Electrochemical Immunosensor Coupled to Cyclone Air Sampler for Detection of *Escherichia coli* DH5α in Bioaerosols

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Abstract
The portable detector ImmunoSMART was combined with the cyclone air sampler to detect the model bioagent *E. coli* DH5α in bioaerosols. The selected capture antibody was immobilized on gold electrodes, antibody-peroxidase conjugate served as a tracer. In buffer, the amperometric immunosensor measured 10^3 to 10^8 CFU/mL within 30 min. Detection in air was realized in a closed aerosol chamber. Automated dissemination of the cells, sampling and measurement allowed remote testing of the cyclone/immunodetector. The level of 150 CFU/L in air was clearly indicated (20 min analysis including 5 min sampling) and independently confirmed using the slit sampler and agar cultivation. The results seem promising for further extended tests in both aerosol chamber and field trial conditions.

Keywords: Bioagents, Sandwich immunoassay, Biosensors, Remote sensing, Aerosol chamber

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1 Introduction

The growing requirements on detection of bioagents originated from field military operations, however, the current interest comes mainly from civil rescue and security services, protection of public buildings and homeland security. As a result, the development of portable, rapid and simple instrumentation based on the bioanalytical detection principles is widely supported [1,2]. For microbial agents, various types of immunochemical devices are preferred for the early response, good sensitivity and continuous monitoring capabilities. The detection is based on the phenotype level and no extraction of the genetic material from the bioagent is required, which is the case for methods based on the polymerase chain reaction (PCR). Immunosensors applied for biodetection purposes operate mainly using various optical systems as transducers [3], surface plasmon resonance (SPR, [4]) e.g. Biacore and Spreeta representing the most popular system and a portable example. Alternative optical systems include fluorescence with evanescent wave excitation based on planar and cylindrical waveguides – the Array Biosensor from Naval Research Laboratory [5] and the Raptor system from Research International [6], respectively.

Electrochemical sensors for bioagents detection represent a promising alternative approach [7,8]; the electrochemical measuring system is highly sensitive, quite cheap and easily miniaturized to portable format. As the measuring element, the screen-printed sensors are widely applied due to easy and reproducible fabrication at both laboratory and mass production scales [9]. Our previous efforts in the field of electrochemical immunosensors for bioagents were focused on *Francisella tularensis* [10,11] as a model microbe belonging to the list of the most dangerous bioagents compiled by Centres of Disease Control. The immunosensors based on screen-printed electrodes were evaluated on liquid samples. Recently, the prototype device ImmunoSMART was tested for detection of microbial cells in bioaerosols. As the cells of *Francisella* are not allowed to be spread in air, we have adopted the strain *Escherichia coli* DH5α as a relatively safe alternative. This seems to be the first attempt to detect the bioaerosol based on this microbial strain using the electrochemical immunosensor coupled to the air sampling cyclone. The preliminary results from this combination are described in this contribution.

2 Experimental

2.1 Chemicals

The antibodies (Ab) specific against *E. coli* included rabbit polyclonal Ab1 (no. 4329-4906, AbD Serotec, 0.5 mg/mL IgG), mouse monoclonal Ab2 (no. ab20386, Abcam, 0.9 mg/mL IgG) and mouse polyclonal Ab3 (kindly provided by Dr. M. Pohanka, Central Military Institute of Health, Těchonín, Czech Rep., antiserum 30 mg/mL protein, purified immunoglobulin 0.7 mg/mL [12]). The mouse monoclonal antibody AL-01 (anti albu-

min, used for reference purposes) was provided by Exbio Praha (Prague, Czech Rep., 10 mg/mL IgG). As tracer for the sandwich immunoassay, the polyclonal rabbit Ab 4329-4906 conjugated to horse radish peroxidase was supplied by AbD Serotec.

Glutaraldehyde (GA, 25% aq. solution) and bovine serum albumin (BSA) were obtained from Sigma. *N*-hydroxysuccinimide (NHS), ethyl-N,N-dimethylaminopropyl carbodiimide (EDC), buffers HBS-N (10 mM HEPES pH 7.4 and 150 mM NaCl) and HBS-P (as HBS-N plus 0.005% Tween-20) were from Biacore AB, Uppsala, Sweden. Phosphate buffer (50 mM sodium phosphate, pH adjusted to 7.0) and phosphate buffered saline (PBS, 50 mM sodium phosphate pH 7.0 and 150 mM sodium chloride) were used for most experiments.

