Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 69 (2006) 232–238
Talanta
www.elsevier.com/locate/talanta

Simultaneous detection of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* with an array-based immunosorbent assay using universal protein G-liposomal nanovesicles☆

Chien-Sheng Chen, Richard A. Durst*

Department of Food Science and Technology, Cornell University, Geneva, NY 14456, USA

Received 8 July 2005; received in revised form 27 September 2005; accepted 27 September 2005
Available online 27 October 2005

Abstract

A novel universal reagent for immunoassays, protein G-liposomal nanovesicles has been developed and successfully used in an immunomagnetic bead sandwich assay for the detection of *Escherichia coli* O157:H7 [C.-S. Chen, A.J. Baeumner, R.A. Durst, Talanta 67 (2005) 205]. To demonstrate the universal capability of protein G-liposomal nanovesicles, this reagent was used to develop an array-based immunosorbent assay for the simultaneous detection of *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*. Both direct and competitive immunoassay formats were used to demonstrate the feasibility of detecting multiple analytes in a single test by using universal protein G-liposomal nanovesicles. Both pure and mixed cultures were examined in the direct immunoassay format. Results indicate that the limits of detection (LODs) of the direct assay for *E. coli* O157:H7, *Salmonella enterica* serovar Typhimurium and *L. monocytogenes* in pure cultures were approximately 100, 500 and 1.5×10^4 CFU/ml, respectively. In mixed cultures, the LODs were approximately 3.1×10^3, 7.8×10^4, and 7.9×10^5 CFU/ml. In the competitive assay format, the LODs for *E. coli* O157:H7, *S. enterica* serovar Typhimurium, and *L. monocytogenes* were approximately 1.5×10^4, 5×10^4, and 1.2×10^5 CFU/ml for the pure cultures. These results showed that protein G-liposomal nanovesicles can be successfully used in a simultaneous immunoassay for several food-borne pathogens, thereby demonstrating that they are effective universal reagents for use in immunoassays.

Keywords: Foodborne pathogens; Simultaneous immunoassay; Protein G-liposomes; Immunoliposomes; Nanovesicles

1. Introduction

Escherichia coli O157:H7, *Salmonella* spp., and *Listeria monocytogenes* are among the most dangerous foodborne bacterial pathogens in terms of human health and disease [2]. *E. coli* O157:H7 is estimated to cause approximately 73,000 illnesses and 61 associated deaths per year in the United States [2], causing hemorrhagic diarrhea and hemolytic uremic syndrome. Ground beef, raw milk, poultry products, fresh apple cider, cold sandwiches, vegetables and drinking water are easily contaminated by *E. coli* O157:H7 [3].

Salmonella is estimated to be responsible for 1.4 million cases of human illness annually and almost 600 deaths, which is approximately 30% of all reported cases of food poisoning in the United States for which the etiology was determined [2,4]. Although the most common sources of *Salmonella* infection are poultry products, outbreaks have also been linked to pork, lamb, beef, dairy products, and vegetables [5,6]. *Salmonella enterica* serovar Typhimurium is responsible for the largest percentage of salmonellosis cases in the United States [4]. Symptoms of *Salmonella* poisoning usually appear 6–48 h after ingestion of the bacteria with the first symptoms being nausea and vomiting followed by abdominal cramps and diarrhea [7].

L. monocytogenes is estimated to be responsible for approximately 2500 cases of human illness and more than 500 deaths per year in the United States [2]. It is especially dangerous for pregnant women, cancer patients, elderly, newborns and immunocompromised persons. *L. monocytogenes* can grow at refrigeration temperature. Dairy products, meats, fish, shellfish,

☆ Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

* Corresponding author. Tel.: +1 315 787 2297; fax: +1 315 787 2397.
E-mail address: rad2@cornell.edu (R.A. Durst).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.09.036

vegetables and ready-to-eat foods have been reported as contamination sources for human listeriosis [8]. A person with listeriosis usually has fever, muscle aches and gastrointestinal symptoms such as nausea or diarrhea. If infection spreads to the nervous system, symptoms such as headache, stiff neck, loss of balance or convulsions can occur [9].

The risk of human illness associated with foods can be prevented by monitoring microbial contamination at points of potential contamination, e.g., in the field of harvest, food processing plants, distribution, or in retail markets [10]. Because conventional culture methods for detection of pathogens are time consuming, results are usually not available until the food has been released to the market or consumed, thereby increasing the risk of uptake or transmission of pathogens [11]. Rapid and sensitive detection of bacterial pathogens is needed for food safety assurance. Since several food types are susceptible to contamination by all of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes*, it would be desirable to detect them simultaneously. Simultaneous detection of these three pathogens have been developed using polymerase chain reaction (PCR) [11,12]. However, PCR requires considerable sample processing prior to analysis, reagents are expensive, and assay development for the simultaneous detection of multiple analytes is arduous [13]. Because antibody-based technologies are highly selective, specific and adaptable to field-deployable devices, immunoassays are widely used in detection systems [14]. To the best of our knowledge, no simultaneous immunoassay for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* has been reported previously. Since three kinds of antibodies are needed to detect these three pathogens, a universal reagent for these immunoassays would be ideal.

