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## **Fast detection of both O157 and non-O157 shiga-toxin producing Escherichia coli by real-time optical immunoassay**

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### ► **To cite this version:**

L. Mondani, S. Delannoy, R. Mathey, F. Piat, T. Mercey, et al.. Fast detection of both O157 and non-O157 shiga-toxin producing Escherichia coli by real-time optical immunoassay. Letters in Applied Microbiology, 2016, 62 (1), pp.39-46. <10.1111/lam.12503>. <hal-01972064>

**HAL Id: hal-01972064**

**<https://hal.science/hal-01972064v1>**

Submitted on 24 Nov 2021

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HAL Authorization

1 **Letters in Applied Microbiology**

2

3 **Title**

4 FAST DETECTION OF BOTH O157 AND NON-O157 SHIGA-TOXIN PRODUCING  
5 *ESCHERICHIA COLI* BY REAL-TIME OPTICAL IMMUNOASSAY

6

7 **Running head**

8 FAST REAL-TIME IMMUNOASSAY FOR STEC DETECTION

9

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26

27 **Significance & impact of the study (<100 words):** This article presents a simple-to-  
28 operate immunoassay for the specific detection of Shiga-toxin producing *Escherichia coli*  
29 (STEC). This approach consists in the on-chip assay detection of viable cells on a  
30 specifically designed antibody microarray. By skipping any enrichment step and  
31 avoiding the use of labelling agent, this approach based on the Surface Plasmon  
32 Resonance imaging of the microarrays turns out to be much faster and more cost-  
33 effective by comparison to standardized methods. (60 words)

34

35 **Abstract (<200 words):**

36 Among bacterial pathogens involved in food-illnesses, seven serogroups (O26, O45,  
37 O103, O111, O121, O145, and O157) of Shiga-toxin producing *Escherichia coli* (STEC),  
38 are frequently identified. During such outbreak, and due to the perishable property of  
39 most foodstuff, the time laps for the identification of contaminated products and  
40 pathogens is thus critical to better circumvent their spread. Traditional detection  
41 methods using PCR or culture plating are time consuming and may present some  
42 limitations. In this study, we present a multiplexed immunoassay for the optical  
43 detection of most commonly enterohemorrhagic *Escherichia coli* serogroups: O26, O45,  
44 O103, O111, O121, O145 and O157:H7 in a single device. **The use of Surface Plasmon**  
45 **Resonance imaging not only enabled the label-free analysis of the samples but gave**  
46 **results in a real-time manner.** A dedicated protocol was set up for the detection of both  
47 low contaminating bacterial concentrations of food samples (5 CFU per 25 g) and post-  
48 enrichment aliquots. **By combining one single device for the detection of O157 and non-**  
49 **O157 STEC in a label-free manner, this rapid approach may have an important economic**  
50 **and societal impact.**

51 (180 words)

52

53

54 **Keywords:** Rapid methods, EHEC (enterohaemorrhagic *E. coli*), Food safety,

55 Microarray, Identification

56

57

58 **Introduction.**

59 Shiga-toxin producing *Escherichia coli* (STEC) are responsible for numerous outbreaks  
60 traced to consumption of contaminated foods and beverages (Rangel et al. 2005, Vogt et  
61 al. 2005, Espie et al. 2006). Among them, *Escherichia coli* O157:H7 has been the first  
62 enterohemorrhagic *E. coli* (EHEC) characterized as a human pathogen in 1982 after an  
63 outbreak involving contaminated burgers in the U.S. (Riley et al. 1983). Several foods  
64 have been identified as potential sources of STEC contamination since this first crisis:  
65 sprouts, leafy greens (lettuce, spinach), hamburgers, undercooked meat, apple juice, raw  
66 milk products and beverages, including tap water for instance (Riley et al. 1983, Griffin  
67 et al. 1991, Swerdlow et al. 1992, Centers for Disease et al. 1993, Centers for Disease et  
68 al. 1996, Como-Sebetti et al. 1997). But the main food source and reservoir of the  
69 pathogen is beef. USDA has proposed rules to carry out verification procedures,  
70 including sampling and testing product components, to ensure control of both  
71 *Escherichia coli* O157:H7 and six other serogroups of Shiga-toxin producing *E. coli*  
72 (STEC) (O26, O45, O103, O111, O121, and O145) (named Top 7 STEC) (Hodges 2012,  
73 USDA 2014). Because *Escherichia coli* is a common bacterium whose species consist of a  
74 very large number of non-pathogenic serovars, the specific detection of STEC  
75 serogroups remains a challenging analysis for food suppliers. When present in a sample,

