SPECIAL GUEST EDITOR SECTION

Surface Plasmon Resonance Biosensors for Detection of Pathogenic Microorganisms: Strategies to Secure Food and Environmental Safety

ALDERT A. BERGWERFF and FRANS VAN KNAPEN
Utrecht University, Institute for Risk Assessment Sciences, Division of Public Health and Food Safety, PO Box 80175, NL-3508 TD Utrecht, The Netherlands

This review describes the exploitation of exclusively optical surface plasmon resonance (SPR) biosensors for the direct and indirect detection of pathogenic microorganisms in food chains and the environment. Direct detection is, in most cases, facilitated by the use of defined monoclonal or polyclonal antibodies raised against (a part of) the target pathogenic microorganisms. The antibodies were immobilized to a solid phase of the sensor to capture the microbe from the sample. Alternatively, antibodies were used in an inhibition-like assay involving incubation with the target organism prior to analysis of nonbound antibodies. The free immunoglobins were screened on a sensor surface coated with either purified antigens or with F_c or F_{ab} binding antibodies. Discussed examples of these approaches are the determination of *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*. Another direct detection strategy involved SPR analysis of polymerase chain reaction products of Shiga toxin-2 genes reporting the presence of *E. coli* O157:H7 in human stool. Metabolic products have been exploited as biomarkers for the presence of a microbial agent, such as enterotoxin B and a virulence factor for the occurrence of *Staphylococcus aureus* and *Streptococcus suis*, respectively. Indirect detection, on the other hand, is performed by analysis of a humoral immune response of the infected animal or human. By immobilization of specific antigenic structures, infections with *Herpes simplex* and human immunodeficiency viruses, *Salmonella* and *Treponema pallidum* bacteria, and *Schistosoma* spp. parasites were revealed using human, avian, and porcine sera and avian eggs. Bound antibodies were easily isotyped using an SPR biosensor to reveal the infection history of the individual. Discussed studies show the recent recognition of the suitability of this type of instrument for (rapid) detection of health-threatening microbes to food and environmental microbial safety.

Guest edited as a special report on "Biosensors: Making Sense of Food" by Harvey Indyk.

Corresponding author's e-mail: a.a.bergwerff@iras.uu.nl

Microorganisms include a wide variety of bacteria, molds (fungi), parasites, and viruses. Pathogenic microorganisms have attracted much attention from the public as consumers of contaminated food and water, which resulted in family or community outbreaks. As a consequence, the media and politicians have played their part in increasing consumer awareness, sometimes leading to mass hysteria. Moreover, authorities have experienced the huge impact of recent examples of bioterrorism on the public. This has led to special interest also from military authorities in the framework of emergency preparedness and protection of the population and military forces in action.

With respect to pathogenic microorganisms, special attention is drawn to a number of zoonotic diseases (1), i.e., microbes transmissible from animals to human, for the following reasons. Most food- and waterborne diseases in humans are zoonotic by nature. Many zoonotic agents have their transmission route through the environment. Both contamination of food/water and environment are also used by bioterrorists to acquire maximum impact in the society.

Microbiological hazards can enter food chains at any point during pre-harvest, production, processing, transport, retailing, domestic storage, or meal preparation. From their introduction on feed or food, highly complex environments can occur in which viruses, bacteria, parasites, or fungi can elude detection and inactivation. Despite greater biological understanding and technological ingenuity, challenges continually arise in the form of familiar pathogens, although new viruses are described regularly, in new foods and as emerging pathogens in traditional foods, because of changes in husbandry, feed and/or food production. Efficient international distribution systems and rapid changes in consumer preferences can facilitate the swift penetration of pathogens through large populations, greatly shortening the reaction time available to public health agencies. Sometimes new routes of infection lead to unexpected explosions in the human population (e.g., through drinking water supply, aerosols in subways or indirect contact with animals; 2).

