

Quantum dot-based array for sensitive detection of *Escherichia coli*

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Abstract A fluorescent quantum dot-based antibody array, used in sandwich format, has been developed to detect *Escherichia coli* O157:H7. Numerous parameters such as solid support, optimal concentration of immunoreagents, blocking reagents, and assay time were optimized for array construction. Quantum dot-conjugated anti-IgG was used as the detecting system. The array allows the detection of *E. coli* O157:H7 at concentrations below 10 CFU mL⁻¹ without sample enrichment, exhibiting an increase of three

orders of magnitude in the limit of detection compared to ELISA. The interference caused by Gram (+) and Gram (-) bacteria was negligible at low concentrations of bacteria.

Keywords Bacteria · *E. coli* · Antibody array · Quantum dot

Introduction

Reliable methods for rapid, sensitive, and selective detection of pathogens are critical to ensure the safety of food supplies and to diagnose infectious bacterial diseases accurately. For certain matrices, particularly in clinical diagnostics, single-cell detection would be desirable, since even such a low concentration of bacteria indicates disease. A good example is the human pathogen *Escherichia coli* O157:H7, a Shiga toxin-producing serotype [1] that has an infectious dose below ten viable cells [2]. *E. coli* O157:H7 is transmitted to humans through contaminated food [3], water [4] and spreads also by a zoonotic or person-to-person transmission route [5], causing severe enteric infections such as diarrhea, hemorrhagic colitis and hemolytic uremic syndrome.

Conventional identification techniques for pathogenic bacteria include among others culture and colony counting [6], and immunological methods [7]. Detection of *E. coli* O157:H7 has been done using enrichment and plating procedures with selective media [8]. Immunological methods have been also extensively used for the detection of *E. coli* O157:H7 due to their potential for high sample throughput. Despite the fact that these approaches are powerful and error-proof, most of them are laborious, complex, and time-consuming. Moreover, they do not afford the necessary specificity towards the target. Alternatively, biosensors

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offer several advantages over existing techniques (e.g., limited hands-on time, high-throughput screening, improved detectability, real-time analysis, and label-free detection). Recently, real-time PCR (polymerase chain reaction) assays have been developed targeting relevant bacteria. Thus, using pathogen-specific probes eight different bacterial strains/species were identified within 2 h [9]. In this line, a microfluidic chip for environmental water enrichment combine with real-time PRC was able to detect *E. coli* O157:H7 with and limit of detection (LOD) of 6 colony-forming units per milliliter (CFU mL⁻¹). Moreover, this system discriminated between the O157:H7 and other non-pathogenic bacteria [10]. However, to date, biosensor and real-time PCR systems cannot detect bacteria at concentrations below 10 CFU mL⁻¹ without bacteria pre-enrichment. Therefore, novel platforms that facilitate rapid, sensitive, and accurate identification of bacteria are needed.

Microarray technology represents a powerful analytical tool for the rapid and parallel detection of multiple analytes in a single device [11]. Antibody microarrays, which exploit the high specificity and sensitivity of antibody–antigen reactions, have enormous potential towards meeting many important criteria for use as bacteria detection method [12, 13]. Several examples of antibody arrays for bacterial cells have already been reported. Gehring et al. developed a sandwich fluorescent immunoassay in a microarray format to detect *E. coli* O157:H7 with LOD of 3.0×10^6 CFU mL⁻¹ [14]. Recently, Kim and co-workers developed a microflow cytometer for the multiplexed detection of *E. coli*, *Listeria*, and *Salmonella* with LOD values of 10³, 10⁵ and 10⁴ CFU mL⁻¹, respectively [15]. Simultaneous detection of pathogens has also been achieved using chemiluminescent antibody arrays, with limits of detection varying from 10³ to 10⁷ CFU mL⁻¹ [16, 17]. Recently, microarrays have been integrated in automated fluidic systems. I.e. *E. coli* O157:H7, *Legionella pneumophila* and *Salmonella typhimurium* were simultaneous detected using a multichannel flow-through chemiluminescence array [18].

Herein, we describe as a proof of concept the development of an antibody microarray that detects *E. coli* O157:H7 at concentrations below 10 CFU mL⁻¹ without bacteria pre-enrichment. Parameters such as detection limit, assay time and immunoreagent concentration of the quantum dot (QD)-based array were compared with those of conventional ELISA (enzyme-linked immunosorbent assay), demonstrating the superior performance of the antibody array. The improved sensitivity of the array can be attributed to the smaller concentration of capture antibody in the array compare to the ELISA and the advantages of incorporating a QD-conjugated anti-IgG as transduction/detection mode.

