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Short communication

A thin layer-based amperometric enzyme immunoassay for the rapid and sensitive diagnosis of respiratory syncytial virus infections

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ABSTRACT

A simple electrochemical sandwich immunoassay involving a polystyrene microarray slide coated with monoclonal capture antibodies and carbon screen-printed sensors (SPS) was designed for the rapid diagnosis of respiratory syncytial virus (RSV). The detection of the antibody-antigen complex formation relied on the use of a horseradish peroxidase conjugate. Its chronoamperometric measurement detection was performed by confining a droplet of H_2O_2/3,3',5,5'-tetramethylbenzidine enzyme substrate/mediator solution within a thin layer between one spot of the microarray and the surface of one screen-printed electrochemical cell. The accumulation of the enzyme product in the thin film of liquid enhanced the electrochemical response which allowed the development of a rapid (25 min) and sensitive thin layer-based amperometric (TLA) enzyme immunoassay. The method was successfully compared to commercially-available immunofluorescent and real-time PCR assays for RSV testing in respiratory secretion clinical samples. This suggests that owing to its rapidity, convenience, low-cost, portability and ability to provide quantified results, the reported concept could be a promising point-of-care diagnostic tool to screen patients with suspected respiratory infection or other types of infectious diseases.

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1. Introduction

The recent influenza A (H1N1) pdm 09 virus strain has raised the issue of the most optimal diagnostic method for the detection of influenza viruses and other viral agents associated with respiratory infections such as respiratory syncytial virus (RSV) and human metapneumovirus [1]. The laboratory diagnosis of respiratory viruses can be made by virus isolation, detection of viral antigens by immunofluorescence (IF) or enzyme-linked immunosorbent assay (ELISA), amplification of viral genome by molecular techniques, demonstration of a rise of antibody levels in serum, or a combination of those approaches [2,3]. Among them, cell culture and IF assays have been traditionally used for the laboratory diagnosis of respiratory viral infections but these assays have a low sensitivity for cell culture and are time-consuming for both diagnostic methods. Because of their excellent sensitivity and specificity, real-time polymerase chain reaction (RT-PCR) methods were mainly used as diagnostic tests during the Influenza A (H1N1) 2009 pandemic. They were performed by hospital laboratories with trained staff and adapted equipment. However, in the specific context of a pandemic, it was observed that the hospital resources can be rapidly saturated and there is a critical need for implementation of point-of-care testing.

Alternative methods based on the direct detection of specific influenza viral antigens using immunochromatographic devices have already shown their value to quickly screen patients with suspected influenza either in clinical labs or in physician's office. Compared to the methods previously mentioned, immunochromatographic assays offer many advantages such as no requirement of instruments, absence of elaborate sample preparation, simple handling and easy analysis result (colored lines on a strip). Because of their rapidity (approximately 15 min), these assays can help to reduce unnecessary costs of diagnostic tests, to decrease inappropriate antibiotic use, to facilitate antiviral treatment and to reduce the duration of treatment in the emergency department or hospitalization. Nevertheless, this technology due to a limited sensitivity, may exclude positive samples for further processing but cannot conclude for negative result. In addition these colorimetric assays are qualitative or semi-quantitative [4]. Therefore, there is a great need to develop an easy-to-use, sample-to-answer platform for rapid and reliable detection of influenza infections [5,6].

Due to their high sensitivity, simple instrumentation, ability to provide quantitative results, excellent implementation on

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microfluidic device, low cost and power requirement, electrochemical detection methods have gained considerable attention in the field of point-of-care immunosensors [7]. Usually, the immunological recognition of the target is converted into a measurable current thanks to the use of an enzyme conjugate that generates an electroactive product close to the electrode surface. The formation of a relatively high local concentration of the enzyme product [8,9] or its involvement in a bioelectrocatalytic reaction [10,11] can lead to significant signal amplification, and thus can improve the sensitivity of the assay. For this purpose, many proof-of-principle solutions involving various modified electrodes or sophisticated detections of the conjugate have been reported. But unfortunately they are seldom compatible with the development of commercially available point-of-care diagnostic kits. Alternatively, another technically easier approach which relies on the accumulation of the enzyme-product in a system of very low volume/surface ratio could be potentially interesting to develop reliable, inexpensive and rapid respiratory antigen detection tests. This improvement of sensitivity by introducing a lower volume of enzyme-substrate has been reported for the development of a disposable screen-printed electrochemical DNA sensor [12]. More recently, a rational study has showed that a significant increase (up to 200 times) in sensitivity may be obtained especially by confining small volumes of the enzyme-substrate solution within a thin-layer between the enzyme-coated planar screen-printed sensor and a coverglass for microscope slides [13].