Authorities are convinced that rapid, versatile, and selective (diagnostic) assays are needed for environmental, feed, and food monitoring. A large portion of the explored monitoring techniques involved the use of biosensors. Indeed, biosensors had already increasingly acquired the attention of

life and pharmaceutical sciences and, recently, that of food and environmental sciences (3–9; Rasooly, this issue). It is clear from current applications and the number of publications in the instrumental field of biosensors, that optical surface plasmon resonance (SPR) instruments have attracted considerable and increasing interest towards securing food and environmental microbial safety.

Detection of Pathogenic Microorganisms

In principle, the presence of microorganisms can be detected directly or indirectly. In the direct assay, the organism itself is detected usually with the application of antibodies reacting with (sub) species- and/or strain-specific antigenic structures. This immunochemical analysis follows time-consuming sample preparation through culturing in selective growth media. In the indirect assay, the presence of the microorganism is suggested by the detection of humoral (immunoglobulins) or cellular (e.g., cytokines) products of an immunological response of the infected host. In most studies, well-defined antigens are used to capture a host's immunoglobulins in any body fluid (serology). The observed binding then reveals the nature of an invasive infestation of a pathogen.

The advantages and disadvantages of indirect and direct pathogen detection are clear: (*1*) individuals are not always immunologically responding to an infection, i.e., differences between low or high immune responders; (*2*) humoral responses are delayed several days or even weeks, possibly leaving a recent infection unnoticed; (*3*) serum antibodies can be found where the causative organism is not detected, as it has been rejected or retracted itself in certain (nonsampled) tissues; and (*4*) serological investigations are very fast and offer better possibilities for high throughput than direct detection. In fact, serology outperforms direct and, in most cases, insensitive detection of tissue parasites, which can only be carried out by histochemistry or digestion techniques and microscopy. Significant differences are also apparent in sample collection and preparation. Bacteria, fungi, and viruses have to be cultured from matrixes to facilitate their detection in enriched solutions, whereas blood is relatively easily collected and prepared for analysis. Here, it should be noted, however, that antibodies cannot only be retrieved from blood, plasma, or serum, but also from muscle (meat juice), milk, colostrums, cerebrospinal fluid, and eggs (10).

Technical Considerations

When monitoring interactions of cells on the biosensor chip, such as in some direct-detection methods, several factors should be considered. The dimensions of capillaries and sensor channels are limited to channel heights of 20 or 60 μm in the flow cell-based instruments of Biacore AB (Uppsala, Sweden). Besides the risk of clogging, attaching and sensor-attached cells experience a shear force created by the laminar flow, which may be stronger than chemical binding forces and prevent binding. Furthermore, the effective penetration depth of the evanescent wave, which arises under conditions of SPR, exponentially decreases with the distance from the predominantly gold layer of the sensor surface. It is almost extinguished at 700 nm. In fact, since the gold-bound surface may already extend to about 100 nm, the dimensions of the bacteria, like those of *E. coli* (5 μ × 1 μm), only contribute partially to the generation of the final SPR response. In addition, depending on the flow rate, particles tend to organize themselves in the center of the solvent flow, preventing their interaction with the ligand on the sensor surface. When associating with ligand, bacterial cells will bind in an unorientated manner, thereby hindering close cellular contact through, e.g., flagella and, therefore, only a few bound cells will contribute to a final response.

Other considerations are also of importance in other affinity assays, such as the enzyme-linked immunosorbent assay (ELISA). In indirect-detection methods, for example, biological fluids usually contain a complex milieu of components in variable amounts, which can affect the assay in a nonspecific manner. The sample matrix may contain soluble receptors and other antigen-binding proteins, which may yield false-positive results. In other instances, antibody-surface ligand interaction may be impaired and may produce false-negative results. Furthermore, antibody-binding proteins, such as complement factors, and binders of nonimmunoglobulin origin may occur at varying concentrations and contribute to unexpected results.

Typically, antigenic structures reflecting the target microorganism are covalently coupled to a solid surface and fish for humoral immune products of the infected host. A serological assay is as good as the antigen preparation and the complex that it can form with its binding partner. In most SPR biosensor configurations, this is of special importance, as the immunoglobulin has to be captured on the flight. In other words, it is not a static assay, as are ELISAs. Bacterial antigen preparations may not be homologous and co-immobilization of the nontargeted antigen may occur. This may be a source for false-positive results. Furthermore, heterophilic antibodies produced in the infected and in the noninfected individual may make reading errors when recognizing the antigen and may provide high background values. Antibodies raised against *Salmonella* spp., for example, may cross-react with antigens of *Citrobacter* spp. and/or *Klebsiella* spp. (11), which are also intestinal inhabitants.