76 STEC have severe impact of human health even at very low contaminating levels (less  
77 than an hundred of CFU per sample) thus requiring a systematic pre-enrichment step  
78 before running any bacterial assay. So far, routine analysis and identification of STEC  
79 serogroups is typically done by culture plating methods, PCR-based analysis, or  
80 immunoassays like ELISA techniques or latex agglutination. Culture methods are based  
81 on plating of pre-enriched samples on media enabling isolation of *E. coli*. Standard  
82 culture procedures require a pre-enrichment step (Beutin et al. 2014); selective  
83 enrichment in solution followed by selective plating before colony isolation and further  
84 characterization (Ge et al. 2009). Due to close metabolic properties of O157 and non-  
85 O157, their differentiation remains challenging although sorbitol consumption turned  
86 out to help somehow in their characterization (Bettelheim 1998) : as only O157 has  
87 been shown to be sorbitol negative, this specificity can be exploited as a phenotypic  
88 sorting parameter on solid culture media. Some chromogenic solid media have also been  
89 described for distinguishing O157 from non-O157 STEC (Tillman et al. 2012). PCR-based  
90 methods showed very specific responses by detecting either O-serogroup and H-type  
91 specific sequences, virulence genes, or both (Perelle et al. 2004, Bugarel et al. 2010,  
92 Fratamico et al. 2011, Fratamico et al. 2012, Wasilenko et al. 2014). Immunological  
93 methods led in different formats have also been done (immuno-magnetic separation,  
94 colony immunoblot, agglutination) with processing times ranging from 20 minutes to 4  
95 hours but systematically requiring an overnight enrichment (Gould et al. 2009, Wang et  
96 al. 2013). Interestingly, label-based immunoassays have also successfully been  
97 described for the detection of clinically relevant STEC serogroups (Lin et al. 2013). In  
98 this end-point analysis, fluorescent nanoparticles enable the identification of pathogenic  
99 *E. coli*. The same year, an alternative label-free immunoassay coupled to Surface  
100 Plasmon Resonance (SPR) imaging allowing the fast detection of viable bacteria present

101 in food samples without preliminary sample enrichment has been published (Bouguelia  
102 et al. 2013). Although other groups described the STEC detection using SPR  
103 (Subramanian et al. 2006, Waswa et al. 2007, Wang et al. 2013), the sensitivities  
104 published so far for the real-time pathogen detection are in the range of  $3.10^3$  to  $10^6$   
105 CFU.mL<sup>-1</sup>, except for Bouguelia *et al.* who managed to detect food-contaminated samples  
106 at very low levels (< 5 CFU per gram of food) (Bouguelia et al. 2013). Their approach is  
107 based on the microarraying, on a gold-covered biochip, of antibodies targeting surface  
108 antigens, followed by bacterial enrichment on-the-chip. The simultaneous monitoring of  
109 the microarray by SPR imaging in a one-step process enabled the continuous monitoring  
110 of the bacterial concentration increase along with semi-quantitative data on the initial  
111 concentrations. More recently, this Culture/Capture/Measure (CCM) process was  
112 successfully applied on stressed *E. coli* strains contaminating food samples (Mondani et  
113 al. 2014). By probing the environment close to the biosensors (few hundreds of  
114 nanometers), SPR imaging also turned out to be a powerful technique for the bacterial  
115 biofilm monitoring (Abadian et al. 2014). With the aim of moving one step forward, we  
116 wish to present a microarray platform dedicated to the analysis of the most common  
117 STEC serogroups, processed in a single culture medium and suitable for samples with or  
118 without an enrichment step. In the specific case where contaminating levels are low (< 5  
119 CFU per sample), the STEC detection is carried during the enrichment phase, thus  
120 significantly decreasing the processing time.