The aim of the present work is to provide a rapid and reliable detection test of respiratory antigens by combining the sensitive thin layer-based amperometric (TLA) enzyme detection concept with the conventionally-used colorimetric sandwich ELISA. The RSV virus is one of the most important viral pathogens that cause respiratory tract infections in the pediatric population [14] and it was selected to optimize the assay format which is the key step depicted in Fig. 1A. It involves, on the one hand, a polystyrene array slide on which the entire sandwich immunoassay takes place and, on the other hand, a disposable screen-printed sensor (SPS) used to electrochemically quantify the amount of attached horseradish peroxidase (HRP) labeled immune complexes, and consequently the concentration of RSV antigen in the sample, by confining a droplet of the hydrogen peroxide/3,3',5,5'-tetramethylbenzidine (H_2O_2/TMB) enzyme-substrate solution within a thin layer. Finally, this assay which can be completed within 25 min was applied in clinical diagnosis of RSV.

2. Materials and methods

2.1. Reagents and solutions

The osmium complex $[Os^{II}(bpy)_2pyCl]PF_6$ was synthesized as previously described Kober et al. [15]. Lyophilized NeutrAvidin was purchased from Pierce. Monoclonal antibodies raised against RSV (MAb) were produced and purified by Argene as well as their biotinylated, HRP- and fluorescein isothiocyanate (FITC)-labeled forms named Bio-MAb, HRP-MAb and FITC-MAb, respectively. Lyophilized bovine serum albumin (BSA, fraction V), Tween® 20, hydrochloric acid were supplied by Sigma while Fetal Calf Serum (FCS) was purchased from Sigma (F2574). The substrate solution containing 1.25 mM TMB, 2.21 mM H_2O_2 in a 0.08 M acetate buffer at pH 4.9 was provided from Moss. The supernatant from a culture medium containing the RSV antigens (Ag) was obtained from a MRC5 cell culture infected by the RSV A strain Long (ATCC VR-26) in Dulbecco's Modified Eagle Medium at +37 °C with 5% of CO_2 for 3 days and its concentration was assessed by RT-PCR (see Section 2.7). Nasopharyngeal patient samples were provided by the laboratory of Virology at Dijon Hospital after testing them for RSV during the 2010–2011 winter and stored at −80 °C until they were used. This panel composed of 14 clinical samples: 7 high positive and 7 negative samples, previously analyzed by IF assay procedure (see Section 2.6). All experiments with RSV were performed in a biosafety level-2 laboratory.

Phosphate buffered saline (PBS; 1.54 mM KH_2PO_4, 5.1 mM Na_2HPO_4 and 130.9 mM NaCl, pH 7.2) and a carbonate-bicarbonate coating buffer (CBCB, $NaHCO_3$,100 mM, Na_2CO_3, 100 mM, pH 9.6) were prepared with deionized and double distilled water.

2.2. Electrochemical equipment and principle of the thin-layer based measurements

Array of 8 × 3 band-electrodes were prepared on a flexible polyester substrate with a semiautomatic screen-printer (Presco, USA) by subsequently printing and baking at 120 °C for 15 min silver-based (418SS) and carbon-based (PF 407A) thermoplastic conductive inks (Acheson Colloid). After the last curing step, two insulating layers (Vinylfast 36-100, Argon) were printed over the array of eight sensors, leaving uncovered the electric contacts and allowing the definition of an electrochemical microcell of a few

