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Chemiluminescent optical fiber immunosensor for the detection of anti-West Nile virus IgG☆

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Abstract

An ELISA-based optical fiber methodology developed for the detection of anti-West Nile virus IgG antibodies in serum was compared to standard colorimetric and chemiluminescent ELISA based on microtiter plates. Colorimetric ELISA was the least sensitive, especially at high titer dilutions. The fiber-optic immunosensor based on the same ELISA immunological rationale was the most sensitive technique.

Keywords: Optical fiber immunosensor; ELISA; West Nile virus; Chemiluminescence

1. Introduction

West Nile virus (WNV) is a mosquito-borne viral pathogen that belongs taxonomically to the Flaviridae family (genus *Flavivirus*). The virus was first identified in 1937 from a febrile adult female from the West Nile District in Uganda [24]. Numerous large epidemics of West Nile fever and meningoencephalitis were recorded in Romania, Russia, Israel and, more recently, in North America [2,4,10,15]. The scientific community is currently invests exerting considerable effort to develop new strategies to prevent, diagnose and treat WNV infection. Recently, it was shown that full prophylactic and therapeutic effects obtained in mice after their treatment with intravenous pooled plasma or immunoglobulins obtained from asymptomatic Israeli blood donors that contained WNV-specific antibodies [1,20]. Molecularly engineered live-attenuated chimeric West Nile/dengue virus vaccines were also used to protect rhesus monkeys from West Nile virus [17].

Laboratory diagnosis of WNV infections is based on the isolation of the virus [6], the detection of viral RNA using reverse transcription polymerase chain reaction (RT-PCR) [21], the detection of antibody by neutralization test (NT) [27] or by enzyme-linked immunosorbent assay (ELISA). Detection of WNV RNA with either RT-PCR or NT has been described [5,7,23], but despite the fact that they demonstrated high sensitivity and specificity, they are time-consuming techniques that must be performed in sophisticated reference laboratories [9].

The serodiagnosis of Flavivirus infection routine is preferentially based on the detection of IgM antibodies by capture or indirect IgG ELISA. Following the 1999 North American WNV outbreak, many laboratories developed immunoassays for the detection of WNV antibodies [26]. Shi and Wong [22] recently reviewed the different serological methods applied to the diagnosis of WNV infection and emphasized the latest

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improvement in WNV antibodies detection with a recombinant antigen microsphere immunoassay [28]. However, flow cytometry based assays remain very expensive in low or middle throughput analysis due to the initial investment in the reading equipment and are always labor intensive. Because most cases of WNV infection are sub-clinical, monitoring the spread of the disease will require the use of very sensitive point-of-care devices like biosensors.

A biosensor couples an immobilized biospecific recognition entity to the surface of a transducer, which 'transduces' a molecular recognition event into a measurable electrical signal [8]. Optical fiber sensors are ideal transducers governed by Snell's law. They have the following advantages [13]: (1) geometric convenience and flexibility; (2) low cost of production; (3) they are inert and therefore non-hazardous; (4) they are free of electric interference; (5) being dielectric, they are protected against atmospheric disturbances; (6) their small volume economizes reagents and enables portability as well as access to difficult areas; (7) they are robust with high tensile strength; (8) their silica composition enables macromolecular conjugation via silanization; (9) they enable solid-phase characterization of the analyte; (10) their potentially long interaction lengths enable remote signal transmission; (11) light transmission is done with minimal loss; (12) high efficiency coupling occurs in the blue region which is ideal for chemiluminescence; (13) optical multiplicity; (14) polyvalence, as an optrode system, they can be easily adapted from one antigen–antibody system to another; (15) they are amenable to mass production; (16) they enable multiple antigen detection via fiber bundles.

Optical fiber biosensors based on biological or chemical luminescence have already shown their ability to detect biological entities such as whole organisms [12], nucleic acids [3], genotoxicants [18] and antibodies [11] with high specificity and sensitivity. One previous report also demonstrated that, optical fiber immunoassays based on chemiluminescence could have some advantages over ELISA mainly concerning the testing time [25].

Immobilization of antigen on the fiber surfaces is usually achieved by silanization by a bi-functional silane (general structure $(MeO)_3Si(CH_2)_nX$) that allows for the covalent attachment of the biomolecule with an appropriate cross-linker or by direct chemical modification of the reactive X group [13]. However, liquid-phase silanization often provides low reproducibility of the silane layer configuration and gas phase preparation, whereas producing better quality surfaces, usually requires vacuum conditions and high temperature to obtain high surface coverage. A recent study showed the possibility of performing a gas-phase silanization procedure at room temperature and atmospheric pressure on silicon oxide [16], which we will modify for our present system.

We report herein the first successful construction of a chemiluminescent anti-WNV IgG optical fiber immunosensor based on the immobilization of inactivated virions on a fiber-glass surface previously functionalized by using argon-phase silanization. Performance of the optical fiber immunoassay is afterwards compared to conventional colorimetric and chemiluminescent ELISA using the same immunological rationale.