Our group has developed a novel universal reagent for immunoassays: protein G-liposomal nanovesicles [1]. Liposomal nanovesicles, i.e., liposomes, are spherical vesicles consisting of phospholipid bilayers surrounding an aqueous cavity. Because each liposomal nanovesicle can contain up to several million fluorescent dye molecules, thereby providing greatly enhanced signals, liposomal nanovesicles have been successfully used as reporter particles in immunoassays [15–18]. Protein G, a cell wall protein obtained from group G *Streptococci*, has the ability to bind to the Fc fragment of most IgGs used in immunoassays [19].

In our previous report, we successfully used the protein G-tagged liposomal nanovesicles in an immunomagnetic bead sandwich assay for the detection of *E. coli* O157:H7 [1]. For demonstrating the universal characteristic of protein G-liposomal nanovesicles, in this report we used this reagent to develop a simultaneous detection of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* with an array-based immunosorbent assay. Since we previously demonstrated the feasibility of using protein G-liposomal nanovesicles in a sandwich assay format, direct and competitive assays using the nanovesicles (Fig. 1) were conducted to detect multiple analytes in a single test. Here, protein G-liposomal nanovesicles bind to all the primary antibodies against *E. coli* O157:H7, *Salmonella* and *L. monocytogenes.*

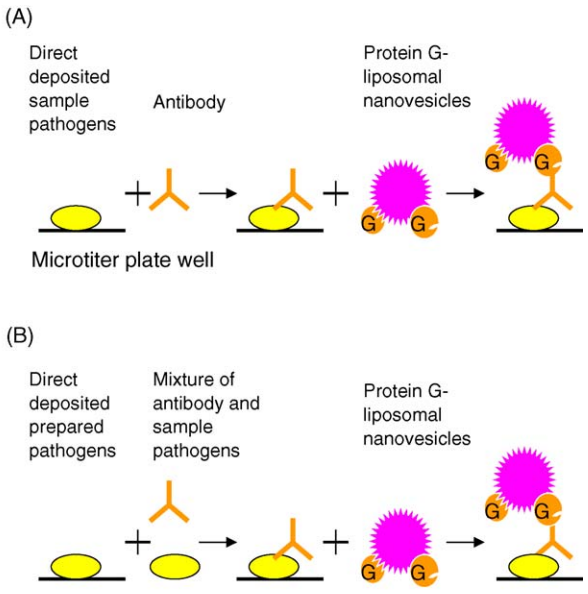

Fig. 1. Schematic representation of the two assay formats used in this study. (A) Direct fluorometric assay: pathogens were adsorbed in the wells of microtiter plates in an array format. Appropriate antibodies were added to the wells and bound to the pathogens. Protein G-liposomal nanovesicles are then added and bind to the Fc fragments of the antibodies. (B) Competitive assay: a preparation of pathogens is adsorbed directly onto the microtiter plate wells in an array format. Sample pathogens are mixed with antibodies before being added to the wells. Both adsorbed and sample pathogens compete for antibody binding. Universal protein G-liposomal nanovesicles are added and also bind to the Fc fragments of captured antibodies.

2. Experimental

2.1. Reagents

Common laboratory reagents were purchased from Sigma–Aldrich Co. (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA). Dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG), and polycarbonate syringe filters of 0.4 and 0.2 μm pore sizes were purchased from Avanti Polar Lipids (Alabaster, AL). Dipalmitoylphosphatidylethanolamine (DPPE) was purchased from Molecular Probes (Eugene, OR). *N*-Succinimidyl-*S*-acetylthioacetate (SATA), protein G (recombinant, *Streptococcus*), Blocker Casein in TBS and sulfo-SMCC were purchased from Pierce (Rockford, IL). Polyclonal antibodies against *E. coli* O157:H7 and *Salmonella* were purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD). Polyclonal antibody against *L. monocytogenes* was purchased from BIODESIGN International (Saco, ME). *E. coli* O157:H7 strain 43895, *S. enterica* serovar Typhimurium strain 14028 and *L. monocytogenes* strain F80287 were kindly provided by Randy Worobo and John Churey, Cornell University, Geneva, NY.

2.2. Preparation of protein G-liposomal nanovesicles

Protein G-liposomal nanovesicles with 0.4 mol% tag were prepared as described previously [1] and further detailed below.