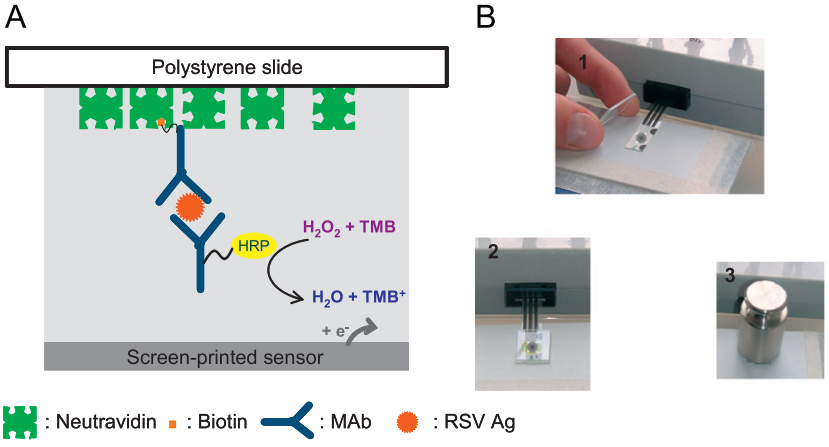


Fig. 1. (A) Schematic representation of the key step of the thin-layer based amperometric immunoassay. (B) Overview of the TLA measurement steps showing (1) on the left the polystyrene immunosensing surface (28.3 mm^2) and on the right the three screen-printed electrodes including a Ag/AgCl reference electrode, carbon working (S=7.07 mm^2) and counter electrodes connected to the potentiostat and covered with a 3-μL droplet of substrate solution, (2) the immunosensing surface carefully positioned upside down on the surface of the SPS so that the droplet wets both the transducer and the spot of the immunoassay solid phase, and (3) the thin layer of liquid is finally flattened with a weight before the chronoamperometric measurement. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

tens of microliters composed of one 7.07 mm^2 working electrode, one pseudo-reference electrode and one auxiliary electrode.

Amperometric measurements were performed with an Autolab potentiostat (PGSTAT 12, Metrohm Autolab B.V., The Netherland) both interfaced to a personal computer equipped with GPES version 4.9 software and connected to the electrochemical cell through a sensor edge connector purchased from Andcare.

2.3. Preparation of the immunosensing microarray slides

Crystal, transparent and amorphous polystyrene (PS) sheets (1.2-mm thick, Goodfellow) and a sealplate adhesive film (Dominique Dutscher, France) were used to prepare the immunoelectrochemical microarray slides shown in Fig. 1B. Briefly, small rectangles of PS (80 mm long and 20 mm wide) were first cut and coated with an adhesive film (60 mm long and 15 mm wide) punched with four holes at regular intervals in order to define four small-volume microcells (28.3 mm^2). To easily separate each immunocell before its thin-layer based measurement, the slide was precut with a scalpel.

Unless otherwise stated, all of the incubations involving the PS microarray slides were performed at 37 °C in a water-saturated atmosphere.

A drop of 20 μL of a 0.5 mg mL^{-1} neutravidin solution was then placed on each sensing microcell and kept overnight in a homemade humidity chamber at 4 °C. Following a quick washing step with PBS, 20 μL aliquots of Bio-MAb (30 μg mL^{-1} in PBS) were spotted on the microarray and incubated for 1 h. Next the microarray was rinsed with PBS containing 0.1% (v/v) Tween ® 20 (PBS-Tween) three times for 3 min and blocked with 20-μL droplets of a 5% FCS in PBS solution for 1 h. After a last washing cycle step (PBS-Tween; 3 × 3 min), the PS microarray was dried and the resulting immunosensing platform was stored at 4 °C until used.

