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Dear Dr Turner,

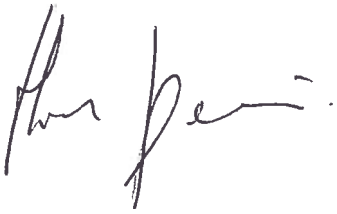
You will find hereafter our manuscript " **Quantification of the trichothecene Verrucarin-A in environmental samples using an antibody-based spectroscopic biosensor**" that we would like to submit for publication in "Biosensors and bioelectronics".

In this paper we describe a new Fourier transform infrared (FTIR) spectroscopic sensor using an attenuated total internal reflection (ATR) grafted with an antibody specific for the mycotoxin verrucarin A.

Corrections in the manuscript have been done according to the received editorial comments and the author guidelines. The manuscript has 4992 words, the preferred positions for tables and figures are indicated between brackets in the text and supplementary data are also provided.

Looking forward to hearing from you soon.

Yours sincerely.



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Quantification of the trichothecene Verrucarin-A in environmental samples using an antibody-based spectroscopic biosensor.

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Abstract

Verrucarin A is a toxic trichotecene mycotoxin that can be produced indoors at very low level by moulds contaminating dwellings and might be associated with several human health problems. In this study a spectroscopic label-free biosensor for verrucarin A is reported This device is based on the high sensitivity of Fourier transform infrared – attenuated reflexion (FTIR-ATR) spectroscopic detection and on the use of a new anti-verrucarin A rat monoclonal antibody, developed and characterized herein, directly grafted at the surface of the infrared element. Both competitive ELISA and FTIR-ATR techniques have been used and compared to detect the verrucarin A in buffer and in complex dust samples obtained from dwellings. After optimization, the competitive ELISA showed a sensitivity of 7.43 ng/ml of verrucarin A in PBS, a dynamic range below one order of magnitude and a good recovery of the mycotoxin spiked in dust samples. The FTIR technique allowed a three orders of magnitude improvement of the detection of the verrucarin A (2.5 pg/ ml in buffer and 6 pg/ml when spiked in dust samples). The dynamic range for its detection extended over five orders of magnitude with a very good accuracy and the percentage of recovery of verrucarin A spiked (1000 ng to 0.1 ng) in dust samples ranged from 97 to 58%. Our results clearly show that this new type of generic biosensor gives better characteristics as compared to classical immunoassays for the detection of this mycotoxin. We demonstrate herein that this biosensor can be very efficiently used in the field of indoor mycotoxin detection.

Keywords:

Biosensors,
Label free
Verrucarin A,
Monoclonal antibodies,
Moulds
Mycotoxins,

1. Introduction

Damp building-related illnesses (DBRI) include a number of respiratory, immunological, and neurological symptoms that have been associated with airborne exposure to indoor fungi (Pestka *et al.,* 2008). Many moulds that flourish in damp indoor environments as for instance *Aspergilus versicolor, Stachybotrys chartarum, Cladosporium spp and Penicillium spp* are potent mycotoxin producers and may play a role in the adverse health effects reported during DBRI. These mycotoxins are in fact secondary metabolites that are produced to give strategic advantages to moulds over encroaching organisms and very often these mycotoxins are found in spores and mycelial fragments of moulds. These spores and mycelia finally set down in dust and can become airborne and inhaled under certain conditions and then cause a number of adverse effects. (Sorenson *et al.,* 1987). Among the mycotoxin plethora, the trichothecenes consist of a family of over 200 structurally related low molecular weight (~200–500D) compounds closely related to sesquiterpenoids with a common 12,13-epoxide-trichothecene ring system (Bamburg, 1983, Kotal *et al.,* 1999).

Macrocyclic trichothecenes conventionally have a cyclic diester or triester ring linking C-4 to C-15 (Grove, 1988, 1993) and are considered to include the most toxic trichothecenes ever observed (Pestka *et al.,* 2008). The macrocyclic trichothecenes including satratoxins, roridins and verrucarins are known to interfere with ribosome function and inhibit protein synthesis (Hernandez and Cannon, 1982), to induce activation of caspase-1 and 3 and apoptosis (Shifrin and Anderson,1999) and also activate inflammasome-associated innate immunity reaction in primary macrophages (Kankkunen *et al.*, 2009). Regarding *Stachybotrys chartarum*, highly toxic macrocyclic trichothecenes are produced by a toxic Stachybotrys chemotype of this fungus (Andersen *et al.,* 2002). Patients with DBRI and children suffering from idiopathic pulmonary hemorrhage often have histories of living in mouldy, water-damaged buildings. Although a causal connection has not been unequivocally proven, these illnesses could be attributed in part to the mycotoxins produced by the indoor growing moulds. (Dearborn *et al.,* 1999, Hossaim *et al.,* 2004, Straus 2009).