Direct SPR Biosensor Detection

In direct analysis, detection relies on antigenic, genetic, or metabolic substances of the microorganisms. For example, Kai et al. (12) demonstrated hybridization of polymerase chain reaction (PCR) products of Shiga toxin-2 genes, reporting the presence of verotoxin-producing *E. coli* O157:H7 at 10^2 colony-forming units (CFUs)/0.1 g human stool using an SPR biosensor. In that study, a biotin-labeled peptide oligonucleotide containing 18 bases was used as sensor probe linked to a streptavidin-containing (SA) sensor chip.

In another study, DNA duplex formation was monitored in mixtures of PCR products of chimeric RNA-DNA primers

specific for *E. coli* O157:H7, verotoxin-2, and *Salmonella* virulence determinant (*invA*) genes (13). Biotinylated verotoxin and *invA* DNA fragments were immobilized using an SA sensor chip to probe produced so-called unilateral protruding DNA (UDP). Specific DNA-hybridization was also monitored to detect single-stranded DNA reflecting human immunodeficiency virus type I (HIV-1) obtained by asymmetric PCR on an SA sensor chip (14). Hybridization was accomplished with at least 60 very reproducible biosensor analysis cycles and demonstrated the presence of 1 to 100 fg HIV-1 *gag* DNA. It was considered one of the most rapid methods, as PCR (15 min) and SPR analysis (10 min) took only 30 min (14).

Fratamico et al. (15) detected immunochemically 2×10^6 CFU *E. coli* O157:H7 per test portion (7×10^7 CFU/mL) in a sandwich assay using a monoclonal anti-*E. coli* O157:H7 as ligand and a polyclonal antibody (Pab) as secondary antibody. Remarkably, this assay could not be improved by immobilizing the antibody through its F_c region by sensor chip-coupled protein A or protein G instead of direct coupling of the capturing antibody. The sensor chip could be used for at least 50 analyses.

In a similar way, *Salmonella* serogroups B, D, and E were detected at 2×10^3 CFU/10 μL test portion (2×10^5 CFU/mL; 16). An anti-*Salmonella* serogroup A, B, D, and E monoclonal antibody (Mab) was immobilized and more than 360 analyses could be performed. The direct detection of bacteria was enhanced dramatically by boiling the bacteria before analysis, and injection of a secondary anti-*Salmonella* Pab, resulting in a sandwich-like assay. Remarkably, like in the *E. coli* O157:H7 assay (15), in this study it was also found that best performance was obtained with a Mab as the first immobilized protein determining specificity, and a Pab as the second immunoglobulin for increased sensitivity. Based on the degree of enhancement of the response by the secondary antibody, the serogroup of the bound *Salmonella* could be predicted. This method has been applied to detect *Salmonella* in avian feces and meat within 8 h of culture at 5.5×10^2 CFU/25 g material in the original sample (17). For this purpose, immunomagnetic separation was applied to extract and concentrate *Salmonella* from homogenized samples before inoculation of the bead-bound *Salmonella* in brain heart infusion or Rappaport-Vassiliadis soy peptone broths, followed by SPR analysis.

