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An immunosensor for syphilis screening based on surface plasmon resonance

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Abstract: In this paper the development of a surface plasmon resonance (SPR) immunosensor for syphilis screening is described. This immunosensor is based on the detection of antibodies in serum against the causative organism *Treponema pallidum*. In order to achieve selectivity a recombinant *Treponema pallidum* membrane protein A (TmpA) was used. This antigen can react with antibodies to *T. pallidum*, present in serum of syphilitic patients.

Reproducible results have been obtained, using a 'sandwich SPR' method: binding of a sandwich antibody to the treponemal antibody after serum incubation was measured in real time while the binding was taking place. The SPR results obtained from ten blind-coded sera corresponded well with classical syphilis tests (*Treponema pallidum* haemagglutination assay (TPHA), fluorescent treponemal antibody-absorbed test (FTA-ABS), venereal diseases research laboratory flocculation test (VDRL) and TmpA-based enzyme-linked immunosorbent assay (TmpA-ELISA)). Preliminary experiments showed that direct measurement of serum (in the 'one step SPR') is not yet possible, probably as a result of non-uniformity of serum samples. The application of latex beads is considered to solve this problem.

Keywords: immunosensor, surface plasmon resonance, syphilis, *Treponema pallidum* membrane protein A.

1. INTRODUCTION

Modern blood banks require an intensive screening for infectious diseases, so there is a great need for a fast and automated screening system. In the case of the serological screening of syphilis the classical methods (*Treponema pallidum* haemagglutination assay (TPHA), fluorescent treponemal antibody-absorbed test (FTA-ABS) and venereal diseases research laboratory flocculation test (VDRL)) have the disadvantage that they cannot be automated (Seidl, 1990). Several enzyme-linked immunosorbent assays (ELISAs) have therefore been developed, based on different

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antigens (IJsselmuiden *et al.*, 1989). These systems have the disadvantage that they are time consuming and often not comparable between laboratories.

Immunosensors are interesting for clinical applications because they offer a number of potential advantages over conventional assay techniques; the detection is quick and the system can easily be automated (Liedberg *et al.*, 1983; Robinson, 1991). Immunosensors based on surface plasmon resonance (SPR) measure changes in the reflection of laser light, caused by a change in refractive index at a metal–liquid interface as a result of antigen–antibody reactions. In this paper the application of an SPR immunosensor for syphilis screening is described.

2. MATERIALS AND METHODS

2.1. Detection system

The SPR sensor is based on the excitation of surface plasmons in thin metal layers (Raether, 1977). In our SPR sensor, excitation takes place in the so-called attenuated total reflection method that makes use of a Kretschmann configuration (Daniels *et al.*, 1988). The system has an adapted, horizontally placed test slide holder for Balzer's disposable glass slides on which a 50 nm gold layer is deposited (University of Twente, Department of Thin Films, The Netherlands). p-polarized laser light from a diode laser with an electronic control unit (DL25, wavelength 780 nm, Spindler & Hoyer) is reflected by the gold layer (Fig. 1).

At a specific angle of incidence of the laser light, electronic oscillations, called plasmons, are generated in the metal and the intensity of the reflected beam is diminished. These plasmons cause a non-propagating evanescent field, which penetrates from the metal surface into the overlying sample fluid to a depth of about half a wavelength (400 nm). Local changes in the refractive index, such as those produced by binding of antibodies to immobilized antigens, disturb this field and the plasmons rise at a different angle of incidence. The angle shift can be detected by measuring a calibrated change in intensity of the reflected beam at a fixed angle of incidence with a pin diode (UDT pin-10DP 128–1, United Detector Technology and Te Lintelo Systems BV). To prevent the influence