2.2 Microorganisms

The microbes were cultivated at FOI using standard procedures, the bacteria were supplied as a suspension of cells in 0.9% sodium chloride. The *E. coli* DH5α strain stock solution contained $1.45 \cdot 10^{10}$ CFU/mL and was stored in a refrigerator for up to 5 days. Content of microbes was determined using the McFarland method [13]. The working solutions were made daily by diluting the stock solution in PBS; the range was from around 10^8 to 1000 CFU/mL using the geometric (10× dilution) scale.

2.3 Surface Plasmon Resonance Measurements

The system Biacore 2000 and measuring chips CM3 were supplied by Biacore AB. The Biacontrol program realized measurements and Biaevaluate data editing and presentation. All experiments were realized at 25 °C and using flow rate of 5 μl/min during immobilization steps and 20 μl/min for biointeraction studies. The activation of carboxy groups of the CM dextran surface was realized according to the manufacturer instructions using 400 mM EDC and 100 mM NHS in water for 10 min. After washing with HBS-P buffer, the individual antibodies dissolved in 50 sodium acetate pH 4.5 were injected to individual channels, the achieved signal was followed and sometimes the injections were repeated in order to achieve the desired immobilization level. Finally, all flow channels (including Fc1 as reference) were blocked using 20 mM ethanolamine pH 9. The interactions of the modified chip with microbial cells were carried out in the HBS-N buffer.

2.4 Electrochemical Immunosensors and Measurements

The exchangeable electrochemical sensor was produced by screen-printing [14]. The 4 gold-paste based working electrodes (1 mm diameter) were placed on an alumina support (8×50 mm). The sensor was produced according to our design by BVT Technologies, Brno and is available as AC8.W1 from this supplier. The gold surface was cleaned with acetone and a self-assembled monolayer was formed during 3-hour incubation with cystamine (10 mg/mL in water). After washing with water, the amino groups were activated for 2 hours with glutaraldehyde (2.5% solution in phosphate buffer) and the antiserum and Ig fraction of Ab3 (15 and 0.5 mg/mL in phosphate buffer, respectively) were attached overnight. For two reference channels, a non-specific antibody AL-01 (0.5 mg/mL, anti human serum albumin) was immobilized in the same way. Thus produced immunosensors were stored in dry conditions in a refrigerator. Prior to use, the measuring area of the immunosensor was incubated for 1 hour with 0.2% bovine serum albumin in order to saturate any non-specific binding sites.

For measurements, the immunosensor was fixed in a thin-layer flow-through cell made from Plexiglas, internal volume was 8 μL. The cell contained also the embedded silver pseudoreference electrode (diameter 3 mm). The immunosensor was connected to the prototype four-channel measuring system ImmunoSMART (Smart, Brno). This system combines the measuring part with a flow system consisting of four miniperistaltic pumps, digital microcontroller and battery [11]. The individual peristaltic pumps (PP) 1 to 4 were coupled to the following solutions: PP1: PBS (also used for the manually inserted liquid sample); PP2: tracer in PBS with 1 mg/mL BSA; PP3: 8 mM H_2O_2 in 100 mM sodium acetate pH 5.0; PP4: 8 mM potassium iodide in 100 mM sodium acetate pH 5.0. The flow-through rate was 20 μL/min and immunoreactions and washing steps were carried out in PBS containing 1 mg/mL albumin. The sample was taken either from a microtube (for calibration curve generation, 15 min incubation in flow) or introduced automatically from the cyclone system (measurements in bioaerosol chamber, described below). After washing (5 min, PBS), the captured microbial cells were further incubated with tracer (anti *E. coli* antibody-peroxidase conjugate, 300—times diluted working concentration) for 10 min followed by 2 min washing. The amperometric response was measured in 8 mM H_2O_2 mixed with a zone of 8 mM potassium iodide; the iodine produced by the peroxidase label was detected amperometrically on the gold-based cathode at −100 mV vs. the silver pseudoreference electrode. The minimum of the cathodic current achieved during a 2 min injection was evaluated. Control of the ImmunoSMART detector was realized through a serial line using the LT_ ImmunoSMART software, data were evaluated using LabTools (both in-house programs developed in Delphi). Each immunosensor was used repeatedly in case of negative detection cycles; after positive detection of target microbes, the immunosensor was replaced by a new one.

