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# **Simultaneous enrichment and detection of low levels of stressed *E. coli* O157:H7 in food matrices.**

Abbreviated running headline: Stressed *E.coli* O157:H7 detection.

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## Abstract

1 **Aims:** Rapid detection of enterohemorrhagic *E. coli* O157:H7 in large range of stress  
2 conditions occurring in food processing.

3 **Methods & Results:** Detection of *E. coli* O157:H7 in various food processing stress  
4 conditions using Surface Plasmon Resonance imaging (SPRi) technique on an antibody  
5 microarray was evaluated. The direct detection method based on the Culture/Capture/Measure  
6 (CCM) process consists of detecting bacteria during an enrichment step, which significantly  
7 decreases the overall assay duration. In optimized culture conditions, this method allows the  
8 specific detection of low CFU/ml in less than seven hours. Detection of bacteria directly in  
9 contaminated food samples were also conducted.

10 **Conclusions:** The CCM technique using an antibody microarray is a label free immunoassay  
11 that allows rapid detection of *E. coli* O157:H7 in both food processing stress conditions and  
12 complex food matrices.

13 **Significance & impact of the study:** The assay is promising for detecting *E. coli* O157:H7 at  
14 different steps of food and drink processing and during storage. SPRi appears to be a suitable  
15 and powerful detection method for routine quality controls in food industry with important  
16 economic and societal impact.

17

18 **Keywords:** Foodborne pathogens, *E. coli* O157:H7, bacterial stress, food matrices, detection,  
19 SPRi.

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21

22

## 23 **Introduction**

24 *Escherichia coli* O157:H7 has been the most common type of enterohemorrhagic *E.*  
25 *coli* (EHEC). Like other EHEC, *E. coli* O157:H7 is a member of Shiga toxin-producing *E.*  
26 *coli* (STEC) pathogens (O'Brien *et al.* 1982). This bacteria is responsible for hemorrhagic  
27 colitis or bloody diarrhea that can potentially evolve into severe diseases such as Hemolytic  
28 Uremic Syndrome (HUS), which can be fatal (US Food and Drug Administration,  
29 <http://www.fda.gov>, 2012). *E. coli* O157:H7 was first identified as a human pathogen in 1982  
30 after an outbreak involving contaminated hamburgers in the USA (Riley *et al.* 1983). It has  
31 been estimated that *E. coli* O157 caused 350 outbreaks from 1982 to 2002 and has been  
32 responsible for more than 63 000 illnesses, approximately 3700 hospitalizations and 68 deaths  
33 in the United States in 2006 (Scallan *et al.* 2001; Rangel *et al.* 2005; Scallan *et al.* 2011). In  
34 Europe, such infections are also on the rise (Health Protection Agency, <http://www.hpa.org.uk>,  
35 2009). *E. coli* O157:H7 is particularly virulent because of its low infectious dose (as few as 10  
36 organisms) (Health Protection Agency, <http://www.hpa.org.uk>, 2009). In addition, *E. coli*  
37 O157:H7 contaminations have a significant economic impact; in the US, it can annually lead  
38 to \$405 million losses in medical costs and lost productivity (Frenzen *et al.* 2005).

39 *E. coli* O157:H7 is a food-borne pathogen. The contaminated food vehicles include  
40 drinking or swimming water, vegetables, and undercooked meat (particularly ground beef).  
41 These sources have all been linked to various outbreaks (Cody *et al.* 1999; Michino *et al.*  
42 1999; Rangel *et al.* 2005; Vogt and Dippold 2005; Espie *et al.* 2006; Center of Disease  
43 Control and Prevention, <http://www.cdc.gov/ecoli/outbreaks.html>, 2012). The dissemination  
44 in the food production chain is particularly due to the fact that bacteria can spread from one  
45 infested consumable item to other uncontaminated products during food processing and  
46 packaging. Furthermore, it is common for products to be processed in a central location and  
47 distributed throughout an entire country, which may result in a national dissemination (Jay *et*

48 al. 2004; Wendel et al. 2009; Sodha et al. 2011). Food contamination by *E. coli* O157:H7  
49 poses a serious threat to the food chain and represents a major public health concern  
50 worldwide.

51

52 In order to prevent the dissemination of contaminated food and water supplies, *E. coli*  
53 O157:H7 needs to be detected as rapidly as possible (i.e. before food shelving). Until today,  
54 detection and identification of STEC O157:H7 is typically done by culture plating methods,  
55 PCR-based analysis, or immunology-based methods like ELISA techniques. According to the  
56 European Commission regulation for bacterial pathogen detection (CE n°2073/2005), the  
57 food industry is required to detect very low numbers of contaminating bacteria in complex  
58 food samples (<50 or <100 CFU/ml of *E.coli* in meat or on fruits respectively, for example).  
59 Standard methods consist of an enrichment phase in order to increase the concentration of  
60 bacteria above the limit of detection (LOD) of the analysis phase thereafter. This 12- to 48-  
61 hour enrichment phase may delay diagnosis and lead to the potential spread of contamination.  
62 Therefore, the limitation of such reference techniques is the time required for the entire  
63 analysis (i.e., an enrichment step followed by the detection phase).