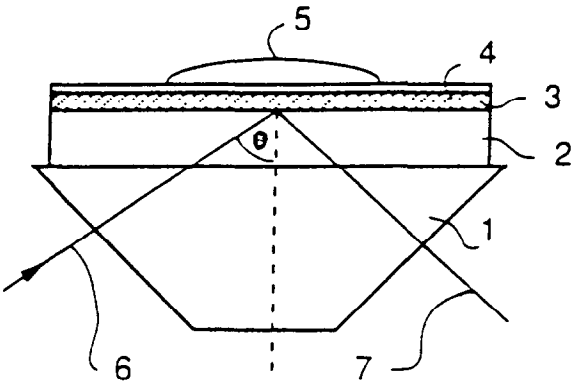

Fig. 1. The Kretschmann configuration. A prism (1) is covered with a glass slide (2), coated with gold (3) and a selective layer (4), and exposed to a sample fluid (5); p-polarized laser light (6) is reflected off the gold-coated slide. At a certain angle of incidence, plasmons are generated in the gold and the intensity of the reflected beam (7) is diminished. Affinity reactions can be detected by measuring the change in intensity of the reflected beam at a fixed angle of incidence.

of light from the surroundings, the laser is modulated by an oscillator from a lock-in amplifier (PARC 5209, single phase, EG&G, Princeton Applied Research). The SPR response is saved on the diskette of a digital oscilloscope (Nicolet 310) and computed in VU-Point (version 1.28, S-Cubed).

2.2. Antigens and sera

Treponema pallidum membrane protein A (TmpA) was obtained from Euro-Diagnostics. Human sera were obtained from RIVM (Bilthoven, The Netherlands): R-1 and R-2 were respectively a standard syphilis positive and negative serum; X-1. . .X-10 were blind-coded sera.

2.3. Coating procedure

Gold-deposited slides were first coated with TmpA and then blocked with gelatin (0·1%, Oxoid) in TRIS buffer (Tris-hydroxymethyl-amino-methane).

2.4. Test procedure

Sera were diluted 1:20 in TRIS buffer containing gelatin (0·1%) and Tween 20 (0·5%, Baker).

In the one-step SPR method, the diluted sera

were put on the TmpA-coated slides and the SPR signal was recorded directly.

In the sandwich SPR method, the diluted sera were incubated on the TmpA-coated slides for one hour. After washing three times with TRIS buffer, unconjugated rabbit anti-human IgG (Dakopatts) was put on the slides and the SPR signal was recorded.

3. RESULTS

Standard syphilis positive (R-1) and negative (R-2) sera were tested with the SPR immunosensor according to the sandwich SPR method. Mixtures of R-1 and R-2 sera in different ratios have been used to measure different concentrations of antibodies (Fig. 2).

Figure 2 shows clearly that real-time detection of binding of anti-human IgG to the treponemal antibody after incubation of serum is feasible. As can be seen, the detection could be achieved within 20 min with reasonable reproducibility between experiments.

The sandwich SPR results of ten blind-coded sera (X-1. . .X-10) were compared with classical syphilis tests (Table 1).

It was clear from the SPR results that sera X-1, X-5 and X-9 were positive and sera X-2, X-3 and X-10 were negative. These results were in good agreement with the traditional tests. The results of X-4 were not so pronounced, either in the classical tests or in SPR: VDRL was negative, ELISA slightly positive and TPHA and FTA-ABS positive. The SPR immunosensor yielded signals just between positive and negative. Sera X-6 and X-8 showed relatively high signals in SPR compared to the classical tests. It is interesting to note that X-6 also gave a positive result with FTA-ABS. Serum X-7, which was positive in the other tests, showed a relatively low SPR signal.

Sera R-1 and R-2 have also been measured directly with the one-step SPR method. Preliminary experiments show that real-time detection in serum of binding of treponemal antibodies to the TmpA coating is not yet feasible. Addition of serum gave rise to an irreproducible non-specific signal. A 100-fold dilution of the serum matrix gave no improvement in discrimination.

4. DISCUSSION

This paper clearly demonstrates the potency of the SPR immunosensor for clinical applications, particularly for syphilis screening. The results show that *T. pallidum* antibodies can be detected in serum by a sandwich SPR procedure. In comparison with the incubation step (1 h), the detection step is very fast; within only 30 s a discrimination between a positive and a negative sample is possible. The reproducibility of the