2. Experimental

2.1. Chemicals and biochemicals

Bovine serum albumin (BSA) (A4503), β-propiolactone (P5648), goat anti-mouse IgG peroxidase-labeled antibodies (A3682), 6-maleimidohexanoic acid *N*-hydroxysuccinimide ester (EMCS) (M9794), (3-mercaptopropyl)trimethoxysilane (A1521) and polyoxyethylene-sorbitan monolaurate (Tween20) (P7949) were purchased from Sigma. Fetal calf serum (FCS) (0.4-001-1) was purchased from Biological Industries Ltd. (Kibbutz Beit Haemek, Israel). Luminescence measurements were carried out using the Western Blot Chemiluminescence Reagent Plus kit from Perkin-Elmer™ Life Science Products (NEL105). Colorimetric assays were performed with 3,3′,5,5′-tetramethylbenzidine (TMB) from Chemicon International Inc. (ES001).

2.2. Preparation of the WNV antigen solution

WNV antigen was prepared according to the method described by Ben-Nathan et al. [1]. Briefly, WNV (Israeli strain, 1952) was grown on Vero cells and at the appropriate cytopathic effect (CPE) (60–70%) the flasks were frozen overnight at −70 °C. After low-speed centrifugation ($1560 \times g$ for 10 min) to remove cell debris, the virus was concentrated 20-fold by high-speed centrifugation ($140{,}000 \times g$ for 90 min) and resuspended in phosphate buffer saline pH 7.2 (PBS) (0.203 g l^{-1} NaH_2PO_4, 1.149 g l^{-1} Na_2HPO_4, 8.5 g l^{-1} NaCl, adjusted to pH 7.2 with 0.1 M HCl/NaOH). The virus was inactivated using β-propiolactone (0.001% final concentration), an alkylating agent that permeates the protein capsid of the viruses and chemically modifies the nucleic acid. To confirm that virus inactivation was complete, the suspension was tested by plaque assay on Vero cells and in 3-day-old suckling mice (Harlan Olac). No viral infectivity could be detected by either methods, confirming complete virus inactivation.

2.3. Preparation of mouse anti-WNV antiserum

Five-week-old BALB/c mice (Harlan Olac) were each injected i.p. with 1×10^4 plaque forming units (PFU) of WNV per mouse. Two weeks later, surviving mice were boosted with 1×10^4 PFU of virus and bled 14 days later. Blood was then centrifuged ($1560 \times g$ for 10 min), the serum collected and then inactivated for 30 min at 56 °C. Thereafter, the samples were stored at −20 °C or used immediately. Control mice were injected with PBS (pH 7.2).

2.4. Detection of anti-WNV IgG antibodies by ELISA

ELISA was performed according to the method of Ben-Nathan et al. [1] with slight modifications. In brief, a volume of 75 μl of the WNV antigen solution (dilution 10^{-2}) was added into each well of a 96-well microtiter plate (MaxiSorp, Nunc). The plate was sealed to avoid evaporation and well-coating of inactivated virions was allowed to take place overnight at 4 °C. After the incubation, the coating buffer was decanted and the plate was washed with PBS (pH 7.2). A blocking step was used, in which 150 μl/well of blocking solution (FCS/BSA 5% (w/v)–1% (w/v) in PBS (pH 7.2) was added to reduce the overall background and increase the sensitivity of the assay. The plate was then incubated for 1 h at 37 °C and the wells washed thrice with PBS–Tween20–BSA (PBST–BSA) (10 g l^{-1} BSA, 0.203 g l^{-1} NaH_2PO_4, 1.149 g l^{-1} Na_2HPO_4, 8.5 g l^{-1} NaCl, 0.05% (v/v) Tween20, adjusted to pH 7.2 with 0.1 M HCl/NaOH). Then 75 μl to each well of diluted serum (dilutions ranging from 10^2 to 10^6) were added in triplicates and the plate incubated 1 h at 37 °C. Sets of three wells were prepared for each concentration measured, and another three wells were used with the control serum. Additional empty wells and non-coated wells were used to check the level of the background signal. The wells were then washed thrice with PBST–BSA (pH 7.2), 75 μl/well of a solution of goat anti-mouse IgG peroxidase-labeled antibodies (10^{-3}) was added and the plate incubated 1 h at 37 °C. After incubation the wells were finally washed thrice with PBST–BSA (pH 7.2) before reading. For the colorimetric ELISA, a total volume of 100 μl of substrate (TMB) was added into each well and the plate was incubated at room temperature for 30 min in the dark. A volume of 50 μl/well of 2N H_2SO_4 was added to stop the chemical reaction. The absorbance at 450 nm was determined with the Labsystems Multiscan RC ELISA reader after 10 s of shaking. The data were collected by using the Labsystems Transmit software, the mean and the standard deviation of the triplicates were calculated for each point and the signal reported in OD 450 units (optical density at 450 nm). In the chemiluminescent ELISA, the microtiter plate was read with a standard luminometer (Thermolabsystems-Luminoskan Ascent) where, before each well measurement, oxidizing reagent and enhanced luminol reagent solutions (NEL105 chemiluminescence reagent kit) were injected into the well in a 1:1 ratio. A total of 20 measurements were performed with a gap of 1 s between each reading. The data were collected with the Luminoskan Ascent Software, the mean and the standard deviation of the triplicates were calculated for each point and the signal reported in RLU (relative light units).