64

65 On the other hand, the large diversity (liquid, solid) and complexity (with various  
66 components) of food matrices where *E. coli* O157:H7 can be found presents a particularly  
67 difficult challenge for bacterial detection in all these various conditions. Indeed, *E. coli* is able  
68 to survive in diverse conditions; these bacteria can grow in a large range of temperatures, pH,  
69 and saline concentrations of culture medium. Typically, *E. coli* O157:H7 can survive and  
70 even grow in sterile freshwater, which stands in contrast to the common misconception that  
71 the organism would die over time in such environments (Vital et al. 2008). Moreover, the

72 detection of low pathogen concentrations in contaminated food, which may also contain  
73 abundant natural flora, requires a high sensitivity and specificity of the technique.

74

75 Finally, stress conditions occurring during food processing (salt addition, low  
76 temperature conservation) can induce morphological transformations and modifications of  
77 gene expressions. As a result, genetic stress responses of *E. coli* O157:H7 have been well  
78 described (Allen et al. 2008; Dong and Schellhorn 2009; Vanaja et al. 2010). However,  
79 despite the interest of rapid detection of *E. coli* O157:H7, little attention has been paid so far  
80 to the impact of stress response on the detection of these bacteria under stressing food  
81 processing conditions. Therefore, there is a growing need for the development of new  
82 methods enabling the rapid, specific, sensitive and faster detection of stressed pathogenic  
83 O157:H7 in food samples.

84 The technique used in this study is based on SPRi detection of bacterial interactions on  
85 antibodies arrayed on a biochip. Surface Plasmon Resonance (SPR) has often been used for  
86 bacterial detection (Fratamico and Strobaugh 1998). Using this approach, an enrichment step  
87 is required prior testing on such antibody microarrays whose detection limits are usually in  
88 the range of 10<sup>4</sup> to 10<sup>9</sup> cfu/mL (Dudak and Boyaci, 2009; Rodriguez-Emmenegger et al.  
89 2011; Skottrup et al. 2008). Recent data have been published on SPR imaging modes enabling  
90 individual object visualization, although with poor optical resolution (Suraniti, 2007). The  
91 detection of bacteria with Surface Plasmon Resonance imaging (SPRi) based on the  
92 Culture/Capture/Measure (CCM) process, very recently described by Bouguelia *et al.*, bears  
93 the major advantage of the direct and consequently rapid detection technique (Bouguelia et al.,  
94 2013): both the “Culture” and “Capture” of viable bacteria were monitored in real-time by  
95 SPRi (“Measure”). The biochip used for this type of detection contains four domains on  
96 which four identical series of antibodies are microarrayed. The multiplexed analysis allows

97 the cross-referencing of different information. The detection of *E. coli* O157:H7 was realized  
98 by using two *E. coli* O157:H7 specific antibodies and a negative control antibody.  
99 Furthermore, in order to reach more robust results, each antibody was spotted in triplicate and  
100 the monitored signals were averaged.

101 Using SPRi with the CCM approach, we showed by this work, that: (i) this  
102 approach is an efficient, label-free method for the detection of *E. coli* O157:H7 at very low  
103 concentrations and (ii) it is directly applicable on a large panel of culture conditions, such as  
104 various stressed samples and complex food matrices.

105

## 106 **Materials and methods**

### 107 **Bacterial strains and counting**

108 *Escherichia coli* O157:H7 CIP 105917 was obtained from Pasteur Institut (Paris, France) and  
109 *Escherichia coli* O157 non-H7 strain 18S+ were from ANSES collection (Maisons-Alfort,  
110 France). *E. coli* cells were grown overnight in optimal culture conditions, at 37°C, 100 rpm in  
111 Tryptic Soy Broth medium (TSB, Fluka). Bacterial counting was carried out after plating and  
112 culture on TSB agar or selective medium (HiCrome agar from Fluka) for food experiments.

### 113 **Bacterial growth in stress conditions**

114 For stress experiments, 10-fold diluted solutions of fresh *E. coli* O157:H7 culture  
115 (approximately  $10^7$  CFU/ml) were grown in TSB in stress conditions overnight. For  
116 temperature stress analyses, cultures were incubated at different temperatures (-20°C, 4°C,  
117 12°C, 37°C, 42°C and 50°C). For high salt concentrations stress conditions, *E. coli* cultures  
118 with addition of NaCl (50 and 90 mg/ml) were performed at 37°C. Then, *E. coli* cultures were  
119 diluted to get a final solution with low CFU per ml (5-23 CFU/ml). These suspensions were  
120 loaded onto an *E. coli*-specific antibody microarray for SPR experiments.

