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Author: György Schneider Miklós Czeller Viktor Rostás Tamás Kovács

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Highlights

- MFC-based biosensoric platform was constructed and tested for antibiogram determination.
- This method is phenotypic and faster than the traditional disk diffusion method.
- Susceptibilities of the tested bacterium strains could be revealed in 4 hours.
- Printed Circuit Board electrodes are integrated into the miniaturised cells.
- Application of PCB technology could promote the spread of MFC-based biosensors.

Microbial fuel cell-based diagnostic platform to reveal antibacterial effect of beta-lactam antibiotics

György Schneider[1,2], Miklós Czeller[2], Viktor Rostás[3], Tamás Kovács[4]

1., Department of Medical Microbiology and Immunology, University of Pécs, Hungary; 7635 Pécs, Szigeti str. 12.; gyorgy.schneider@aok.pte.hu;

2., Bioplex Research and Development Ltd., Hungary, 7635 Pécs, Rózsahegyi str 6.; czeller.miklos@gmail.com

3., R.V.M. Engineer Ltd., Hungary, 7362 Vásárosdombó, Rákóczi str. 19.; rostas.viktor@rostaskkt.hu

4., Nanophagetherapy Center, Enviroinvest Corp., Hungary, 7632 Pécs, Kertváros str. 2.; kovacst@enviroinvest.hu

Corresponding author:

Dr. György Schneider: gyorgy.schneider@pte.aok.hu, Tel.: +36303312751,

Postal address: University of Pécs, Department of Medical Microbiology and Immunology, 7624 Pécs, Szigeti str. 12.

Running title: **Microbial fuel cell-based diagnostic platform**

Abstract

Beta-lactam antibiotics comprise the largest group of antibacterial agents. Due to their bactericidal properties and limited toxicity to humans they are preferred in antimicrobial therapy. In most cases, therapy is empiric since susceptibility testing in diagnostic laboratories takes a relatively long time. This paper presents a novel platform that is based on the microbial fuel cell (MFC) technology and focuses on the early antibiogram determination of isolates against a series of beta-lactam antibiotics. An advantage of the system is that it can be integrated into traditional microbiological diagnostic laboratory procedures. Tested bacterium suspensions are uploaded into the anodic chambers of each miniaturized MFC unit integrated into a panel system, containing different antibiotic solutions. Electronic signals gained in each MFC unit are continuously monitored and are proportional to the metabolic activity of the presenting test bacterium. Using this method, antibiotic susceptibility can be evaluated in 2-4 hours after inoculation. Hereby we demonstrate the efficacy of the platform in antibiogram determination by testing the susceptibilities of *Escherichia coli* strain ATCC 25922 and *Staphylococcus aureus* strain ATCC 29213 against 10 beta-lactam antibiotics (penicillin, ampicillin, ticarcillin, cefazolin, cefuroxime, cefoperazone, cefepime, cefoxitin, cefaclor, imipenem). This paper also presents the construction of the background instrumentation and the panel system into which a printed circuit board (PCB) based electrode was integrated. Our results suggest that MFC based biosensors have the potential to be used in diagnostics for antibiogram determination.

Keywords:

55 Microbial fuel cell; biosensor; diagnostic, beta-lactam; susceptibility, printed circuit
56 board.

57

1. Introduction

The spread of antibiotic resistance is one of the most significant health care issues or our times [1]. This process is chiefly generated by inadequate antibiotic prescription habits. In most cases empiric antibiotic therapies are used due to the fact that clinicians do not wait for results from microbiological diagnostic laboratories. If time-consuming specimen processing could be reduced to a manageable level (a couple of hours), a targeted therapy could be initiated earlier, which could eventually limit, the presently used empiric therapy.

