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ORIGINAL ARTICLE

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

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

Keywords
EHEC (enterohaemorrhagic *Escherichia coli*), food safety, identification, microarray, rapid methods.

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Abstract

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

Introduction

Shiga-toxin producing *Escherichia coli* (STEC) are responsible for numerous outbreaks traced to consumption of contaminated foods and beverages (Rangel *et al.* 2005; Vogt and Dippold 2005; Espie *et al.* 2006). Among them, *E. coli* O157:H7 has been the first enterohemorrhagic *E. coli* (EHEC) characterized as a human pathogen in 1982 after an outbreak involving contaminated burgers in the U.S. (Riley *et al.* 1983). Several foods have been identified as potential sources of STEC contamination since this first crisis: sprouts, leafy greens (lettuce, spinach), hamburgers, undercooked meat, apple juice, raw milk products and beverages, including tap water for instance (Riley *et al.* 1983; Griffin and Tauxe 1991; Swerdlow *et al.* 1992; Centers for Disease and Prevention 1993, Centers for Disease and Prevention 1996, Como-Sebetti *et al.* 1997). But the main food source and reservoir of the

pathogen is beef. USDA has proposed rules to carry out verification procedures, including sampling and testing product components, to ensure control of both *E. coli* O157:H7 and six other serogroups of STEC (O26, O45, O103, O111, O121 and O145) (named Top 7 STEC) (Hodges 2012; USDA 2014). Because *E. coli* is a common bacterium whose species consist of a very large number of nonpathogenic serovars, the specific detection of STEC serogroups remains a challenging analysis for food suppliers. When present in a sample, STEC have severe impact of human health even at very low contaminating levels (less than an hundred of CFU per sample) thus requiring a systematic pre-enrichment step before running any bacterial assay. So far, routine analysis and identification of STEC serogroups is typically done by culture plating methods, PCR-based analysis or immunoassays like ELISA techniques or latex agglutination. Culture methods are based on plating of pre-enriched samples on media enabling isolation of *E. coli*. Standard culture procedures require a pre-enrichment step (Beutin and Fach 2014); selective enrichment in solution followed by selective plating before colony isolation and further characterization (Ge and Meng 2009). Due to close metabolic properties of O157 and non-O157, their differentiation remains challenging although sorbitol consumption turned out to help somehow in their characterization (Bettelheim 1998): as only O157 has been shown to be sorbitol negative, this specificity can be exploited as a phenotypic sorting parameter on solid culture media. Some chromogenic solid media have also been described for distinguishing O157 from non-O157 STEC (Tillman *et al.* 2012). PCR-based methods showed very specific responses by detecting either O-serogroup and H-type specific sequences, virulence genes, or both (Perelle *et al.* 2004; Bugarel *et al.* 2010; Fratamico *et al.* 2011; Fratamico and Bagi 2012; Wasilenko *et al.* 2014). Immunological methods led in different formats have also been done (immuno-magnetic separation, colony immunoblot, agglutination) with processing times ranging from 20 min to 4 h but systematically requiring an overnight enrichment (Gould *et al.* 2009; Wang *et al.* 2013). Interestingly, label-based immunoassays have also successfully been described for the detection of clinically relevant STEC serogroups (Lin *et al.* 2013). In this end-point analysis, fluorescent nanoparticles enable the identification of pathogenic *E. coli*. The same year, an alternative label-free immunoassay coupled to Surface Plasmon Resonance (SPR) imaging allowing the fast detection of viable bacteria present in food samples without preliminary sample enrichment has been published (Bouguelia *et al.* 2013). Although other groups described the STEC detection using SPR (Subramanian *et al.* 2006; Waswa *et al.* 2007; Wang *et al.* 2013), the sensitivities published so far for the real-time pathogen detection are in the range of $3{\cdot}10^3$ to 10^6 CFU ml^{-1}, except for Bouguelia *et al.* (2013) who managed to detect food-contaminated samples at very low levels (<5 CFU per gram of food). Their approach is based on the microarraying, on a gold-covered biochip, of antibodies targeting surface antigens, followed by bacterial enrichment on-the-chip. The simultaneous monitoring of the microarray by SPR imaging in a one-step process enabled the continuous monitoring of the bacterial concentration increase along with semi-quantitative data on the initial concentrations. More recently, this Culture/Capture/Measure (CCM) process was successfully applied on stressed *E. coli* strains contaminating food samples (Mondani *et al.* 2014). By probing the environment close to the biosensors (few hundreds of nanometers), SPR imaging also turned out to be a powerful technique for cell analysis or bacterial biofilm monitoring (Abadian *et al.* 2014a,b). With the aim of moving one step forward, we wish to present a microarray platform dedicated to the analysis of the most common STEC serogroups, processed in a single culture medium and suitable for samples with or without an enrichment step. In the specific case where contaminating levels are low (<5 CFU per sample), the STEC detection is carried during the enrichment phase, thus significantly decreasing the processing time.