2.5. Preparation of optical fibers modified with WNV antigen

All fibers used were SFS400/440B Superguide G UV-Vis all silica fibers (Fiberguide Industries, Stirling, USA) with an original numerical aperture (NA) of 0.22. Their core, which is 400 μm in diameter (refractive index of 1.457 at 633 nm), is surrounded by a 40 μm silica cladding (refractive index of 1.44 at 633 nm), followed by a 150 μm-thick silicon buffer and finally a 210 μm-thick black Tefzel jacket. The length of any single fiber used in our experiments was 25 cm in length. The black Tefzel jacket and silicon buffer were stripped away mechanically using a fiber-stripping tool (Micro-Strip, from Micro-Electronics Inc., USA), leaving a 1 cm nude optical fiber core tip. In order to prepare the fibers for the silanization procedure, they were soaked successively in distilled water and ethanol for 10 min each to remove major contaminants. Then a 5 min chemical etching in 48% (v/v) hydrofluoric acid was used in order to guarantee the complete removal of the silica cladding. Afterwards, the fiber tips were soaked in a 1:1 methanol/97% HCl (v/v) solution for 30 min to purify the newly exposed fiber core from micro-contaminants. Finally, the optical fiber tips were exposed to 95% (v/v) sulfuric acid for 30 min to enhance the exposure of hydroxyl groups on the silica surface. A quick rinse with distilled water followed by drying by airflow was applied between all the previously described steps to clean the surface from the previous solutions. The silanization procedure was then performed by disposing the fibers in a reactor chamber. The different steps performed to reach the covalent binding of the antigen to the optical fiber are shown in Fig. 1A. After exposure of the hydroxyl groups (step 1), a total volume of 10 μl of pure mercaptosilane was manually injected in the bottom of the reactor and a flow of 0.5 l/min of argon was allowed to flow into the reactor bulk for a total duration of 30 min at room temperature and ambient pressure. In that way, silane molecules directly reacted to the fiber surface by gas phase deposition (step 2). After silanization the fibers were rinsed 10 min in ethanol to remove the unattached silane molecules and placed in PBS (pH 7.2). The fibers were then dipped in a 1:9 (v/v) DMSO/PBS (pH 7.2) solution containing 2 mM EMCS for a total duration of 60 min at room temperature. The maleimide group of the EMCS reacted strongly with the thiol group of the silane and a covalent bond was created (step 3). The fibers were rinsed in DMSO to remove the non-bound cross-linker molecules and then dipped in 75 μl of WNV antigen solution (10^{-2}) for 60 min at room temperature. The ester group of the EMCS reacted with an amino group presented at the surface of the inactivated virions, creating an amide bond and thus attaching covalently the antigen to the fiber surface (step 4). The modified fibers were either used immediately or stored at 4 °C.

2.6. Detection of anti-WNV IgG antibodies using the optical fiber immunosensors

The steps carried out in the immunoassay procedure are shown in Fig. 1B. The West Nile virus-conjugated fibers were dipped for 20 min in 150 μl of blocking solution (step 5), FCS/BSA (5% (w/v)–1% (w/v)) in PBS (pH 7.2) then rinsed and washed thrice with PBS (pH 7.2). The fibers were then