At present, the cheap and easily interpretable Kirby-Bauer disk diffusion method is used by most laboratories for revealing the susceptibility of the tested infectious agent [2, 3]. The advantage of this traditional growth-based method is that it is phenotypic nevertheless, it takes a relatively long time (24-48 hours) till the result becomes available to the clinician. Molecular based methods, such as simple and multiplex PCR, DNA sequencing, macro- and microarray are also applied in well-equipped laboratories in order to routinely detect the presence of certain resistance genes [4]. Presence of the detected gene, however, does not necessarily mean that it is also functional, as demonstrated earlier [5, 6] in the case of beta-lactams which comprise the largest group of antibacterial agents, with dozens of derivatives available for clinical use [7]. The popularity of these agents results from their bactericidal action and lack of toxicity. These drugs target and inhibit cell wall biosynthesis and since most clinically relevant bacteria have cell walls, beta-lactam agents act against a broad spectrum of gram-positive and gram-negative bacteria [7]. Many factors (species of bacteria, type and number of

penicillin binding proteins, structural characteristics, resistance mechanisms, etc.), however, contribute to a beta-lactam's spectrum, and for this reason laboratory testing should anticipate therapy in order to choose the best drug of choice. It is of vital importance since the clinician has to know whether the infection-causing agent in question is susceptible to a certain antibiotic or not. To identify this, a fast phenotypic method is needed.

Microbial fuel cells (MFCs) are electrochemical devices in which metabolic activities of the tested microorganisms can be monitored. If microbes in the anodic chamber are physiologically active, they will establish a potential difference between the anode and the cathode assuring a driving force for an electron flow. MFCs are thought to be potent devices for harvesting the energy from wastes and for this reason they can assure sustainable energy production. Only recently have they been considered as potential biosensors [8, 9, 10], where microorganisms in the anode compartment act as biocatalysts and the electrodes and proton exchange membrane serve as transducers. The signal is gained directly as a measurable electric signal. In comparison with other existing sensors (i.e. pH, temperature), MFC biosensors work as small bioreactors with high selectivity [11]. Miniaturization is critical for the development of MFCs for biosensor applications. Not only miniaturization but also the establishment of an efficient electron transfer between the bacterium and the anode is a key factor for proper functioning. This can be facilitated by different methods [12, 13].

In this paper we demonstrate the applicability of an MFC-based biosensor for fast determination of the susceptibility of two tested pathogenic bacteria against 10 different beta-lactam antimicrobial agents belonging to different classes and subclasses. Although,

miniaturized two-chamber MFC systems in µl scale range have been reported earlier [14, 15, 16], none of them applied the printed circuit board (PCB) technology for establishing a graphite electrode system. Since this technology is ideal for large scale electrode production it can promote the spread of MFC based biosensor applications in the future. The advantage of the system in the determination of the antibiotic susceptibility testing is that it is (1) phenotypic, (2) results can be interpreted within 1-4 hours after inoculation, (3) susceptibility of an infectious agent against several antibiotics can be determined parallel. These could result in an earlier targeted antibiotic therapy and reduce unnecessary antibiotic usage that is not only a waste of money, but also contributes to the spread of antibiotic resistance among bacteria.

2. Materials and methods

2.1. Platform design, production and assembly

The panel and the complete platform (Figure 1.) were designed by the 3D modeling software Solid Edge ST5 (Siemens PLM). Compartment bodies of the anodic and cathode chambers were microfabricated of plexi on the CNC routers TMV850 and EMR610. Between the two chambers activated Nafion N115 was applied as proton selective membrane and external sides of the chambers were covered by PCB (type FR4) containing the integrated electrodes (Figure 1c-d.). These special PCB-based sides, that did not only contain the electrodes but also assured connection between the chambers and the instrumental part of the platform, were designed by the Expedition Enterprise PCB design software (Mentor Graphics, USA). Production was carried out according to the

126 resulting Gerber file. The PCB was manufactured with the standard PCB manufacturing
127 process. FR4 (woven fiberglass cloth with an epoxy resin) was applied as a base material
128 for the board and copper thickness was 17.5 μm on both sides. Instrumental side of the
129 copper surface was coated with ENIG (electro less nickel immersion gold), while inner
130 sides (anodic and cathode chamber side) with graphite (Figure 3a.). Between the two
131 sides true hole VIAs assured electric connection. (Information about the manufacturing
132 process: http://www.eurocircuits.com/)
133 After production each part of the platform -except the Nafion membrane- containing the
134 miniaturized MFC units were washed in acetone for 15 minutes, in sterile distilled water
135 for three times, once in 96 % ethanol for 15 minutes, air dried at 75°C for 15 minutes and
136 assembled by sandwiching them (Figure 1c-d.) under a laminar flow box to assure
137 germfree conditions. After construction, cathode chambers were filled up with 0.02 M
138 potassium hexacyanoferrate (III) solution, while the anodic chambers with 200 μl-s of 0.1
139 M phosphate puffer-based medium containing the proper antibiotic with more than two-
140 fold higher concentration than the established minimal bactericidal concentration. Filled
141 up panels were closed with caps and stored at 4°C-s till usage.
142