2.5 Measurements in the Aerosol Chamber

The in-house constructed aerosol testing chamber available at FOI, Umea was used. It was a metal box with internal volume of 62 m^3, the tested systems have available power supply and ethernet link. The external glass windows allow visual control and personnel can enter to the

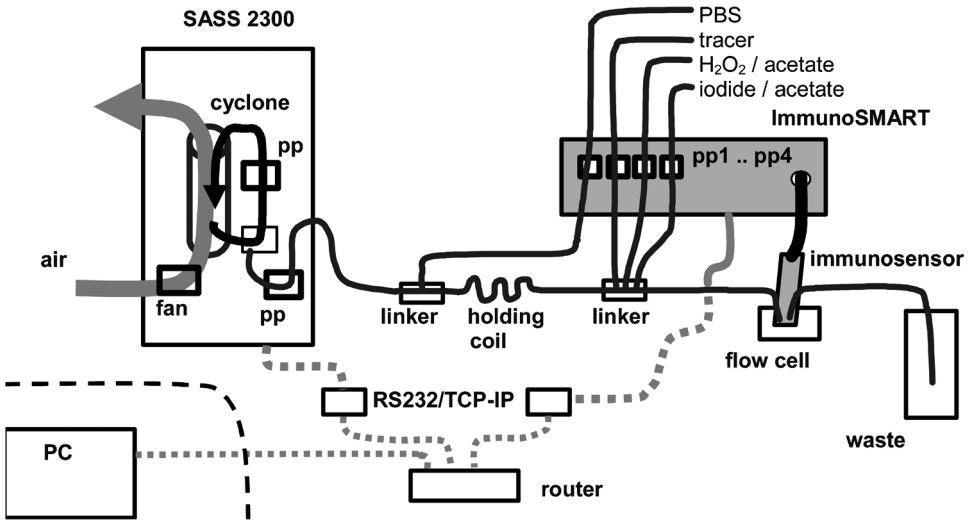

Fig. 1. Combination of the air sampler SASS 2300 (consisting of air-pumping fan, cyclone unit, liquid sample container and two peristaltic pumps pp) with the electrochemical detector ImmunoSMART (four peristaltic pumps pp1 to pp4 and digitally controlled potentiostat) for measurements with exchangable immunosensors. The flow system consists of two linker elements; the first one is merging the acquired sample from the cyclone with the PBS path from the immunodetector pp1; the sample is delayed in the holding coil and passes through the second linker (mixing with other reagents from pp2 to pp4) to the flow-through cell containing the immunosensor. The serial interfaces of both instruments are converted to the TCP-IP network and routed to the external computer located outside the chamber. The thick arrow indicates flow of air in the cyclone, thinner lines corresponds to the flow system and dashed lines indicate communication paths.

chamber through double door. The spreader system for dissemination of microbial agents allows to quickly (within few seconds) spread the source suspension of microbes (*E. coli* DH5α, concentration approx. 10^{10} CFU/mL) within the whole volume with the help of rotating ventilators. The start and duration of dissemination were controlled externally as well as ventilators and HEPA filters allowing decontamination of the air inside the chamber. The chamber was closed and no persons were present inside during dissemination of microbes and before the decontamination of air. All persons entering the chamber were properly equipped with protective suit and wear the chemical protective mask according to the safety regulations.

For sampling of the aerosol inside the chamber, the portable air sampler SASS 2300 (Research International, Seattle, WA, USA) was adopted. The sampler was battery-operated and remotely controlled through a serial link and serial/ethernet converter. It is a wetted wall cyclone system, the aspirated air (rate of the air fan – Figure 1, left, was 325 L/min) was contacting the circulating PBS. The total volume flowing within the cyclone was set at 5 mL, it was pumped using the internal peristaltic pump (upper pp pump of SASS 2300 in Figure 1). Inside the cyclone system, the present microbes became captured in the circulating solution. During operation of the cyclone, this volume of liquid became recirculated approximately 6–20 times per min. The sample acquired during the 5 min sampling interval was used for further immunosensor measurements.