In an attempt to improve detection, an inhibition assay was explored for detection of *E. coli* O157:H7 (18) and *Salmonella* (6; and this study). In the case of *E. coli*, bacterial cells were mixed with anti-*E. coli* O157:H7 antibodies and centrifuged; the supernatant was assayed for unreacted IgG by injection over a surface coated with protein A (18). Although this approach showed an enhanced sensitivity (10^6 CFU/mL) compared to the whole bacteria injection method, it is less sensitive relative to ELISA for *E. coli* O157:H7. In a review, it was reported that an inhibition assay was used to detect *Salmonella* at 4×10^6 CFU/mL (6). In that study, however, instead of centrifugation, ultrafiltration was used to separate unbound antibodies from cell-bound variants. Immobilized anti-Fab immunoglobulins were then used to capture free anti-*Salmonella* antibodies. In an ongoing project in our laboratory, we are currently applying an inhibition assay to detect *Salmonella* spp. as well. In this study, 20 μL 20-fold diluted polyvalent somatic (anti-serogroups A to S) antibodies was incubated with *Salmonella*-containing medium for 30 min. Antibodies were fractionated by centrifugation and nonbound antibodies were allowed to bind somatic antigens immobilized to the sensor surface. In this way, approximately 10^6 CFU/mL *S. enteritidis*, *S. goldcoast*, *S. livingstone*, and *S. typhimurium* as pure cultures could be detected, which has to be improved for screening purposes (nonpublished results, 2005).

Another serious food pathogen, *Listeria monocytogenes*, was detected by measuring free and unbound polyclonal IgG type antibodies as well (19). The antibodies were separated from the *Listeria*-bound variants by centrifugation and injected on a surface coated with covalently linked anti-Fab antibodies. A number of 120 analyses were performed over a 2-week interval before significant loss of binding capacity was observed. Of note, centrifugation was executed by gradually increasing speed from 50 to 3200 × *g* to prevent dissociation of bound antibodies and, in this way, 10^5 bacteria/mL were detected within 30 min (19). It must be noted that, in this study, pure cultures suspended in phosphate-buffered saline were used and that this sensitivity was not demonstrated in contaminated food samples nor with cells suspended in selective growth media.

Indirect SPR Biosensor Detection

To perform serology, well-defined and pure antigens are needed. Until now, antigenic structures included lipopolysaccharides (LPS), polysaccharides (PS), peptides, proteins, or neoglycoproteins (20–22). Antigens were isolated from corresponding microorganisms, or were produced by recombinant DNA techniques or chemically synthesized. The antigens were then immobilized to a solid support, such as a sensor chip. SPR technology offers the possibility to inject a secondary antibody to improve selectivity and sensitivity and to isotype the bound antibodies in terms of (secretory) IgA, IgE, IgG, IgM, and IgG_1 through IgG_4. This information can disclose the infection moment, as, e.g., IgM expression occurs before IgG expression (20).

Anti-HIV-1 antibodies were captured on surfaces containing 9 different oligopeptides of 15–40 amino acid residues in length (23). A concentration of 4.9 ng/mL Mab with high affinity for HIV-1 (strain IIIB) gp120 V3 loop peptide was readily detected. Various dilutions of heat-inactivated (56°C, 35 min) human sera were made in an aqueous buffer containing 10 mM HEPES pH 7.4, 150 mM NaCl, 3.4 mM EDTA, and 0.05% surfactant P20 (HBS buffer). The surface allowed 90 analyses, and biosensor results correlated well ($r^2 = 0.88$) with those of a conventional peptide ELISA (23). A similar biosensor assay was used to detect anti-HIV-1 antibodies against the V3 loop of HIV_{MN} in HBS buffer-diluted cerebrospinal fluid (CSF) of seropositive

patients, demonstrating the robustness of the method with respect to type of sample matrix (24).

Herpes simplex virus (HSV) infections could be detected and assigned to type 1 or type 2 using synthetic, biotinylated oligopeptides reflecting glycoprotein B-1 or glycoprotein B-2, respectively (22). The tagged peptides were immobilized on an SA sensor chip, which were exposed to human sera diluted 1:100 in HBS buffer. Compared to a Western blotting assay, the HSV-1 specific antibodies were detected with 83% sensitivity (SE) and 67% specificity (SP), whereas HSV-2 specific antibodies were detected with 86% SE and 100% SP (22).