Because of their high toxicity, the detection of trichothecenes indoors is an important task which can be hampered by their very low concentrations and their presence on “difficult matrix” (e.g., wallpapers, dust, etc.) that may interfere with the analytical methods used and therefore prevent the development of preventive strategies for this kind of indoor pollution. Recently we described a new type of generic biosensor based on the high sensitivity of Fourier transform infrared (FTIR) spectroscopy and on an attenuated total internal reflection (ATR) element transparent in the infrared (Voué *et al.,* 2007). This attractive infrared (IR) configuration allows the spectrometric analysis of compounds in solution when they are brought in contact with the ATR element while the FTIR provides quantitative informations as well as spectroscopic signatures allowing the precise determination of the interacting molecules, an information usually not accessible using standard sensors which only indicate the mass loading of the surface. The chemical signature of the analyte bound is of major importance and helps to circumvent the drawbacks related to possible non-specific interactions. Obviously the specificity of this FTIR-ATR configuration is based on the use of specific ligand-receptor couples and provides thus a label-free technology. Hence a germanium or silicon crystal can be used as ATR element and the grafting of an organic layer directly at the surface of the crystal and of bifunctionnal spacer molecules directly allows

the covalent binding of receptors which will finally capture and concentrate the targeted ligands at the surface of the ATR crystal.

We previously used this FTIR-ATR configuration for the competitive detection of the small molecule 2,4-Dinitrophenol (Gosselin *et al.,* 2009) and for the direct detection in a complex medium of factor VIII molecules using phospholipid membrane fragments grafted to the organic layer (Goldzstein *et al*., 2009). Here we present the development of an FTIR-ATR immunosensor for the direct detection of the macrocyclic trichothecene Verrucarin A.

2. Materials and methods

2.1. Reagents

Unless otherwise stated, the purchased chemicals were of analytical grade and were used as received. Ver A, Roridin A, Ovalbumin (OVA), Bovine Serum Albumin (BSA), Methanol, H_2SO_4 and H_2O_2 were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS, 0.010 M pH 7.4) solution was prepared from Sigma Aldrich dry powder.

2.2. Production of a Verrucarin A rat monoclonal antibody.

Rat mAbs were produced as initially described in Lebacq-Verheyden *et al.*, 1983. All experiments were performed in accordance to the local ethics committee of the WIV-ISP.

2.3. Preparation of dust extracts.

Dust samples collected during environmental audits were used. To prepare the dust extracts, 50 mg of dust in a microtube were extracted in 1 ml of PBS by agitating during 16 hours at room T°. Supernatants were then clarified by centrifugation (2000g for 15 min) and stored at -20°C until use. To prepare the spiked dust extracts, various concentrations of Ver A diluted in 100 µl of PBS were added to 50 mg of dust in a microtube. After drying, the material was extracted asdescribed above.

2.4. ELISA

A quantitative competitive ELISA was developed using a selected Ver A specific antibody (F24-1G2). Microplates were coated as previously with 0.5 µg of BSA-Ver A (or OVA-Ver A) in 100 µl of borate buffer per well. Plates were washed in PBS and saturated with PBS supplemented with 10% of Foetal Calf Serum (300 µl/well) for 2 h at 37°C. After washing in PBS, serial twofold dilutions of VerA in 50 µl of PBS or dust were applied. Wells devoid of Ver A were used as positive (Max) or negative controls (Blank). Immediately 50 µl of F24-1G2 diluted at 1 µg/ml in PBS 0.1 % Tween 20 1% BSA were added in each well except for the negative controls. Plates were incubated for 1h at 37°C and then washed in PBS 0.1% Tween 20. Peroxidase-labelled mouse anti-rat IgK (1 µg/ml in PBS, 0.1% Tween 20, 1 % BSA, 100 µl/well) was then added and the plates were incubated for 2h at 37°C. Plates were finally washed and developed by the addition of 100 µl of TMB (Sigmafast, Sigma-Aldrich). The reaction was stopped with 50 µl of 2N H_2SO_4 and O.D. was read at 450 nm. The percentage of inhibition was calculated as follows: % Inh = (1- (OD_{492} Sample - OD_{492} Blank)/ (OD_{492} Max - OD_{492} Blank))*100. Non-linear least squares (ordinary fit) regression were carried out and the best fit parameters were calculated for each data set using the GraphPad Prism software.

2.5. Procedure to obtain the VerA reference IR spectra.

To determine the IR fingerprint of the Ver A, a layer of pure Ver-A was concentrated onto the ATR crystal on the sampling area. A good consistency was observed between the chemical composition of the Ver A and the adsorption peaks of the infrared spectra. In brief, the infrared spectra of the Ver A showed one strong intensity band at 1716 cm^{-1}due to an ester C=O bond and two low bands at 1635 and 1582 cm^{-1} corresponding to C=C functions. A broad absorption band around 3500 cm^{-1} for Ver A indicating the presence of OH groups and three stretching vibrations of CH_3 and CH_2 between 3000 and 2850 cm^{-1} were also observed. The vibrations involving the stretching of the C-O bonds gave several strong infrared bands below 1000 cm-1 indicating the presence of different compounds (carboxylate, cyclopentanone and aromatic structures). Several other bands at 1083 cm^{-1} (C-O-C), 2158 cm^{-1} (-C-CH_3), 1269 (C-O), and 1209 cm^{-1} (C-O-H), 1188, 1126, 1083, 1029, 996, 967, 879, 820 cm^{-1} were also found.