Results and discussion

Antibody microarraying and biochip processing

In order to specifically detect and identify one—or several—STEC serogroups present in a sample, polyclonal antibodies were electrochemically arrayed on the SPR biochip. Thanks to the small size of each feature (<500 μm in diameter), two identical series of antibodies (anti-*E. coli* O26, O45, O103, O111, O121, O145, O157:H7, and control antibodies) could be spotted on a single device, enabling the analysis of two samples loaded in two chambers fitting each array (Fig. 1). In order to assess the inter-spot variability during the microarraying, each antibody was arrayed in duplicate. The selectivity of the antibody response was also compared to background signals measured on negative controls (anti-KLH and anti-botulinum toxin B monoclonal antibodies). According to the CCM method (Bouguelia *et al.* 2013), samples were spiked with measured concentrations of STEC strains from various serogroups, and directly deposited on the biochip located in a thermalized SPR imager (37°C) without further operation. Regions of interest corresponding to individual microarrayed antibodies were defined and individual responses were monitored during bacterial on-chip culture. Sharp SPR specific responses were observed

at different characteristic times, thus giving semi-quantitative information on the initial pathogen concentration (Bouguelia *et al.* 2013; Mondani *et al.* 2014). Figure 2 shows the specificity of our method for anti-O145 antibodies. Raw data were monitored on the antibody microarray after spiking with 2 ± 1 CFU ml^{-1} of *E. coli* O145:H- ED28. A specific increase in the signal corresponding to anti-O145 antibodies was recorded after approx. 8 h, which corresponds to the growth of 2 ± 1 CFU ml^{-1} present at the beginning of the experiment, and confirmed by plating and counting on agar plates. The similar responses between each spot arrayed with anti-O145 antibodies confirmed the low inter-spot variability. SPR responses monitored on irrelevant anti-O antigen antibodies, anti-KLH (NC1), anti-Btox (NC2) and polypyrrole (NC3 spots) are slightly increasing upon time, probably due to a shift of the culture medium optical index upon bacterial culture. Similar results and specific responses were obtained after medium spiking with the bacterial strains listed in Table 1.