After the sampling period, the output capture solution from the sampler was aspirated at the fixed rate of 15 mL/min (Figure 1, lower pp pump of SASS 2300) to a holding coil and then slowly pumped through the flow cell with the immunosensor for 2 min using one of the peristaltic minipumps (pp1) of ImmunoSMART. Further incubation of microbes mixed with tracer continued for additional 5 min (pp2) giving the total contact time of the immunosensor with the captured microbial cells of 7 min.

The ImmunoSMART device was also battery-operated and connected to the serial/ethernet link for a remote control. The schema of the measuring system is provided in Figure 1.

The details of the principal part of the sampler/detector combination are shown in Figure 2. The program LT_ImmunoSMART managed synchronization of the sampler and detector and controlled all timing, washing, incubation steps and data recording; the individual working parameters were edited as a working script thus allowing simple programming of performance and reproducibility of measuring procedures.

Further embedded equipment of the aerosol chamber included several temperature sensors and humidity sensor, all chamber systems were controlled through an own interface developed in LabView. The reference measurement of viable microbes in the air inside the chamber was realized using an own system of agar plate slit samplers, consisting of 12 slit sampler units (BC-AM-11, Biotrace, F. Baker), mass flow meter (EL-FLOW, Bronkhorst) and vacuum pump (17 L/min). The individual slit samplers are switched on using remotely controlled solenoid valves [15]. The incoming air passes through a narrow slit and contacts the rotating Petri dish plate with solid agar cultivation medium, the captured microbes are

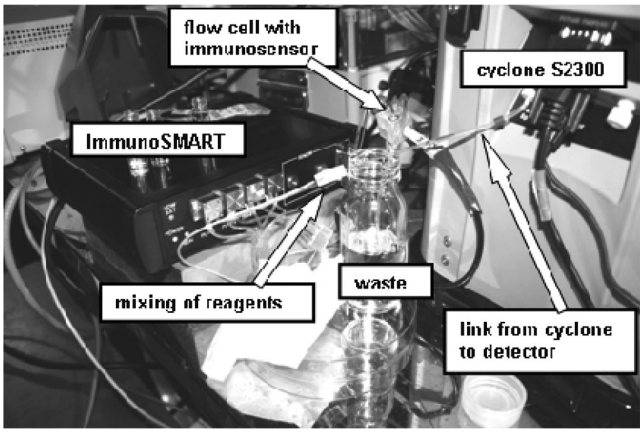

Fig. 2. Photography of the ImmunoSMART detector (left, black box with four miniperistaltic pumps at the front board) and the air sampler SASS 2300 (right, the rear part is shown). In between these units, coils of the flow system and the flow-through cell are shown.

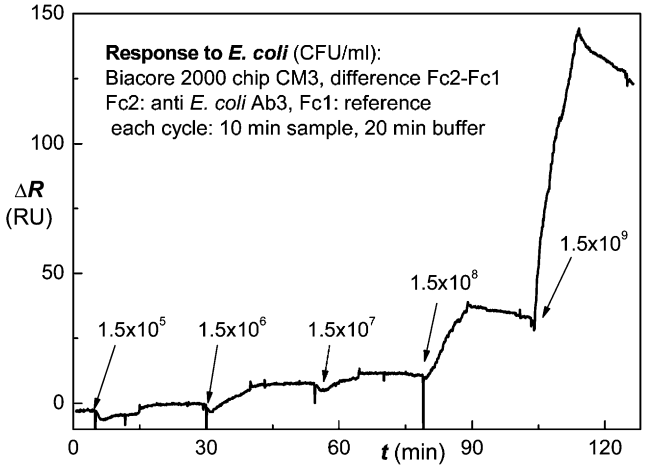

Fig. 3. Testing of the anti *E. coli* polyclonal mouse antibody (Ab3) immobilized on the Biacore chip CM3 (path Fc2) in the presence of increasing concentrations of *E. coli* DH5α cells. The trace corresponds to the difference signal resulting after subtraction of the reference channel Fc1.

cultivated overnight and the counted number of colonies corresponds to the number of CFU per liter of air. Each agar plate represented 30 sectors exposed for 8 s to the air, i.e. 2 min of recording during the dissemination experiment per plate and 24 min in total. Upper limit of the method is 250 colonies per sector, counting was realized either visually or the digitized image of the plate was processed in the program ProtoCOL SR/HR (Synbiosis). All particles in the air were continuously counted using the MetOne 1 (Met One, Grants Pass, USA) system with discriminating particle sizes of 0.5–1–2–3–5–10 μm per individual channels.