Schistosoma mansoni, *S. haematobium*, or *S. japonicum* parasitic infections were detected serologically using human serum diluted 1:40 with HBS buffer containing 0.5% surfactant P20 (20). Diluted samples were injected over a sensor surface coated with neoglycoproteins (20). These xenobiotic proteins were constructed from bovine serum albumin conjugated with Lewis^x, GalNAcβ1-4GlcNAc and GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNAc structures. The neoglycoproteins were amine-coupled to the sensor's dextran layer and allowed at least 500 analysis cycles with excellent reproducibility of the measurements. For each sample, the total antibody responses as well as the specific IgG and IgM immunoglobulin responses were determined in a single run (20).

Membrane protein A from *Treponema pallidum*, the syphilis causative bacterium, was coated on gold-deposited slides and brought in contact with human sera for 1 h (25). The sera were 20-fold diluted in TRIS buffer containing 0.1% gelatin and 0.5% Tween 20. After washing, the slide was incubated with antihuman IgG antibodies to accomplish a sandwich-type assay. The attachment of antibodies was then analyzed in an SPR system configured without a flow system (25). Analysis of 10 blind-coded sera showed very good comparison with traditional tests for 3 clearly negative and 3 clearly positive sera, whereas the results of the remaining 4 samples were somewhat ambiguous, as were results from the conventional assays used in parallel (25).

We showed the first SPR analysis of antibodies in animal sera to determine past or current infections in chickens (21). For that purpose, *Salmonella enteritidis* (H:*g,m*) and *S. typhimurium* (H:*i* and H:*1,2*)-specific flagellar antigens were expressed as fusion proteins in *E. coli.* Isolated and purified antigens were amine-coupled to the sensor surface. To reduce nonspecific binding, sera were diluted 40 times in HBS buffer containing 0.5% (m/v) carboxymethyl dextran and 0.35 M NaCl. Besides sera and plasma, fresh chicken blood prevented from clotting was analyzed successfully for the presence of anti-*Salmonella* immunoglobulins as well (21).

In a nonpublished study, 383 chicken sera from differently treated flocks were screened using the biosensor assay and by an ELISA based on the same flagellar antigens. In the *Salmonella*-free flock, the H:*g,m* showed 100% SP, but false positives were found using H:*i* (18 out of 98 samples) and H:*1,2* (33 out of 98 samples) antigens (Table 1). However, 100% relative accuracy and 100% relative specificity was found for 30 sera collected from *S. gallinarum*-vaccinated chickens, which were negative in these assays, as expected. Relative accuracy (AC), SE, and SP were determined according to EN ISO 16140:1999. Flocks vaccinated with either *S. enteritidis* or a mixture of *S. enteritidis* and *S. typhimurium* showed good SP for the biosensor method, but a reduced and improved SE for H:*g,m* and for H:*i* and H:*1,2*, respectively, compared to the ELISA. Similar results were found for animals from flocks infected with either *S. enteritidis* or *S. typhimurium* (Table 1).

Although more than 300 analyses could be run, it was considered insufficient to screen routinely large animal populations at the farm or at the slaughter line. Furthermore, an assay detecting all food safety-relevant *Salmonella* serovars was desired. For this reason, somatic (O) antigens were isolated and purified from well-selected *Salmonella* serovars, which reflect *Salmonella* serogroups B, C, D, and E (results not shown). These LPS were coupled separately or as a mixture to sensor chips and as binding partners for immunoglobulins predicting *Salmonella* infections in chickens and pigs. The chips were regenerated at least 1500 times without significant loss of reproducibility of the analyses. In collaboration with the Animal Sciences Group (Wageningen University and Research Centre, Lelystad, The Netherlands), work is in progress to analyze more than 20 000 porcine samples in 2005 to test this assay for its merits compared to conventional ELISA.