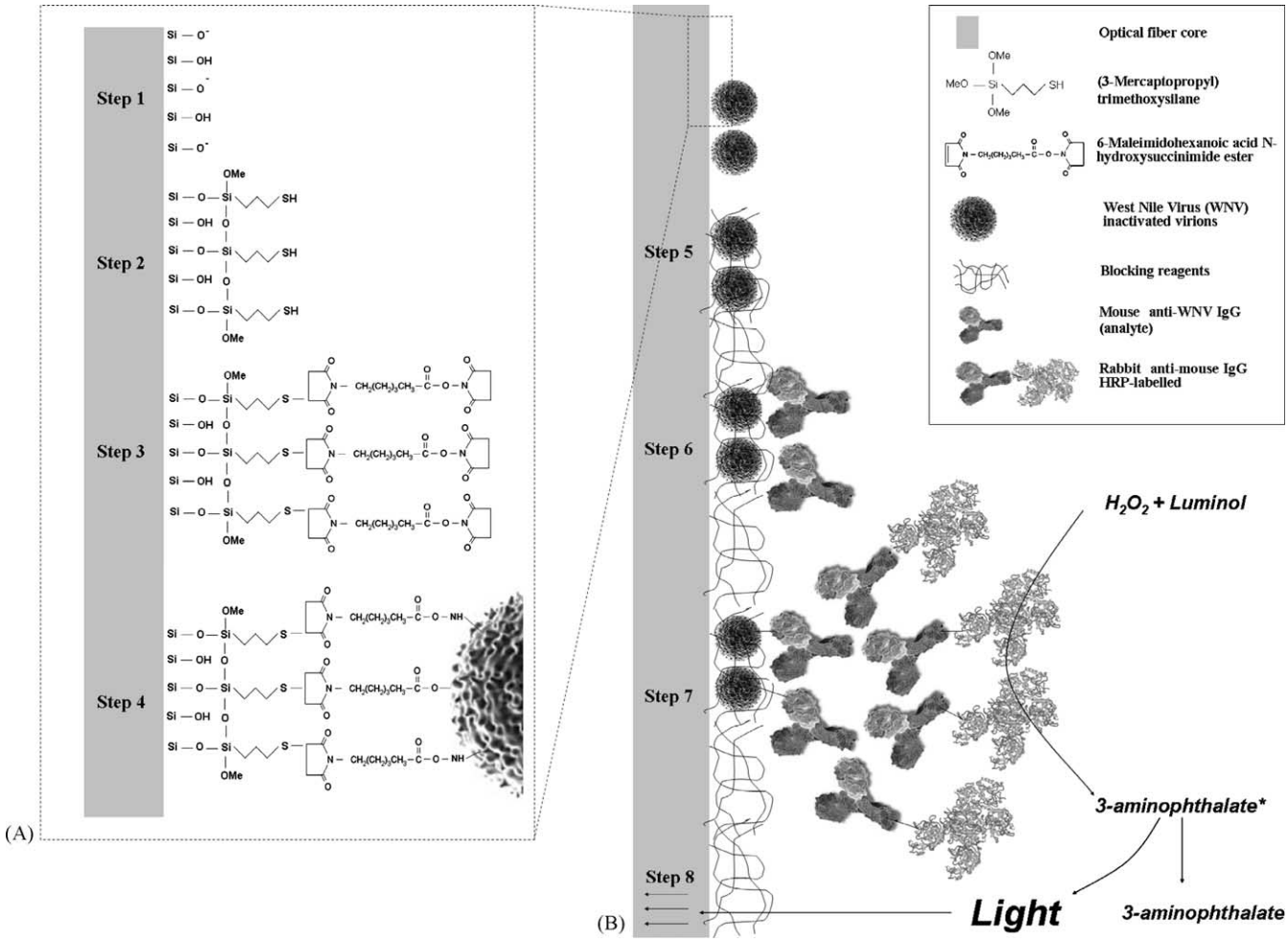

Fig. 1. Optical fiber modification and immunoassay rationale. (A) Modification of the optical fiber core: the hydroxyl groups exposed on the fiber core by exposure to sulfuric acid (step 1) allow the covalent attachement of bi-functional silane molecules (step 2). The thiol group of the silane is then allowed to react with the maleimide function of the bi-functional crosslinker (step 3). Finally the ester moiety of the crosslinker allows the covalent linking of the WN virions through an amino group (step 4). (B) Immunoassay steps: after adding the blocking reagents to the surface to minimize non-specific binding (step 5), the serum sample is tested for anti-WNV IgG presence (step 6) followed by the chemiluminescent detection of mouse IgGs (step 7). The luminous signal is finally captured by the fiber core itself (step 8), measured and analyzed. Picture of the WN virion is adapted from [14].

placed 20 min in 75 μl diluted serum solutions at dilutions ranging from 10^2 to 10^6 (step 6). Two sets of three replicates were prepared for each experimental point. The first one was used with WNV antiserum and the second one with control serum (negative control). Additional unmodified fibers were used to check the level of the background signal. The tips were rinsed and washed once with PBST–BSA for 10 min, and then thrice for 3 min each. Subsequently, the fibers were dipped into 75 μl of a solution containing the goat anti-mouse IgG peroxidase-labeled antibodies (10^{-3}) for 20 min, and finally, rinsed and washed once with PBST–BSA for 10 min and then thrice for 3 min each (step 7) before reading (step 8).

2.7. Signal measurement for the chemiluminescent optical fiber immunoassay

The instrument set-up for the fiber chemiluminescence measurements has been previously described [19]. A Hamamatsu HC135-01 Photo Multiplier Tube (PMT) Sensor Module was used for chemiluminescence measurements, combining the sensitivity of a photomultiplier tube with the intelligence of a micro-controller. The detector is optimized to the blue light region and includes a 21 mm diameter active area convenient to gather light radiation without any optical focusing elements for luminescence measurements. The instrument set-up was placed in a light-tight box. The far end of the fiber was held by a fiber holder (FPH-DJ, Newport) and placed into an adjustable single-fiber mount (77837, Oriel). To prevent damage to the photon-counting unit by environmental light, a manual shutter (71430, Oriel) was placed in front of the detector. To move the slide shutter, a workshop-made lever was placed outside the box. To receive and treat data, a specific driver was developed using LabView (Version 3.1, National Instruments Corporation), which allowed monitoring of the chemiluminescent signal and data handling in real time. The immunosensor optical

fiber probes were placed in a 400 μl sample tube containing the combined oxidizing reagent and enhanced luminol reagent solutions (NEL105 chemiluminescence reagent kit) in a 1:1 (v/v) ratio. The chemiluminescence readings were integrated for 1 s and each measurement was obtained by taking a mean value of photon counts during 15 s. The responses of the controls were also checked by the same procedure. Mean of the triplicates and the standard deviation was calculated for each dilution and reported as RLU (relative light units).

3. Results

After demonstrating briefly the advantages of optical fiber silanization in argon flow, the characterization of the different immunoassays employed in the detection of anti-WNV IgG – namely, colorimetric ELISA, chemiluminescent ELISA and chemiluminescent optical fiber immunoassay – was performed based on their respective calibration curves. The modelization curves allowed afterwards the determination of essential parameters like the dynamic range, the sensitivity and the lower detection limits of the different procedures.

3.1. Advantages of fiber silanization in argon flow

This method of silanization was previously reported for the modification of a flat piece of silicon oxide surface [28] and we successfully adapted this methodology to the modification of optical fiber silica tips. Our small reactor allowed the parallel modification by mercaptosilane of 25 fibers in 30 min at room temperature and local pressure. Control of the surface modification was performed by X-ray microanalysis and of the fiber tip surface, which clearly showed the presence of sulfur atoms belonging to the mercaptosilane (data not shown). The results observed in the optical fiber immunoassays following this procedure (Fig. 2C) were relatively homogeneous and suggest that the fiber silanization was reproducible. This methodology thus allows us to obtain a reproducible and convenient procedure to achieve functionalized silane layers on optical fiber tips without any increase of temperature or pressure commonly used in gas-phase deposition and this in a very short time (30 min against 6 h usually recommended).