2.4. Thin-layer based chronoamperometric enzyme immunoassay

Either 20 μL of lysed and subsequently PBS diluted supernatant of cell culture containing the RVS antigens or 20 μL of nasopharyngeal specimen were dropped in duplicate on the immunosensing microarray and incubated for 10 min. The immunocells were rinsed three times for 1 min with PBS-Tween and further incubated with 20 μL of HRP-MAb. After a 10-min incubation, excess of HRP-conjugate was washed away with PBS-Tween (3 × 1 min). Each immunocell was individualized and then kept immersed in PBS until the thin-layer based amperometric detection was carried out. For this purpose, the adhesive film was removed from the immunocell which sensing surface was previously delimited with a pencil ring and a 3-μL droplet of the H_2O_2-TMB solution was cast onto the surface of the single-use screen-printed sensor. Then, the immunocell was carefully placed upside down and flattened with a 200-g weight so that the HRP-labeled complex got in contact with the substrate solution. The enzymatic reaction was allowed to proceed in the thin-layer for 5 min after which the accumulated product was finally measured by chronoamperometry ($E = -0.1$ V versus pseudo Ag/AgCl for 90 s). The absolute value of resulting cathodic current corresponding to the reduction of the radical intermediate TMB^+ form at $t = 60$ s was taken as the analytical response.

2.5. Colorimetric enzyme immunoassay

Unless otherwise stated, all of the incubations were performed at 37 °C and followed by a washing cycle consisting of three incubations for 3 min in 300 μL of PBS-Tween. Each series of experiments were carried out in duplicate and included a PBS negative control.

Immobilization of the RSV capture antibody was carried out by incubating 100 μL of the MAb (3 μg mL^{-1} in CBCB) in MaxiSorp™ flat-bottomed polystyrene microwells (NUNC) for 1 h. Next, the walls of the microwells were rinsed and saturated with 200 μL of a PBS solution containing 5% FCS for 1 h. Once washed, they were further incubated with 100 μL of the lysed and PBS-diluted supernatant of cell culture containing the RVS antigens for 45 min. Subsequently, 100 μL of the HRP-MAb conjugate (0.7 μg mL^{-1} in PBS) were added and incubated for 45 min. Following this step, the microwells were washed again and then filled with 100 μL of the H_2O_2/TMB substrate solution. After enzyme incubation in the dark at room temperature for 30 min, the reaction was stopped by the addition of 100 μL of HCl (0.25 M). Finally, the optical density was read at 450 nm with a spectrophotometer (Labsystem Multiscan).

2.6. IF assay procedure

Nasopharyngeal specimens were first centrifuged at 180 *g* for 10 min and washed with PBS two or three times to remove the excess of mucus. For the cell culture, when the CPE has affected 50% of cells, the cells were scraped. The cell suspension was centrifuged at 200 *g* for 10 min, the supernatant was eliminated and cells were washed two times with PBS.

Thirty microliter of the resulting suspension was spotted in duplicate on the wells of a slide for immunofluorescence purposes (Argene, ref. 31–010).The slides were allowed to dry in a laminar air flow cabinet at room temperature. When the preparations were completely dry, the cells were fixed on the slides by using cold acetone for 20 min. 30 μL of RSV-specific FITC-MAb (Argene, ref. 17–042) was further placed on each well and the slides were incubated for 15 min at 37 °C in a moist chamber. Next, excess antibody was removed by successively dipping the slides in PBS (5 min), in distilled water (single in/out motion to eliminate PBS crystals) and the slides were air-dried at room temperature. One drop of mounting fluid was added to the center of each well, and a coverslip was placed over the mounting fluid before the entire well area of the slide was scanned with an fluorescence microscope (Olympus BX41) with appropriate filters for optimized visualization with FITC. RSV was detected by its characteristic granular, bright apple green fluorescence within the cytoplasm of infected cells which contrasted with the red background staining of uninfected cells.

2.7. Real-time TaqMan RT-PCR conditions

Total nucleic acids were isolated by placing 200 μL of samples or virus cell culture supernatant in a NucliSens® easyMAG instrument (Biomérieux) and by using NucliSens® easyMag Reagents (Biomérieux ref. 280110) with the specific B protocol extraction. 50 μL of silica has been added during extraction step. Total nucleic acids were eluted in 50 μL of elution buffer (Biomérieux) and 10 μL of the extracts were used for nucleic acid amplification.

A real-time duplexed RT-PCR was performed on a SmartCycler® 2.0 (Cepheid) to detect RSVA in the FAM channel (530 nm) and RSVB in the HEX channel (560 nm). Briefly, 1 μL mixture containing enzymes with diluted RT Omniscript 1/10 (Qiagen ref. 205113) and HotStarTaq™ (Qiagen ref. 203205) was added to 14 μL of premix (Argene ref. 71–007).The reaction conditions were designed as follows: reverse transcription at 50 °C for 30 min, HotStarTaq polymerase activation at 95 °C for 15 min, and 45 cycles of denaturation (95 °C for 10 s), annealing/extension (54 °C for 30 s) and elongation (72 °C for 10 s).