121

## 122 **Results and discussion**

### 123 *Antibody microarraying and biochip processing*

124 In order to specifically detect and identify one –or several- STEC serogroups present in a  
125 sample, polyclonal antibodies were electrochemically arrayed on the SPR biochip.  
126 Thanks to the small size of each feature (< 500 µm in diameter), two identical series of  
127 antibodies (anti-*E. coli* O26, O45, O103, O111, O121, O145, O157:H7 and control  
128 antibodies) could be spotted on a single device, enabling the analysis of two samples  
129 loaded in two chambers fitting each array (figure 1). In order to assess the inter-spot  
130 variability during the microarraying, each antibody was arrayed in duplicate. The  
131 selectivity of the antibody response was also compared to background signals measured  
132 on negative controls (anti-KLH and anti-botulinum toxinB monoclonal antibodies).  
133 According to the CCM method (Bouguelia et al. 2013), samples were spiked with  
134 measured concentrations of STEC strains from various serogroups, and directly  
135 deposited on the biochip located in a thermalized SPR imager (37°C) without further  
136 operation. Regions of interest corresponding to individual microarrayed antibodies  
137 were defined and individual responses were monitored during bacterial on-chip culture.  
138 Sharp SPR specific responses were observed at different characteristic times, thus giving  
139 semi-quantitative information on the initial pathogen concentration (Bouguelia et al.  
140 2013, Mondani et al. 2014). Figure 2 shows the raw data monitored on the antibody  
141 microarray after spiking with 2 +/-1 CFU.mL<sup>-1</sup> of *E. coli* O145:H- ED28. A specific  
142 increase of the signal corresponding to anti-O145 antibodies was recorded after  
143 approximately 8 hours, which corresponds to the growth of 2 +/-1 CFU.mL<sup>-1</sup> present at  
144 the beginning of the experiment, and confirmed by plating and counting on agar plates.  
145 The similar responses between each spot arrayed with anti-O145 antibodies confirmed  
146 the low inter-spot variability. SPR responses monitored on irrelevant anti-O antigen  
147 antibodies, anti-KLH (NC1), anti-Btox (NC2) and polypyrrole (NC3 spots) are slightly  
148 increasing upon time, probably due to a shift of the culture medium optical index upon

149 bacterial culture. Similar results and specific responses were obtained after medium  
150 spiking with the bacterial strains listed in table 1.

151

152 *Assessment of specificity for the detection of a large repertoire of STEC*

153 In order to explore the specificity of the antibody-based approach for the detection of  
154 naturally encountered STEC, different samples of each STEC strain were analysed (two  
155 *E.coli* O26:H11, two *E.coli* O45:H2, three *E.coli* O103:H2, two *E.coli* O111:H-, one *E.coli*  
156 O121:H19, two *E.coli* O145:H- and three *E.coli* O157:H7) as well as non-STECS strains  
157 (*E.coli* O4:H5 and *E.coli* O132:H18) (Table 1). A couple of strains depicting each STEC  
158 serogroups was chosen as representative of the typical O and H (H+ and H-) antigens  
159 diversity of EHEC. Each strain was sequentially analysed on antibody microarrays  
160 functionalized with both specific serogroups (anti-*E. coli* O26, O45, O103, O111, O121,  
161 O145 and O157:H7 antibodies) and negative controls spots. No cross-reactivity was  
162 observed with strains harbouring O-antigens other than O26, O45, O103, O111, O121,  
163 O145 and O157, which confirms such approach gives low levels of false positive  
164 responses. SPR responses on irrelevant anti-O antigen antibodies spots were similar to  
165 the signals monitored for the general negative controls (anti-KLH, anti-Btox and  
166 polypyrrole).

167

168 *General protocol suitable for any STEC serogroup detection at both low contaminating*  
169 *levels and in enriched samples*

170 With the aim of proposing one single assay for the detection of any STEC serogroups  
171 (O157 and non-O157 strains), we developed a standardized protocol for sample  
172 incubation and characterization on the chip (table 2). Each strain depicting the Top 7  
173 STEC was assayed in TSB medium spiked at different concentrations and both O157 and

174 non-O157 strains were successfully detected. One series of assays (few CFU.mL<sup>-1</sup> per  
175 sample) corresponds to the minimum concentration range encountered in naturally  
176 contaminated samples, while the other series (concentrations > 15,000 CFU.mL<sup>-1</sup>) is  
177 similar to the expected concentrations found after an enrichment step led on a  
178 contaminated sample. Interestingly, this approach successfully enabled the specific  
179 detection of every STEC serogroups assayed for any initial concentration assayed. On a  
180 quantitative point of view, signal analysis of each curve enabled the measurement of  
181 detection times only for positive antibody features. Detection times measured for each  
182 concentration are consistent with the detection of viable *E. coli* dividing and multiplying  
183 on the biochip, as longer detection times were obtained with the lowest seeding STEC  
184 concentrations. With the aim of counting contaminating levels, it has been shown that  
185 calibration curves can be plotted to extract initial concentrations from detection times  
186 (Bouguelia et al. 2013). Interestingly, detection times measured for low seeding  
187 concentrations (1 CFU.mL<sup>-1</sup>) showed low disparity between the tested strains (detection  
188 time at 8h20 +/- 2 hours). Although an highly specific detection of several samples  
189 defining STEC serogroups was noticed on the antibody microarray (O26:H11; O45:H2;  
190 O111:H-; O145:H-), some cross-reactivity of few STEC (O103:H2, O111:H-, O121:H19  
191 and O157:H7) was observed, more frequently when the analysis was run with low  
192 bacterial concentrations. Such side effect might be due to the use of polyclonal  
193 antibodies, containing a mixture of antibodies with some of them being specific to  
194 epitopes common to several serogroups. As already mentioned, this cross-reactivity was  
195 not observed on strains belonging to other serogroups than the Top7 STEC serogroups,  
196 thus demonstrating that the use of polyclonal antibodies does not favour the appearance  
197 of false-positive responses. Eventually, the situation where natural non-pathogenic *E.*  
198 *coli* flora (O127:H6) was present in an STEC O157:H7 contaminated sample was also