3 Results and Discussions

3.1 Characterization of Antibodies

For capturing the target microbe at the immunosensing surface of electrode, the appropriate antibody should be selected. The surface plasmon resonance system Biacore provides option for a real-time observation of the interaction between immobilized antibody and microbial cells in solution. The recommended EDC/NHS activation procedure was carried out on all flow channels (Fc) of the CM3 chip, the achieved activation levels were 66 to 120 RU. The 10-times diluted antiserum Ab3 was injected to Fc2 for 10 min providing 7.1 kRU of immobilized protein. Similarly, Ab2 (dil. 4 times) was linked to Fc3 resulting in 2.2 kRU and Ab1 (dil. twice) was immobilized in Fc4 providing 450 RU during 5-times repeated 10 min injections. Even though the immobilized amounts of antibodies were rather different, this was not considered as problem as the achieved binding capacities were anyway more than sufficient considering the expected interaction with large bacterial cells. For the same reason, the chip CM3 was chosen as it is recommended for interactions involving large particles as microbes.

The binding of antibodies with *E. coli* DH5α was tested for 10^3 to 10^8 CFU/mL using HBS-N as the working buffer; even at the highest amounts of the microbe, the responses for channels with Ab1 and Ab2 were not distinguishable from the reference line Fc1. The temporary signal changes were 20 to 80 RU for the given amounts, respectively, but the signal always immediately returned to the baseline in the absence of microbes in the flowing solution. The specific response was only observed for Ab3, the differential signal shown in Figure 3 clearly indicates microbes captured by the antibody and only slowly dissociating from the formed immunocomplex. Regarding direct immunosensing of microbial cells, these presented data from Biacore generally correspond to our previous results obtained using the piezoelectric immunosensor [12]; directly operating biosensors could achieve sufficiently low detection limits for microbes only through signal enhancing steps and complex sensing strategies [16]. Thus, Ab3 was chosen for further experiments and construction of electrochemical immunosensors.

3.2 Performance of the Electrochemical Immunosensor

The electrochemical immunosensor employed the sandwich assay format schematically shown in Figure 4. The antibody layer was covalently immobilized on the gold electrode surface; the target microbes captured during incubation with sample were subsequently labelled with the peroxidase-antibody tracer during the second incubation. The assay was heterogeneous, as washing steps were included after incubations. Finally, the surface bound peroxidase activity was determined electrochemically, the enzymatically-produced iodine was measured at a negative potential. The concept was quite similar as used previously for detection of *Francisella* [10,14]. The initial optimization experiments concerning incubation times with

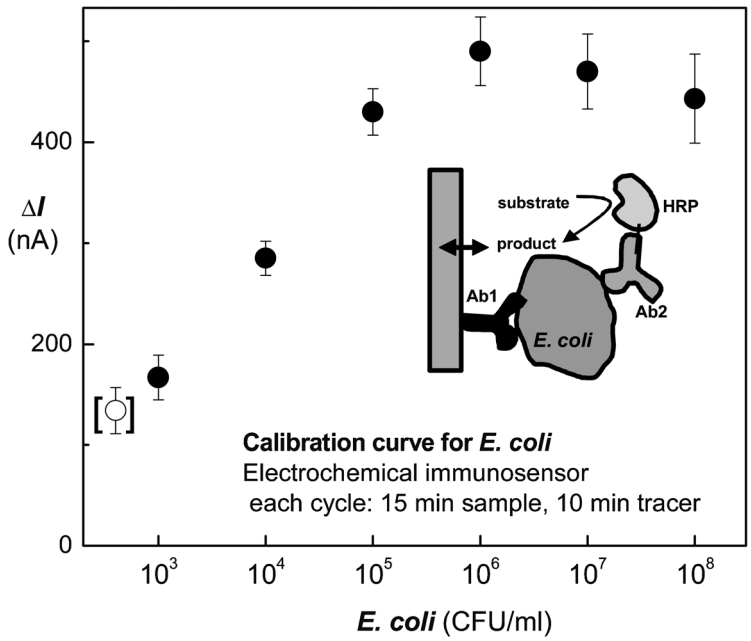

Fig. 4. Calibration curve of the electrochemical immunosensor based on immobilized Ab3 for *E. coli*. The error bars represent estimated deviations from repeated experiments ($n=3$), the empty point in brackets indicates the background signal in the absence of microbial cells. The schematic view of the sandwich assay realized on the gold sensing electrode is shown as the inset.