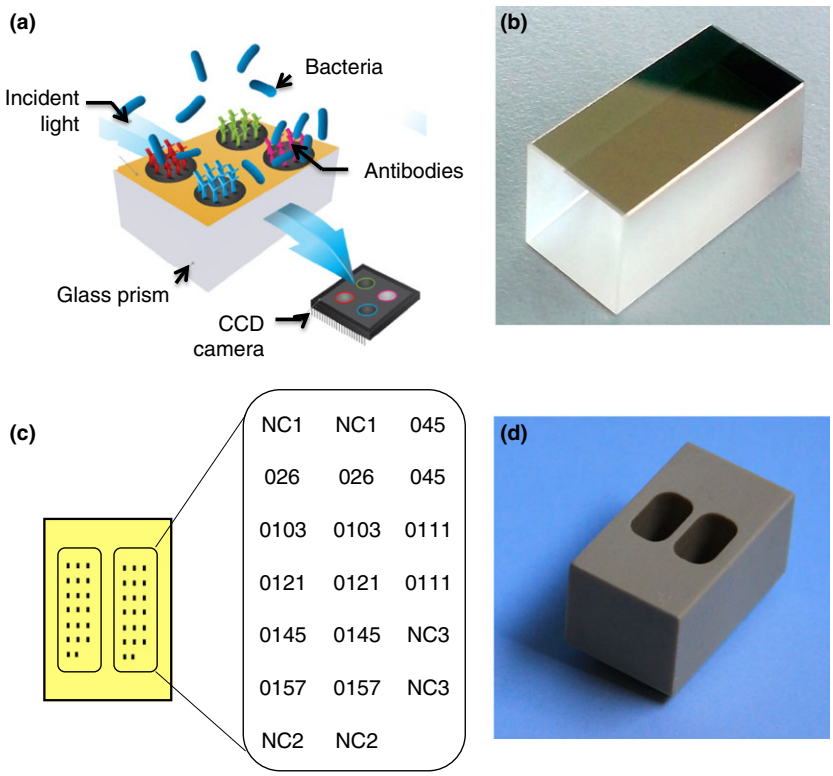

Figure 1 Microarray design and analysis by Surface Plasmon Resonance Imaging (SPRi). (a) Schematic representation of the device and instrumental setup. (b) Photograph of a SPRi biochip. The gold layer is 25 × 12 mm large and 50 nm thick. The glass prism height is 12 mm. (c) Mapping of the microarrayed antibodies. The biochip contains two identical series of antibodies. One sample is then loaded on each series. OXX legends are corresponding to *Escherichia coli* O-antigen specific antibodies. NC (Negative Controls) spots are antibodies nonspecific to *E. coli* and are used to assess the nonspecific response. Each antibody is arrayed in duplicate. (d) Photograph of the two separate chambers fitting the microarrayed antibodies. The volume of liquid added to the chamber is 500 μL.

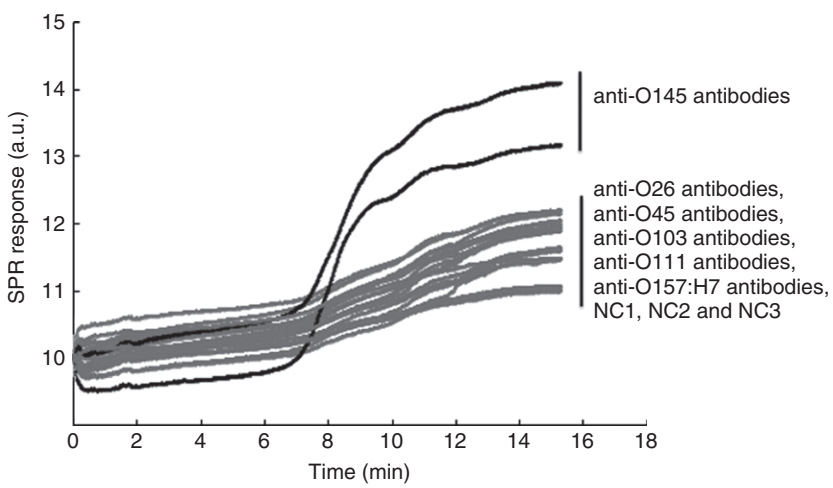

Figure 2 SPR responses plotted upon time after bacteria incubation on a microarray. 500 μl of TSB sample was spiked with 2 ± 1 CFU ml^{-1} of *Escherichia coli* O145:H-ED28 and incubated at 37°C in the SPR imager. Raw data of every individual antibody spot was plotted to assess both the response specificity and the inter-spot variability.