199 tested. The real-time immunoassay enabled the specific detection of a STEC serogroup  
200 present at low levels regarding a non-STEC strain (1:7 ratio). Such results confirm the  
201 suitability of this approach for the detection of STEC present at low concentrations  
202 (about 10 CFU per sample) even though harmless *E. coli* strain is present at higher  
203 levels. The detection time (8 hours) was consistent with the values observed in single-  
204 strain samples contaminated at similar levels, which shows that the overall process was  
205 not delayed by the presence of several *E. coli* strains in a single sample.

206

207 *Real-time optical immunoassay with food samples contaminated with E. coli O157:H7*

208 With the aim of testing this approach in situations as close as possible to real conditions,  
209 contaminated food samples were processed and tested on the STEC serogroups specific  
210 antibody biochip. Because meat is a common sample where STEC contamination occurs  
211 (Riley et al. 1983), a portion of ground-beef meat (25 g) was spiked with only few  
212 *Escherichia coli* O157:H7 cells (table 3). After sample processing according to  
213 normalized dilution procedures (ISO6887 standard) followed by on-chip analysis, the  
214 bacteria were successfully detected in these spiked samples, with no cross-reaction or  
215 matrix inhibition. The result was obtained a few hours after sample dilution, with no  
216 need for specific sample preparation, extraction or handling after incubation.

217 As a conclusion, the antibody array method was able to detect and identify *E. coli* strains  
218 carrying the regulated Top 7 STEC O-antigens (O157, O145, O121, O103, O111, O45,  
219 O26) therefore it could be used as a rapid screening approach to identify presumptive  
220 positive samples while a confirmation step with conventional immunological or PCR  
221 tests will definitely confirm the serogroup. On the contrary to other immunoassays  
222 involving labelling steps and end-point analysis (Lin et al. 2013), our label-free approach  
223 allows real-time analysis, faster detection and a reduced cost by avoiding expensive

224 labels. We did not observe any bias due to bacterial mobility (strains lacking flagella),  
225 virulence factors (stx1, stx2 and eae). The antibody microarray specificity was  
226 challenged on 15 different bacterial strains defining the top-7 EHEC serogroups. Our  
227 results showed no cross-reactivity with non-EHEC serogroups although some STEC  
228 strains cross-reacted with other polyclonal anti-STE C antibodies. The presence of  
229 polyclonal antibodies targeting a large repertoire of antigens is a plausible explanation  
230 of this minor side effect. On the contrary to regular culture methods where the  
231 discrimination of O157 from non-O157 STEC using dedicated culture medium is still  
232 controversial (Vimont et al. 2007), our approach enabled the characterization of any  
233 assayed STEC serogroups with simple culture medium (TSB) incubated at 37°C. This  
234 real-time detection is based on the continuous and label-free monitoring of bacterial  
235 growth and is thus highly specific to viable bacteria. For this reason, dead cells would  
236 not interfere with the biochip and does not lead to false positive as it may happen for  
237 PCR-based techniques. More importantly, this method showed similar results for any  
238 chosen concentration of bacteria. One can reasonably expect that such analysis based on  
239 sample analysis during the enrichment phase could pave the way to an alternative  
240 confirmation method. For instance, it could be led by combining our approach with a  
241 method enabling, for instance, the Shiga toxin gene detection from enriched aliquots  
242 processed on the antibody microarray. In such case, our approach could be used as a  
243 fast and cost effective pre-screening approach where only positive samples are then  
244 fully characterized by PCR-based gene analysis.