2.6. Functionalization of the biosensors and grafting of the VerA-specific mAb.

The cleaning, activation, grafting of the octadecyltrichlorosilane self-assembled monolayer and of the spacer N-Succinimidyl (4-azidophenyl) 1,3' –dithiopropionate (NHS) molecules onto silicon crystals were realized according to the procedure already described (Gosselin *et al.,* 2009) leading to a multitracking biosensor with the appropriate microfluidic. Each experiment was performed on a single track under a semi-continuous flow. Five hundred µl of the Ver A-specific rat mAb (F24-1G2) at a concentration of 0.1 mg/ml were injected with a flow rate of 12 µl/min during 30 min. The track was then washed with PBS at a flow rate of 50 µl/min in order to remove the unbound antibodies. The binding of the F24-1G2 mAb to the sensor surface and its stability was quantified and monitored on-line from the FTIR intensity of some specific absorption bands. Indeed four distinct bands are characteristic of the appropriate anchoring of the mAb at the sensor surface: the amide I and amide II absorption bands of the antibody and the stretching bands v(C=O) and v(C-O). Since the coupling of the antibody at the activated crystal surface requires the hydrolysis of the activated ester of the N-succinimidyl group of the azoture molecule, the two amide bands appear in positive mode as they correspond to the binding of the mAb, while the two stretching bands appear in negative mode as they monitor the hydrolysis of the reactive function of azoture NHS molecules. During the binding of the antibody the absorbance of the amide II band increased and remained constant during PBS rinsing of the flow cell. After binding of the antibody, a BSA solution (5 mg/mL in PBS) was injected in the flow cell at a flow rate of 25 µL/min (continuous), in order to saturate the remaining available bifunctional molecules. After 30 minutes, the cell was washed with the buffer to remove the excess of BSA.

2.7. IR measurement

IR spectra were recorded on an FTIR 6700 infrared spectrophotometer (ThermoFischer) equipped with a Mercury-cadmium-telluride (MCT) detector at a resolution of 4 cm^{-1} with a mirror speed of 0.6329 cm/s. For each measurement 32 scans were collected and averaged to obtain the final absorbance

spectrum. The spectrometer was continuously purged using an air dryer (Parker-Zander, Germany) at a flow rate of 30 standard cubic feet per hour (SCFH). The processing of the spectra was done using the OMNIC 7.3 software (Thermo Electron Corporation, USA). The functionalized surfaces were placed in an ATR flow cell (Specac, UK) connected to a Watson-Marlow 403U/VM2 peristaltic pump (Farmount, UK). The typical flow speed was 12 µL/min for the binding steps and about 200 µl/min for the rinsing steps. The infrared element was a triangular-shaped silicon crystal (4.8 mm X 4.8 mm X 45 mm, ACM, France). With this geometry, a single reflection occurs and fifteen tracks can be used on a single crystal.

Once correctly functionalized and saturated and after determination of the background infrared signal the sensor was ready to detect the Ver A and to monitor its binding versus time. Each time, a washing step allowed to confirm that the analyte of interest was well anchored. To set up the blank 80 µl of PBS were injected at a flow rate of 12 µl/min. Then a succession of experimental samples (80 µl) containing increasing concentrations of the analyte were injected in the flow cell at a flow rate of 12 µL/min and the binding of the Ver A to the specific antibody was monitored as a function of time by recording IR spectra for each different sample. Between each sample the flow cell was washed with 600 µl of PBS at a flow rate of 200 µl/min.