sample and with the tracer were carried out using a fixed amount of 10^5 CFU/mL of *E. coli*. The incubation with the sample was studied from 1 to 60 min and the highest response (difference of signals measured in specific and non-specific channels) was achieved for 15 min, longer incubation did not improve the useful signal but a substantial decrease was observed. The 10 min interval was adopted for incubation of the immunosensor with tracer; again, the response was in the saturation area. These parameters were used for generation of the calibration curve of the developed immunosensor for *E. coli* (Figure 4).

The limit of detection was around 10^3 CFU/mL considering the fluctuations of the background signal and the assay was linear up to 10^6 CFU/mL; for higher amounts of *E. coli*, a gradual decrease of signal was observed due to oversaturation of the binding surface similarly as for immunosensors for other microbes [14].

3.3 Detection of *E. coli* in Aerosol Chamber

The model detection of *E. coli* in aerosols was the main goal of this research. Most detection systems focused on bioaerosols are commonly tested using spores of the gram-positive bacterium *Bacillus globigii* which is a safe variant representing *B. anthracis*. *B. globigii* is allowed to be spread in closed chambers and even in field trial facilities for testing purposes. As our previous research of electrochemical immunosensors was focused on the gram-negative bacterium *F. tularensis*, which must not be spread in the air neither in closed nor open environments, the relatively safe strain *Escherichia coli* DH5α was chosen as model gram-negative microbe suitable for spreading in a closed air chamber. Our previous efforts for immunosensing of microbial cells always focused on liquid samples containing the target microbes. However, for detection in real situations, the sampling of bioaerosols in air must be realized. For this purpose, the portable air sampler SASS 2300 operating on the cyclone principle was purchased. Its construction and microcontroller-based operation allowed on-line integration with our electrochemical immunodetector ImmunoSMART and even remote control of both systems, required for operation in the closed chamber. The serial interfaces available for communication in both devices can be easily transported on intranet/internet networks using common RS232/TCP-IP converters; the wired links were used here, but wireless options can be considered for future use in open space. The chosen communication hardware and developed software allowed robust and completely reliable remote device control and uninterrupted data collection. Furthermore, the rechargeable battery power packs embedded in both devices provided sufficient energy sources for whole-day experiments (8 to 10 working hours).

After initial performance tests, the dissemination experiments were started. The systems SASS 2300 and ImmunoSMART were placed inside the chamber and fresh working solutions were filled to the containers. The chamber was closed and operation of HEPA filters for 1 hour removed most particles from the air in the chamber (below 100 particles/L of air). Afterwards, one sampling/measurement interval was performed in order to achieve the background signal; the final trace of current from the specific channels is shown in Figure 5, the upper set of

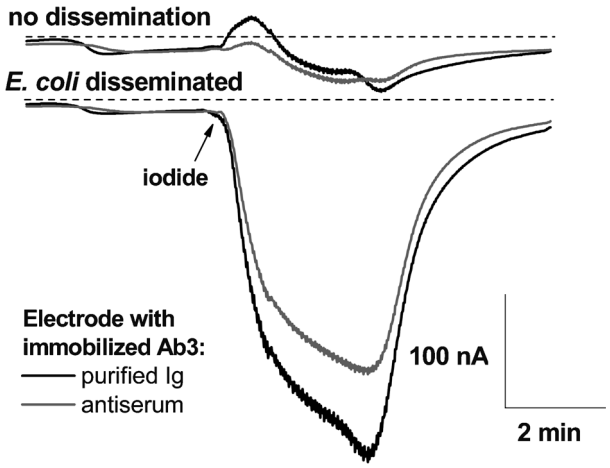

Fig. 5. Traces from testing of the air-sampler/immunodetector systems combination for detection of *E. coli* DH5α cells in air inside the aerosol chamber. The signal vs. time plots correspond to the measured currents in the presence of substrate mixture hydrogen peroxide/iodide (injection point marked by the arrow) which becomes converted to iodine in the enzyme reaction of peroxidase used as a label (see Inset of Figure 4). The upper set of traces represents control blank signal in the absence of *E. coli*, the lower traces indicate positive detection of disseminated microbes. As immunorecognition elements, either crude antiserum or purified immunoglobuline fraction were immobilized on the sensing electrodes.