Materials and methods

General methods and instruments

The pH and conductivity of all buffers were measured with a pH meter 540 GLP and a LF 340 conductimeter, respectively (WTW, Weilheim, Germany). Capture antibody dilutions were spotted onto slides using a BioOdyssey Calligrapher MiniArrayer (Bio-Rad Laboratories, Inc. USA). Measurements were recorded on a *ScanArray Gx PLUS* (Perkin Elmer, USA). Quantum dot-conjugated anti-IgG fluorescence was measured using a Fluorimeter® Gemini XPS (Molecular Devices Sunnyvale, CA, USA). ELISA washing steps were carried out using a SLY96 PW microplate washer (SLT Labinstruments GmbH, Salzburg, Austria). Absorbances were read using a SpectramaxPlus (Molecular Devices) at a single wave length mode of 595 nm. The standard curves were analyzed with a quadratic polynomial equation using the software Softmax-Pro v2.7 (Molecular Devices) and GraphPad Prism v 4 (Graphpad Software Inc., San Diego, CA).

Materials, chemicals and immunoreagents

Poly-L-lysine coated microscope slides were purchased from Polysciences, Inc. (Eppelheim, Germany) and the nitrocellulose slides from Schott (Jena, Germany). Carboxyl quantum dots were from Invitrogen (Qdot® 605 ITK™; Eugene, OR). Capture antibody anti-*E. coli* O157:H7 was from Affinity Bioreagents (PA1-7213; Golden, CO, USA). Detection antibody anti-*E. coli* was purchased from Abcam (ab20425; Abcam, Cambridge, UK). The anti-rabbit IgG and chemicals were obtained from Sigma-Aldrich (Buchs, Switzerland).

Bacterial samples

E. coli O157:H7 (KPL, Gaithersburg, MD, USA, Cat. 50-95-90). *E. coli* K12, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were a kind gift from the group Unitat de Microbiologia Aplicada of the Universitat Autònoma de Barcelona.

Buffers

Phosphate-buffered saline (PBS), 10 mM, is the phosphate buffer, 0.8% saline solution, pH 7.5. PBST is PBS with 0.05% Tween 20. Borate buffer is 10 mM sodium borate, pH 7.4. Coating buffer is 50 mM carbonate-bicarbonate buffer, pH 9.6. Citrate buffer is a 40 mM solution of sodium citrate, pH 5.5. The ELISA substrate solution contains 0.01% tetramethylbenzidine and 0.004% H₂O₂ in citrate buffer.

QD conjugation to anti-IgG

The conjugation protocol has been described elsewhere [19]. The QD: anti-IgG: *N*-Ethyl-*N'*-dimethylaminopropyl-carbodiimide hydrochloride (EDC) ratio used was 1:3:1500. Briefly, carboxyl-coated QD605 stock solution (8 μ M, 62.5 μ L) was blended in 400 μ L 10 mM borate buffer and mixed with the anti-IgG (6.7 μ M, 225 μ L). EDC (5.2 mM, 136 μ L) was added to the reaction mixture that was gently stirred for 2 h at room temperature (RT). The reaction was quenched by addition of borate buffer (50 mM, 50 μ L, pH 9.0). QD-conjugated anti-IgG was separated from free anti-IgG and free quantum dots using a Superdex™ 200 prep grade column (GE Healthcare, Uppsala, Sweden) following the manufacturer's instructions. Bradford test absorbance of QD605 was measured to calculate its concentration $Abs=(\epsilon_{638}=8 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1})$. The molar ratio of antibody to QD in the conjugate was 1:10 (Fig. 1).

Antibody array preparation

Capture antibody to *E. coli* O157:H7 (500 μ g mL^{-1} , PBS) was spotted onto poly-L-lysine or nitrocellulose substrates using a BioOdyssey Calligrapher MiniArrayer in a high-humidity chamber (60%) and maintained at 20 °C for 2 h. Each glass slide contained 18 wells. A 3×4 spot matrix was printed on each well. The capture antibody was printed in triplicate on each array.

Slide processing

Slides were washed four times with PBST and blocked for 30 min with a 2% solution of gelatine in PBS

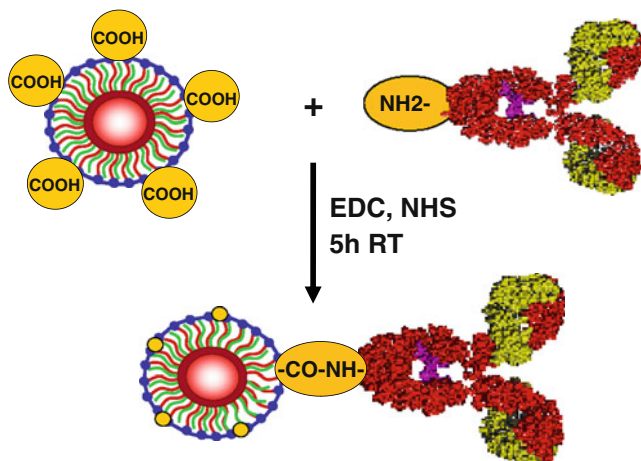


Fig. 1 QD conjugation to anti-IgG. QDs were covalently linked to anti-IgG by using the EDC chemistry. By a strict control of the reaction time, we obtained a reproducible anti-IgG:QD ratio of 1:10