3. Results and discussion

3.1. Ver A specific mAb and Ver A specific ELISA

SN from the growing hybridomas were first screened for their binding to OVA-Ver A. This assay detected between 5 to 10% positive hybridomas in each fusion experiment. To select the hybridomas recognizing the free toxin, positive hybridomas were then rescreened using the same assay but in the presence or absence of 10 µg/ml of free Ver A as inhibitor. This procedure led to the selection of a single hybridoma, F24-1G2 (figure 1A) which was thereafter subcloned, produced *in vitro* and purified. The F24-1G2 specificity was first analysed using an indirect ELISA on microplates coated with unlabelled or labelled OVA or BSA with VerA or Roridin A (Ror A), a trichothecene with a very similar structure to Ver A but only differing in one substitution at CI3 (C=O to C-C-CH_3(-OH)). As shown on figure 1B, the F24-1G2 mAb efficiently bound to the Ver A in the context of the OVA or BSA carrier while Ror A labelled or unlabelled OVA or BSA were not at all recognized demonstrating the specificity of the F24-1G2 mAb. To detect the free Ver A in solution we then developed a competitive ELISA based on the interaction between the F24-1G2 mAb in solution and BSA labelled Ver A coated on the microplates. **[Put here figure 1]** We optimalized the different steps of this assay, namely the coating of the BSA-Ver A, the concentration of the F24-1G2 mAb, the incubating buffers, the incubating time and the peroxydase substrate in order to achieve the best sensitivity. The results for this competitive assay with the free Ver A are shown in figure 1C and can be modelled by a sigmoid equation whose parameters are the log EC_{50} (the logarithm of the inhibitor concentration giving 50 % of inhibition) and the slope of the curve at its inflexion point (Hill slope). In this case the EC_{50} was 12.26 $\pm$ 1.09 ng/ml and the hill slope was 2.769 $\pm$ 0.579. The EC_{20} (the inhibitor concentration giving 20 % of inhibition) was 7.43 $\pm$ 0.87 and can be considered as the limit of detection of the assay while the EC_{80}-EC_{20} range (dynamic range) was 20.25 – 7.43 ng/ml. This dynamic range is known to depend on the avidity of the antibody and more particularly on the affinity constant of the antigen-antibody complex in the solid and the solution phase and of the amount of antigen in the solid phase (Pesavento *et al.*, 1998). Typically, competitive ELISAs show lower dynamic range as compared to sandwich ELISA and this low dynamic range obviously impairs the usefulness of this assay. The inter-assay coefficient of variation for this assay was 29%. In contrast to the Ver A, the free Ror A was not able to inhibit the binding of the F24-1G2 to the BSA-Ver A, confirming the results obtained with the indirect ELISA and demonstrating further the specificity of the F24-1G2 mAb. Since Ver A in the environment will be present in complex dust matrix leading to possible interference in the assay, we compared the ELISA curves obtained from Ver A diluted in PBS or in an environmental dust matrix. As shown in figure 1D, the curves obtained were very similar with a EC_{50} and a hill slope of 24.89 ng/ml and 1.882 for the Ver A diluted in PBS and 14.82 ng/ml and 1.887 for the Ver A diluted in the dust matrix. The Ver A percentage of recovery was evaluated using two dust sample spiked with 250 ng of Ver A. These samples were extracted and the concentrations of Ver A in each sample were determined four times using the competitive ELISA on BSA-Ver A coated microplates. As shown in table 1, the percentages of recovery for the two spiked samples were 92.25 $\pm$ 14.86 % and 64.57 $\pm$ 16.12 % and the CVs were 16.1 and 24.97 demonstrating the good recoveries for this specific assay. **[Put here table 1]**

3.2. Ver A detection using an anti ver-A antibody based biosensor.

Preliminary experiments showed that the infrared region which gave the least perturbations in the Ver A spectra when the PBS was injected in the flow cell was the $CH_{2,3}$ stretching vibration around 3000 cm^{-1}. Preliminary experiment were performed to characterize the precision of the FTIR-ATR sensor and its reproducibility (inter-day variation) and investigated the variation of the infrared signal around 3000 cm^{-1} upon binding of increasing Ver A concentrations to the crystal grafted with the specific antibody (F24-1G2). By monitoring the $CH_{2,3}$ stretching region, the preliminary results showed a rather low reproducibility upon binding of the Ver A onto the different lanes of the sensor (figure 2A). **[Put here figure 2]** Yet by plotting these peak areas observed during the binding of the target as function of the Ver A concentration we nevertheless observed a linear relationship using a semi-logarithmic scale for three series of experiments. In fact the binding of the Ver A was clearly dependent upon the quantity of receptors presents at the sensor surface and therefore the observed peak area obtained in the $CH_{2,3}$ stretching vibration, corresponding to the amount of the Ver A bound to the sensor, needed to be normalized. Since the quantity of the mAb bound to the sensor surface can be determined by evaluating the peak area of the amide bands (NH_2) after the grafting step (Voué et al., 2007), the NH-2 peak area of each track was used for the normalization. Therefore the experimental normalized peak area was defined as the ratio between the peak area measured during the Ver A binding step in the $CH_{2,3}$ stretching vibration and the peak area of the amide bands measured after the receptor binding step. When this normalisation procedure was used, all the data points corresponding to the three series of experiments merged together (figure 2B) and the associated correlation coefficient of the linear equation was close to 1 (R^2= 0.98). These results clearly demonstrate that the quantification of the Ver A is directly affected by the quantity of specific antibody molecules present on the surface of the crystal. Similarly, an excellent repeatability (intra-day variation) was observed (see figure 2C) when PBS or a low concentration of Ver A (0.24 ng/ml) was used. As can be seen on the figure 2C the results obtained during five consecutive cycles of detection on the same track were very stable, demonstrating the robustness and the low variability of the IR biosensor.