Acetylthioacetate (ATA)-tagged liposomes were prepared first and then conjugated to maleimide-modified protein G.

The liposome encapsulant, a 150 mM SRB solution, was prepared in 0.02 M HEPES buffer. A solution was prepared containing 7.2 μmol of DPPE and a volume fraction of 0.7% triethylamine in chloroform. This solution was reacted with 14.3 μmol of SATA to form DPPE–ATA. Then 40.3 μmol of DPPC, 4.2 μmol of DPPG, and 40.9 μmol of cholesterol were dissolved in a mixture of chloroform/methanol (3:1, by volume). To this lipid solution, 3.6 μmol of DPPE–ATA was added and mixed thoroughly. Using vacuum rotary evaporation, the organic solvent was removed yielding a thin dry lipid film. Three milliliters of the SRB encapsulant were then added to hydrate the lipid mixture. The hydrated lipid suspension was subjected to freeze/thaw cycles by alternately placing the sample vial in a dry ice/acetone bath and 50 °C water bath. Once the lipid mixture was fully hydrated, it was allowed to stand for 4 h at 50 °C. Finally, the liposomes were extruded through polycarbonate syringe filters with 0.4 and 0.2 μm pore size in series. To remove unencapsulated dye, the liposomes were gel filtered on a Sephadex G-50-150 column.

Protein G was modified with a maleimide group by incubation with 15 times the molar quantity of sulfo-SMCC dissolved in dimethyl sulfoxide (DMSO). The reagents were allowed to react for 2 h at room temperature. Hydroxylamine hydrochloride was used to deacetylate the ATA groups on the liposome to yield the reactive sulfhydryl groups. For this reaction, 0.1 ml of 0.5 M hydroxylamine hydrochloride solution was added per 1 ml of the liposome solution. The reaction was allowed to proceed at room temperature in the dark for 2 h. For conjugation, the thiol groups on the liposome surface were reacted with the maleimide group-modified protein G at pH 7 for 3.5 h at room temperature and then overnight at 4 °C. All unconjugated thiol groups were quenched with ethylmaleimide solution. The protein G-liposomal nanovesicles were then purified by Sepharose CL-4B equilibrated with Tris-buffered saline (TBS).

2.3. Bacterial inoculum preparation

Bacterial strains were inoculated into tryptic soy broth (TSB) and incubated for 20 h at 37 °C at 200 rpm. The TSB for *L. monocytogenes* contains 0.6% yeast extract. They were then serially diluted with TBS buffer. The population of bacteria in the dilution tubes was determined by spread plate counts on tryptic soy agar. (*Caution*: These bacteria are pathogenic and should be handled with extreme care as required for BSL-2 organisms. See http://bmbl.od.nih.gov/sect3bsl2.htm for more information.)

2.4. Direct fluorometric assays on pure cultures

FluoroNune MaxiSorp microtiter plate wells (Nunc, Rochester, NY) were coated in an array format with 100 μl of serially diluted *E. coli* O157:H7, *S. enterica* serovar Typhimurium and *L. monocytogenes* at 37 °C for 2.5 h. After removing excess cultures, the wells were blocked at 37 °C for 1 h with Blocker Casein in TBS (25 mM Tris, 150 mM NaCl, pH 7.4). The wells were then washed once with Blocker Casein in TBS with 0.05% Tween-20 (BCT). Twenty-five micrograms per milliliter of *E. coli* antibody, 20 μg/ml of *Salmonella* antibody and 22.5 μg/ml of *Listeria* antibody were added to the appropriate wells, respectively. After incubating 1.5 h at room temperature, unbound antibodies were removed and the wells were then washed three times with BCT. Protein G-liposomal nanovesicles were then added to each well and incubated for 0.5 h, gently shaking at room temperature. Unbound protein G-liposomal nanovesicles were removed and the wells were then washed three times with BCT. To lyse the bound protein G-liposomal nanovesicles, 100 μl of 30 mM *n*-octyl glucopyranoside (*n*-OG) were added to each well and shaken at room temperature for 5 min. The fluorescence signal was read in a Cytofluor fluorescence plate reader (Cytofluor, PerSeptive Biosystems) using an excitation filter of 530 nm and emission filter of 590 nm.

2.5. Direct fluorometric assays of mixed cultures

The protocol was the same as that described above but, instead of pure cultures, mixed cultures of *E. coli* O157:H7, *S. enterica* serovar Typhimurium, and *L. monocytogenes* were coated on the plate wells. Here, serially diluted *E. coli* O157:H7 contained 10^6 CFU/ml of *S. enterica* serovar Typhimurium and 10^6 CFU/ml of *L. monocytogenes*. Likewise, serially diluted *S. enterica* serovar Typhimurium and *L. monocytogenes* also contained 10^6 CFU/ml of the other two pathogens.