Table 1 List of *Escherichia coli* strains used for the immunoassay

			Gene coding		
Serotype	Strain	Source	stxl	stx2	eae
E. coli O4 :H5	MB05	ANSES (France)	–	–	–
E. coli O26:H11	H19	USA	+	–	+
	90·0105	USA	+	–	+
E. coli O45:H2	A9619-C2-83	USA	+	–	+
	2010C-4211	USA	+	–	+
E. coli O103:H2	ED172	Italy	+	–	+
	336S89	Belgium	+	–	–
	85-250	Canada	+	–	+
E. coli O111: H-	Ec1-Can	Canada	+	–	+
	Ec564	Canada	+	+	+
E. coli O121:H19	MB57	ANSES (France)	–	–	+
E. coli O132:H18	NV118	ANSES (France)	–	–	–
E. coli O145:H-	ED28	Italy	+	–	+
	VTH34	Spain	–	+	+
E. coli O157:H7	EC1	ANSES (France)	+	+	+
	8624	USA	–	+	+
	CIP105917	Institut Pasteur	–	–	–

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

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

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

With the aim of proposing one single assay for the detection of any STEC serogroups (O157 and non-O157 strains), we developed a standardized protocol for sample incubation and characterization on the chip (Table 2). Each strain depicting the Top 7 STEC was assayed in TSB medium spiked at different concentrations and both O157 and non-O157 strains were successfully detected. One series of assays (few CFU ml^{-1} per sample) corresponds to the minimum concentration range encountered in naturally contaminated samples, while the other series (concentrations >15 000 CFU ml^{-1}) is similar to the expected concentrations found after an enrichment step led on a contaminated sample. Interestingly, this approach successfully enabled the specific detection of every STEC serogroups assayed for any initial concentration assayed. On a quantitative point of view, signal analysis of each curve enabled the measurement of detection times only for positive antibody features. Detection times measured for each concentration are consistent with the detection of viable *E. coli* dividing and multiplying on the biochip, as longer detection times were obtained with the lowest seeding STEC concentrations. With the aim of counting contaminating levels, it has been shown that calibration curves can be plotted to extract initial concentrations from detection times (Bouguelia *et al.* 2013). Interestingly, detection times measured for low seeding concentrations (1 CFU ml^{-1}) showed low disparity between the tested strains (detection time at 8 h 20 min ± 2 h). Although an highly specific detection of several samples defining STEC serogroups was noticed on the antibody microarray (O26:H11; O45:H2; O111:H-; O145:H-), some cross-reactivity of few STEC (O103:H2, O111:H-, O121:H19 and O157:H7) was observed, more frequently when the analysis was run with low bacterial concentrations. Such side effect might be due to the use of polyclonal antibodies, containing a mixture of antibodies with some of them being specific to epitopes common to several serogroups. As already mentioned, this cross-reactivity was not observed on strains belonging to other serogroups than the Top7 STEC serogroups, thus demonstrating that the use of polyclonal antibodies does not favour the appearance of false-positive responses. Eventually, the situation where natural nonpathogenic *E. coli* flora (O127:H6) was present in an STEC O157:H7 contaminated sample was also tested. The real-time immunoassay enabled the specific detection of a STEC serogroup present at low levels regarding a non-STEC strain (1 : 7 ratio). Such results confirm the suitability of this approach for the detection of STEC present at low concentrations (about 10 CFU per sample) although harmless *E. coli* strain is present at higher levels. The detection time (8 h) was consistent with the values observed in single-strain samples contaminated at similar levels, which shows that the overall process was not delayed by the presence of several *E. coli* strains in a single sample.