### 121 **Food sample preparation.**

122 The food analyzed came from a local supermarket. For food spiking experiments, *E. coli*  
123 O157:H7 cultures previously grown overnight in TSB at 37°C were used after dilution for  
124 food inoculation. For water analysis, an *E. coli* culture 10-fold diluted in fresh tap or spring  
125 water (Evian®) was incubated overnight at room temperature. Then, series of 10-fold  
126 dilutions were done in TSB (until 5-20 CFU/ml) for loading on *E. coli* specific antibody  
127 microarray and SPR experiment. For fruit and vegetable analyses, 1 ml of a diluted fresh  
128 culture (several 10-fold dilutions until reaching 50-100 CFU/ml) was dropped and dried under  
129 microbial safety cabinet on an apple peel or on salad previously washed with 70%  
130 ethanol/water. The dried sample was re-suspended in 1 ml TSB for SPRi analysis. For milk  
131 analysis, an *E. coli* culture 10-fold diluted in fresh pasteurised 2% milk was incubated  
132 overnight at 4°C (□□□□□□CFU/ml). Then, this solution was serially 10-fold diluted in  
133 TSB before SPRi experiments (until reaching approximately 150 CFU/ml). For ground beef  
134 analysis, a fresh *E. coli* culture was diluted several times to a final bacterial concentration of  
135 hundreds CFU/ml (approximately 550 CFU/ml). 25 g of ground beef samples were spiked  
136 with 1 ml of this diluted culture and then incubated at 4°C overnight. The ground beef was  
137 then added to 250 ml of TSB and vigorously homogenised in a micro-perforated filter bag  
138 (BagPage®, Dutscher, Brumath, France) with a Bagmixer® 400<sup>cc</sup> (Interscience). Then, 1 ml  
139 of solution was collected from the filtered part of the bag and 200 µl were loaded on a biochip  
140 for SPRi analysis. 4 different contaminated samples (different initial contamination  
141 concentrations of *E. coli*) were tested simultaneously on a biochip (4 reaction chambers).  
142 Dozen of samples were tested however, to be closer to industrial conditions, data shown come  
143 from one single ground beef sample.

#### 144 **Antibodies microarraying on SPR biochips.**

145 For specific *E. coli* O157:H7 detection, affinity purified goat anti-*E. coli* O157:H7 polyclonal  
146 antibody was purchased from Kirkegaard & Perry Laboratories Inc. (BacTrace®),

147 Gaithersburg – MA, USA). A purified monoclonal antibody against *E. coli* O157:H7 was  
148 obtained from Acris antibodies (Herford, Germany). A purified monoclonal antibody against  
149 *E. coli* O157 was obtained from Abcam (United Kingdom). A monoclonal anti-Botulinum  
150 toxin antibody (gift from L. Bellanger CEA-Marcoule, France) was used as a general negative  
151 control (IgG control, NC). 200  $\mu$ l of antibodies (0,5 mg/ml) were washed twice with 100  $\mu$ l of  
152 phosphate-buffered saline (PBS, pH 7.4) by centrifugation (15000 g for 15 min at 4°C) on  
153 size-excluding membranes (Vivaspin, 30-kDa cut off, Vivascience, Hannover, Germany) and  
154 re-suspended in 200  $\mu$ l of PBS. Before running the electrochemical arraying, proteins were  
155 covalently coupled to pyrrole monomers (N-Hydroxysuccinimide-Pyrrole, NHS-Pyrrole) as  
156 described elsewhere (Roupioz *et al.*, 2009). SPRi biochips were purchased from Horiba  
157 Scientific (Palaiseau, France). Antibody-pyrrole conjugates ( $1 \mu\text{mol.l}^{-1}$ ) were arrayed on gold  
158 covered biochip by electro-chemical polymerization in presence of pyrrole. Triplicates of  
159 each protein were spotted on the gold surface. The spot size of each antibody feature is about  
160 400  $\mu\text{m}$  in diameter. After microarraying, biochips were washed with PBS and stored in PBS  
161 at 4°C. Before each SPRi experiment, the biochip was blocked for 15 minutes at room  
162 temperature with 1 % (w/v) Bovine Serum Albumin (Sigma-Aldrich, Saint Quentin Fallavier,  
163 France) in PBS, and then washed with culture medium.

#### 164 **SPRi experiments.**

165 SPRi measurements were performed using a SPRi-Lab+™ system (Horiba Scientific,  
166 Palaiseau, France). Using the commercial software, regions of interest (ROI) corresponding to  
167 individual features microarrayed on the biochips were defined. The SPR signal was monitored  
168 with a CCD camera, and reflectivity changes of each ROI were followed and plotted over  
169 time. Regarding to the data analysis and assessment of positive responses, reflectivity  
170 responses were plotted after bacterial spiking and responses were considered as positive when  
171 reaching >1% reflectivity increase above negative controls. In some highly complex food

172 matrices, some heterogeneous responses were observed within the microarrayed antibodies.  
173 When negative controls could not be used as baselines for the assessment of positive  
174 responses, the initial slope of each antibody response was plotted and assumed as positive  
175 when the reflectivity signal reached >1% increase above the initial slope. On the contrary to  
176 most SPR-based molecular assays, bacterial detection involves significant reflectivity  
177 increases, sometimes exceeding this linearity range for quantitative measurements.  
178 Nevertheless, only initial reflectivity shifts are handled for the identification of positive  
179 responses, independently of plateaus reached at the end of the experiments. A multiple  
180 reaction-chamber (4x200  $\mu$ l) piece was micro-machined in a massive PEEK ingot. Four  
181 identical series of proteins were arrayed on each biochip to fit each chamber, thereby allowing  
182 quadruplicate analysis. 200  $\mu$ l of bacterial solutions were loaded in thermalized (37°C)  
183 biochips and chambers were closed by a porous film. Assays were run in standard growth  
184 conditions (TSB, 37°C). Graphs of  $\Delta R$  along time (figures 1, 3, 4) were plotted on Origin data  
185 analysis and graphing software.