2.6. Competitive assays

FluoroNune MaxiSorp microtiter plate wells were coated in an array format with 100 μl of 10^5 CFU/ml *E. coli* O157:H7, 10^6 CFU/ml *S. enterica* serovar Typhimurium and 10^7 CFU/ml *L. monocytogenes* at 37 °C for 2 h. Here, the pathogens served as a surface-bound capture agent for antibodies in competition with sample pathogens. After removing excess cultures, the wells were blocked at 37 °C for 1 h with Blocker Casein in TBS. One microliter of antibody against *E. coli* O157:H7, *Salmonella*, or *L. monocytogenes* was mixed with 98 μl of serially diluted cultures and 1 μl of 5% Tween-20. The antibody concentrations were 71.4, 160, 225 μg/ml, respectively. The mixtures were incubated at 37 °C for 1 h and then added to the appropriate wells. After 1 h incubation at 37 °C, unbound antibodies were removed and the wells were then washed twice with BCT. Protein G-liposomal nanovesicles were then added to each well and incubated for 0.5 h, gently shaking at room temperature. Unbound protein G-liposomal nanovesicles were removed and the wells were then washed three times with BCT. To lyse the bound protein G-liposomal nanovesicles, 100 μl of 30 mM *n*-OG was added to each well and shaken at room temperature for 5 min. The fluorescence signal was read in a Cytofluor fluorescence plate reader as before.

3. Results and discussion

3.1. Direct fluorometric assays on pure cultures

Three serially diluted pathogens were adsorbed on the wells of microtiter plates in an array format. Appropriate antibodies

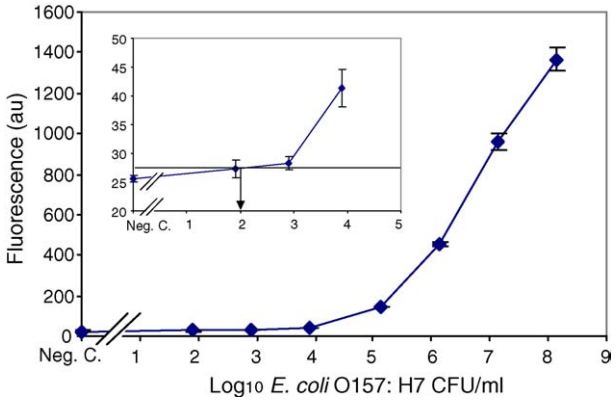

Fig. 2. Dose–response curve for pure *Escherichia coli* O157:H7 cultures in a direct array-based immunosorbent assay using protein G-liposomal nanovesicles. (Error bars represent the standard deviations of triplicate measurements.) The inset expanded curve shows location of the LOD. The straight horizontal line is three standard deviations higher than the mean intensity at zero concentration (negative control), and the intersection of this line with the dose–response curve is the LOD as indicated by the vertical arrow.

were applied to the wells and bound to the pathogens. Protein G-liposomal nanovesicles would bind to the Fc fragments of antibodies, and then the bound liposomes were lysed by a detergent solution. Finally, the quantity of the bacteria in the sample was quantified by measuring the fluorescence intensity of the fluorescent dye molecules released by the lysis.

The analytical sensitivity and detection limit were determined from the dose–response curves. As shown in all of the full dose–response curves for the three pathogens (Figs. 2–4), the fluorescent signals increase with increasing concentration of the pathogens, showing broad dynamic ranges. The dynamic range of the assay for *E. coli* O157:H7 is six orders of magnitude from 10^2 to 10^8 CFU/ml, *S. enterica* serovar Typhimurium is five orders of magnitude from 10^3 to 10^8 CFU/ml and *L. monocytogenes* is four orders of magnitude from 10^4 to 10^8 CFU/ml. The limit of detection (LOD) is defined as the lowest concentration of analyte producing a fluorescence intensity that is three standard deviations higher than the mean intensity at zero concentration (negative control). According to this definition, the LODs of these assays for *E. coli* O157:H7, *S. enterica* serovar

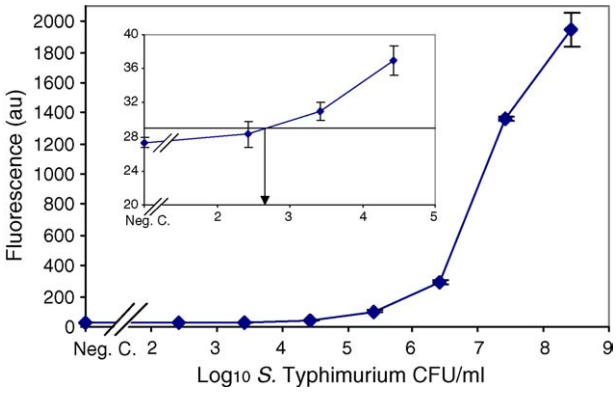

Fig. 3. Dose–response curve for pure *Salmonella enterica* serovar Typhimurium cultures in a direct array-based immunosorbent assay using protein G-liposomal nanovesicles. Inset curve again shows the location of the LOD.