A positive sample is defined if a Ct value is displayed in FAM or in Hex channel. If there are Ct calculated in the FAM channel, the interpretation is subtype A, in the HEX channel, subtype B and if Cts are calculated in FAM and in HEX channel it is a coinfection of subtype A and B.

3. Results and discussion

3.1. Characterization of the thin-layer based amperometric detection

Our first concern was to propose a simple and reproducible readout process. To this end, the TLA detection of the HRP label was carried out as follows: once deposited on the electrochemical cell, the 3-μL droplet of the enzyme-substrate solution was uniformly flattened by carefully positioning the sensing spot of the polystyrene microarray slide and then positioning a 200-g weight on the screen-printed sensor (SPS) so that the droplet wetted both the transducer and the immunosensing surface (Fig. 1B). With the aim to further use the slide as a platform to perform the immunoassay, the polystyrene material, known to be well adapted to immobilize proteins, was selected. On the other hand, the use of a weight was mainly envisioned to create reproducible thin layers. The reproducibility of the process was investigated by determining the cyclic voltammetric responses (scan rate of 50 mV s^{-1}) of a solution of $[Os(bpy)_2pyCl]^+$ (Os^{II}, 10^{-4} M in PBS). An average anodic peak current i_p of 175 ± 10 nA corresponding to the reversible oxidation of the Os^{II} into Os^{III} was obtained with a relative standard deviation of 5.8% from 8 random single-use SPS. The main source of error was probably related to the manual character of the procedure. The thickness of the thin layer was also estimated from the cyclic voltammetry of the Os^{II} solution according the method reported by Limoges et al. [13]. Upon rising the scan rate from 10 to 200 mV s^{-1}, the transition in voltammetry from a thin layer to a semi-infinite diffusion layer ($l=4\times0.446(DRT/Fv)^{1/2}$) allowed us to calculate a film thickness of 27 μm from the following values: $D=4.5\times10^{-6}$ cm^2 s^{-1}, $R=8.314$ J mol^{-1} K^{-1}, $T=293$ K, $F=96500$ C mol^{-1} and $v=50$ mV s^{-1}. This measured value which is about 2.5 times smaller than the previously reported film thickness obtained between a planar screen-printed cell and a glass coverslip for microscope slide can be principally explained by the use of a weight. Positioned on the top of the system and initially aimed at improving the reproducibility of the measurement, this weight enhanced the adhesive forces between the liquid, the planar electrochemical cell, and the PS slide and then created thinner films of liquid in which the enzyme-product would be even more concentrated.

3.2. Optimization of the immunoassay conditions

The key step of the TLA enzyme immunoassay of RSV antigens is depicted in Fig. 1A. Its procedure is an adaptation of the ELISA protocol which basically involved five main steps: (1) immobilization of monoclonal capture antibodies (MAb), (2) capture of the RSV Ag which is (3) then sandwiched with a secondary HRP-labeled antibody (HRP-MAb), (4) incubation of the H_2O_2/TMB enzyme substrate and (5) colorimetric detection of the enzyme-generated product. Because of its strong reactivity with 2 epitope-binding sites on the RSV target antigen (F0 and F1 subunits of the fusion protein present on all RSV strains), the selected MAb is both used as primary- and secondary antibody. The measurement of the activity of the peroxidase label anchored in the immune complex involved the most widely used and sensitive chromogenic TMB cosubstrate [16–18]. In the presence of hydrogen peroxide and HRP, TMB is efficiently oxidized through two one-electron steps into a blue colored charge-transfer complex (TMB^+) which amount is usually measured by absorption spectrophotometry after its acidification into a stable yellow diamine product (TMB^{2+}). However, TMB^+ is also electroactive owing to its benzidine function (aromatic amines) and it can be detected using an electrochemical technique [19]. Because of its simplicity to further design point-of-care devices, the chronoamperometry technique was selected in this work to monitor the catalytic reduction of H_2O_2 by the bound-HRP label in the presence of TMB in the thin layer. Instead of taking place on the top of the screen-printed sensor, the immunoassay was deliberately carried out on the polystyrene slide to allow the incorporation of relatively thick biocomponent layers during immunofunctionalization and blocking without hampering the electrochemical detection. However, it was shown that the capture MAb could not be directly physical absorbed on the PS microarrays slide according to the RSV ELISA protocol owing to a significant loss of activity. Hence, the attachment of biotinylated MAb on neutravidin-coated PS slides was investigated and selected to further prepare immunosensing platforms which could be stored at least 4 weeks without any loss of activity. Finally, we took advantage of the dramatic increase in sensitivity achieved by accumulating the enzyme product in the film to reduce the incubation times and thus to complete the entire assay within 25 min as described in Materials and methods Section 2.4.