245

## 246 **Materials and methods**

247 *Bacterial strains and culture conditions*

248 *Escherichia coli* strain O157:H7 CIP 105917 was provided by the Institut Pasteur (Paris,  
249 France); *E. coli* strains O4:H5 MB05, O26:H11 (H19 and 90.0105), O45:H2 (A9619-C2-83  
250 and 2010C-4211), O103:H2 (ED172, 336S89 and 85-250), O111:H- (Ec1-Can and  
251 Ec564), O121:H19 MB57, O132:H18 NV118, O145:H- (ED28 and VTH34) and O157:H7  
252 (EC1 and 8624) were from ANSES collection (Maisons-Alfort, France). The bacterial  
253 characterizations are summarized in table 1. All *E. coli* strains were grown overnight in  
254 optimal culture conditions, at 37°C, 100 rpm in Tryptic Soy Broth medium (TSB, Fluka).  
255 Brain Heart Infusion (BHI) was purchased from BIOKKAR Diagnostics (Beauvais,  
256 France). Turbidity of liquid cultures was adjusted with a McFarland densitometer (Grant  
257 Instruments, Cambridge) to 1.0 McFarland (approximately  $3.10^8$  CFU.mL<sup>-1</sup>). Serial  
258 dilutions were performed in PBS to obtain the desired bacterial concentrations.  
259 Bacterial counting was carried out after plating and culture on Trypticase Soy Agar  
260 plates (AES, France) according to standard protocoles.

261

262 *Antibodies microarraying on SPR biochips.*

263 For specific *E. coli* detection, affinity-purified goat anti-*E. coli* O26, O45, O103, O111,  
264 O121, O145 and O157:H7 polyclonal antibodies were purchased from Kirkegaard &  
265 Perry Laboratories Inc. (BacTrace®, Gaithersburg – MA, USA). A monoclonal anti-KLH  
266 and a monoclonal anti-Botulinum toxin (anti-Btox) (gifts from L. Bellanger CEA-  
267 Marcoule, France) were used as general negative controls (respectively NC1 and NC2).  
268 Before running the electrochemical arraying (Grosjean et al. 2005), proteins were  
269 washed and covalently coupled to pyrrole monomers (N-Hydroxysuccinimide-Pyrrole,  
270 NHS-Pyrrole) as described elsewhere (Suraniti et al. 2007, Mondani et al. 2014).  
271 Polypyrrole was also arrayed and used as a reference control (NC3). SPRi biochips  
272 (obtained from Prestodiag, Villejuif, France) are made of optical glass, covered by a 50

273 nm thick gold layer deposited on a 2 nm thick Cr. Antibody-pyrrole conjugates (1  
274  $\mu\text{mol.L}^{-1}$ ) were robotically arrayed on gold covered biochip by electro-chemical  
275 polymerization in presence of a pyrrole excess (20 mM in acetonitrile). Duplicates of  
276 each protein were spotted on the gold surface. Electro- copolymerization of both free  
277 pyrrole and pyrrole-modified proteins on the biochips was carried out in a pipette tip  
278 filled with the solution to be polymerized and containing a platinum wire used as a  
279 counter electrode. The pipette tip was moved at the vicinity of the gold layer, till an  
280 electrical contact was enabled between the working (gold surface) and counter  
281 (platinum wire) electrodes. The polymerization on the prism gold layer was performed  
282 with a 100 ms electric pulse at a 2.0 V bias independently of any reference electrode.  
283 This electrochemical process ensures the gentle and long lasting grafting of  
284 biomolecules on SPR biochips. The spot size of each antibody feature is about 300-500  
285  $\mu\text{m}$  in diameter. Following the microarraying, biochips were washed twice with PBS and  
286 stored in PBS at 4°C. Before SPRi experiment, the biochip was blocked for 15 minutes at  
287 room temperature with 1 % (w/v) Bovine Serum Albumin (Sigma-Aldrich, Saint Quentin  
288 Fallavier, France) in PBS, and then washed with culture medium.

289

#### 290 *SPR imaging and analysis*

291 SPR measurements were performed using a MonoPresto SPR imager prototype  
292 (Prestodiag, Villejuif, France) based on PlasmIA™ (Plasmonic ImmunoAssay)  
293 technology, data were processed with a dedicated software (Kinetika v3.0.5), and  
294 Regions Of Interest (ROI) corresponding to individual spots microarrayed on the  
295 biochips were defined. The SPR signal was monitored with a CCD camera, and grayscale  
296 changes of each ROI were followed and plotted over time. Regarding data analysis, first-  
297 order derivative of each curve was realized and the maximum of the derivative