(100 μ L/well). After washing, as described above, concentrations of heat-killed *E. coli* O157:H7 varying from 10^5 to 1 CFU mL^{-1} were added to each well (100 μ L/well, PBS). Following a 30-min incubation, slides were washed and detection antibody to *E. coli* O157:H7 was added (8 μ g) in PBST for 30 min (100 μ L/well). Slides were washed again and subsequently incubated with QD-conjugated anti-IgG. After 30 min, slides were washed and dried with N_2 . Measurements were recorded on a ScanArray Gx PLUS with a Cy3 optical filter with 10 μ m resolution. The laser power and PMT were set to 90% and 70%, respectively. The spots were measured by F543_Mean-B543 (Mean Cy3 foreground intensity minus mean Cy3 background intensity). Fluorescence intensity values were expressed in relative units as average and standard deviation of three replicate wells. The standard curve was fitted to a quadratic polynomial equation as indicated by Herman et al [20] and the LOD was calculated using the statistical approach reported by Long and Winefordner [21]. All steps were performed at RT.

Sandwich ELISA

Ninety-six-well microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were coated overnight at 4 °C with 100 μ L/well of the capture antibody to *E. coli* O157:H7 at 1 μ g mL^{-1} in 50 mM carbonate buffer, pH 9.6. The plates were washed five times with 300 μ L of PBST. Serial concentrations of heat-killed *E. coli* O157:H7 diluted in 100 μ L of PBS were added and incubated for 30 min. After washing as described above, 100 μ L of HRP-labeled detection antibody to *E. coli* were added to each well (0.5 μ g mL^{-1} ; PBST) and incubated for 30 min. Following washing, a substrate solution for peroxidase (100 μ L/well; 40 mM sodium citrate pH 5.5 containing 0.01% tetramethylbenzidine, 0.004% H_2O_2) was added to the wells and left for 30 min at room temperature. Enzymatic reaction was stopped with 4 N H_2SO_4 (50 μ L/well). The absorbance was measured at 450 nm. The standard curve was fitted to a quadratic polynomial equation as described in the Slide Processing. All steps, but the coating, were performed at RT.

Cross-reactivity

To evaluate the cross-reactivity of the *E. coli* antibodies with other common pathogenic bacteria heat inactivated *E. coli* K12 (CECT 433), *P. aeruginosa* (CECT 110), *S. aureus* (CECT 5190), at 10^6 , 10^4 , and 10^2 CFU mL^{-1} , were subjected to the same slide/ELISA processing protocol.

Results

Development of the antibody array platform

The correct choice of a solid surface is a critical factor in the development of a protein array. The main requirements of the solid support are having high binding capacity, ability to retain antibody activity and high signal-to-noise ratio. Currently, there are a myriad of surface chemistries available for antibody arrays. From all of them, we evaluated the performance of nitrocellulose (diffusion-based), epoxy-silane (covalent binding) and poly-L-lysine (physical adsorption). For such purpose, we spotted serial dilutions of Qd-conjugated anti-IgG onto the different surfaces. As reported by other authors [16, 22], we observed that nitrocellulose slides performed poorly compared to epoxy-silane or poly-L-lysine due to high slide noise. Between, epoxy-silane and poly-L-lysine, the latest gave the greatest degree of binding along with minimal background (signal-to-noise ratio, 1.3:1, 20:1, 25:1, for nitrocellulose, epoxy-silane and poly-L-lysine, respectively). Therefore, we selected poly-L-lysine as substrate.

Choosing the appropriate spotting buffer is another relevant aspect that is normally disregarded in many studies. It is a common practice to use buffers containing glycerol [23, 24] to prevent microspot evaporation. In a comparative study performed with PBS buffer (10 mM, pH 7.5) containing a 10% of glycerol, we found that inclusion of a surfactant significantly diminished the signal of QD-conjugated anti-IgG spotted onto poly-L-lysine (data not shown). Moreover, spots without glycerol did not evaporate when incubated for 2 h at 60% humidity. We also tested PBS against buffers of different composition and pH (carbonate buffer 50 mM pH 9.6; PBS buffer (pH 8.5/0.01% sodium dodecyl sulfate). Nevertheless, PBS provided the most reproducible spot morphology (data not shown).

To reduce the non-specific binding of other immunoreagents in subsequent steps, we explored several blocking buffers in a 2D assay format, which allowed us to simultaneously adjust the optimal concentrations of the capture and the detection antibodies. Since albumins are known to non-specifically interact with QDs [23, 25], those proteins were discarded as blocking agents. We assayed 2% polyvinylpyrrolidone, 2% casein and 2% gelatine in PBS. Capture antibody was spotted at concentrations varying from 500 to 62.5 $\mu\text{g mL}^{-1}$. Detection antibody was added at 320, 160, 80 and 40 $\mu\text{g mL}^{-1}$. Experiments were carried out at a constant bacteria concentration of 10^7 CFU mL^{-1} and in its absence. We chose PBS as buffer, given that detergents inhibit the antibody–bacteria interaction. Results from this analysis indicated that 2% gelatine reduced non-specific interactions from 57% to 21% (see Fig. 2). Optimal