3.3. Performances of the immunoassay

With the aim of a comparative study, the sandwich immunoassay of RSV antigens was performed using both the colorimetric ELISA and the thin-layer based amperometric immunoassay. Based on optimal assays conditions, PBS dilutions of a lysed supernatant of cell culture containing the RSV antigens were detected in duplicate. The current responses and the optical densities were normalized to the zero RSV Ag concentration (PBS blank assay). Fig. 2 compares the log–log calibration plots of RSV Ag obtained with the colorimetric (○) and the electrochemical (■) approaches. The comparison of their sensitivity (i.e. the slopes of the calibration plots using linear scales) only indicates 1.5-higher sensitivity for the ELISA method.

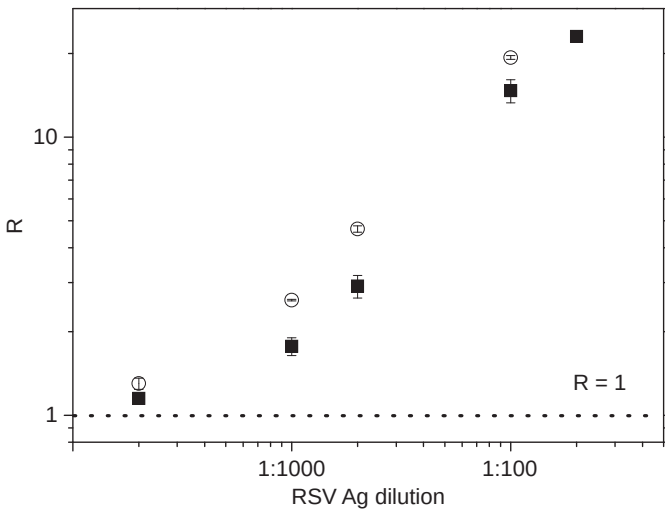
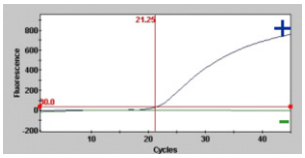
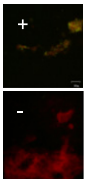
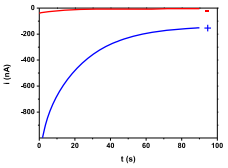


Fig. 2. Calibration plots of RSV Ag obtained for ○ the colorimetric- and ■ electrochemical-based assay. The *R* values correspond to the optical densities and the current responses normalized to the zero RSV concentration (PBS blank assay corresponds to an optical density $OD=0.134$ and an absolute value of the chronoamperometric current $i=11$ nA). The error bars represent the standard error of two measurements. The cell culture sample containing the RSV Ag was tested by RT-PCR (see Materials and methods Section 2.7) and the obtained Ct value of 15.45 cycles corresponds to roughly 10^7 RSVs/PCR.

Table 1
RT-PCR, IF and TLA analytical data obtained for the RSV determination for a positive (+) and a negative (−) control and comparison of the three techniques for the RSV testing in nasopharyngeal clinical specimens ($n=14$).