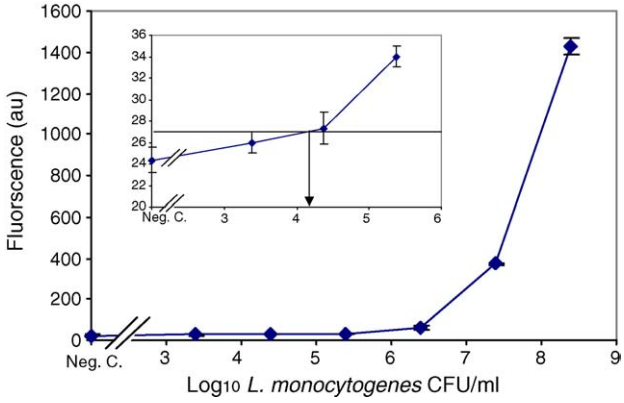

Fig. 4. Dose–response curve for pure *Listeria monocytogenes* cultures in a direct array-based immunosorbent assay using protein G-liposomal nanovesicles. Inset curve again shows the location of the LOD.

Typhimurium and *L. monocytogenes* were determined to be approximately 100, 500 and 1.5×10^4 CFU/ml, respectively (as shown in the insets of Figs. 2–4). The detection limits of enzyme-linked immunosorbent assays (ELISAs) for *E. coli* O157:H7, *S. enterica* serovar Typhimurium and *L. monocytogenes* have all been reported to be approximately 10^4 to 10^5 CFU/ml [20–23]. The direct fluorometric immunosorbent assay described herein has better or comparable detection limits than ELISAs which are also performed in immunosorbent assay formats. The high sensitivities observed in our studies are in part attributable to the advantages offered by dye-encapsulating liposomes. Because each liposome contains a very large number of fluorescent dye molecules, the signals generated from the binding events are greatly enhanced, thereby resulting in higher sensitivity.

3.2. Direct fluorometric assays for mixed cultures

Because analyte is adsorbed directly onto the microtiter well in a direct fluorometric immunosorbent assay, pure analyte is usually required to achieve maximum adsorption efficiency. To examine the performance of direct fluorometric assays for mixed cultures and the specificity of the antibodies, dose–response curves of *E. coli* O157:H7, *S. enterica* serovar Typhimurium or *L. monocytogenes* were obtained in the presence of the other two bacteria. Serially diluted *E. coli* O157:H7, *S. enterica* serovar Typhimurium or *L. monocytogenes* was spiked into a solution containing 10^6 CFU/ml of the other two bacteria.

The dose–response curve of *E. coli* O157:H7 in the presence of *S. enterica* serovar Typhimurium and *L. monocytogenes* (Fig. 5) shows a dynamic range of more than five orders of magnitude from 10^3 to 10^8 CFU/ml and greatly increased sensitivity above 10^5 CFU/ml. This dynamic range is only one order of magnitude less than the dose–response curve of pure *E. coli* O157:H7 (Fig. 2). Because the fluorescent signal increases with an increasing concentration of *E. coli* O157:H7 in the presence of 10^6 CFU/ml *S. enterica* serovar Typhimurium and *L. monocytogenes*, the experiment also demonstrated the specificity of the *E. coli* O157:H7 antibody without any significant cross-reactivity to *S. enterica* serovar Typhimurium and *L. monocytogenes*. Fig. 5 also shows that the LOD of this assay for *E. coli* O157:H7 in the

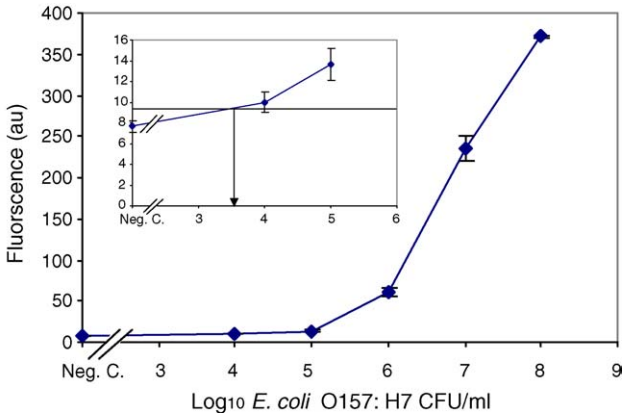

Fig. 5. Dose–response curve for *E. coli* O157:H7 in the presence of 10^6 CFU/ml of both *S.* Typhimurium and *L. monocytogenes* in a direct array-based immunosorbent assay using protein G-liposomal nanovesicles. Inset curve again shows the location of the LOD.