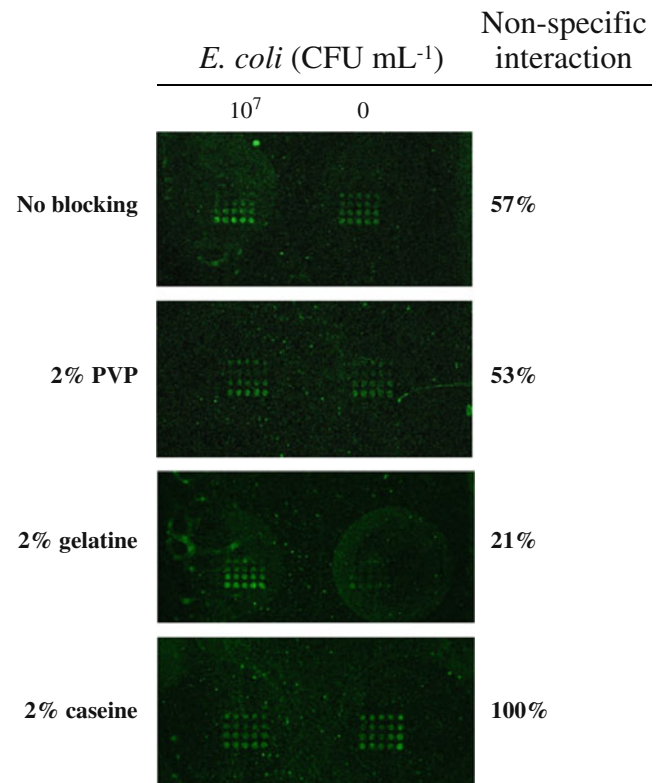


Fig. 2 Blocking agent study. PVP, casein and gelatine at 2% in PBS were tested to reduce the unspecific signal due to immunoreagent interactions. Non-specific interaction is indicated as the percentage of dividing the fluorescence without bacteria by that obtained with 10^7 CFU mL^{-1} of *E. coli*

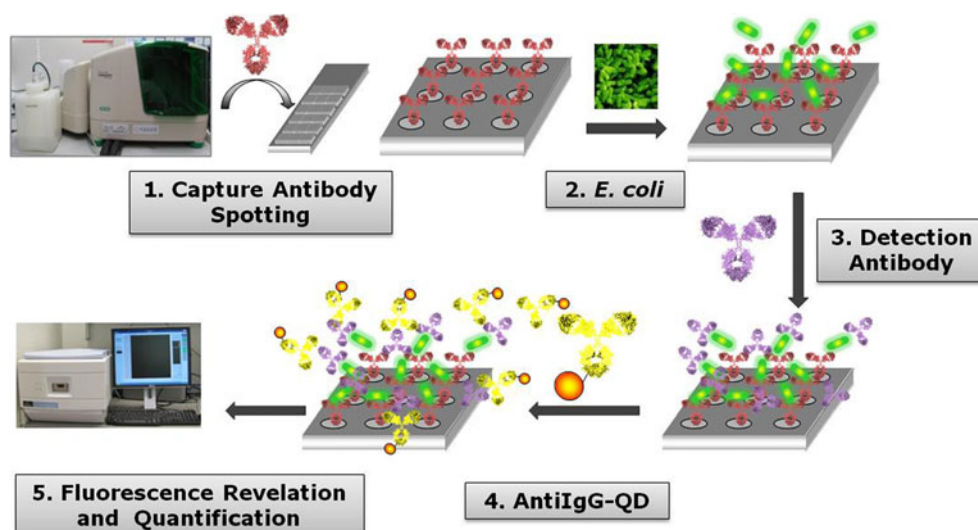
capture and detection antibody concentrations were set at 500 and 40 $\mu\text{g mL}^{-1}$, respectively.

Detection of *E. coli* O157:H7

Once the optimized conditions of the assay were established (Fig. 3; Table 1), we evaluated the capacity of the array to detect *E. coli* O157:H7. As shown in Fig. 4, the bacteria exhibited a dose-dependent response from 10^5 to 1 CFU mL^{-1} . Higher concentrations of bacteria displayed the same fluorescence as 10^5 CFU mL^{-1} , indicating saturation at that point. Low variability was observed between slides in experiments performed four times in different days. The estimated LOD was $1 \pm 0.001\%$.

The antibody microarray was then compared to a conventional ELISA. We carried out the ELISA assay using the same bacteria stock and antibodies as those employed in the array. Detection in the ELISA was accomplished by a horseradish-peroxidase-catalyzed reaction, instead of the fluorescence emission of QDs. Table 2 shows that by using the method proposed here, the time of assay was reduced from 15 to 2 h. What is more, antibody array LOD was three times lower than that of the ELISA.