Table 2 Screening of O157 and non-O157 Shiga-toxin producing *Escherichia coli* (STEC) on an antibody microarray

Strains		CFU/	Targeted antigens							Negative controls			
Serotype	Other nomenclature	sample 500 μl	Anti-O26	Anti-O45	Anti-O103	Anti-O111	Anti-O121	Anti-O145	Anti-O157:H7	Anti-Btox	Anti-KLH	Ppy	Detection time
O4:H5	MB05	1475	–	–	–	–	–	–	–	–	–	–	–
O26:H11	H19	24 900	+	–	–	–	–	–	–	–	–	–	3 h
		2	+	–	–	–	–	–	–	–	–	–	7 h 45 min
	90·0105	20 750	+	–	–	–	–	–	–	–	–	–	3 h
		2	+	–	–	–	–	–	–	–	–	–	7 h 45 min
O45:H2	A9619-C2-83	8500	–	+	–	–	–	–	–	–	–	–	3 h 30 min
		1	–	+	–	–	–	–	–	–	–	–	8 h 30 min
	2010C-4211	3800	–	+	–	–	–	–	–	–	–	–	3 h
		1	–	+	–	–	–	–	–	–	–	–	12 h 30 min
O103:H2	ED172	11 350	–	–	+	–	+	+	–	–	–	–	3 h
		1	–	–	+	–	+	+	–	–	–	–	8 h
	85-250	16 500	–	–	+	–	+	–	–	–	–	–	3 h
		1	–	–	+	–	+	–	–	–	–	–	7 h
	336S89	22 250	–	–	+	–	+	–	–	–	–	–	3 h
		2	–	–	+	–	+	–	–	–	–	–	4 h 30 min
O111:H-	Ec1-Can	7850	–	–	–	+	–	–	–	–	–	–	3 h 30 min
		1	–	–	–	+	–	–	–	–	–	–	8 h
	Ec564	14 500	–	–	–	+	–	–	–	–	–	–	3 h 15 min
		1	–	–	–	+	–	–	+	–	–	–	7 h 30 min
O121:H19	MB57	1332	–	–	–	–	+	+	–	–	–	–	2 h 40 min
		1470	–	–	–	–	+	+	–	–	–	–	2 h 50 min
O132:H18	NV118	103	–	–	–	–	–	–	–	–	–	–	–
O145:H-	ED28	22 600	–	–	–	–	–	+	–	–	–	–	4 h
		2	–	–	–	–	–	+	–	–	–	–	8 h
	VTH34	30 350	–	–	–	–	–	+	–	–	–	–	2 h 30 min
		3	–	–	–	–	–	+	–	–	–	–	6 h 30 min
O157:H7	O157 Ec1	8250	–	–	–	–	–	–	+	–	–	–	2 h 15 min
		1	–	–	–	+	–	–	+	–	–	–	6 h 30 min
	8624	120 050	–	–	–	–	–	–	+	–	–	–	2 h
		12	+	–	–	–	–	–	+	–	–	–	8 h
	CIP 105917	493	+	–	–	+	–	–	+	–	–	–	6 h 30 min
		5	+	–	–	+	–	–	+	–	–	–	7 h
Mix O127:H6 (E2348/69)/ O157:H7 (8624)		7315/11	–	–	+	–	–	–	+	–	–	–	8 h

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

Real-time optical immunoassay with food samples contaminated with *Escherichia coli* O157:H7

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

As a conclusion, the antibody array method was able to detect and identify *E. coli* strains carrying the regulated Top 7 STEC O-antigens (O157, O145, O121, O103, O111, O45, O26) therefore it could be used as a rapid screening approach to identify presumptive positive samples while a confirmation step with conventional immunological or PCR tests will definitely confirm the serogroup. On the contrary to other immunoassays involving labelling steps and end-point analysis (Lin *et al.* 2013), our label-free approach allows real-time analysis, faster detection and a reduced cost by avoiding expensive labels. We did not