143 **2.2. Bacterial strains, growth media, and antibiotic solutions**

144 Throughout the study two quality control strains were used: *Staphylococcus aureus*
145 ATCC 29213 and *Escherichia coli* ATCC 25922. Both strains were directly purchased
146 from the American Type Culture Collection (ATCC, USA). They were routinely grown
147 on cathion adjusted Mueller-Hinton II medium (Beckton-Dickinson, USA) with or
148 without Agar (Oxoid, USA). To inoculate the MFC based platforms, direct colony

suspensions were used and administered to the equivalent of a 0.5 McFarland standard. This and all subsequent solutions were made up of 0.1 M phosphate buffer (PBS: 0.05 M Na_2HPO_4 and 0.05 M NaH_2PO_4), pH 7.0. Anodic chamber buffer contained 0.5 M glucose and 0.1 mM methylene blue. Cathode chambers were filled up with 0.02 M potassium hexacyanoferrate (III) solution. Each antibiotic (Sigma, Hungary) was administered to the 10 ml aliquots of PBS from its stock solution before usage as listed below (solvent):

100 mg/ml for penicillin G (water); 100 mg/ml for ampicillin (water); 200 mg/ml for ticarcillin (water); 1 mg/ml for cefazolin (water); 1mg/ml for cefuroxime (10% methanol); 1 mg/ml cefoperazone (water); 1mg/ml for cefepime (water); 100 mg/ml for cefoxitin (water); 1mg/ml for cefaclor (methanol); 1mg/ml for imipenem (water).

2.3. Antibiotic susceptibility testing by disk diffusion, MIC and MBC determination

Instructions of the Clinical and Laboratory Standards Institute [1] served as guidance for traditional antibiotic determination experiments (Table 1). Briefly: midlog culture of the bacterium was centrifuged and re-suspended in PBS. Its optical density was adjusted to the equivalent of a 0.5 McFarland (OD_{600} 0.2), and 100 µls were dispensed on a Mueller-Hinton II agar medium. Filter-paper disks impregnated with the proper antibiotics (Table 1.) (BioRad, USA) were placed on the fresh culture and were let grow for 20-24 hours at 37°C. Susceptibilities were evaluated according to the size of the clearing zones as resistant (R), moderately susceptible (M) and sensitive (S).

In order to control the antibiotic solutions applied in the anodic chambers of the MFC system, the minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations

were evaluated by applying the microplate dilution method in 100 µl end volumes. Dilutions were carried out in Mueller-Hinton II medium and simultaneously in glucose containing (0.5 M) PBS solution. 200 µls of antibiotic stock solutions were mixed in the first tube and a two-step serial dilution was carried out. After dilution, 100 µl bacterium suspension (0.5 McFarland) was added to each test-tube and incubated for 24 hours at 37°C. Breakpoints for MIC were determined visually. For MBC determination, 100 µls of the suspensions were spread on the surface of blood agar plates, and after an overnight (ON) incubation those minimal antibiotic concentrations were accepted to be bactericidal where no living bacterium cells were visually detected.

2.4. Susceptibility testing by MFC based platform

Each panel of the platform contained twelve miniaturized MFC-based sensor units. Ten of them contained different antibiotics in different concentrations (Table 1.), while the remaining two units did not contain any antimicrobial agents. Anodic chambers were inoculated with 50 µl bacterium suspension (0.5 McFarland) by using a multichannel micropipette. The last chamber was left uninoculated and served as a negative control. After inoculation, the anodic chambers of the panel were closed by using a cover strip. After sealing the panel, it was inserted into one of the 10 slots of the background instrument and fastened. By locking the door, the panel was fixed in the tempered interior of the instrument and measuring spring-pins assuring electric connection between the chambers and the electric background were mechanically pressed against the outer golden surface of the PCB. After starting, measurements can be tracked with the help of an application based on the software Microsoft Visual Studio.