This SPR biosensor assay is now also used to test egg yolks to identify infected flocks or to screen efficacy of vaccination protocols (work in progress). For this purpose, yolk homogenates were 5-fold diluted in HBS buffer containing 1% (m/v) carboxymethylated dextran, 0.85 M NaCl, and 0.05% (v/v) Tween-80 and applied to a Biacore 3000 instrument equipped with sensor chips coated with LPS isolated from *S. enteritidis*. While egg yolks of nonexposed laying hens gave approximately 20 response units (RUs), egg yolks from chickens exposed to 10^8 CFU *S. enteritidis* at age 20 weeks, gave 97–5461 RU in time up to 2 weeks after infection (N = 10). Pre-ovulatory follicles collected from 20 week-old chickens, which were exposed to 10^8 CFU at age of 16 weeks, were analyzed in an identical way. Averaged response of the follicles from the control group animals was 29 RUs (N = 8), whereas the follicles of the challenged animals showed responses between 789 and 4687 RUs, with an average of 2586 RUs (N = 5). Remarkably, and although animal experiments are not completely comparable, egg yolk IgY responses, in terms of RU, seemed to be almost an order of magnitude higher than those by their immunoglobulin counterparts in serum from similarly challenged broilers.

Detection of Microbial Metabolic Products

Detection of bacterial toxins can be considered as analysis of metabolic products revealing a history of microbial contamination. As possible bioterrorism agents, appropriate and sensitive biosensor methods are desired for fast detection of botulinum and shiga toxins, and *Staphylococcus*

Table 1. Results of SPR biosensor analysis of 383 chicken sera samples according to the biomolecular interaction assay (BIA; 21) using a Biacore 3000[a]

		Salmonella-free		*S. gallinarum* vaccinated		*S. enteritidis* vaccinated		*S. enteritidis*/ *S. typhimurium* vaccinated		*S. enteritidis* infected		*S. typhimurium* infected	
Antigen	Biosensor response	ELISA+	ELISA–	ELISA+	ELISA–	ELISA+	ELISA–	ELISA+	ELISA–	ELISA+	ELISA–	ELISA+	ELISA–
H:*g,m*	BIA+	0	0	0	0	13	0	20	0	4	7	1	2
	BIA–	7	91	0	30	7	0	14	3	7	80	14	81
H:*i*	BIA+	0	18	0	0	1	9	1	7	2	51	1	18
	BIA–	4	76	0	30	2	8	1	28	1	44	4	75
H:*1,2*	BIA+	3	30	0	0	1	3	0	5	1	63	0	30
	BIA–	4	61	0	30	2	14	1	31	0	34	1	67

[a] Results were compared to an ELISA, which used identical antigens as capturing ligands coated to the wells of the plates. Sera were obtained from broilers, which were either free form *Salmonella*, vaccinated with *S. gallinarum*, *S. enteritidis*, or a mixture of *S. enteritidis* and *S. typhimurium*, or which were challenged with *S. enteritidis* or *S. typhimurium*. Number of samples is noted in each cell.

enterotoxin B (SEB) in water, food, and air. It must, however, be noted that most studies address biosensor analysis of toxins to prevent food-intoxications rather than to reveal the corresponding producing organism, and therefore these studies are not discussed here. The SEB toxin produced by *Staphylococcus aureus*, which regularly contaminates food production lines, was successfully detected in milk and meat at 10 ng/mL (26). The method used a secondary antibody to gain sensitivity and specificity in 8 min analysis cycles. SEB was also identified at 1 ng/mL in milk and mushroom homogenates by a combination of SPR analysis and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (SPR-MS; 27, 28). MS analysis of the sensor-captured molecules revealed whether unwanted cross-reactivity or nonspecifically bound biomolecules occurred, and disclosed information on the degree of degradation of the captured toxins. Interestingly, with respect to the biological warfare hazard, SPR-MS is able to reveal whether the Pab-captured toxins were genetically or chemically modified (28).

We developed an SPR biosensor assay for the detection of *Streptococcus suis* (nonpublished results, 2005), which is one of the most important swine pathogens worldwide and is also threatening the health of abattoir workers and pork handlers, as shown by the summer 2005 outbreak in Southeast Asia (29). In an immunobiosensor assay, selective-growth Todd-Hewit culture broths are screened for the presence of a proteinaceous extracellular factor (EF), which is considered a key virulence factor in the pathogenesis of *S. suis* serotype 2 infections (30). Despite its favorable molecular mass (110 kDa), an inhibition assay had to be designed to increase sensitivity and selectivity relative to the assay capturing EF directly on the sensor. Following removal of cells and concentration and purification over an ultrafilter, culture medium was incubated with murine anti-EF antibody before injection over a sensor chip coated with EF antigen. Following this injection, an antibody against murine antibodies was used to enhance the biosensor response. In this way, 4 ng/mL EF was easily detected and the assay was used to screen field samples, including tonsil samples of noninfected and infected pigs, with good correlation with PCR and selective growth results (nonpublished results, 2005).