Dilution curves of the Ver A in buffer or in a complex dust matrix were established and the figures 3A and B shows the regression plot of the peak area of the $CH_{2,3}$ stretching region for increasing concentrations of target, before and after normalization respectively. The calibration curves obtained in buffer or in dust were in both case linear showing very good regression coefficients ($R^2 \geq 0.99$ for the four curves). Moreover the calibration curves of the Ver A diluted in buffer or in dust are very close indicating that the quantification of the Ver A in this complex environmental media was minimally affected showing therefore a very low matrix effect using this biosensor. **[Put here figure 3]**

The LOD was calculated according to the normalized threshold defined as being three times the standard deviation (SD) of the IR signal observed during the injection of PBS in the flow cell. The highest deviation observed in the $CH_{2,3}$ stretching region was 1.3 10^{-2} absorbance corresponding to the threshold equal to 3.9 10^{-2} absorbance and 5.4 10^{-2} after normalization. Excellent detection limits were reached corresponding to 2.5 pg/ml of Ver A in PBS and 6 pg/ml in the dust matrix. These LOD are three order of magnitude below those obtained using an ELISA based on the same mAb demonstrating

the very high sensitivity of this biosensor. Moreover it showed a powerful dynamic range extending over five orders of magnitude of Ver A concentration (as compared to one order for the ELISA), a very interesting characteristic indeed for the analysis of field samples showing high variations in their concentrations. These results are in the same range or even better than those reported recently about the biosensing detection of food mycotoxins. For instance an enzyme-linked-immunomagnetic-electrochemical assay based on deoxynivalenol specific Fab fragments demonstrated a working range between 100 and 4500 ng/ml (Romanazzo *et al.,* 2010) and a surface plasmon resonance immunoassay with a deoxynivalenol specific mAb presented an IC50 value of 14.9 ng/ml. A quartz-crystal microbalance piezoelectric biosensor using polyclonal antibodies specific for the ochratoxin A showed a detection limit of 8 ng/ml (Vidal *et al., 2009*) while a magnetic aptasensor for the indirect detection of the same toxin showed a linear response in the range of 0.78 – 8.74 ng/ml and a LOD of 0.07 ng/ml (Bonel *et al.,* 2011). Finally a signal transduction by ion nona-gating (STING) sensor for the ultrasensitive detection of the HT-2 toxin reported recently demonstrated a LOD of 100 fg/ml (Actis *et al., 2010*).

[Put here table 2]

Finally we determined the percentages of recovery for a series of dust samples spiked across a large concentration range of Ver A. Three measurements for each sample spiked with the various Ver A concentrations were recorded on different days. As shown in table 2, the percentages of recovery for the spiked samples were situated between 78 to 102%. Only one sample (dust 2 spiked with 0.1 ng/ml) showed a lower (58%) recovery. The coefficients of variation (% CV) were always below 25% even for the samples spiked at low level indicating a good reproducibility between the measurements recorded on different days.

4. Concluding remarks

We have developed and characterized a monoclonal antibody-based FTIR-ATR biosensor for the direct detection of the mycotoxin Ver A. This study is the first, to our knowledge, focusing on the biosensing detection of indoor macrocyclic trichothecenes, As compared to a classical competitive ELISA using the same mAb, the spectroscopic detection allowed an improvement of the sensitivity by three orders of magnitude. Moreover it showed an impressive dynamic range extending over five orders of magnitude of Ver A concentration as compared to one order for the ELISA. The sensitivity of the ver A detection was minimally affected when the mycotoxin was spiked in an environmental dust matrix demonstrating the capability of the sensor to detect the Ver A in the environment. Finally an excellent recovery of the Ver A spiked in environmental dust matrix was obtained with the spectroscopic detection. Our results clearly demonstrate that this mAb-based FTIR-ATR biosensor is a promising tool that could be translated to many biosensing applications for the control of mycotoxins present at low concentrations in the environment.

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Legend to the figures

Figure 1: A) Screening of the OVA-Ver A positive hybridoma SN in the presence of free Ver A. SN from hybridomas containing antibodies recognizing the OVA-Ver A were tested using an indirect ELISA on OVA-Ver A coated microplates in the absence (shaded histogram) or in the presence (open histogram) of 10 µg/ml of free Ver A. The arrow indicates the hybridoma secreting antibodies of which the binding to the bound Ver A was inhibited by free Ver A. B) Analysis of the F24-1G2 specificity using an indirect ELISA on microplates coated with unlabelled OVA or BSA or Ver A or Ror A labelled OVA or BSA. C) Detection of free Ver A but not Ror A using an F24-1G2 based-competitive ELISA on BSA-Ver A coated microplates. D) Comparison of the ELISA curves obtained from Ver A in PBS or in a dust matrix using the F24-1G2 based-competitive ELISA on OVA-Ver A coated microplates.