Specimen n°	RT-PCR	IF	TLA
			
2	+ (25.4)[a]	+ (2)[b]	+ (97 ± 17)[c]
3	+ (23.2)	+ (3)	+ (292 ± 40)
5	+ (21.2)	+ (3)	+ (177 ± 17)
14	+ (21.8)	+ (1)	+ (215 ± 6)
17	+ (23.8)	+ (3)	+ (1126 ± 30)
26	+ (23.0)	+ (3)	+ (313 ± 21)
64	+ (23.1)	+ (2)	+ (407 ± 31)
80	+ (31)	–	− (7 ± 1)
13	–	–	− (8 ± 1)
23	–	–	– (6 ± 0.8)
24	–	–	– (7 ± 0.5)
27	–	–	– (6 ± 0.1)
78	–	No cells	– (5 ± 0.1)
79	–	–	– (7 ± 0.2)

[a] Cycle threshold value
[b] Number of fluorescent cells (1), weak; (2), medium; and (3), strong
[c] Average absolute value of the chronoamperometric current measured in nA ($E=-0.1$ V vs. Ag/AgCl; $t=60$ s; $n=2$). Cutoff for $i=10$ nA

Moreover, according to the calibration curves, the detection limits, determined as the RSV Ag dilution leading to a signal corresponding to that of the blank plus three standard deviations, were 1:6000 for the spectrophotometric method and 1:5000 for the electrochemical detection. These very similar detection limits demonstrate that good analytical performances can be obtained quickly with the TLA immunoassay (25 min instead of 2 h required for the ELISA test), which is critical to further develop rapid and reliable portable analytical devices to screen respiratory viruses.

3.4. Detection of RSV in respiratory samples

To evaluate its capability for the RSV antigen testing in clinical specimens; the TLA enzyme immunoassay was compared with two classical laboratory diagnosis methods, including IF tests and TaqMan RT-PCR. To this end, the remainder of fourteen nasopharyngeal samples, which had adequate volumes to perform the three tests in duplicate, was analyzed and the results are summarized in Table 1. At the top of this latter, are shown the data obtained with the three techniques and their interpretation for both a negative (PBS) and a positive (RVS Ag from cell culture) control which were introduced in each set of experiment. A sample was defined RSV positive if a Ct value in RT-PCR or a characteristic granular and bright apple green fluorescence of the cell cytoplasm in IF assay was displayed. The absolute value of the cutoff for the TLA immunoassay ($i=10$ nA) was calculated from the mean ten negative control determinations plus three times the standard deviation. Of the 14 specimens tested, 8 were positive by at least one assay (upper part of the table) while 6 were negative by the three techniques (lower part of the table). Among the 8 positive samples, all of them tested positive by RT-PCR, whereas 7 tested positive by both immunoassays. Indeed, the specimen n° 80 that had tested weakly positive by RT-PCR was found to be negative by IF and TLA tests, thus confirming that immunoassays are less sensitive than RT-PCR using the assay parameters applied in this work to detect RSV in specimens with low viral titer. On the other hand, the electrochemical approach developed in this paper led to the same results as those obtained with the fluorescent test which is yet to be completed within 2 h–2 h 30 min and requires technical expertise for the subjective interpretation of the results obtained in laboratories with expensive and fragile equipments. By both reaching the sensitivity of the fluorescent assay and circumventing its main drawbacks, the TLA immunoassay is a promising approach to develop reliable point of care rapid antigen tests.

4. Conclusions

An amperometric immunoassay based on the concentration of the enzyme-label product in a thin film of liquid has been successfully developed for the sensitive RSV testing in clinical respiratory samples. The entire assay took place on a homemade polystyrene slide before reading each spot of the microarray by placing it upside down onto the surface of the screen-printed electrochemical cell containing the enzyme substrate. The performance of the assay was very simple and the detection was completed within 25 min. Moreover, contrary to IF, this assay is well-adapted for decentralized early diagnosis insofar as it is possible to develop a portable multichannel potentiostat that unable to easily align and clamp the polystyrene microarray slide on the top of a matching array of screen-printed sensors. Finally, the proposed concept, which is a significant improvement over traditional methods for the detection of respiratory viruses, is currently extended to the diagnosis of influenza viruses A and B with the aim to further provide a microarray for the differential diagnosis of respiratory viruses.

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