Besides detection of pathogenic microorganisms, many SPR biosensor studies have appeared that study the adhesion or interaction of pathogens with surface biomolecules (31–37). However, discussion of these papers is considered outside the scope of this review.

Conclusions

Progress is rapid in the application of SPR to secure food and environmental safety, including determination of a range of important community health-threatening microbial entities, including bacteria, parasites, and viruses. This overview of current literature and ongoing projects demonstrates that the discussed SPR technology can detect microbial agents with a

good possibility for identification at the species, subspecies, and strain level. In addition, the platform provides concentration-dependent responses, so that titers (serology) or the number of agents can be determined. In serology, SPR analysis offers the possibility to determine the isotypes, which result from a humoral response and which gives insight into the infection history of the host.

The optical SPR biosensors promise multiple, simultaneously analyzed analytes with potential for high-throughput screening. The current integration of SPR technology, e.g., in the slaughter process to monitor *Salmonella* contamination, is ongoing, but is still in its infancy; there is no reason, though, to renounce a full and advanced integration of these instruments in quality and safety management systems, which are introduced to increase feed, food, and environmental safety.

Acknowledgments

We are grateful to G. Bokken, T. Companjen, B. Jongerius, J. Kok, O. van der Kolk, T. Sowar El Dahab, E. Thomas, and J.-W. Verhaag (Division of Public Health and Food Safety, Utrecht University), and B. Swildens (Department of Farm Animal Health, Utrecht University) for providing their preliminary results.

References

(1) Meslin, F.X., Stohr, K., & Heymann, D. (2000) *Rev. Sci. Tech. Off. Int. Epiz.* **19**, 310–317

(2) Koopmans, M., Wilbrink, B., Conyn, M., Natrop, G., Van der Nat, H., Vennema, H., Meijer, A., van Steenbergen, J., Fouchier, R., Osterhaus, A., & Bosman, A. (2004) *Lancet* **363**, 587–593

(3) Rand, A.G., Ye, J., Brown, C.W., & Letcher, S.V. (2002) *Food Technol.* **56**, 32–39

(4) Ivnitski, D., Abdel-Hamid, I., Atanasov, P., & Wilkins, E. (1999) *Biosens. Bioelectron.* **14**, 599–624

(5) Karlsson, R. (2004) *J. Mol. Recognit.* **17**, 151–161

(6) Medina, M.B. (1997) *Food Test. Anal.* **3**, 14–16, 36

(7) Koubová, V., Brynda, E., Karasová, L., Škvor, J., Homola, J., Dostálek, J., Tobiška, P., & Rošický, J. (2001) *Sens. Actuators B* **74**, 100–105

(8) Patel, P. (2002) *TRAC-Trends Anal. Chem.* **21**, 96–115

(9) O'Kennedy, R., Leonard, P., Hearty, S., Daly, S., Dillon, P., Brennan, J., Dunne, L., Darmaninsheehan, A., Stapleton, S., Tully, E., Quinn, J., & Chakraborty, T. (2005) in *Rapid Methods for Biological and Chemical Contaminants in Food and Feed*, A. Van Amerongen, D. Barug, & M. Lauwaars (Eds), Wageningen Academic Publishers, Wageningen, The Netherlands, pp 85–104

(10) Indyk, H.E., & Filonzi, E.L. (2003) *J. AOAC Int.* **86**, 386–393

(11) Wray, C., & Wray, A. (Eds) (2000) in *Salmonella in Domestic Animals*, CAB International, Oxon, UK

(12) Kai, E., Ikebukuro, K., Hoshina, S., Watanabe, H., & Karube, I. (2000) *FEMS Immunol. Med. Microbiol.* **29**, 283–288