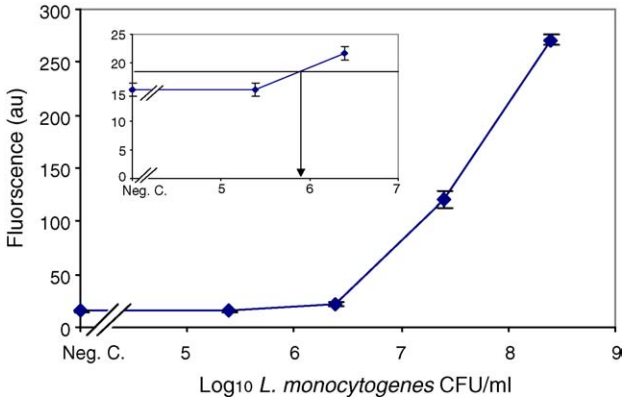

Fig. 7. Dose–response curve for *L. monocytogenes* in the presence of 10^6 CFU/ml of both *S.* Typhimurium and *E. coli* O157:H7 in a direct array-based immunosorbent assay using protein G-liposomal nanovesicles. Inset curve again shows the location of the LOD.

presence of *S. enterica* serovar Typhimurium and *L. monocytogenes* was approximately 3.1×10^3 CFU/ml, which still showed a lower LOD compared to a standard ELISA for *E. coli* O157:H7.

The dose–response curve of *S. enterica* serovar Typhimurium in the presence of *E. coli* O157:H7 and *L. monocytogenes* (Fig. 6) shows a dynamic range of more than four orders of magnitude from 10^4 to 10^8 CFU/ml, which is two orders less than the dose–response curve of pure *S. enterica* serovar Typhimurium (Fig. 3). Fig. 6 also shows the specificity of the *S. enterica* serovar Typhimurium antibody without any significant cross-reactivity to *E. coli* O157:H7 and *L. monocytogenes.* The LOD of this assay for *S. enterica* serovar Typhimurium in mixed cultures is approximately 7.8×10^4 CFU/ml (Fig. 6), which is still comparable to ELISA [22,23].

The dose–response curve of *L. monocytogenes* in the presence of *E. coli* O157:H7 and *S. enterica* serovar Typhimurium (Fig. 7) shows a dynamic range of over three orders of magnitude from 10^5 to 10^8 CFU/ml, which is one order of magnitude less than the dose–response curve of pure *L. monocytogenes* (Fig. 4). Likewise, Fig. 7 also shows the specificity of the *L. monocytogenes* antibody without any significant cross-reactivity to *E. coli* O157:H7 and *S. enterica* serovar Typhimurium. As seen also in Fig. 7, the LOD of this assay for *L. monocytogenes* in mixed cultures was determined to be approximately 7.9×10^5 CFU/ml, which is only slightly less sensitive than commercially available ELISA [21].

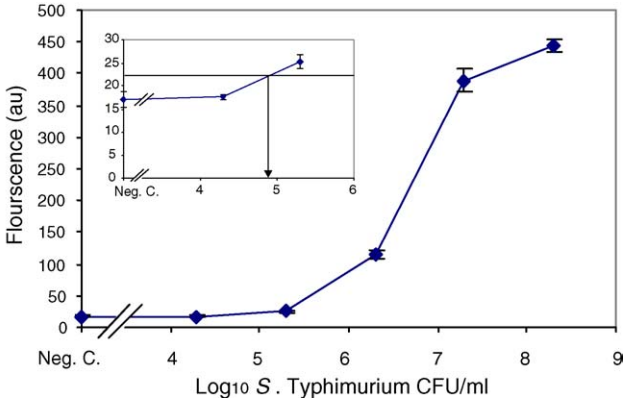

Fig. 6. Dose–response curve for *S.* Typhimurium in the presence of 10^6 CFU/ml of both *E. coli* O157:H7 and *L. monocytogenes* in a direct array-based immunosorbent assay using protein G-liposomal nanovesicles. Inset curve again shows the location of the LOD.

Whether in the pure or mixed cultures, the *E. coli* O157:H7 assay was always the best among the three pathogens in terms of sensitivity. The *L. monocytogenes* assay, on the other hand, was always the least sensitive. This disparity in overall sensitivity among the assays of three pathogens is probably attributable to differences in antibody affinities. Yu and Bruno also addressed the same issue for the detection of *E. coli* O157:H7 and *S.* Typhimurium [24].