298 corresponding to the inflection point of kinetic curve was characterized as the detection  
299 time. Two identical series of proteins were arrayed on each biochip and combined to a  
300 reaction chamber, thereby allowing duplicate analysis. A double reaction-chamber was  
301 used to simultaneously run two experiments on a single device. Samples were prepared  
302 as described in the following section and loaded without any further treatment on the  
303 biochip: 500  $\mu$ L of bacterial solutions were loaded in thermalized (37°C) SPR reader and  
304 chambers were closed by a porous film to limit evaporation. Then, real-time monitoring  
305 of signals corresponding to individual ROI were recorded and displayed. After 16h, the  
306 experiment was stopped, the sample was neutralized by adding a diluted bleach solution  
307 (1/10) and data were processed to extract the detection times. Control viability  
308 experiments were simultaneously carried out for each SPR assay.

309

#### 310 *Sample preparation*

311 Detection of *Escherichia coli* O157:H7 was also assessed in an artificially-contaminated  
312 ground beef sample: ground beef with 15% fat was purchased from a local supermarket  
313 (Monoprix, Villejuif). 25 g portions of meat were weighted and artificially contaminated  
314 with tenfold serially-diluted bacterial solutions (in PBS) to obtain the expected final  
315 contaminating levels. Both control and spiked ground meat were then stored for 2 days  
316 at 2-8°C to mimic usual stress conditions. Bacterial counts were performed by plating  
317 the inoculum in duplicate on TSA plates. The artificially contaminated samples were put  
318 into 4 sterile blending bags including a fluidic connector (Prestodiag, Villejuif, France).  
319 After dilution by adding 225 mL of buffered peptone water, bags were homogenized for  
320 60 seconds using a peristaltic blender (BioMérieux, France). The bags were then sealed  
321 and connected using biochips arrayed with anti-*E. coli* polyclonal antibodies as  
322 previously described. The samples and kit were placed on a Monopresto SPR imager in

323 an incubator at 37°C (France Etuves, France), and continuous reading of the signal was  
324 performed overnight.

325

326

### 327 **Acknowledgements**

328 We acknowledge the Pasteur Institute (Centre de Ressources Biologiques de l'Institut  
329 Pasteur - Paris, France) for providing the *E. coli* O157:H7 CIP105917 strain. This work  
330 was supported by the NRBC-E program (Colitrack project) of the CEA and by the Labex  
331 ARCANE program (ANR-11-LABX-0003-01).

332

333

### 334 **Conflict of interest**

335 No conflict of interest to declare.

336

337

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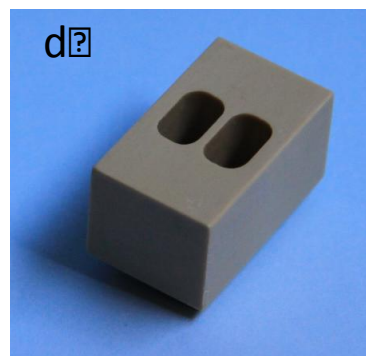
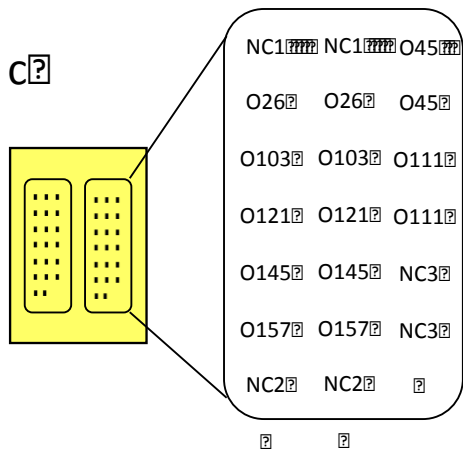
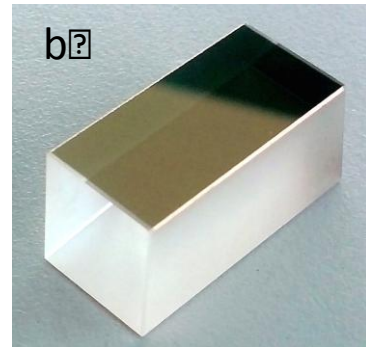
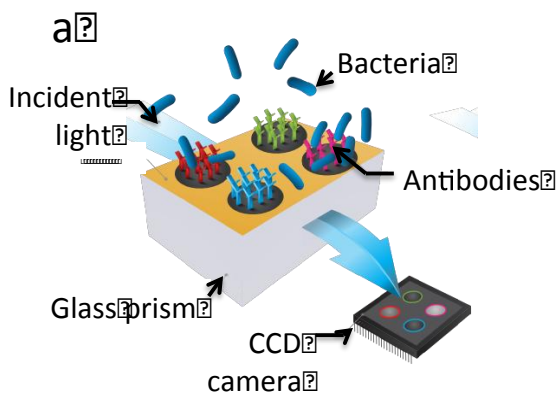
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450 **Figure and table captions**

