by
335 adding antibodies also with magnets (similarly to the standard IMS). Red pathogens and
336 antibodies are attracted to each other and, while flowing in the device, will be deviated by the

337 magnet. When trapped, the piezoelectric sensor will detect the shock that will then turn on the
338 light to warn of pathogen presence. Once pathogens are detected, the right outlet of the
339 channel is closed, the left one opened and the magnet removed. All the trapped particles will
340 finally flow in the left outlet thus are separated from the other particles.

341

342 *B.2. Separation of all the “bad” particles using DLD and LEGO[®]*

343 After this first separation and detection step, children should notice that other “bad” particles
344 (brown particles in Figures 4 and 6) remain in the water sample and cannot be separated by
345 IMS since they don't have the corresponding antibodies in this activity. The last step of this
346 experience thus consists of trying to remove all the “bad” particles with another technique,
347 the Deterministic Lateral Displacement (DLD) presented previously. Microchannels and
348 posts used in our laboratory are here represented by a rectangular vase (IKEA[®], Rektangel)
349 and LEGO[®] board with cylindrical LEGO[®] posts of diameter $D = 7.8$ mm to shape the
350 obstacles (Figure 11).

351

352 The positions of the posts are crucial to separate “good” from “bad” particles. In this paper,
353 the following configuration is proposed:

- 354 - Gap between two posts $G=1.7$ cm
- 355 - $\epsilon=0.37$. This parameter can easily be determined by measuring the angle θ between
356 the first blue line with the vertical axis. ϵ can be deduced given θ and based on
357 Equation (2).

358 Based on Equation (1), the critical diameter of this system is thus 1.47 cm. As presented
359 in Figure 8, red and brown particles with a diameter around 1.6 cm are larger than the
360 critical diameter and are thus deviated in the device to follow the blue path. Yellow (1.1

361 cm in diameter) and green (0.8 cm in diameter) particles are smaller than the critical
362 diameter and follow a straight path within the LEGO[®] device.

363

364 It can be noted that such macroscopic experiments cannot be performed in water.
365 Microfluidics is characterized by laminar flow and thus slow fluid motion. To reproduce
366 this phenomenon, viscous media have to be considered. While glycerol is considered in
367 some studies [24-26], in the present paper and for safety reasons, diluted shower gel is
368 used. Depending on the product used and especially its viscosity, it can be used pure,
369 without dilution, but if the viscosity is too high, particles will need a very long time to
370 pass through the LEGO[®] device. If so, a slight dilution with tap water can solve the
371 problem. The shower gel should be carefully introduced in the vase to avoid air bubbles
372 to be trapped in the liquid. Due to the high viscosity of the solution, air bubbles require a
373 long time to rise and hinder any visualization in the vase. The liquid should be carefully
374 introduced by using for instance the LEGO[®] board to pour the liquid against and avoid
375 bubble formation. Finally, it is important to mention the higher the device, the larger the
376 displacement between large and small particles.

377

378 *C. Detection with insect magnifier*

379 After separation, the number of “bad” particles trapped by IMS are counted by fluorescence.
380 All the trapped particles are placed within a fake fluorescent microscope composed of an
381 insect magnifier for children placed in a black-painted cardboard box to see the fluorescence
382 of the fluorescent magnetic beads (the fluorescence of beads is hardly visible with daylight)
383 (Figure 9).

384 Even though simplified in comparison with the real process for labelling pathogens, this
385 approach allows children to be introduced to complex notions such as antibodies,
386 fluorescence, microscopy while being able to run the whole experiment on their own.

387

388 **V. Conclusion**

389 This paper presents a new and original approach to introduce children major scientific
390 challenges. A recreational and interactive procedure is proposed to define notions of safe
391 drinking water, pathogens, separation, detection and microfluidics. By simplifying and
392 magnifying laboratories procedures, the next work-force generation can enjoy being part of
393 the research world by visualizing, testing, running experiments and analysing results related
394 to this water problem. The procedure has been developed as a story, starting from the
395 presence of particles in water that require magnifying techniques to be visualized, then a first
396 separation procedure (Immuno-Magnetic-Separation) specific to one particle/antibody
397 combination. The several advantages offered by microfluidics are then introduced in the
398 context of waterborne pathogen separation. Once all the components containing in this
399 activity are completed (FIMO[®] beads, LEGO[®] board, etc.), the duration of this “story” is
400 about 30 minutes. The activity can easily be shortened by not presenting all the modules
401 proposed in the paper. The total cost of each module is kept as low as possible (around £15
402 for the vase, £20 for the LEGO[®], £10 for FIMO[®], £20 for the magnetic beads, £20 for the
403 shower gel, £10 for the cardboard box and the insect magnifier, £25 for the piezoelectric
404 sensor). The activity presented in this paper is easy to run and can involve children from the
405 beginning (particle modelling, etc.) to introduce complex notions in a fun and interactive
406 manner. Such activities are of prime interest to master children with the science world,
407 interesting and increasingly growing research topics and perhaps promote scientific
408 vocations.

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414

415 **Conflict-of-interest statement**

416 The authors declare no commercial or financial conflict of interest.

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