3.2. Calibration curves

The calibration curve describes the general behavior of the IgG detecting system. The curves were built on a set of dilutions of the analyte solution ranging from 10^{-2} to 10^{-6}. For each experimental point in the three methodologies, the same amount of antigen (inactivated WN virions), of analyte (mouse anti-WNV antiserum) and of secondary antibodies (anti-mouse antibodies) were used in order to allow a suitable comparison. The regression curves were calculated by analyzing the data in SigmaPlot 8.2 (Systat) and by applying

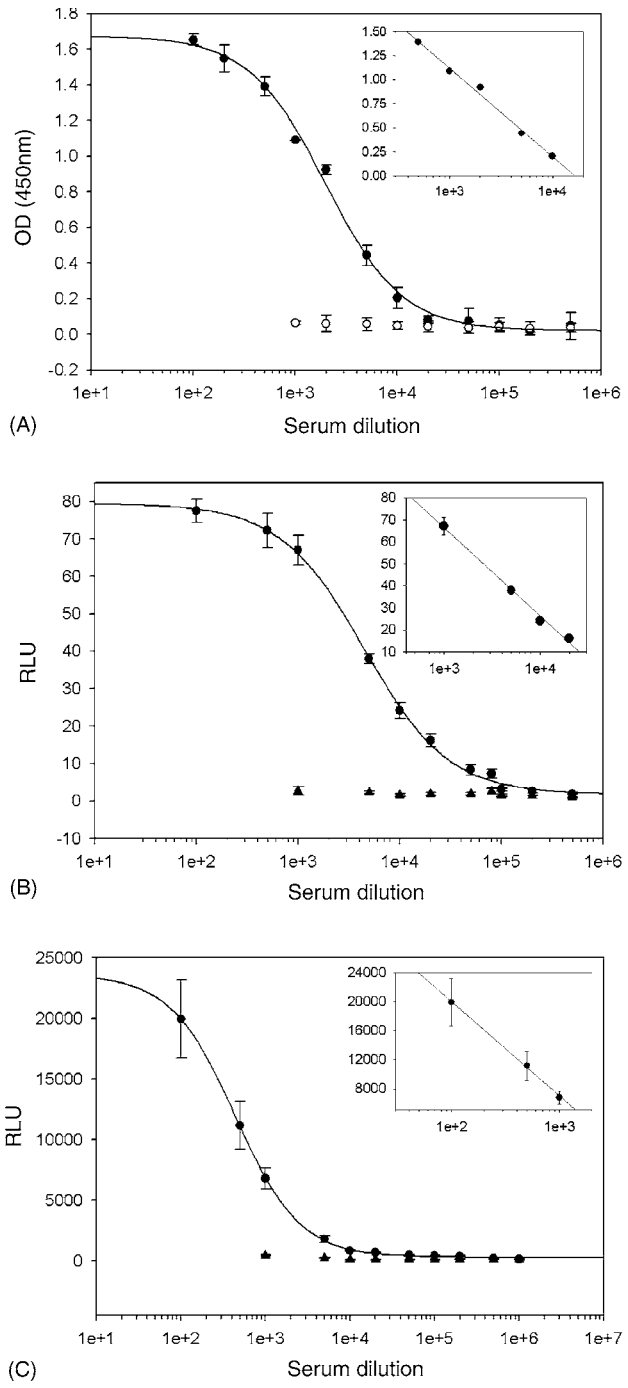

Fig. 2. Calibration curves. The curves were obtained by using serum dilutions ranging from 10^2 to 10^6. The result of the linear regression in the middle part of the curve is represented in each upper right corner: (●) positive serum and (▲) control serum. (A) Calibration curve for the colorimetric ELISA, (B) calibration curve for the chemiluminescent ELISA and (C) calibration curve for the optical fiber immunoassay.

the logistic sigmoid four-parameter curve regression model described by the following equation:

$$y = y_0 + \frac{a}{1 + \left(\frac{x}{x_0}\right)^b} \quad (1)$$

Table 1
Mathematical models and performance description of the methodologies

Characteristics	Colorimetric ELISA	Chemiluminescent ELISA	Optical fiber immunoassay
Curve mathematical modeling parameters (Eq. (1))			
a	1.65	77.62	23,315.96
b	1.16	1.05	1.13
x_0	1,970	4,486.01	439.97
y_0	0.020	1.79	304.31
r^2	0.99	0.99	0.99
Linear domains parameters (Eq. (2))			
c	−0.92	−40.00	−13,036.88
d	3.87	186.34	46,100.96
r^2	0.99	0.99	0.99
c^*	n/a	n/a	−349.19
d^*	n/a	n/a	2,206.57
r^{2*}	n/a	n/a	0.97
Performance criteria of the methodology			
Linear range (titer)	1:500–1:10,000	1:1,000–1:20,000	1:100–1:2,000 1:10,000–1:1,000,000
Sensitivity (normalized signal/titer)	0.55	0.50	0.55 0.01
Lower detection limit (titer) (Eq. (3))	1:10,000	1:500,000	1:1,000,000
Maximum response (RLU or Abs)	1.65	79.41	23,620.27

Results are shown in Fig. 2 and the mathematical parameters are reported in Table 1. The regression model applied showed to be a good description of the relation between the serum titers and the reported signal as shown by the r^2 values (near 1) and the Durbin–Watson statistic values that show the independency of the residuals (around 2). The maximum response for a theoretical non-diluted serum sample can be calculated by adding the values of a and y_0. All three methodologies also revealed a good reproducibility of the measurement merely at high serum dilutions. This demonstrates that the deviation observed in the signal is not due to non-specific binding of the secondary antibodies, but is more likely correlated to the attachment efficiency of the anti-WNV antibodies to the antigen surface. In each procedure, experimental points based on control serum showed a low, homogenous and linear-based signal.