Table 3 Detection of *Escherichia coli* O157:H7 contaminated meat

Sample	Strain	Inoculum (CFU/25 g)	Result	Time-to-result (hours)
Ground Beef	*E. coli* O157:H7	2·5 ± 1	+	9
Ground Beef	*E. coli* O157:H8	2·5 ± 1	+	12
Ground Beef	*E. coli* O157:H9	0	–	–
Ground Beef	*E. coli* O157:H10	0	–	–

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

observe any bias due to bacterial mobility (strains lacking flagella), virulence factors (stx1, stx2 and eae). The antibody microarray specificity was challenged on 15 different bacterial strains defining the top-7 EHEC serogroups. Our results showed no cross-reactivity with non-EHEC serogroups although some STEC strains cross-reacted with other polyclonal anti-STEC antibodies. The presence of polyclonal antibodies targeting a large repertoire of antigens is a plausible explanation of this minor side effect. On the contrary to regular culture methods where the discrimination of O157 from non-O157 STEC using dedicated culture medium is still controversial (Vimont *et al.* 2007), our approach enabled the characterization of any assayed STEC serogroups with simple culture medium (TSB) incubated at 37°C. This real-time detection is based on the continuous and label-free monitoring of bacterial growth and is thus highly specific to viable bacteria. For this reason, dead cells would not interfere with the biochip and does not lead to false positive as it may happen for PCR-based techniques. More importantly, this method showed similar results for any chosen concentration of bacteria. As other methods require up to 48 h for sample preparation before getting into analysis, one can reasonably expect that such analysis based on sample analysis during the enrichment phase could pave the way to an alternative confirmation method. For instance, it could be led by combining our approach with a method enabling, for instance, the Shiga-toxin gene detection from enriched aliquots processed on the antibody microarray. In such case, our approach could be used as a fast and cost-effective prescreening approach where only positive samples are then fully characterized by PCR-based gene analysis.

Materials and methods

Bacterial strains and culture conditions

Escherichia coli strain O157:H7 CIP 105917 was provided by the Institut Pasteur (Paris, France); *E. coli* strains O4:H5 MB05, O26:H11 (H19 and 90·0105), O45:H2 (A9619-C2-83 and 2010C-4211), O103:H2 (ED172, 336S89 and 85–250), O111:H- (Ec1-Can and Ec564), O121:H19 MB57, O132:H18 NV118, O145:H- (ED28 and VTH34) and O157:H7 (EC1 and 8624) were from ANSES collection (Maisons-Alfort, France). The bacterial characterizations are summarized in Table 1. All *E. coli* strains were grown overnight in optimal culture conditions, at 37°C, 100 rev min^{-1} in Tryptic Soy Broth medium (TSB, Fluka). Brain Heart Infusion (BHI) was purchased from BIOKKAR Diagnostics (Beauvais, France). Turbidity of liquid cultures was adjusted with a McFarland densitometer (Grant Instruments, Cambridge, UK) to 1·0 McFarland (approx. $3{\cdot}10^8$ CFU ml^{-1}). Serial dilutions were performed in PBS to obtain the desired bacterial concentrations. Bacterial counting was carried out after plating and culture on Trypticase Soy Agar plates (AES, France) according to standard protocols.