Fig. 3 Schematic diagram of the antibody array strategy for the detection of *E. coli* O157:H7



This improvement can be attributed to the smaller amount of capture antibody used in the array compared to the ELISA. Additionally, detection provided by QD cannot be disregarded either. Nowadays, it is well-known that the superior characteristics of these nanoparticles as detection elements can increase, among many, the sensitivity of the microarray technology [26, 27].

Evaluation of cross-reactivity

To determine the specificity of the antibody array, we performed cross-reactivity tests against safe handle *E. coli* K12 (Gram-negative) and the pathogenic *S. aureus* (Gram-positive) and *P. aeruginosa* (Gram-negative). As shown in Fig. 5, the specificity of the *E. coli* O157:H7 antibody pair increased when decreasing the bacteria concentration with the exception of *S. aureus*, which showed interference independent of the concentration.

Discussion

In the past years, DNA and RNA microarrays have been successfully employed for pathogen detection [28–31]. Despite the fact that this strategy requires previous PCR

amplification, literature still shows a preference for nucleic acid over protein arrays. This is due to the more complex nature of proteins, which raises additional challenges in the fabrication of microarrays. In this context, a critical factor in protein arrays is choosing the most suitable type of reactive surface. This surface must preserve the native conformation, stability, and functionality of the proteins plus providing good reproducibility and homogeneity when attaching the proteins onto the solid substrate. Several surfaces have been employed such as two-dimensional slides onto which proteins and antibodies can be bind either by physical adsorption or covalent binding. As an alternative, diffusion-based of proteins into three-dimensional gel/membrane-coated surfaces can be used. In this study, we evaluated the performance of the three strategies: antibody covalent attachment to epoxy-silane modified slides, non-specific interaction onto poly-L-lysine and diffusion into nitrocellulose. We observed clear differences between two- and three-dimensional surfaces. Although a three-dimensional matrix preserves the protein activity and structure, we found that nitrocellulose showed a higher background than the two-dimensional surfaces: poly-L-lysine and epoxy-silane. This phenomenon has been attributed to non-specific antibody binding, limited protein mobility and inefficient washing and/or blocking due to the

Table 1 Antibody array fabrication and slide processing

Surface	Poly-L-lysine
Capture antibody	0.75 ng; 10 mM PBS pH: 7.5
Spotting buffer	10 mM PBS; 60% humidity
Incubation time	2 h; 60% humidity
Blocking	2% gelatine, 10 mM PBS
<i>E. coli</i> O157:H7	10^5 – 1 CFU mL ⁻¹ ; 10 mM PBS
Detection antibody	0.8 µg, 10 mM PBS, 0.05% Tween 20
Anti-IgG:QD ratio	1:10; 8 ng anti-IgG; 10 mM PBS, 0.05% Tween 20
Washing steps	10 mM PBS, 0.05% Tween 20

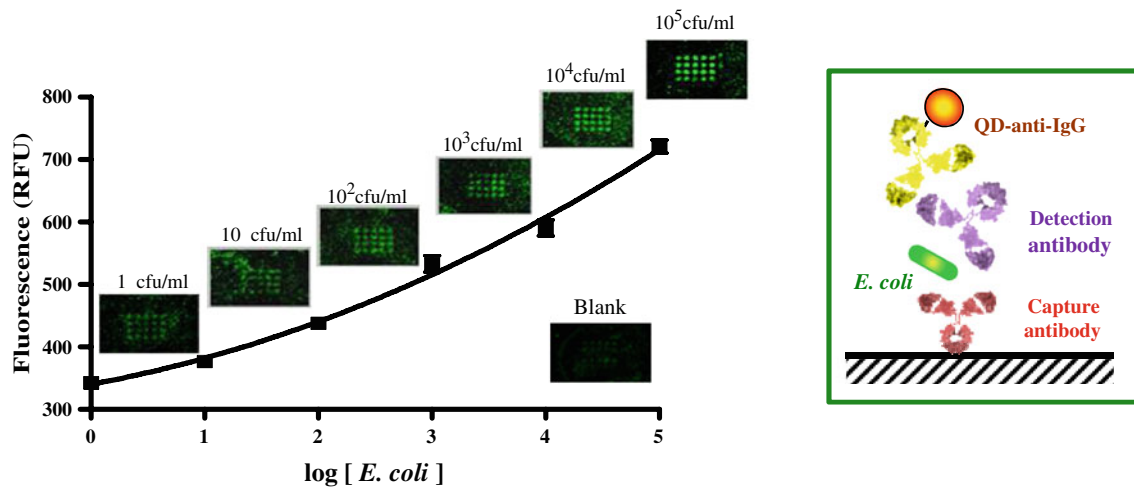


Fig. 4 Dose-response curve for *E. coli* O157:H7 in a sandwich array-based assay. The standard curve was fitted to a quadratic polynomial equation as indicated by Herman et al [20] and the LOD was