traces labeled "no dissemination". The duplicate traces represent signals from channels with immobilized serum and IgG of the capture antibody Ab3; no significant difference was observed.

To achieve shorter detection cycle, the sampling of air was carried out for 5 min. To transfer the captured microbes concentrated in a liquid sample, the holding coil was filled by the peristaltic pump from the cyclone during a 15 s interval. The sample from the holding coil was afterwards transferred to the flow cell of ImmunoSMART using its pump PP1 and a 2 min injection time (Figure 1). Afterwards, the tracer was immediately passed through the flow cell for 5 min using PP2. A 2 min washing with PBS from PP1 followed, sensor was electrically connected to the potentiostat inside ImmunoSMART and the working potentials were applied, PP3 introduced acetate with hydrogen peroxide for 2 min (baseline recording) and the mixed flow from PP4 introduced iodide; the enzyme reaction was initiated and carried out for 2 min, PP4 was switched off and after another 2 min PP3 was switched off and the measuring cycle was completed by a 5 min washing using PBS from PP1. Thus, the individual measuring cycle lasted for 20 min.

This negative control was always realized with a new sensor. Afterwards, the dissemination was initiated, the microbial suspension was spread inside the chamber as aerosol for 2 min (remotely controlled nebulizer was used), and after the first minute of dissemination the detection cycle of cyclone/immunodetector was initiated. The described testing procedure resulted after numerous unsuccessful trials. Initially, negative detections were obtained from the immunodetector for several trials, and it was confirmed that negligible counts of viable *E. coli* existed in the air. This fact was due to rather low humidity (25 %) inside the chamber; placing of wet tissues inside the chamber increased the humidity level up to 35–37 % and this helped to successfully detect *E. coli* using both the immunodetector and the agar slit sampling systems. A typical traces of signal from the ImmunoSMART representing the "successful" positive detection are presented in Figure 5, the lower part marked "*E. coli* disseminated".

To relate the "positive detection" achieved using the immunosensor to the real content of *E. coli* cells present in the bioaerosol, the time-profile of viable cells in the air was followed independently using the slit sampler; the data corresponding to the immunosensor response shown in Figure 5 are presented in Figure 6. Surprisingly, the amount of cells in air was generated for a very short period and quickly after ending of the dissemination (within 2 min), the count of cells reached the zero level. Consequently, the sampling realized by the cyclone for the second half of the chosen period was not able to capture any cells.

The profile of cells (CFU) content in the air during the 5 min sampling interval (Figure 6) allowed to calculate the mean level equal to 23.0 CFU/L; during other dissemination trials the levels equal to 18.8, 39.2, 63.9, 63.4 and 20.1 CFU/L of air were also positively detected. Based on the fan rate (325 L/min) and sampling interval (5 min), the profile from Figure 6 provides 37 400 captured cells (as CFU) or the final content in the capture buffer of 7500 CFU/mL. The absolute amount of cells incubated inside the cell with the immunosensor can be estimated from the flow rates 20 μl/min of pumps pp1 and pp2 (Figure 1) and the contact time of 7 min. This corresponds to 140 μl, i.e. 1050 CFU passed during incubation through the cell. The initial fast transfer (at 15 mL/min) of sample from the cyclone to the immunosensor flow system with holding coil, linkers, flow cell and tubings is not considered as the contact time was too short (< 15 s). This level fits well to the calibration curve of the electrochemical immunosensor (Figure 4) where contents from 10^3 to 10^4 CFU/mL were clearly indicated.

From the practical point of view, the peak generated content of *E. coli* in the air was 150 CFU/L, and this can be considered as an easy to detect level using the proposed combination of air sampler and immunosensor. For future, the detectable limits can be improved, as the difference between signals in Figure 5 is quite large; furthermore, a longer sampling of aerosols using the cyclone will help to detect lower levels, too.