3.3. Competitive assays

In competitive assays, a preparation of pathogens is adsorbed directly onto the microtiter plate wells in an array format. Sample pathogens are mixed with antibodies before being added to the wells. Both adsorbed and sample pathogens compete for the binding of the antibodies. Finally, universal protein G-liposomal nanovesicles are added to the wells to bind the antibodies bound to adsorbed pathogens on the wells and thereby produce the signals. Unlike the direct fluorometric assays, the detected signals are inversely related to the pathogen concentration in the samples.

As shown in the dose–response curves (Figs. 8–10), the fluorescent signals decrease with increasing concentration of the pathogens, showing a dynamic range over three orders of magnitude from 10^4 to 10^7 CFU/ml for *E. coli* O157:H7 and *S. enterica* serovar Typhimurium. However, the dynamic range of *L. monocytogenes*, while also three orders of magnitude, is from 10^5 to 10^8 CFU/ml. The limit of detection is defined as the lowest concentration of analyte producing a fluorescence intensity that is three standard deviations lower than the mean intensity at zero concentration (negative control). According to this definition, the LODs of this assay for *E. coli* O157:H7, *S. enterica* serovar Typhimurium, and *L. monocytogenes* were determined to be approximately 1.5×10^4, 5×10^4,

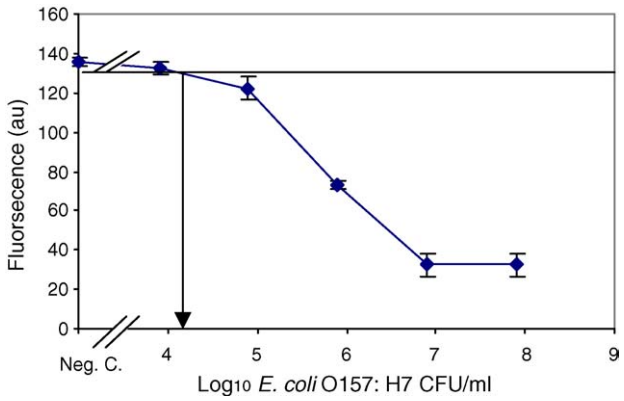

Fig. 8. Dose–response curve for *E. coli* O157:H7 in a competitive array-based immunosorbent assay using protein G-liposomal nanovesicles.

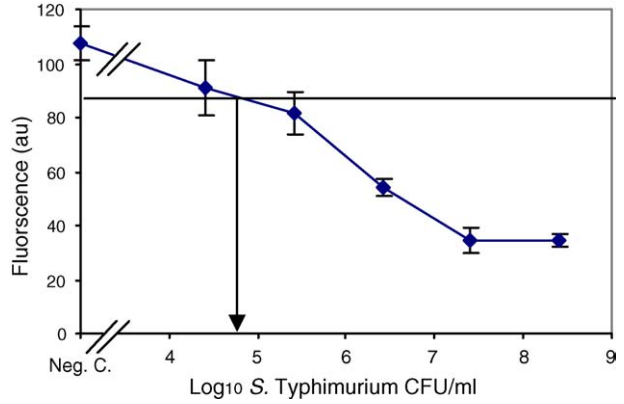

Fig. 9. Dose–response curve for *S.* Typhimurium in a competitive array-based immunosorbent assay using protein G-liposomal nanovesicles.

and 1.2×10^5 CFU/ml, respectively. These LODs are also comparable to standard ELISAs [20–23]. Although the competitive assays are faster than the direct assays (∼3 h shorter), they are less sensitive than direct assays. One possible explanation is that direct assays have a higher avidity due to a higher local concentration of antigen than do competitive assays. While the binding of the antibody to antigen in competitive assays is limited by diffusion in the liquid phase, antigen in direct assays is immobilized on a solid phase, thereby providing a higher local antigen concentration and increasing the chance that any dissociated antibodies will rebind to neighboring antigens [25]. Although less likely, another possible explanation is that direct fluorometric assays required time for the sample adsorption on the wells during which the bacteria may be also enriched, thereby producing higher signals.

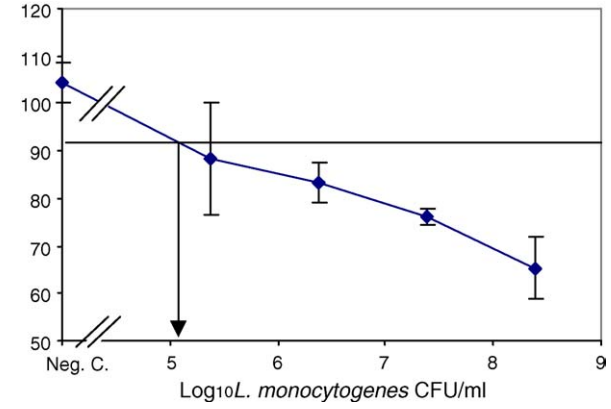

Fig. 10. Dose–response curve for *L. monocytogenes* in a competitive array-based immunosorbent assay using protein G-liposomal nanovesicles.