complexity of three-dimensional surfaces [32]. On the other hand, planar surfaces showed good performance, demonstrating that non-covalent chemistries can work as well as covalent ones. Therefore, we chose poly-L-lysine as working surface since it provided better and more reproducible degree of binding than epoxy-silane. Spotting buffer is another factor that cannot be disregarded when developing a protein array. Small differences in pH and salt composition can have a great effect on the assay. This is what we observed when we tested different buffers. PBS 10 mM pH 7.5 provided a good spot morphology. On the other hand, when carbonate buffer (50 mM pH 9.6) and PBS buffer (pH 8.5/0.01% sodium dodecyl sulfate) were used, spots could barely be seen. The presence of a 10% glycerol in the spotting buffer produced the same effect. Blocking of the surface after capture antibody immobilization cannot be overseen either. The main purpose is to prevent unspecific adsorption of analytes/immunoreagents onto the surface. In our case, 2% gelatine in PBS significantly reduced non-specific interactions in a 36%. We found that bacteria, detection antibody, and QD-anti-IgG steps were straighter forward. Nevertheless, in our experience, each array requires different conditions and what works for the *E. coli* assay, could not work for another

Table 2 Array versus ELISA

	Array	ELISA
LOD (CFU mL ⁻¹)	1±0.001	1.1×10 ³ ±2.4
Immunoreagent		
Capture antibody (μg)	7.5×10 ⁻⁴	0.1
Detection antibody (μg)	0.8	0.05
Assay time (h)	2	15

calculated using the statistical approach reported by Long and Winefordner [21]. Results correspond to the average and standard deviation of four assays run on 4 different days in duplicate ($n=12$)

bacteria, or more generally, for another antibody array. Thus, we are convinced of the necessity of carrying out a careful optimization of each array to achieve good sensibilities.

It was difficult for us to imagine how 1 CFU mL⁻¹ could be quantified by taking 100 μL per well. This could be the result of the lysis treatment performed on the bacteria. The stock of *E. coli* O157:H7 was commercial and its concentration was well defined: 10⁹ CFU mL⁻¹. We assumed that the concentration diminished by ten in each dilution we made. However, bacteria had been heat-killed; therefore, some fragmentation might had occurred. As a result, a LOD of 1 CFU mL⁻¹ could correspond to the detection of a bacteria fragment. Nevertheless, we could assume that concentrations below 10 CFU mL⁻¹ are being detected.

QD-based detection system also plays an important role in the excellent LOD achieved in this work. Until recently,

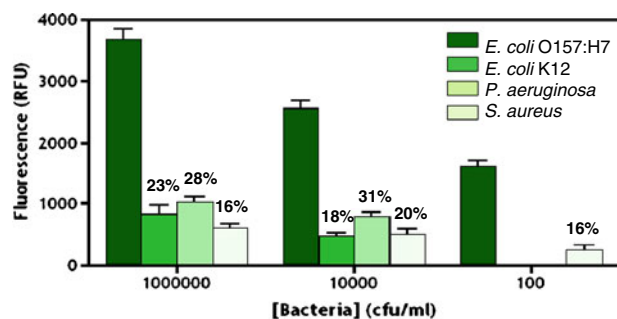


Fig. 5 Cross-reactivity obtained to characterize the specificity of the antibody array for *E. coli* O157:H7. Two Gram-negative bacteria, *E. coli* K12 and *P. aeruginosa*, and one Gram-positive bacterium, *S. aureus*, were tested. Cross-reactivity at different concentrations of bacteria is expressed as a percentage of the fluorescence of the cross-reactant divided by the fluorescence of *E. coli* O157:H7

microarrays have used fluorescent dyes as reporters for detection [14, 33]. In the last years, the incorporation of fluorescent nanoparticles has allowed generating enhanced signal-to-noise ratios. In this context, the capability of QDs to improve assay sensitivity compared to traditional organic dyes as has been demonstrated [27, 34, 35]. Recently, QDs have been applied for bacteria detection in immunoassays [36, 37]. However, works regarding the application of QD for pathogen detection in a microarray platform are still scarce with LOD above 10^6 CFU/ml [14]. These LOD values have only been improved by enrichment with magnetic beads and in those cases, detection is done in solution and not onto an array platform [36–38]. In this context, the present work considerably improves the characteristics of the arrays that have been developed so far.

An important issue regarding multiplexing antibody microarrays is cross-reactivity due to the actual deficit in the market of antibodies with good specificity and affinity. Antibodies developed against bacteria, cross-reactions could be the result of common epitopes between different bacteria strains, and in some cases, between different bacterial species. In the present study, to evaluate specificity, we analyzed the non-specific interaction of several bacteria on the array. We found that at high concentrations of bacteria the three species tested: *E. coli* K12, *S. aureus* and *P. aeruginosa*, cross-reacted with *E. coli* O157:H7. The non-specific interaction diminished at 100 CFU mL⁻¹. Nevertheless, we still observed a small interference of *S. aureus* in the assay, which makes us guess that if more bacterial strains/species were tested, cross-reactivity would be observed in several cases, at least. Therefore, this lack of specificity of *E. coli* O157:H7 commercial antibodies constitute the main drawback to develop a multiplexed assay for the simultaneous detection of several bacteria. In relation to this statement, the work by Magliulo and co-workers has shown that it is possible to achieve the multiplexed detection of four bacteria species (*E. coli* O157:H7, *Yersinia enterocolitica*, *S. typhimurium*, and *Listeria monocytogenes*) without any cross-reactivity by producing specific antibodies against each bacterium plus by using a suitable detection technique [39]. Thus, this work emphasizes that suitable antibodies are a critical factor to accomplish multiplexing.