186

## 187 **Results**

188 The technique based on SPRi detection of bacterial interactions on antibodies arrayed on a  
189 biochip, used in this study, allow to observe a significant signal increase when bacteria were  
190 captured and grown on specific antibody spots on the biochip.

191

### 192 *E. coli* O157:H7 specific detection

193 Detection of *E. coli* O157:H7 is shown in figure 1a. Similar results were obtained from  
194 triplicates for each antibody (specific and control antibodies). A significant increase of the  
195 specific O157:H7 antibody signal was recorded after 350 min, which corresponds to the  
196 growth of 63 $\pm$ 17 bacteria/ml (as present at the beginning of the experiment).

197 In order to test the specificity of the *E. coli* O157:H7 biochip, similar experiments were  
198 conducted with another bacterial genus (*Salmonella enterica* Enteritidis) and non-O157 *E.*  
199 *coli* strains. In each case, the perfect superposition of the specific and negative control signals  
200 monitored throughout the experiment shows the specificity of the biochip (Suppl.1).  
201 Detections were also done on *E.coli* O157 non-H7 (figure 1 b). Signals from *E.coli* O157 and  
202 *E.coli* O157:H7 specific antibodies were recorded. The differences of reflectivity ( $\Delta R\%$ )  
203 recorded for *E.coli* O157 and *E.coli* O157:H7 antibodies were particularly distant after an  
204 *E.coli* O157:H7 culture. On the contrary, during an *E.coli* O157 non-H7 culture, the  $\Delta R\%$   
205 recorded were similar.

206

#### 207 Semi-quantitative assessment of contaminating concentrations.

208 The bacterial growth detected via the SPRi method is an exponential phenomena, thus by  
209 analogy to the cycle threshold (Ct) calculated for quantitative PCR, a time threshold (Tt) was  
210 determined for each experiment. This characteristic time corresponds to the time necessary to  
211 reliably detect the presence of growing bacteria (Suppl 2). Measured Tt was plotted according  
212 to contaminating concentrations and showed the semi-quantitative character of the SPRi  
213 detection system (figure 2).

214 The exploration of the standard curve shows a linear domain between  $10^2$  and  $10^5$  CFU/ml,  
215 which corresponds to a time threshold of 390 to 20 min, respectively. Higher concentrations  
216 of bacteria were detected in approximately 20 min and lower concentrations in less than 450  
217 min.

218

#### 219 Detection of bacteria exposed to various food processing stress conditions

220 Several stress conditions often encountered in food processing were tested. Detection  
221 experiments were carried out after culture at different temperatures and in presence of high

222 salt concentrations in order to mimic refrigerated or undercooked food and convenience food,  
223 respectively.

224 In order to investigate the performances of SPRi detection on bacteria stressed by  
225 refrigeration, tests were performed after culture at 4°C. A Tt of 400 min similar to those  
226 obtained in optimal conditions (37°C), was measured with this *E. coli* O157:H7 culture,  
227 showing the weak effect of refrigeration (figure 3 a).

228 In the same manner as before, series of tests after growth were conducted at different  
229 temperatures ranging from -20°C (in order to reproduce the conservation conditions of frozen)  
230 to 50°C (in order to mimic undercooked meat). The calculated Tt are reported in Table 1.  
231 These results show that regardless of the stress temperature from 4 to 50°C (4, 12, 37, 42 or  
232 50°C), there is no significant effect on Tt values (i.e., Tt values are in the same range of those  
233 measured in optimal conditions). However, a longer delay was recorded when bacteria were  
234 previously incubated at -20°C (average Tt = 595min).

235 Cultures in the presence of different concentrations of NaCl (in TSB complemented with 0, 50,  
236 90 mg/ml,) were also tested. The results are shown in figure 3b. Similar to the Tt measured in  
237 optimized conditions, 17+/-6 and 3+/-1 CFU/ml of *E. coli* O157:H7 previously grown in the  
238 presence of 50 and 90 mg/ml of NaCl were detected in 325 and 335 min, respectively, with  
239 the SPRi system. The data are reported in Table 1 and show the efficiency of the SPRi system  
240 on salt-stressed bacteria.

241

#### 242 Detection of bacteria in various food matrices

243 In order to explore the performance of CCM closer to industrial food processing conditions,  
244 detection assays were subsequently done directly on food matrices.

245 Assays in spring and tap water were realized. The method allowed the detection of 10 to 20  
246 CFU/ml of *E. coli* O157:H7 in spring water and in tap water (Table 1) in 445 and 475 min,

247 respectively, which was slightly higher than the Tt recorded in optimal culture conditions  
248 (culture media).

249 A contaminating dose of *E.coli* (around 70 CFU/ml) was dried on an apple peel or on salad  
250 and detected in 440 and 315 min, respectively, which is similar to values obtained under  
251 optimal conditions.