Figure 2: A) F24-1G2 based FTIR-ATR biosensor detection of the Ver A in three different series of experiments recorded on different days. Each measurement corresponding to the peak area of the $CH_{2,3}$ stretching vibration around 3000 cm^{-1} is represented by a different shape of symbols and each symbol is the mean value of spectra during the IR experiments detection. The squares correspond to the first measurement, the circles to the second and the triangles to the third measurement. B) Normalization of the biosensor measurements. The three series of experiments presented in A were normalized using a ratio between the peak area of the $CH_{2,3}$ stretching vibration and the peak area of the amide bands measured after the receptor binding step. The filled symbols match the open symbols presented in figure 2 A (one type of symbol per experiment). The slope of the corresponding linear equation fitted is equal to $8.5\ 10^{-1}$ +/- $4.6\ 10^{-2}$. C) Consistency in the repeatability of the Ver A detection using the F24-1G2 based FTIR-ATR biosensors and recorded with the same track. Each signal refers to the increase of the amplitude of the peak around 3000 cm^{-1}. This increase is considered with respect to the baseline corresponding to the rinsing step. Each point represented by a symbol is the mean value of monitored spectra. The filled squares represent IR response in a PBS sample with Ver A at 0.24 ng/ml. The open circles are the mean value of the normalized peak area reseted each time with respect to a background spectrum.

Figure 3: A) IR dilution curves obtained using Ver A diluted in PBS or in the dust matrix using the F24-1G2 based FTIR-ATR biosensors.

The open squares and the filled circles represent the dilution curves of Ver A diluted in PBS and in dust extract respectively. The corresponding linear equations fitted (plain line for the PBS and dotted line for the dust) are relatively close. Indeed, the slopes in the PBS and in the dust are respectively equals to $5.01\ 10^{-3}$ +/- $1.1\ 10^{-4}$ and $4.22\ 10^{-3}$ +/- $1.8\ 10^{-4}$.

B) Normalized IR dilution curves corresponding to the measurements presented in figure 3 A. The symbols used are the same as in figure 3A. The corresponding linear equations are also very close and the slope is equal to $6.67\ 10^{-1}$ +/- $2.2\ 10^{-2}$ in PBS (plain line) and $5.60\ 10^{-1}$ +/- $2.4\ 10^{-2}$ in dust media (dashed line).

Supplementary Material

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Figure 1

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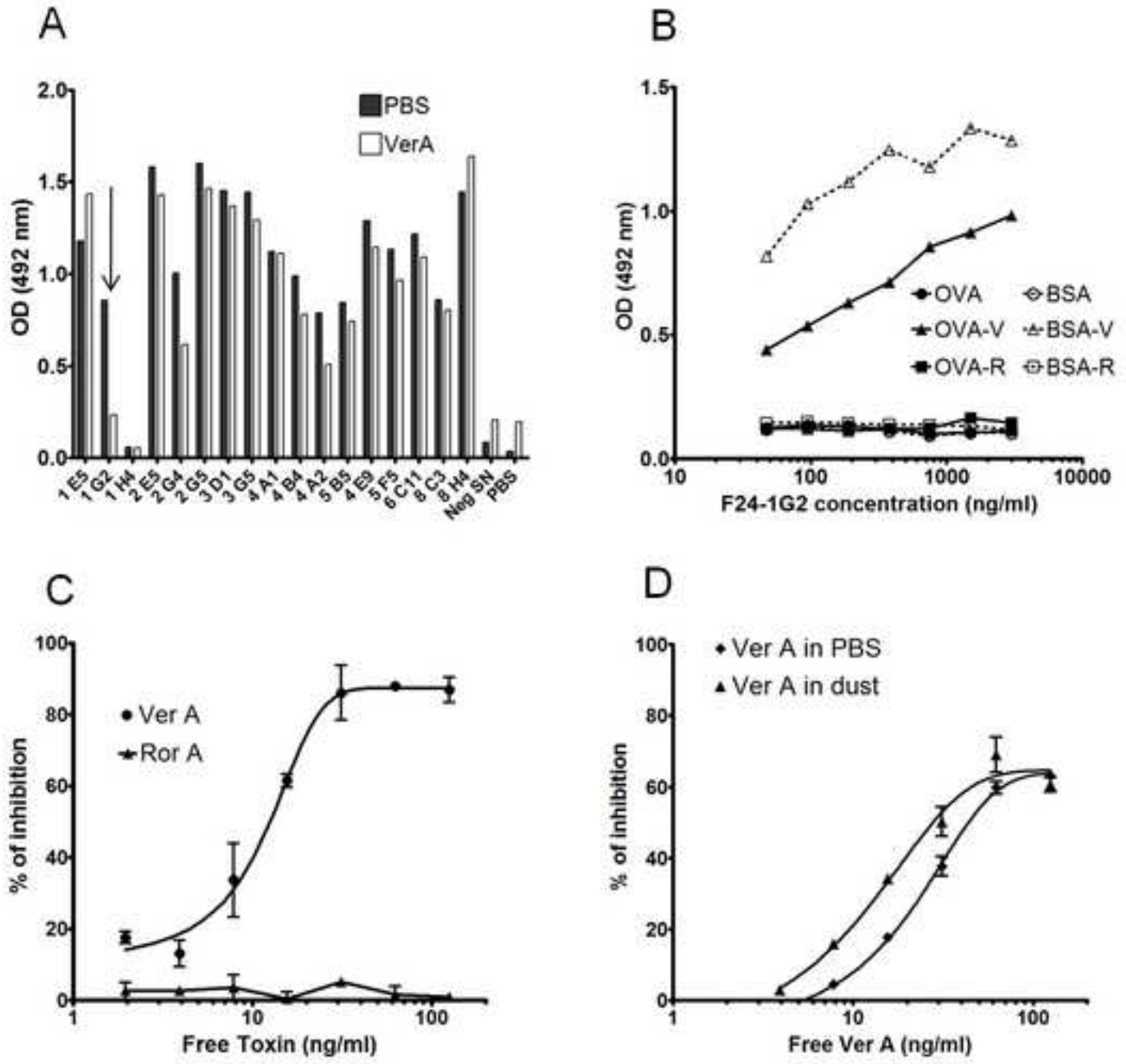