3.3. Dynamic ranges

The dynamic range (or working range) gives the titer range where the detection system shows a proportional relation between the serum titer and the measured signal. Clinical diagnostics usually requires a serum dilution that belongs to this range in order to guarantee the putative detection of comparable amounts of target antibodies. The dynamic ranges can be extracted from the previous results by applying a two-parameter linear regression in the medium part of the curves by the following equation:

$$y = c\log(x) + d \quad (2)$$

Dynamic ranges can be visualized in Fig. 2 in the top right corner of their corresponding curve and the mathematical data are reported in Table 1. In this study, the dynamic ranges were 1:500–1:10,000 for the colorimetric ELISA and 1:1000–1:20,000 for the chemiluminescent ELISA and 1:100–1:2000 for the optical fiber immunoassay. However, the latter one clearly showed another linear range from 1:10,000 to 1:1,000,000 that was not observable in the chemiluminescent ELISA calibration curve (r^2 value equals 0.84 for the last three points before the detection limit). This particular part of the sigmoid is shown in Fig. 3. All these curves showed a very good reliability according to their high r^2 values. We can conclude from these results that both the colori-

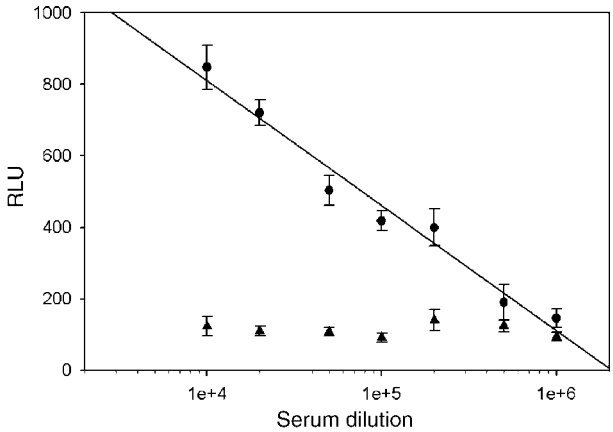

Fig. 3. Optical fiber immunoassay working range at very low serum titer: (●) positive serum and (▲) control serum. This working range was only visible in the optical fiber immunoassay and allowed theoritically a very low working dilution. It ranged approximately from 10^4 to 10^6 serum dilutions.

metric ELISA and the fiber immunoassay are the most reliable methodologies for anti-WNV IgG detection at highly concentrated sera, while chemiluminescent ELISA is for medium concentrated sera. The optical fiber immunoassay remains the only reliable procedure at low serum titers and thus theoretically offers the possibility to work with a smaller volume of serum than any ELISA diagnosis procedure. Consequently, it can help to lower the volume of initial blood sampling and can constitute a major advantage if numerous tests have to be run on the same serum sample. Moreover, the fiber-based procedure was shown to be less time-consuming than ELISA (approximately 2 h against 4 h, respectively, after antigen binding) thus, constituting a real gain in time for high-throughput diagnosis.

3.4. Sensitivity

The parameter called sensitivity, takes into account the power of discrimination of the system between two different serum dilutions located within the working range. It is calculated in terms of signal units per log of titer and is particularly relevant if a relative quantification of the analyte needs to be performed. In order to enable a clear comparison of the three methodologies, each value of their respective dynamic range was normalized by dividing it by the maximum response of the system and reported as percent of maximum response according to the serum titer (Fig. 4). The sensitivity is then equal to the slope module of the linear regression curve reported. Whereas, the sensitivities are relatively close in high/medium serum concentrations for the chemiluminescent ELISA, the optical fiber immunoassay and the colorimetric ELISA (0.50, 0.55 and 0.55, respectively), the fiber immunoassay shows a relatively low sensitivity at lower concentrations (0.01) with this calculation method. However, the signal amplitude at low titers in the optical fiber immunoassay remains largely sufficient to discriminate between two experimental points as previously shown in Fig. 3 (from 200 to 800 RLU). This may be explained by the fact that the chemiluminescent reaction occurs close to the fiber surface and that small variations of the collected light can be measured with the biosensor.