252

253 Similar to the detection in water, direct detection of bacteria after overnight culture at 4°C in  
254 pasteurized 2% contaminated milk was realized. The Tt for detection of approximately 160  
255 CFU/ml of *E. coli* O157:H7 in milk was 180 min, which is even quicker than the Tt obtained  
256 in standard culture conditions (figure 4a). In the same way, after resuspension and  
257 homogenization of contaminated ground beef conserved overnight at 4°C, detection of *E. coli*  
258 O157:H7 was tested. A positive signal was obtained by SPR when the ground beef  
259 enrichment suspension reaches approximately 560 CFU/ml. As reported in this study (figure  
260 4b) the 200 min measured Tt indicated that detection can be performed in less than seven  
261 hours in different food matrices

262

## 263 **Discussion**

264 Numerous studies have shown that *E. coli* O157:H7 are the cause of major foodborne  
265 infection outbreaks (Soborg et al., 2013). Food and drinks are the most important source of  
266 contamination (McCollum et al., 2010; Slayton et al., 2011). The European Commission  
267 regulation notified limit doses for each microorganism and each food sample category  
268 (European union official journal, 2005). However, the early detection of pathogens in  
269 complex food matrices, which would limit the cases of contamination, remains challenging.  
270 Furthermore, most food products are perishable and the contaminating concentration of  
271 bacteria could be very low, so an early and sensitive detection of contamination is necessary

272 to prevent dissemination. Thus, the aim of food industry quality and safety services is to  
273 detect, as rapidly as possible, a few bacteria in food samples (depending on the limit requested  
274 by laws). New methods enabling the faster and sensitive detection of pathogenic bacteria in  
275 food samples are a priority for public health.

276

277 In the current study, it was proposed to use SPRi detection to track *E. coli* O157:H7 in  
278 food. SPRi signal analysis has shown the quantitative aspect of this approach between  $10^2$  and  
279  $10^5$  CFU/ml. With high contaminating concentrations of bacteria, the media is saturated and  
280 the measured delay corresponds to the minimal delay necessary for bacterial fixation on  
281 antibodies at the surface of the biochip (around 20 min). Therefore, the  $T_t$  is dependent on the  
282 contaminating concentration of the sample. However, even for very low contaminating  
283 concentrations of a sample, the SPRi detection system allows detection of bacteria. Indeed, 5  
284 CFU/ml of *E. coli* O157:H7 can be specifically and reliably detected in less than seven hours  
285 in optimal conditions, a much shorter time in comparison to the one or few days necessary for  
286 typical detection reference methods (PCR and culture plating, respectively). Due to the direct  
287 detection of bacteria during the enrichment step, the SPRi detection system is less time-  
288 consuming compared to other methods which need an enrichment step before the analysis.

289 Another advantage of this technology is the easy handling of the system; the prepared food  
290 sample (a simple homogenization for solid food sample) was directly added in the SPRi setup.  
291 Furthermore, SPRi analysis is non-destructive for the sample, thus enabling the use of the  
292 enriched sample at the end of the SPRi analysis for an alternative detecting experiment, such  
293 as PCR. Moreover, thanks to the multiplexed approach, many different antibodies can be  
294 arrayed on the biochip surface. In this manner, the information provided by different  
295 antibodies can be cross-referenced for more robust results and could even be used for the  
296 simultaneous detection of different pathogens. Furthermore, the nature of antibody specificity

297 towards its target provides this method with a high level of specificity. For example, results of  
298 *E.coli* O157 non-H7 detection show that although positive signals are recorded, the  
299 multiplexed approach allows the differentiation between signals recorded during an *E.coli*  
300 O157 non-H7 culture and those given by an *E.coli* O157:H7 culture (figure 1).

301 Little is known about *E. coli* O157:H7 detection performance under stress conditions.  
302 Only a few recent studies referenced enumeration assays on stressed bacteria (plating or PCR  
303 enumeration assays) (Fusco et al. 2012; Smith et al. 2013). However, it is well known that  
304 stress can induce numerous morphological and/or genetic modifications (Lombardo et al.,  
305 2004).

306 The present study shows that different food processing conditions (refrigeration,  
307 undercooking, and presence of salt) do not alter the detection of *E. coli* O157:H7 by the CCM  
308 approach. The efficiency of the method to rapidly detect bacteria previously conserved at  
309 temperatures ranging from 4°C to 50°C was also proven. This observation is important  
310 because raw or undercooked meat is a main source of *E. coli* O157:H7 contamination (Rangel  
311 et al. 2005; Health Protection Agency, <http://www.hpa.org.uk>, 2009; Center of Disease  
312 Control and Prevention, <http://www.cdc.gov/ecoli/outbreaks.html>, 2012). Although the rapid  
313 detection of pathogens in frozen products is not as crucial due to their later date of expiration,  
314 detection assays on frozen sample were also completed. In these experiments, *E.coli* O157:H7  
315 was detected with a delay, certainly due to the period necessary to restart growth after  
316 freezing.

317 In the food industry, particularly in meat processing, salt is a common additive. The majority  
318 of convenience foods and cooked meats also contain salt in a range from 0 mg/ml to 35 mg/ml  
319 (~190 mg/g in salted cod). However, little attention has been paid to the effect of stress  
320 induced by salt on *E. coli* O157:H7 detection efficiency. Harris *et al.* showed that high salt  
321 concentration can even increase toxicity of *E. coli* O157:H7 by enhancing Shiga toxin 2

322 production (Harris et al. 2012). The Tt measured after growth with 50 and 90 mg/ml of NaCl  
323 addition were similar to those measured in optimized conditions. After culture in presence of  
324 higher concentrations of salt (extreme conditions in food processing), a shift of Tt was  
325 observed, yet *E. coli* O157:H7 was nonetheless still detected (data not shown).