Figure 2

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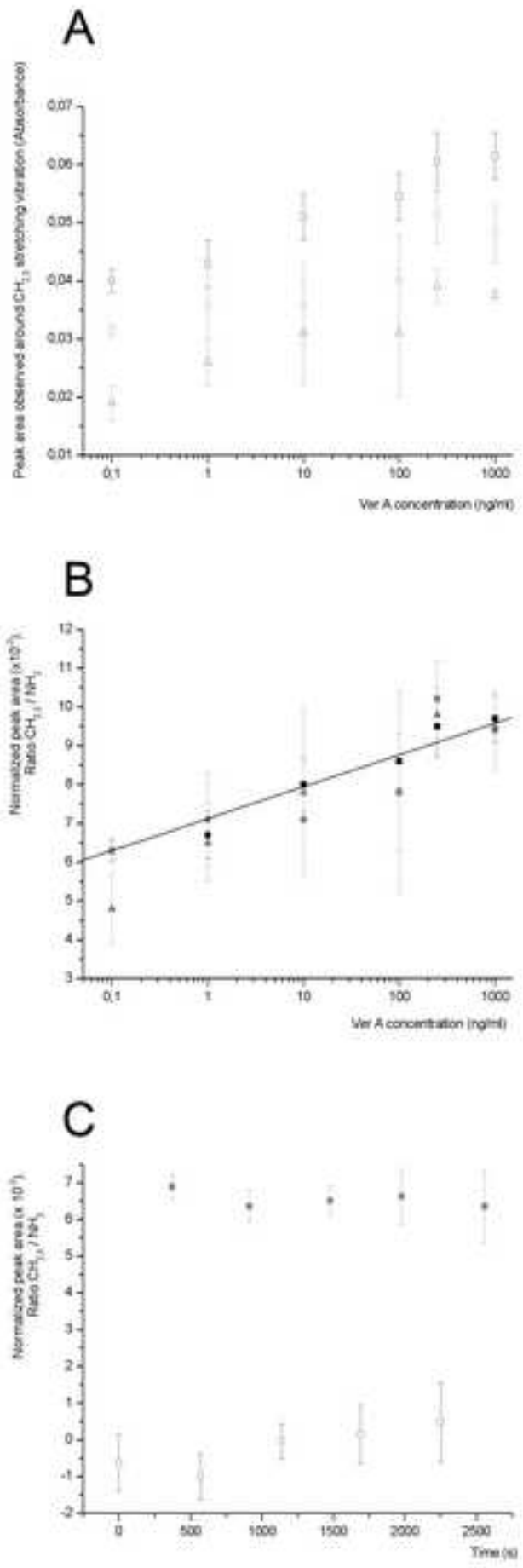