(13) Miyachi, H., Yano, K., Ikebukuro, K., Kono, M., Hoshina, S., & Karube, I. (2000) *Anal. Chim. Acta* **407**, 1–10

(14) Bianchi, N., Rutigliano, C., Tomassetti, M., Feriotto, G., Zorzato, F., & Gambari, R. (1997) *Clin. Diagn. Virol.* **8**, 199–208

(15) Fratamico, P.M., Strobaugh, T.P., Medina, M.B., & Gehring, A.G. (1998) *Biotechnol. Tech.* **12**, 571–576

(16) Bokken, G.C.A.M., Corbee, R.J., van Knapen, F., & Bergwerff, A.A. (2003) *Fems Microbiol. Lett.* **222**, 75–82

(17) Corbee, R.J., Bokken, G.C.A.M., van Knapen, F., & Bergwerff, A.A. (2005) (in preparation)

(18) Fratamico, P.M., Strobaugh, T.P., Medina, M.B., & Gehring, A.G. (1997) in *Book of Abstracts, 214th ACS National Meeting,* American Chemical Society, Las Vegas, NV, September 7–11, AGFD-017

(19) Leonard, P., Hearty, S., Quinn, J., & O'Kennedy, R. (2004) *Biosens. Bioelectron.* **19**, 1331–1335

(20) van Remoortere, A., van Dam, G.J., Hokke, C.H., van den Eijnden, D.H., van Die, I., & Deelder, A.M. (2001) *Infect. Immun.* **69**, 2396–2401

(21) Jongerius-Gortemaker, B.G., Goverde, R.L., van Knapen, F., & Bergwerff, A.A. (2002) *J. Immunol. Methods* **266**, 33–44

(22) Wittekindt, C., Fleckenstein, B., Wiesmuller, K., Eing, B.R., & Kuhn, J.E. (2000) *J. Virol. Methods* **87**, 133–144

(23) VanCott, T.C., Loomis, L.D., Redfield, R.R., & Birx, D.L. (1992) *J. Immunol. Methods* **146**, 163–176

(24) Lucey, D.R., VanCott, T.C., Loomis, L.D., Bethke, F.R., Hendrix, C.W., Melcher, G.P., Redfield, R.R., & Birx, D.L. (1993) *J. Acq. Immun. Def. Synd.* **6**, 994–1001

(25) Sevars, A.H., Schasfoort, R.B., & Salden, M.H. (1993) *Biosens. Bioelectron.* **8**, 185–189

(26) Rasooly, A. (2001) *J. Food Prot.* **64**, 37–43

(27) Nedelkov, D., Rasooly, A., & Nelson, R.W. (2000) *Int. J. Food Microbiol.* **60**, 1–13

(28) Nedelkov, D., & Nelson, R.W. (2003) *Appl. Environ. Microbiol.* **69**, 5212–5215

(29) Staats, J., Feder, I., Okwumabua, O., & Chengappa, M. (1997) *Vet. Res. Commun.* **21**, 381–407

(30) Vecht, U., Wisselink, H., Jellema, M., & Smith, H. (1991) *Infect. Immun.* **59**, 3156–3162

(31) Verdonck, F., Cox, E., Vancaeneghem, S., & Goddeeris, B.M. (2004) *FEMS Immunol. Med. Microbiol.* **41**, 243–248

(32) Medina, M.B. (2004) *Int. J. Food Microbiol.* **93**, 63–72

(33) Holmes, S.D., May, K., Johansson, V., Markey, F., & Critchley, I.A. (1997) *J. Microbiol. Methods* **28**, 77–84

(34) Medina, M.B. (2001) *Int. J. Food Microbiol.* **69**, 199–208

(35) Pourshafie, M.R., Marklund, B.I., & Ohlson, S. (2004) *J. Microbiol. Methods* **58**, 313–320

(36) Amano, A., Nakamura, T., Kimura, S., Morisaki, I., Nakagawa, I., Kawabata, S., & Hamada, S. (1999) *Infect. Immun.* **67**, 2399–2405

(37) Wann, E.R., Gurusiddappa, S., & Hook, M. (2000) *J. Biol. Chem.* **275**, 13863–13871