3.5. Lower detection limit

The lower detection limit is a parameter that describes the lower analyte dilution that can be efficiently distinguished by the detecting system. It is calculated by taking into account, for a definite dilution, the difference of the signal level between the experimental points with positive or control sera, and is then modulated by the standard deviation of the control. The lower detection limit can be calculated by the following equation:

$$\sum \frac{y(x_0)}{n_1} - \sum \frac{z(x_0)}{n_2} > 3\sqrt{\frac{n_2 \sum z(x_0)^2 - (z(x_0))^2}{n_2(n_2-1)}} \quad (3)$$

where $y(x)$ and $z(x)$ are the functions relative to the positive serum and the control serum in the system, respectively, x_0 the serum titer and n_1 and n_2 the number of replicates for the positive serum and the control serum, respectively. If the formula is verified for the specific serum titer then the point is validated. After application of this rule, the lower detection limit for the colorimetric ELISA, the chemiluminescent ELISA and the optical fiber immunoassay were reported as 1:10,000; 1:500,000 and 1:1,000,000, respectively. The effect of the background signal can be observed by using a ratio between the signal value of the anti-WNV IgG containing serum (signal) and the control serum (noise). Results are reported in Fig. 5 as signal-to-noise ratio as a func-

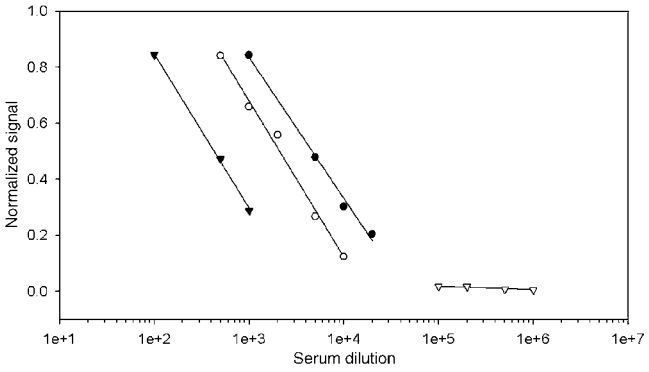

Fig. 4. Linear range and sensitivity of the procedures: (●) chemiluminescent ELISA; (▼) colorimetric ELISA; (◯) optical fiber immunoassay low titer linear range; (▽) optical fiber immunoassay high titer linear range. These curves are obtained by dividing the relative signal in each methodology by its maximum response. It clearly shows that sensitivity of the three methods are comparable in high serum titer and that optical fiber immunoassay displays a lower sensitivity for low serum titers.

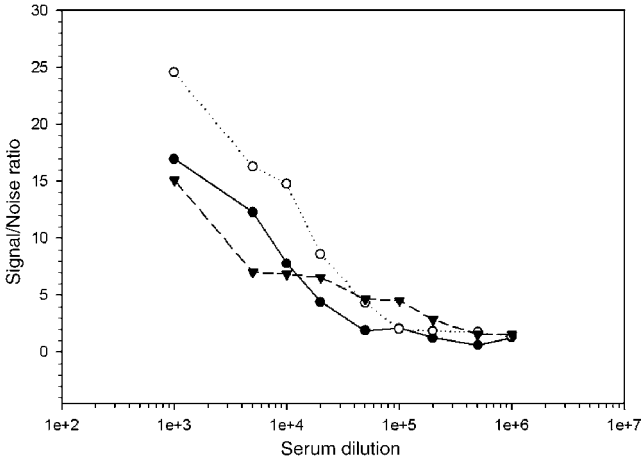

Fig. 5. Evolution of the signal-to-noise ratio: (◯) chemiluminescent ELISA; (●) colorimetric ELISA; (▼) optical fiber immunoassay. Signal values (anti-WNV IgG containing serum) are divided by the noise values (control serum) in order to follow the evolution of the background signal. The colorimetric methodology shows a faster decrease of the ratio than the chemiluminescent methodologies pointing the fact that the background signal has a greater influence at low serum titers in this procedure.

tion of the serum dilution. We can see that the colorimetric ELISA gives already a ratio around 2 for a titer of 1:50,000, whereas a comparable ratio is only reached at 1:200,000 and 1:400,000, respectively for both chemiluminescent ELISA and the fiber immunoassay. The chemiluminescent procedures are thus shown to be clearly more specific and less subject to a background noise. It is interesting to note that the optical fiber immunoassays showed a greater lower detection limit than ELISA despite the fact that the theoretical antigen surface coverage was 6.5× greater in the microtiter wells than on the exposed fiber core (approximately 79 and 12 mm^2, respectively) and might therefore result on less-bound antigen. Two major hypotheses could explain the improved results observed in the optical fiber immunoassay over the chemiluminescent ELISA: the fact that WN virions were covalently attached to the fiber and not 'simply' adsorbed on the surface may increase the stability of the antigen–antibody complex; on the other hand, the peroxidase-mediated chemiluminescent reaction occurs very close to the fiber surface and may enhance the light collection efficiency.

4. Conclusions

We have demonstrated that an anti-WNV IgG optical fiber immunoassay can be created by covalent modification of the core of an optical fiber tip with inactivated WN virions. We showed that the first step involving the activation of the silica surface by silanization can be achieved by a fast and convenient gas-phase deposition in argon flow in ambient conditions. The comparison between the optical fiber immunoassay and the standard ELISA procedures revealed that: (1) colorimetric ELISA and chemiluminescent ELISA are a reliable system for high and medium IgG containing serum concentrations, respectively; (2) the optical fiber immunoassay is the only procedure to have a reliable working range at very high serum dilutions with a greater sensitivity; (3) chemiluminescent procedures are at least 50–100 times more sensitive than the colorimetric setup enabling the detection of very low analyte concentrations; (4) chemiluminescent optical fiber immunoassays showed some advantages over chemiluminescent ELISA, such as a gain in the time of procedure, and enabled a lower volume of original serum sampling because of its specific very low working range with a comparable specificity. These results support the previous study made by Starodub and coworkers that enhanced some of the advantages of using fiber-optic immunosensors based on enhanced chemiluminescence [25]. The fiber-optic immunosensor is amenable to be field-operational. Indeed, the authors have already made a field-operable fiber-optic photodetector that has been used in the field [19] and if these fiber probes are commercialized they will be simpler to use as the antigen (or antibody) will already be pre-bound for use with only some rapid dipping steps needed.