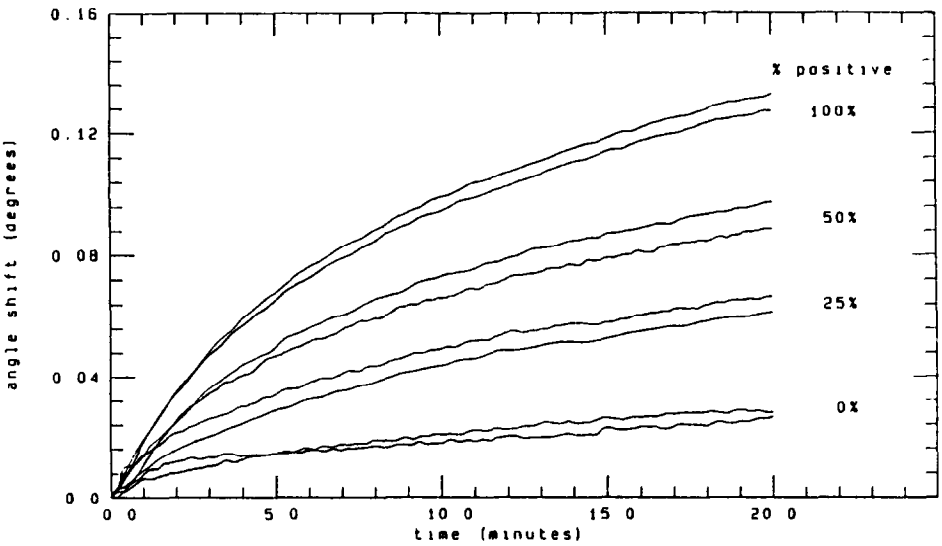

Fig. 2. SPR signals (in duplicate), recorded during the sandwich assay of a standard syphilis positive serum sample (R-1, 100% positive), a standard negative serum sample (R-2, 0% positive) and mixtures of R-1 and R-2 in different ratios.

TABLE 1 Comparison of the sandwich SPR results with traditional syphilis test results. Figures represent qualitative scores (TPHA; FTA-ABS), reciprocal dilution, which just gave positive results (VDRL), or optical density measurements at a standard dilution (1:20, TmpA-ELISA). SPR results are expressed as percentages of the standard positive serum R-1.

Serum code	*TPHA*	*FTA-ABS*	*VDRL*	*TmpA-ELISA*	*SPR*
X-1	2	3	4	48	65
X-2	0			12	5
X-3	0			12	0
X-4	1	2	0	26	10
X-5	3	3	32	124	120
X-6	0	1	0	15	20
X-7	2	3	4	69	25
X-8	0			14	15
X-9	1	1	1	70	90
X-10	0			13	−5

system is reasonable, especially when we take into account that coating procedures and serum incubations are not yet optimized. From the comparison of SPR with traditional tests it can be concluded that SPR results below 10% are negative and those above 25% are positive. Apparently, however, between 10 and 25% it cannot be determined whether the sample is positive or negative.

With regard to the relatively high concentrations of specific IgG in serum, real-time detection by the one-step SPR method should be possible. However, in the case of syphilis screening, this appeared not to be feasible under the test conditions as a result of non-specific matrix effects, probably due to non-uniformity of serum samples. Direct detection can only be performed if there is a pronounced difference between the refractive index of the binding antibodies and the bulk refractive index of the serum. Perhaps the concentration of treponemal antibodies in serum is too low compared with an ineradicable background signal. Another explanation is that the TmpA coating procedure is not optimal, resulting in an insufficient refractive index change when treponemal antibodies bind to the TmpA coat.

In comparison with the sandwich SPR method, the one-step SPR has several advantages: fast detection, with no washing or separation steps required. It would also be possible to perform the one-step test in remote locations (e.g. the doctor's surgery). On the other hand, in the sandwich SPR method the second step, using a sandwich antibody, serves as an amplification step. The advantage of this procedure is that it is less influenced by non-uniformity of serum samples from different patients. In comparison with ELISA, conjugation of sandwich antibodies to, for instance, enzymes or other labels is not required.

The aim of our future research is to realize a one-step test. Several approaches can be followed. Currently, we have developed an SPR competition assay based on latex beads of submicrometre size (Schasfoort, 1990). For application of this test to syphilis screening, treponemal antibodies must be coated on latex particles. When these particles are mixed with a serum sample, a competition will take place between antibodies on the latex and free antibodies from the serum for binding to the TmpA coat. As binding of latex to the surface changes the refractive index dramatically, non-specific signals of the serum will probably dissipate. Generally, latex particles are versatile tools to improve sensitivity and can be applied to convert immunoassays to SPR immunosensors.

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