Conclusions

In this study, we have developed a QD-based antibody array for the detection of *E. coli* O157:H7. The excellent detectability achieved is the result of both a meticulous optimization of the assay parameters and the detection accomplished with QDs. Compared with a conventional ELISA method, the array shows an improvement of three

orders of magnitude in the LOD and a significant reduction of the assay time. Cross-reactivity studies show little interference of other bacteria when working at concentrations of 100 CFU mL⁻¹. The strategy described here can be applied for the simultaneous detection of multiple pathogens using multicolour QDs. For this purpose, antibodies to be used in the array must be suitable to ensure specificity. Moreover, pair of antibodies for each bacterium must perform well in the same assay conditions. Unfortunately, nowadays, the market does not provide bacteria antibodies with such characteristics. Until this happens, this study constitutes a proof of concept of the potential of QD-based arrays for bacteria detection.

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References

- Bell C (2002) Approach to the control of entero-haemorrhagic *Escherichia coli* (EHEC). *Int J Food Microbiol* 78:197–216
- Griffin PM, Tauxe RV (1991) The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol Rev* 13:60–98
- Dorn CR, Angrick EJ (1991) Serotype O157:H7 *Escherichia coli* from bovine and meat sources. *J Clin Microbiol* 29:1225–1231
- Hrudey SE, Payment P, Huck PM, Gillham RW, Hrudey EJ (2003) A fatal waterborne disease epidemic in Walkerton, Ontario: comparison with other waterborne outbreaks in the developed world. *Water Sci Technol* 47:7–14
- Mead PS, Griffin PM (1998) *Escherichia coli* O157:H7. *Lancet* 352:1207–1212
- Allen MJ, Edberg SC, Reasoner DJ (2004) Heterotrophic plate count bacteria—what is their significance in drinking water? *Int J Food Microbiol* 92:265–274
- Van Dyck E, Ieven M, Pattyn S, Van Damme L, Laga M (2001) Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by enzyme immunoassay, culture, and three nucleic acid amplification tests. *J Clin Microbiol* 39:1751–1756
- Manafi M, Kremsmaier B (2001) Comparative evaluation of different chromogenic/fluorogenic media for detecting *Escherichia coli* O157:H7 in food. *Int J Food Microbiol* 71:257–262
- Hansen WL, Beuving J, Bruggeman CA, Wolffs PF (2010) Molecular probes for the diagnosis of clinically relevant bacterial infections in blood cultures. *J Clin Microbiol* 48(12):4432–4438
- Dharmasiri U, Witek MA, Adams AA, Osiri JK, Hupert L, Bianchi S, Roelke DL, Soper SA (2010) Enrichment and detection of *Escherichia coli* O157:H7 from water samples using an antibody modified microfluidic chip. *Anal Chem* 82:2844–2849
- Ligler FS, Sapsford KE, Golden JP, Shriver-Lake LC, Taitt CR, Dyer MA, Barone S, Myatt C (2007) The array biosensor: portable, automated systems. *Anal Sci* 23:5–10
- Angenendt P (2005) Progress in protein and antibody microarray technology. *Drug Discov Today* 10:503–511