In the chambers, elevated MBC-s were applied that were 3 to 5 times more than the highest CLSI MIC values (Table 1.).

2.5. Data acquisition (DAQ) system

The system contained four DAQ boards and an integrated personal computer (PC) background. A four-stage signal conditioning was carried out during the measurements (Figure 3.). At the first stage, a voltage follower circuit was applied with high input impedance (JFET input, 10^{12} Ohm input resistance), while a buffer circuit for ADC (analogous to digital converter) was integrated in the second stage. The 16 Bit analog-to-digital converter (ADC) was installed at the 3rd stage. After conversion, data were processed by the Field-Programmable Gate Array (FPGA) in stage 4. The communication and other tasks (temperature control, fixture control) were also controlled by the FPGA. A simple USB communication between the PC and the DAQ boards assured a connection. Each input module contained a separate resistor. Their role was to ensure short circuits between the anodic and cathode chambers, thus they helped detect the metabolic activity of presenting bacteria. All functions were controlled by the PC module. The application allowed the user to collect and illustrate the measured data.

Signal processing in the digital part was carried out by using FPGA. A relatively big averaging - 1024 or bigger - was used for good signal/noise ratio.

2.6. Measurements and calculations

The current generated by each individual miniaturized MFC unit was monitored real time. For this purpose 128 external resistors (R) were inserted into the electric circuits

between the electrodes of each MFC unit. Potential drop (V) across the resistor was recorded every 10 seconds.

3. Results and discussion

3.1. Design, construction of the MFC based platform

The biosensor platform used in this study consisted of (1) a microfabricated panel system, and (2) an instrumental background (Figure 1.). The panel system was based on a two-chamber microbial fuel cell (MFC). The metabolic activity of the presenting bacteria was monitored in the anodic chamber. Incubation occurred in the tempered (37°C) part of the background instrument during measurements, since the system was planned to monitor metabolic activities of human pathogenic bacteria. This part of the instrument also contained all the mechanics which assure a physical connection between the MFC units and the DAQ system. DAQ system itself was situated in a non-tempered part and due to practical considerations it was divided into four major levels (Figure 3.). Measuring capacity of the system was 128, which means that this version could monitor 128 MFC units parallel. Parallel evaluation of 10 panels (120 individual MFC units) could be carried out.

3.2. Electrode construction

Requirements which had to be fulfilled during electrode design were the effective electron transfer between the bacterium cell and the electrode surface as well as a stable and flexible contact between the MFC units and the DAQ system.

Most reported µl-scale MFCs employed gold as an anode material, although they were prone to high internal resistances, relatively lower power densities and high fabrication costs [16]. Since traditional carbon electrodes are bulky for sub-100 µl applications and thin carbon cloth electrodes are difficult to handle in large scale [16], we have applied PCB based carbon electrode family manufactured by the standard PCB manufacturing process. FR4 was chosen as board material since it is non-toxic, non-flammable and cheap. Due to its good conductivity, copper was applied for coating, but since it is toxic to bacteria it was layered with graphite on the inner sides (Figure 3a.).

Establishment of an electron transfer from the microbial cell to the electrode is a basic requirement for the proper functioning of the MFC system. Electrons can only be transferred from the cell membrane either *via* the physical transfer of reduced compounds (mediators), or *via* membrane bound redox enzymes. Regardless of the mechanism, the electron transfer outside the cell must lead to redox active species that is capable of electronically linking the bacterial cell to the electrode [17]. This electric activity was also reported to clinically important bacterium species, like *Escherichia coli* [18], *Proteus vulgaris* [19], *Klebsiella pneumoniae* [20], *Staphylococcus aureus* [21], *Pseudomonas aeruginosa* [22] etc., however, an effective electron transfer between the bacterial cell and anode could only be detected in the presence of a proper mediator.

In our system we applied methylene blue, which was formerly reported to be effective at different Gram- and Gram+ pathogenic bacteria. The two strains tested were

internationally accepted reference strains for antibiotic resistance susceptibility tests [23] and produced around 20 mVs if the mediator was present in the system. No significant electrochemical activities were detected in a short period of time (in 2-4 hours) without adding the mediator.