Figure 3

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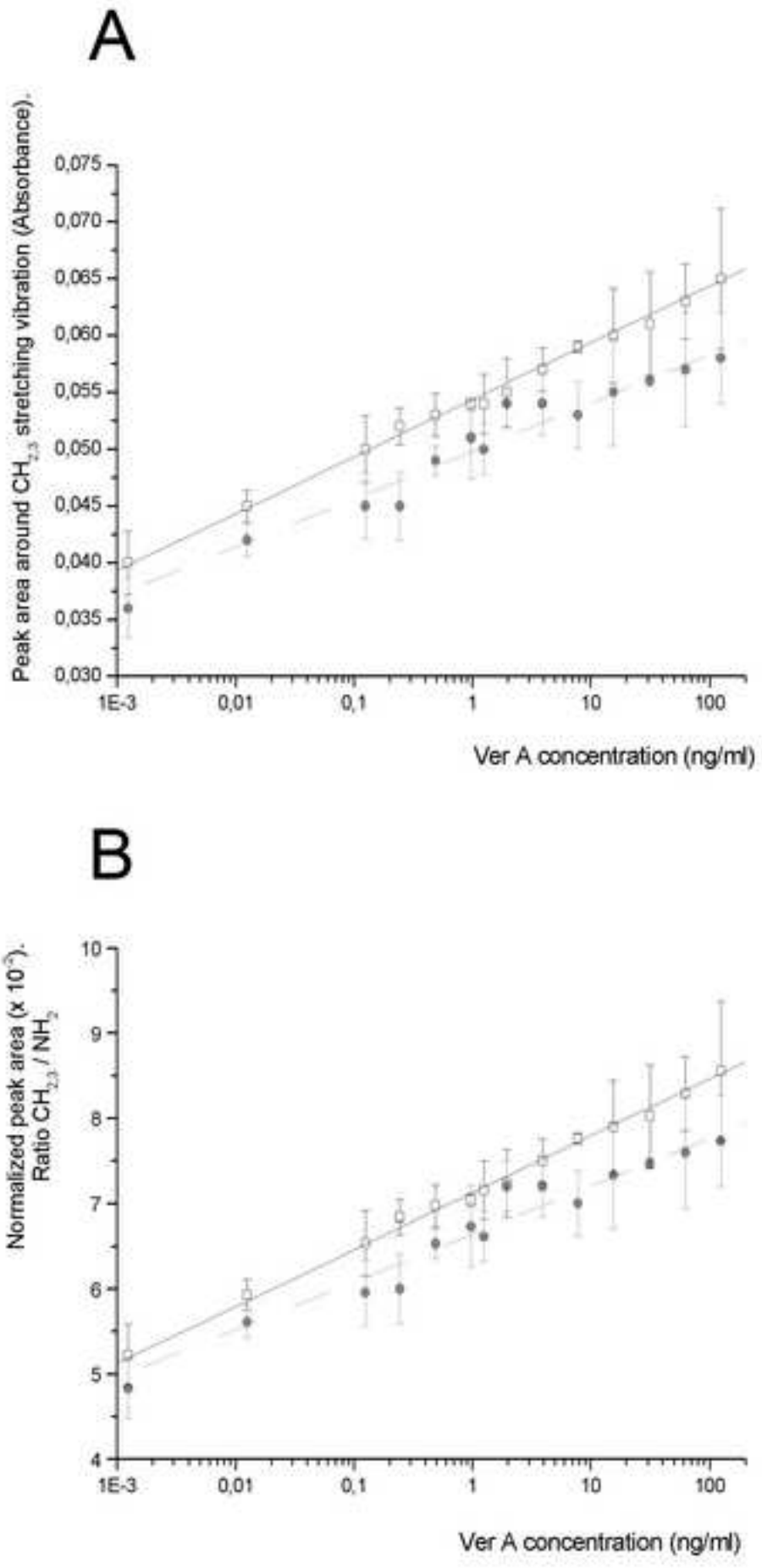

Table 1

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Table 1: CVs and recovery percentages of Ver A spiked in dust samples as determined by ELISA

Spiked dust Samples	Conc* (ng/ml)	Estimated Conc (ng/ml)	SD	Mean Estimated (ng/ml)	SD	CV %	Recovery %	SD
Sample 1	250	232.6 178.6 265.0 246.3	36.1 46.6 120.0 75.9	230.63	37.14	16.10	92.25	14.86
Sample 2	250	130.4 133.0 165.3 217.0	29.2 32.3 63.7 40.1	161.43	40.31	24.97	64.57	16.12

* 50 mg of dust were spiked with 250 ng of Ver A and then extracted in a total volume of 1 ml as described in the materials and methods section

Table 2

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Table 2: CVs and recovery percentages of Ver A spiked in dust samples as determined by the FTIR-ATR biosensor

Spiked dust Samples	Conc* (ng/ml)	Mean Estimated (ng/ml)	SD	CV %	Recovery %	SD
Sample 3	0.1	0.08	0.008	10	78	6
	1	0.85	0.15	17	85	12
	10	8.7	2	23	87	17
	100	86	21	24	86	17
	250	256	27	11	102	9
	1000	952	79	8	95	7
Sample 4	0.1	0.058	0.003	6	58	3
	1	0.8	0.09	11	80	9
	10	7.8	1.4	18	78	14
	100	87	12	14	87	12
	250	231	8	4	92	3
	1000	971	91	9	97	9

* 50 mg of dust were spiked with the indicated concentration of Ver A and then extracted in a total volume of 1 ml as described in the materials and methods section