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References

[1] D. Ben-Nathan, S. Lustig, G. Tam, S. Robinzon, S. Segal, B. Rager-Zisman, J. Infect. Dis. 188 (2003) 5–12.
[2] G.L. Campbell, A.A. Marfin, R.S. Lanciotti, D.J. Gubler, Lancet Infect. Dis. 2 (2002) 519–529.
[3] J.R. Epstein, A.P. Leung, K.H. Lee, D.R. Walt, Biosens. Bioelectron. 18 (2003) 541–546.
[4] M. Giladi, E. Metzkor-Cotter, D.A. Martin, Y. Siegman-Igra, A.D. Korczyn, R. Rosso, S.A. Berger, G.L. Campbell, R.S. Lanciotti, Emerg. Infect. Dis. 7 (2001) 659–661.
[5] T.L. Hadfield, M. Turell, M.P. Dempsey, J. David, E.J. Park, Mol. Cell. Probes 15 (2001) 147–150.
[6] C.N. Huang, B. Slater, R. Rudd, N. Parchuri, R. Hull, M. Dupuis, A. Hindenburg, Emerg. Infect. Dis. 8 (2002) 1367–1371.
[7] D.J. Johnson, E.N. Ostlund, B.J. Schmitt, J. Vet. Diagn. Invest. 15 (2003) 488–493.
[8] T. Konry, A. Novoa, S. Cosnier, R.S. Marks, Anal. Chem. 75 (2003) 2633–2639.
[9] P. Koraka, H. Zeller, M. Niedrig, A.D.M.E. Osterhaus, J. Groen, Microb. Infect. 4 (2002) 1209–1215.
[10] R.S. Lanciotti, J.T. Roehrig, V. Deubel, J. Smith, M. Parker, K. Steele, B. Crise, K.E. Volpe, M.B. Crabtree, J.H. Scherret, R.A. Hall, J.S. MacKenzie, C.B. Cropp, B. Panigrahy, E. Ostlund, B. Schmitt, M. Malkinson, C. Banet, J. Weissman, N. Komar, H.M. Savage, W. Stone, T. McNamara, D.J. Gubler, Science 286 (1999) 2333–2337.
[11] B. Leshem, G. Sarfati, A. Novoa, I. Breslav, R.S. Marks, Luminescence 19, in press.
[12] Y. Liu, J. Ye, Y. Li, J. Food Prot. 66 (2003) 512–517.
[13] R.S. Marks, E. Bassis, A. Bychenko, M.M. Levine, Opt. Eng. 36 (1997) 3258–3264.
[14] S. Mukhopadhyay, B.S. Kim, P.R. Chipman, M.G. Rossmann, R.J. Kuhn, Science 302 (2003) 248.
[15] B. Murgue, S. Murri, H. Triki, V. Deubel, H.G. Zeller, Ann. N.Y. Acad. Sci. 951 (2001) 117–126.
[16] E. Pavlovic, A.P. Quist, U. Gelius, S. Oscarsson, J. Colloid Interface Sci. 254 (2002) 200–203.
[17] A.G. Pletnev, M.S. Claire, R. Elkins, J. Speicher, B.R. Murphy, R.M. Chanock, Virology 314 (2003) 190–195.
[18] B. Polyak, E. Bassis, A. Novodvorets, S. Belkin, R.S. Marks, Sens. Actuators B: Chem. 74 (2001) 18–26.
[19] B. Polyak, E. Bassis, A. Novodvorets, S. Belkin, R.S. Marks, Water Sci. Technol. 42 (2000) 305–311.
[20] B. Rager-Zisman, D. Ben Nathan, Isr. Med. Assoc. J. 5 (2003) 691.
[21] P.Y. Shi, E.B. Kauffman, P. Ren, A. Felton, J.H. Tai, A.P. Dupuis, S.A. Jones, K.A. Ngo, D.C. Nicholas, J. Maffei, G.D. Ebel, K.A. Bernard, L.D. Kramer, J. Clin. Microbiol. 39 (2001) 1264–1271.
[22] P.Y. Shi, S.J. Wong, Exp. Rev. Mol. Diagn. 3 (2003) 733–741.

[23] K. Shirato, T. Mizutani, H. Kariwa, I. Takashima, Microbiol. Immunol. 47 (2003) 439–445.
[24] K. Smithburn, T. Hughes, A. Burke, J. Paul, Am. J. Trop. Med. Hyg. 20 (1940) 471–492.
[25] N.F. Starodub, P.J. Arenkov, A.N. Starodub, V.A. Berezin, Sens. Actuators B: Chem. 18 (1994) 161–165.
[26] G. Tardei, S. Ruta, V. Chitu, C. Rossi, T.F. Tsai, C. Cernescu, J. Clin. Microbiol. 38 (2000) 2232–2239.
[27] E.G. Westaway, Virology 26 (1965) 528–537.
[28] S.J. Wong, V.L. Demarest, R.H. Boyle, T. Wang, M. Ledizet, K. Kar, L.D. Kramer, E. Fikrig, R.A. Koski, J. Clin. Microbiol. 42 (2004) 65–72.