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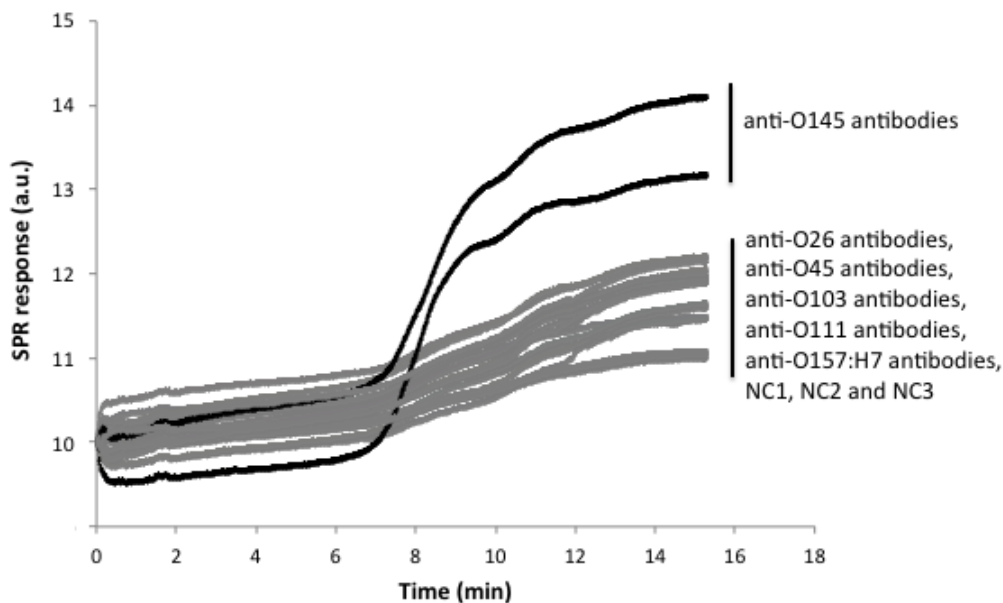
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**Figure 1**

455 **Figure 1:** Microarray design and analysis by Surface Plasmon Resonance Imaging  
456 (SPRi). (a) Schematic representation of the device and instrumental setup. (b)  
457 Photograph of a SPRi biochip. The gold layer is 25 mm x 12 mm large and 50 nm thick.  
458 The glass prism height is 12 mm. (c) Mapping of the microarrayed antibodies. The  
459 biochip contains two identical series of antibodies. One sample is then loaded on each  
460 series. OXX legends are corresponding to *E. coli* O-antigen specific antibodies. NC  
461 (Negative Controls) spots are antibodies non specific to *E. coli* and are used to assess the  
462 non-specific response. Each antibody is arrayed in duplicate. (d) Photograph of the two  
463 separate chambers fitting the microarrayed antibodies. The volume of liquid added to  
464 the chamber is 500  $\mu\text{L}$ .  
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**Figure 2**

468 **Figure 2:** SPR responses plotted upon time after bacteria incubation on a microarray.  
469 500  $\mu\text{L}$  of TSB sample was spiked with 2  $\pm$  1 CFU.mL<sup>-1</sup> of *E. coli* O145:H- ED28 and  
470 incubated at 37°C in the SPR imager. Raw data of every individual antibody spot was  
471 plotted to assess both the response specificity and the inter-spot variability.

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Serotype	Strain	Source	Gene coding		
			<i>stx1</i>	<i>stx2</i>	<i>eae</i>
<i>E. coli</i> O4:H5	MB05	ANSES (France)	-	-	-
<i>E. coli</i> O26:H11	H19	USA	+	-	+
	90.0105	USA	+	-	+
<i>E. coli</i> O45:H2	A9619-C2-83	USA	+	-	+
	2010C-4211	USA	+	-	+
<i>E. coli</i> O103:H2	ED172	Italy	+	-	+
	336S89	Belgium	+	-	-
	85-250	Canada	+	-	+
<i>E. coli</i> O111:H-	Ec1-Can	Canada	+	-	+
	Ec564	Canada	+	+	+
<i>E. coli</i> O121:H19	MB57	ANSES (France)	-	-	+
<i>E. coli</i> O132:H18	NV118	ANSES (France)	-	-	-
<i>E. coli</i> O145:H-	ED28	Italy	+	-	+
	VTH34	Spain	-	+	+
<i>E. coli</i> O157:H7	EC1	ANSES (France)	+	+	+
	8624	USA	-	+	+
	CIP105917	Institut Pasteur	-	-	-