326 Drinkable water, contaminated vegetables and complex food matrices like dairies and meats  
327 have been associated with infection outbreaks (Licence et al. 2001; Olsen et al. 2002; Vogt  
328 and Dippold 2005; Espie et al. 2006; Grant et al. 2008; Wendel et al. 2009; Slayton et al.  
329 2011)). Detection assays were done after *E. coli* O157:H7 culture in water. The measured Tt  
330 in water were slightly higher by comparison to those recorded in optimal condition cultures.  
331 These shifts could be associated to the adaptation time necessary after a culture media  
332 modification (water/TSB). The CCM approach was also conducted on contaminated  
333 vegetables and the measured Tt were similar to those recorded in optimal condition cultures.  
334 These results showed the efficiency of the SPRi setup in these conditions. Finally, analyses  
335 were done in complex food matrices (milk and ground beef) in order to evaluate the impact of  
336 the complexity of a food matrix on the method and to test the efficiency of the SPRi method  
337 in the presence of abundant natural flora. In these conditions, the CCM approach allowed for  
338 specific and reliable detection of the presence of 100 to 1000 CFU/ml of *E. coli* O157:H7 in a  
339 contaminated sample in less than seven hours. In all food experiments, a linear drift is  
340 observed on all curves including control curves. This drift might be due to the sample  
341 complexity (proteins, fatty substances), which impact the medium reflectivity, and may also  
342 be particularly due to the presence of the bacterial natural flora of the sample. However, this  
343 drift does not impact the detection of the exponential events occurring on the antibodies array.  
344 These experiments showed the efficiency of the method, particularly for direct detection in  
345 refrigerated food samples.

346

347 In summary, the CCM process used in this study is a suitable and easy-to-operate  
348 detection method for food industry diagnostics, independently of sample conditioning method.  
349 Another advantage of this technique is the non-damaging protocol, which allows for  
350 complementary analyses to be made. The rapid detection of pathogenic bacteria in samples is  
351 needed in various other fields ranging from clinical (Manu-Tawiah et al. 2001; WHO 2007) to  
352 environmental (Touron et al. 2005; Velusamy et al. 2010). To assess the performance of this  
353 biochip system in other biological fields, the development of microarrays specific to other  
354 pathogenic targets of interest are currently being developed. A multiplexed biochip specific  
355 for different pathogens of a selected matrix (with combinations of different antibodies), such  
356 as clinical bacterial strains or environmental strains of human fluid or soil/air samples is also  
357 considered. Indeed, with combinations of different antibody responses, numerous pathogens  
358 can be efficiently detected with the same biochip.

359

## 360 **Supporting information**

361 Two figures in PDF format, about specificity of *E.coli* O157:H7 biosensor and how to  
362 determine the time threshold of detection analysis are available in supplemental information.

363

364

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372

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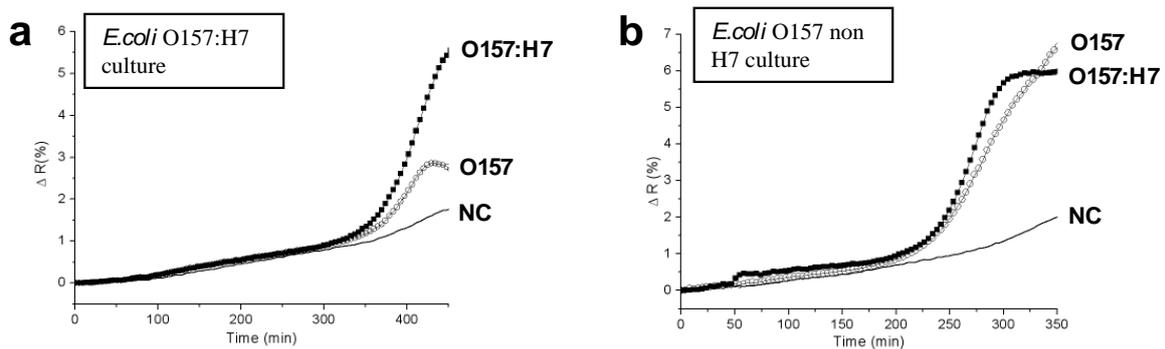
## Tables and Figures

**Tab.1:** Detection time threshold of *E.coli* O157:H7 after growth in different stress conditions.

Stress Conditions	Temperature					NaCl			Water		Salad	Apple
	-20°C	4°C	12°C	42°C	50°C	50mg/ml	90mg/ml	spring	tap			
CFU/ml at t0	5	5-20	10	10	5	10-23	2-5	10	20	~70	~70	
Detection time (min)	595*	390*	440**	375**	455**	320*	340*	445*	475**	315**	440**	

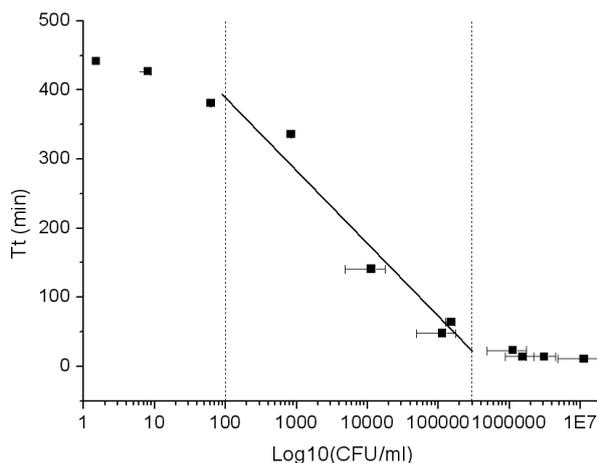
Detection after overnight culture in TSB at different temperatures, with different concentrations of NaCl added to TSB (salt stress), after an overnight culture in water (spring and tap water), and after drying on vegetables.