4. Conclusion

Simultaneous detection of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* with an array-based immunosorbent assay performed by protein G-liposomal nanovesicles showed sensitivities comparable to, or better than, standard ELISAs. Universal protein G-liposomal nanovesicles were successfully used in both direct and competitive assays and only required 30 min of incubation for antibody coupling at room temperature. Our results demonstrated that protein G-liposomal nanovesicles are effective universal reagents for use in immunoassays. In this study, pure and mixed cultures of pathogens were used to demonstrate the feasibility of using protein G-liposomal nanovesicles in the simultaneous detection assays. In the future, the assays should be tested in food matrices commonly associated with *E. coli*, *Salmonella*, and *Listeria* outbreaks.

Acknowledgements

The authors thank Dr. Randy Worobo and John Churey for supplying the *E. coli* O157:H7. This research was supported in part by the Cornell University Agricultural Experiment Station Federal Formula Funds, Project No. NYG 623498, received from Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture.

References

[1] C.-S. Chen, A.J. Baeumner, R.A. Durst, Talanta 67 (2005) 205.
[2] P.S. Mead, L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin, R.V. Tauxe, Emerg. Infect. Dis. 5 (1999) 607.
[3] P.M. Griffin, R.V. Tauxe, Epidemiol. Rev. 13 (1991) 60.
[4] S.J. Olsen, L.C. MacKinnon, J.S. Goulding, N.H. Bean, L. Slutsker, MMWR CDC Surveill. Summ. 49 (2000) 1.
[5] H.E. Ekperigin, K.V. Nagaraja, Vet. Clin. N. Am. Food Anim. Pract. 14 (1998) 17.
[6] J.V. Campbell, J. Mohle-Boetani, R. Reporter, S. Abbott, J. Farrar, M. Brandl, R. Mandrell, S.B. Werner, J. Infect. Dis. 183 (2001) 984.
[7] J.Y. D'Aoust, J. Maurer, J.S. Bailey, Food Microbiology: Fundamentals and Frontiers, 2nd ed., ASM Press, Washington, DC, 2001.
[8] P. Gilot, C. Hermans, M. Yde, J. Gigi, M. Janssens, A. Genicot, P. Andre, G. Wauters, J. Infect. 35 (1997) 195.
[9] J.M. Farber, P.I. Peterkin, Microbiol. Rev. 55 (1991) 476.
[10] L.R. Beuchat, J.H. Ryu, Emerg. Infect. Dis. 3 (1997) 459.
[11] S. Kawasaki, N. Horikoshi, Y. Okada, K. Takeshita, T. Sameshima, S. Kawamoto, J. Food Protect. 68 (2005) 551.
[12] A.A. Bhagwat, Int. J. Food Microbiol. 84 (2003) 217.
[13] P. Belgrader, W. Benett, D. Hadley, G. Long, R. Mariella Jr., F. Milanovich, S. Nasarabadi, W. Nelson, J. Richards, P. Stratton, Clin. Chem. 44 (1998) 2191.
[14] M.T. McBride, S. Gammon, M. Pitesky, T.W. O'Brien, T. Smith, J. Aldrich, R.G. Langlois, B. Colston, K.S. Venkateswaran, Anal. Chem. 75 (2003) 1924.

[15] S. Park, R.A. Durst, Anal. Biochem. 280 (2000) 151.
[16] H.A.H. Rongen, H.M. Vanderhorst, G.W.K. Hugenholtz, A. Bult, W.P. Vanbennekom, P.H. Vandermeide, Anal. Chim. Acta 287 (1994) 191.
[17] J.A. Ho, R.A. Durst, Anal. Biochem. 312 (2003) 7.
[18] L. Locascio-Brown, A.L. Plant, V. Horvath, R.A. Durst, Anal. Chem. 62 (1990) 2587.
[19] L. Bjorck, G. Kronvall, J. Immunol. 133 (1984) 969.
[20] I. Abdel-Hamid, D. Ivnitski, P. Atanasov, E. Wilkins, Biosens. Bioelectron. 14 (1999) 309.
[21] R. Capita, C. Alonso-Calleja, B. Moreno, M.C. Garcia-Fernandez, Int. J. Food Microbiol. 65 (2001) 75.
[22] C. Goodridge, L. Goodridge, D. Gottfried, P. Edmonds, J.C. Wyvill, J. Food Protect. 66 (2003) 2302.
[23] R.R. Beumer, E. Brinkman, F.M. Rombouts, Int. J. Food Microbiol. 12 (1991) 363.
[24] H. Yu, J.G. Bruno, Appl. Environ. Microbiol. 62 (1996) 587.
[25] E. Harlow, D. Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999.