13. Wingren C, Borrebaeck CA (2009) Antibody-based microarrays. *Meth Mol Biol* 509:57–84
14. Gehring AG, Albin DM, Bhunia AK, Reed SA, Tu SI, Uknalis J (2006) Antibody microarray detection of *Escherichia coli* O157:H7: quantification, assay limitations, and capture efficiency. *Anal Chem* 78:6601–6607
15. Kim JS, Anderson GP, Erickson JS, Golden JP, Nasir M, Ligler FS (2009) Multiplexed detection of bacteria and toxins using a microflow cytometer. *Anal Chem* 81:5426–5432
16. Karoonuthaisiri N, Charlermroj R, Uawisetwathana U, Luxananil P, Kirtikara K, Gajanandana O (2009) Development of antibody array for simultaneous detection of foodborne pathogens. *Biosens Bioelectron* 24:1641–1648
17. Wolter A, Niessner R, Seidel M (2008) Detection of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Legionella pneumophila* in water using a flow-through chemiluminescence microarray readout system. *Anal Chem* 80:5854–5863
18. Karsunke XY, Niessner R, Seidel M (2009) Development of a multichannel flow-through chemiluminescence microarray chip for parallel calibration and detection of pathogenic bacteria. *Anal Bioanal Chem* 395:1623–1630
19. Mukundan H, Xie H, Anderson AS, Grace WK, Shively JE, Swanson BI (2009) Optimizing a waveguide-based sandwich immunoassay for tumor biomarkers: evaluating fluorescent labels and functional surfaces. *Bioconjug Chem* 20:222–230
20. Herman RA, Scherer PN, Shan G (2008) Evaluation of logistic and polynomial models for fitting sandwich-ELISA calibration curves. *J Immunol Meth* 339:245–258
21. Long GL, Winefordner JD (1983) Limit of detection: a closer look at the IUPAC definition. *Anal Chem* 55:712–724
22. Olle EW, Messamore J, Deogracias MP, McClintock SD, Anderson TD, Johnson KJ (2005) Comparison of antibody array substrates and the use of glycerol to normalize spot morphology. *Exp Mol Pathol* 79:206–209
23. Gokarna A, Jin LH, Hwang JS, Cho YH, Lim YT, Chung BH, Youn SH, Choi DS, Lim JH (2008) Quantum dot-based protein micro- and nanoarrays for detection of prostate cancer biomarkers. *Proteomics* 8:1809–1818
24. Macbeath GS, Schreiber SL (2000) Printing proteins as microarrays for high-throughput function determination. *Science* 289:1760–1763
25. Hanaki K, Momo A, Oku T, Komoto A, Maenosono S, Yamaguchi Y, Yamamoto K (2003) Semiconductor quantum dot/albumin complex is a long-life and highly photostable endosome marker. *Biochem Biophys Res Commun* 302:496–501
26. Geho D, Lahar N, Gurnan IP, Huebschman M, Herrmann P, Espina V, Shi A, Wulfkuhle J, Garner H, Petricoin ER, Liotta LA, Rosenblatt KP (2005) Pegylated, streptavidin-conjugated quantum dots are effective detection elements for reverse-phase protein microarrays. *Bioconjug Chem* 16:559–568
27. Rousserie G, Sukhanova A, Even-Desrumeaux K, Fleury F, Chames P, Baty D, Oleinikov V, Pluot M, Cohen JH, Nabiev I (2010) Semiconductor quantum dots for multiplexed bio-detection on solid-state microarrays. *Crit Rev Oncol Hematol* 74:1–15
28. Park TJ, Yoo SM, Keum KC, Lee S (2009) Microarray of DNA-protein complexes on poly-3-hydroxybutyrate surface for pathogen detection. *Anal Bioanal Chem* 393:1639–1647
29. Jaing C, Gardner S, McLoughlin K, Mulakken N, Alegria-Hartman M, Banda P, Williams P, Gu P, Wagner M, Manohar C, Slezak T (2008) A functional gene array for detection of bacterial virulence elements. *PLoS ONE* 3:e2163
30. Miller MB, Tang YW (2009) Basic concepts of microarrays and potential applications in clinical microbiology. *Clin Microbiol Rev* 22:611–633
31. Uttamchandani M, Neo JL, Ong BN, Moochhala S (2009) Applications of microarrays in pathogen detection and biodefence. *Trends Biotechnol* 27:53–61
32. Seurynck-Servoss SL, White AM, Baird CL, Rodland KD, Zangar RC (2007) Evaluation of surface chemistries for antibody microarrays. *Anal Biochem* 371:105–115
33. Gonzalez RM, Seurynck-Servoss SL, Crowley SA, Brown M, Omenn GS, Hayes DF, Zangar RC (2008) Development and validation of sandwich ELISA microarrays with minimal assay interference. *J Proteome Res* 7:2406–2414
34. Hahn MA, Tabb JS, Krauss TD (2005) Detection of single bacterial pathogens with semiconductor quantum dots. *Anal Chem* 77:4861–4869
35. Liu Y, Brandon R, Cate M, Peng X, Stony R, Johnson M (2007) Detection of pathogens using luminescent CdSe/ZnS dendron nanocrystals and a porous membrane immunofilter. *Anal Chem* 79:8796–8802
36. Yang L, Li Y (2006) Simultaneous detection of *Escherichia coli* O157:H7 and *Salmonella typhimurium* using quantum dots as fluorescence labels. *Analyst* 131:394–401
37. Zhao Y, Ye M, Chao Q, Jia N, Ge Y, Shen H (2009) Simultaneous detection of multifoed-borne pathogenic bacteria based on functionalized quantum dots coupled with immunomagnetic separation in food samples. *J Agric Food Chem* 57:517–524
38. Su XL, Li Y (2004) Quantum dot biolabeling coupled with immunomagnetic separation for detection of *Escherichia coli* O157:H7. *Anal Chem* 76:4806–4810
39. Magliulo M, Simoni P, Guardigli M, Michelini E, Luciani M, Lelli R, Roda A (2007) A rapid multiplexed chemiluminescent immunoassay for the detection of *Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Salmonella typhimurium*, and *Listeria monocytogenes* pathogen bacteria. *J Agric Food Chem* 55:4933–4939