\* average of two different experiments (three specific antibodies signals for each). \*\* one experiment. Results are compared to 12 experiments led in optimal conditions (TSB, 37°C) : 5-35 CFU/mL were detected with a Tt of 315-420 min.

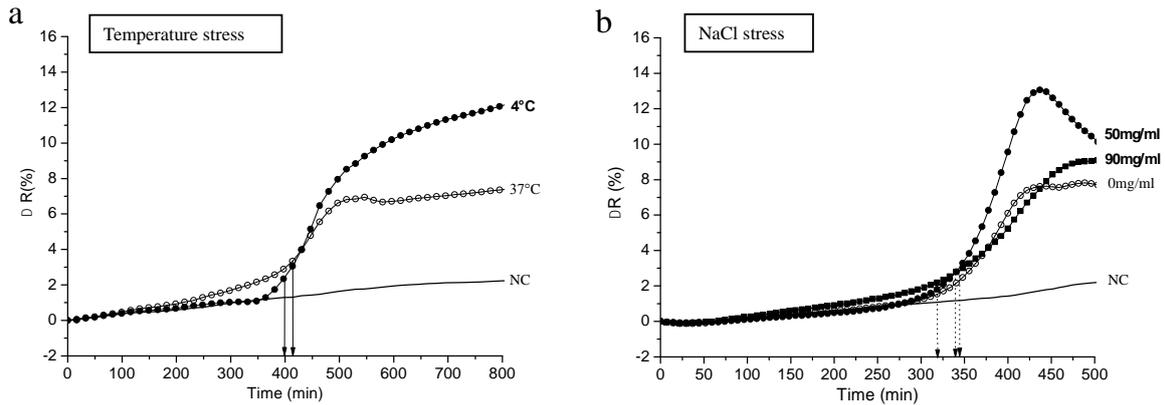


**Fig. 1:** Specificity of the *E.coli* O157:H7 biochip

(a) Analysis of an *E.coli* O157:H7 culture (63 +/- 17 CFU/mL at the beginning of the experiment). (b) Analysis of an *E.coli* O157 non H7 culture (53 +/- 23 CFU/mL at the beginning of the experiment). O157:H7 (line with black circles) : anti-O157:H7 antibodies. O157 (line with empty circles) : anti-O157 antibodies. NC (solid line) : negative control antibodies.

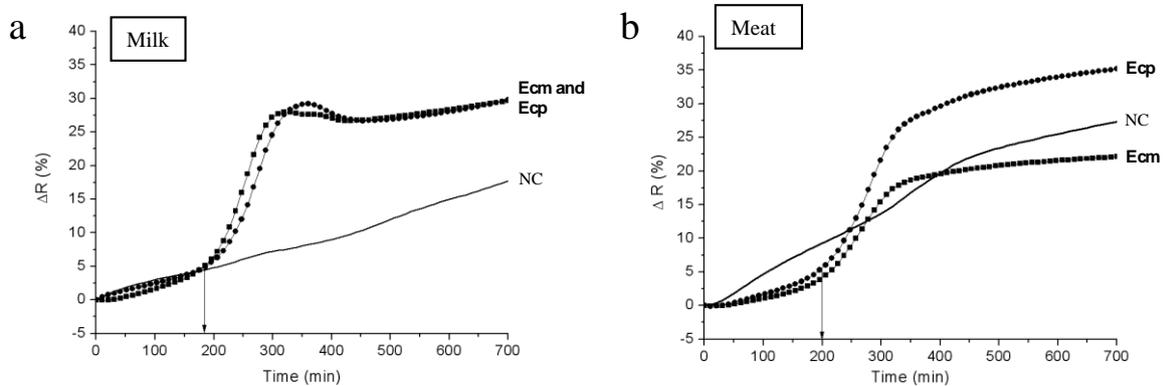


**Fig. 2:** Standard curve for semi-quantitative measurement. Curve of Tt depending on the number of bacteria present at the beginning of the experiment.