Antibodies microarraying on SPR biochips

For specific *E. coli* detection, affinity-purified goat anti-*E. coli* O26, O45, O103, O111, O121, O145 and O157:H7 polyclonal antibodies were purchased from Kirkegaard & Perry Laboratories Inc. (BacTrace®, Gaithersburg, MA). A monoclonal anti-KLH and a monoclonal anti-Botulinum toxin (anti-Btox) (gifts from L. Bellanger CEA-Marcoule, France) were used as general negative controls (respectively NC1 and NC2). Before running the electrochemical arraying (Grosjean *et al.* 2005), proteins were washed and covalently coupled to pyrrole monomers (N-Hydroxysuccinimide-Pyrrole, NHS-Pyrrole) as described elsewhere (Suraniti *et al.* 2007; Mondani *et al.* 2014). Polypyrrole was also arrayed and used as a reference control (NC3). SPRi biochips (obtained from Prestodiag, Villejuif, France) are made of optical glass, covered by a 50 nm thick gold layer deposited on a 2 nm thick Cr. Antibody-pyrrole conjugates (1 μmol l^{-1}) were robotically arrayed on gold-covered biochip by electro-chemical polymerization in presence of a pyrrole excess (20 mmol l^{-1} in acetonitrile). Duplicates of each protein were spotted on the gold surface. Electro- copolymerization of both free pyrrole and pyrrole-modified proteins on the biochips was carried out in a pipette tip filled with the solution to be polymerized and containing a platinum wire used as a counter electrode. The pipette tip was moved at the vicinity of the gold layer, till an electrical contact was enabled between the working (gold surface) and counter (platinum wire) electrodes. The polymerization on the prism gold layer was performed with a 100 ms electric pulse at a 2·0 V bias independently of any reference electrode. This electrochemical process ensures the gentle and long-lasting grafting of biomolecules on SPR biochips. The spot size of each antibody feature is about 300–500 μm in diameter. Following the microarraying, biochips were washed twice with PBS and stored in PBS at 4°C. Before

SPRi experiment, the biochip was blocked for 15 min at room temperature with 1% (w/v) Bovine Serum Albumin (Sigma-Aldrich, Saint Quentin Fallavier, France) in PBS, and then washed with culture medium.

SPR imaging and analysis

SPR measurements were performed using a MonoPresto SPR imager prototype (Prestodiag, Villejuif, France) based on PlasmIA™ (Plasmonic ImmunoAssay) technology, data were processed with a dedicated software (KINETIKA ver. 3.0.5, Thermo Fisher Scientific, Waltham, MA), and Regions Of Interest (ROI) corresponding to individual spots microarrayed on the biochips were defined. The SPR signal was monitored with a CCD camera, and greyscale changes of each ROI were followed and plotted over time. Regarding data analysis, first-order derivative of each curve was realized and the maximum of the derivative corresponding to the inflection point of kinetic curve was characterized as the detection time. Two identical series of proteins were arrayed on each biochip and combined to a reaction chamber, thereby allowing duplicate analysis. A double reaction chamber was used to simultaneously run two experiments on a single device. Samples were prepared as described in the following section and loaded without any further treatment on the biochip: 500 μl of bacterial solutions were loaded in thermalized (37°C) SPR reader and chambers were closed by a porous film to limit evaporation. Then, real-time monitoring of signals corresponding to individual ROI were recorded and displayed. After 16 h, the experiment was stopped, the sample was neutralized by adding a diluted bleach solution (1/10) and data were processed to extract the detection times. Control viability experiments were simultaneously carried out for each SPR assay by monitoring cell mortality observed in control sample incubated in similar conditions.

Sample preparation

Detection of *E. coli* O157:H7 was also assessed in an artificially contaminated ground-beef sample: ground beef with 15% fat was purchased from a local supermarket (Monoprix, Villejuif). 25 g portions of meat were weighted and artificially contaminated with tenfold serially diluted bacterial solutions (in PBS) to obtain the expected final contaminating levels. Both control and spiked ground meat were then stored for 2 days at 2–8°C to mimic usual stress conditions. Bacterial counts were performed by plating the inoculum in duplicate on TSA plates. The artificially contaminated samples were put into four sterile blending bags including a fluidic connector (Prestodiag, Villejuif, France). After dilution by adding 225 ml of buffered peptone water, bags were homogenized for 60 s using a peristaltic blender (BioMérieux, Lyon, France). The bags were then sealed and connected using biochips arrayed with anti-*E. coli* polyclonal antibodies as previously described. The samples and kit were placed on a Monopresto SPR imager in an incubator at 37°C (France Etuves, France), and continuous reading of the signal was performed overnight.

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Conflict of Interest

No conflict of interest to declare.

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