The main challenge from these experiments was the impossibility of stabilizing *E. coli* DH5α cells in the bioaerosol for prolonged (> 5 min) periods.

Another interesting result from the agar plates experiments was quite heavy contamination of the air in the chamber from the spores of other microbes commonly disseminated in the chamber previously; thus (Figure 6,

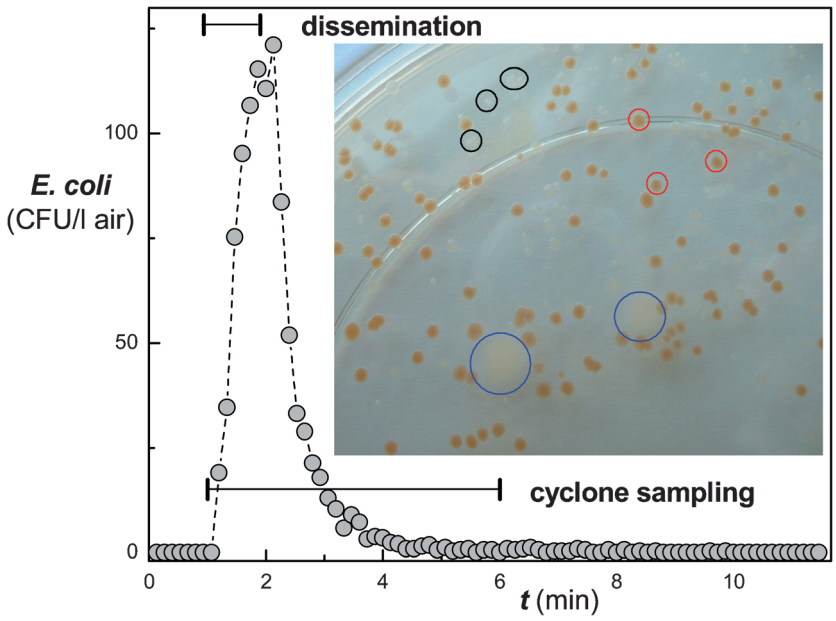

Fig. 6. Independent monitoring of viable cells in the air inside the aerosol chamber using the slit sampler with rotating agar plates. The dissemination of *E. coli* cells and cyclone sampling intervals (corresponding to Figure 5) are marked. The inset photography presents typical agar sampling plate after overnight cultivation; the black circles indicate colonies of *E. coli DH5α*, red and blue circles mark colonies of contaminating *B. globigii* and *thuringiensis*, respectively.

the inserted photography), the small (1 mm) white colonies corresponding to *E. coli* were accompanied with numerous slightly greater (2 mm) orange-yellowish colonies corresponding to *B. globigii* and large (5 mm) light-yellowish colonies of *B. thuringiensis*. Evidently, the commonly adopted cleaning and disinfection of the chamber interior and the present instrumentation was not completely effective. However, the presence of such microbial contaminants did not contributed to the low control signal of reference measurements (Figure 5, upper traces) thus indicating negligible specificity of the employed immunoreagents towards gram-positive *Bacillus* species.

4 Conclusions

The portable electrochemical immunosensor ImmunoSMART developed in laboratory was successfully combined with the commercial cyclone-based air sampler SASS 2300. This combination provided a reliable working system suitable for sampling bioaerosols in order to detect the target microbe – *E. coli* DH5α. Due to safety reasons, the dissemination of this model bioagent was realized in a closed aerosol chamber, where all parameters are properly monitored. It appeared that such microbial cells, which do not form spores, were rather difficult to detect due to very limited stability in the air, most probably limited by the environmental humidity. However, proper synchronization of the dissemination and sampling events allowed realizing the preliminary tests.

The parallel independent monitoring of microbes in air using the slit sampler and agar cultivation plates demonstrated the presence of 150 CFU/L in air (peak value) and this level was clearly indicated by the tested air sampler/immunodetector system. Thus, this level of target microbes represents the current demonstrated limit of detection, which in combination with the reasonable short time of analysis – 20 min for one cycle – seems to be promising for future extended tests in both aerosol chamber and field trial conditions.

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