**Fig. 3:** SPR detection of stressed *E.coli* O157:H7.

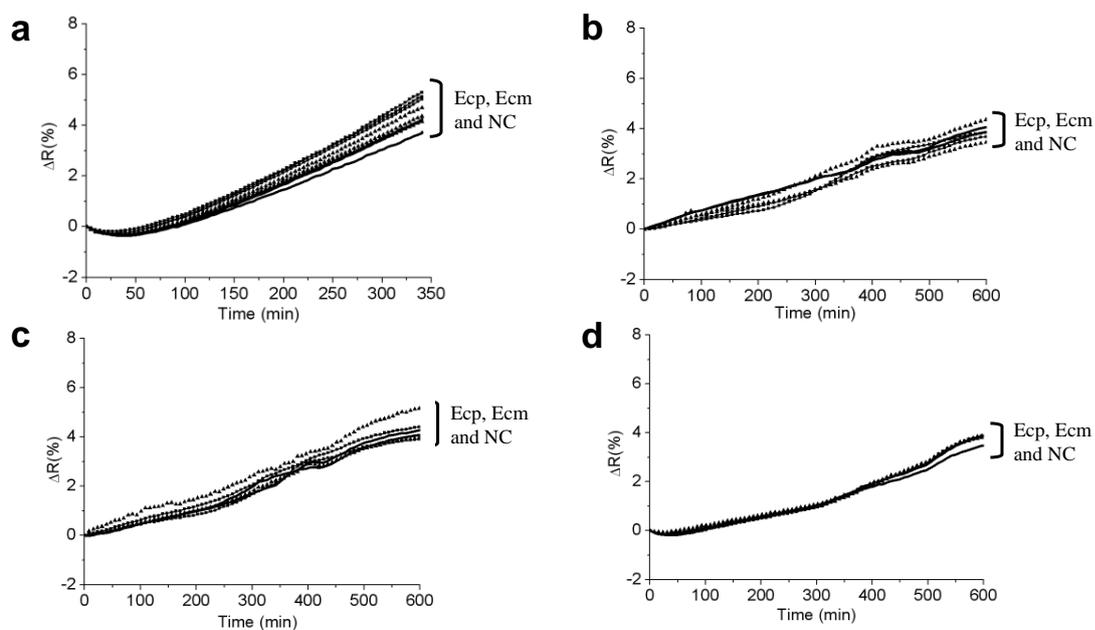
Detection after an overnight culture of *E.coli* O157:H7 in stressed (lines with solid symbols) and in optimal conditions (TSB, 37°C – lines with empty symbols). The solid lines represent negative control (NC) detection signals. Each curve represents an average of triplicate. Vertical arrows show the time necessary to reliably detect *E.coli* O157:H7 ( $T_t$ ). **(a)** Detection of *E.coli* O157:H7 after 4°C stress. Monitoring of growth after an overnight culture at 4°C (black circles) and in optimal conditions (TSB, 37°C- empty circles) of *E.coli* O157:H7 (14 $\pm$ 6 and 22 $\pm$ 5 CFU/mL at the beginning of the experiment, respectively). **(b)** Detection of *E.coli* O157:H7 after salt stress. Monitoring of growth after an overnight culture of *E.coli* O157:H7 in growth medium with 0 (line with empty circles), 50 (line with black circles) and 90 mg/ml (line with black squares) of added salt (NaCl) (19 $\pm$ 6, 17 $\pm$ 6 and 3 $\pm$ 1 CFU/mL at the beginning of each experiment, respectively).



**Fig. 4:** Detection of *E.coli* O157:H7 in complex food matrices.

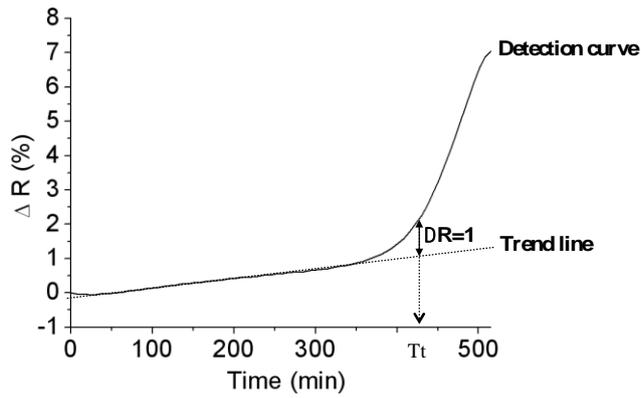
Line with black squares correspond to *E.coli* O157:H7 monoclonal antibody signals (Ecm), line with black circles to *E.coli* O157:H7 polyclonal antibody signals (Ecp) and solid line to negative control (NC) detection signals. Vertical arrows show the  $T_t$ . **(a)** Detection of *E.coli* O157:H7 bacteria stressed at 4°C in milk (160 $\pm$ 65 CFU/mL at the beginning of the experiment). **(b)** Detection of *E.coli* O157:H7 bacteria stressed at 4°C in meat (560 $\pm$ 600 CFU/mL at the beginning of the experiment).

## Supplemental information



**Fig S1:** Specificity of *E. coli* O157:H7 biosensor

—■— = Ecp: anti-*E. coli* O157:H7 polyclonal antibodies; — = Ecm: anti-*E. coli* O157:H7 monoclonal antibodies; ▲▲▲ = NC: negative control antibodies. **(a)** Culture of *Salmonella* Enteritidis (455+/-271 CFU/mL). **(b)** Culture of *E. coli* O26:H32 (339+/-135 CFU/mL). **(c)** Culture of *E. coli* O121:H19 (453+/-91 CFU/mL). **(d)** Culture of *E. coli* O103 (128+/-55 CFU/mL). *E. coli* O26:H32, *E. coli* O121:H19 and *E. coli* O103 were provided by P. Fach team at ANSES and were cultivated in same conditions than *E. coli* O157:H7.



**Fig S2:** Determination of the Time threshold ( $T_t$ ). The  $T_t$  correspond to the time necessary to increase the signal of an average of three curves corresponding to the reflectivity of three Ecp spots on the array, by 1% from the DR of the trend curve. A  $DR > 1\%$  is significant for bacterial detection, it is largely superior to the SPR detection threshold (Bouguelia et al., 2013; Daniel et al., 2013).