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**Table 1. List of *E. coli* strains used for the immunoassay**

477

Strains		cfu/sample	Targeted Antigens						Negative Controls			Detection time	
			Anti-O26	Anti-O45	Anti-O103	Anti-O111	Anti-O121	Anti-O145	Anti-O157:H7	Anti-Btox	Anti-KLH		Ppy
O4:H5	MB05	1475	-	-	-	-	-	-	-	-	-	-	-
		24900	+	-	-	-	-	-	-	-	-	-	3h
O26:H11	H19	2	+	-	-	-	-	-	-	-	-	-	7h45
	90.0105	20750	+	-	-	-	-	-	-	-	-	-	3h
O45:H2	A9619-C2-83	8500	-	+	-	-	-	-	-	-	-	-	3h30
		1	-	+	-	-	-	-	-	-	-	-	8h30
O103:H2	2010C-4211	3800	-	+	-	-	-	-	-	-	-	-	3h
		1	-	+	-	-	-	-	-	-	-	-	12h30
O111:H-	ED172	11350	-	-	+	-	+	+	-	-	-	-	3h
		1	-	-	+	-	+	+	-	-	-	-	8h
	85-250	16500	-	-	+	-	+	-	-	-	-	-	3h
		1	-	-	+	-	+	-	-	-	-	-	7h
O111:H-	336S89	22250	-	-	+	-	+	-	-	-	-	-	3h
		2	-	-	+	-	+	-	-	-	-	-	4h30
O111:H-	Ec1-Can	7850	-	-	-	+	-	-	-	-	-	-	3h30
		1	-	-	-	+	-	-	-	-	-	-	8h
O111:H-	Ec564	14500	-	-	-	+	-	-	-	+	-	-	3h15
		1	-	-	-	+	-	-	-	+	-	-	7h30
O121:H19	MB57	1332	-	-	-	-	+	+	-	-	-	-	2h40
		1470	-	-	-	-	+	+	-	-	-	-	2h50
O132:H18	NV118	103	-	-	-	-	-	-	-	-	-	-	-
O145:H-	ED28	22600	-	-	-	-	-	+	-	-	-	-	4h
		2	-	-	-	-	-	+	-	-	-	-	8h
O145:H-	VTH34	30350	-	-	-	-	-	+	-	-	-	-	2h30
		3	-	-	-	-	-	+	-	-	-	-	6h30
O157:H7	O157:Ec1	8250	-	-	-	-	-	-	+	-	-	-	2h15
		1	-	-	-	+	-	-	+	-	-	-	6h30
O157:H7	8624	120050	-	-	-	-	-	-	+	-	-	-	2h
		12	+	-	-	-	-	-	+	-	-	-	8h
	CIP105917	493	+	-	-	+	-	-	+	-	-	-	6h30
		5	+	-	-	+	-	-	+	-	-	-	7h
Mix O127:H6 (E2348/69) O157:H7 (8624)		7315	-	-	+	-	-	-	+	-	-	-	8h

478

479 **Table 2. Screening of O157 and non-O157 STEC on an antibody microarray.**

480 **Table 2:** Samples contaminated with low and high concentrations of STEC (O26:H11;  
 481 O45:H2, O103:H2; O111:H-; O121:H19; O145:H-; O157:H7) and non-STEC (O127:H6;  
 482 O132:H18; O4:H5) were processed on the antibody microarray. Detection times were all  
 483 consistent with the initial spiking levels. Expected positive responses with dedicated  
 484 antibodies are shaded (dark grey) while cross-reacting effects are shown in light grey  
 485 boxes.

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Sample	Strain	Inoculum <sup>2</sup> (CFU/25g)	Result	Time-to-result <sup>2</sup> (hours)
Ground Beef	<i>E. coli</i> O157:H7	2.5+1	+	9
Ground Beef	<i>E. coli</i> O157:H7	2.5+1	+	12
Ground Beef	<i>E. coli</i> O157:H7	0	-	-
Ground Beef	<i>E. coli</i> O157:H7	0	-	-

488

489 **Table 3. Detection of *E. coli* O157:H7 contaminated meat.**

490 **Table 3:** 25 g of ground meat were spiked with low levels of *E. coli* O157:H7 and  
 491 processed according to protocols standardized by the EU when assayed on the anti-body  
 492 microarray.

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