Although Zhang et al. [24] demonstrated that a Darwinian type evolution can occur in fuel cell environments, however such a "trained" *E. coli* culture was able to reach only one tenth of that power output that could be reached in the presence of a proper redox mediator [25]. Recent trials to enhance electron transfer by modifying the surface of carbon based electrodes have been only partially successful [12, 13, 26], and currently, it seems that the use of effective mediators have to be considered in such MFC based biosensor applications where basic requirements are (i) the monitor of metabolic activities of different species and (ii) rapidity.

3.3. Comparison of antibiotic susceptibility testing by disk diffusion and MFC based biosensor

In order to evaluate the MFC based measurements antibiotic susceptibility tests were carried out with the traditional phenotypic Kirby-Bauer disk diffusion method [2, 3] parallel to MFC measurements, which is the golden standard of growth-based methods for antibiogram determination. Both *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 strains were used as test organisms that are official reference strains for antibiotic resistance evaluations [1].

Setting the initial bacterium suspensions of both reference strains onto 0.5 McFarland (OD_{600}=0.2), which is the usual starting suspension for the Kirby-Bauer method and

equals with $2x10^7$ CFU/ml, assured synchronization before starting the measurements with the two methods. Since 50 µls were administered to the MFC units from this suspension, the starting colony forming units (CFUs) in the anodic chambers (250 µl volume) were $1x10^6$ ($4x10^6$ CFU/ml). Without any antibiotic pressure (positive control) the metabolic activities of *S. aureus* and *E. coli* generated maximum 15-20 mVs in 1-4 hours (Figure 4.). If the initial CFU was only one tenth ($2x10^6$) of this CFU the maximum voltage was reached slowly (Figure 4.).

In the negative control units of the MFC system, where no bacteria and no antibiotics were present, only the open circuit voltage (≈20 mV) could be detected presented by a flat signal on the monitor.

A similar flat signal (no metabolic activity) characterized those antibiotic containing units of the MFC panel in which an effective antimicrobial agent was present. In our experimental setup, with one exception (penicillinG, *E. coli*) this flat signal was observed since most investigated beta-lactams were antibacterial on the two tested bacterium strains. Results were confirmed by the Kirby-Bauer disk diffusion method in all cases, where definite clearing zones around the disks impregnated with efficient antibiotics could be observed on the surface of inoculated agar plates (Figure 4.). Although both results indicated antibacterial effects, it is important to highlight that the disk diffusion method could not differentiate between bactericidal and bacteriostatic effects. Non-proliferating, or suppressed but living bacteria can be present around the disk if the antibacterial agent is only bacteriostatic. In contrast, the flat signal in the MFC system is a clear indication of the tested antimicrobial agent being bactericidal, since due to the lack of metabolic activity no signal is generated. This indicates the limitation of the

MFC-based platform, by which antibacterial effect could only be revealed if the tested agents in the anodic chambers were bactericidal. Beta-lactams are a typical group of antibiotics possessing this property. Based on the above, the MFC system can be adequate to test resistances against aminoglycosides, fluoroquinolones, nitrofurantoins etc. that are also considered to be typically bactericidal.

Applied antibiotic concentrations (Table 1.) were at least three times higher than their MIC values against the two tested bacteria [1]. Tests were carried out in Mueller Hinton II or in PBS, these concentrations proved to be bactericidal as demonstrated by the microdilution method (data not shown). In every case the parallel Kirby-Bauer disk diffusion method confirmed the antibiogram evaluation of the MFC based method, while in the first case, results could be evaluated only after 24 hours using the MFC-based method results were already available after 3-4 hours.

Number of bacteria in the anodic chamber is critical for the proper function of the system. Best results can be observed if bacterial cell numbers are minimum 10^6 CFU/ml in the anodic chamber, when bacteria are fairly active establishing anaerobic conditions in the chambers. This is a basic requirement for proper MFC function [27]. As a result of this lag phase, onset of measurable voltage generation takes nearly one hour after inoculation. Quian et al. [15] in their µl-scale system did not observe any significant lag period in voltage generation which is typically reported for macroscopic MFCs and also characteristic of our system. A possible explanation is that they applied bacterial cultures at an optical density of 0.4-0.6 (measured at 600 nm) for inoculation and disclosed any contact with air from their system and also from the sample upload step. Since we could not disclose this latter from our system, the above described nearly one hour

acclimatization phase could be detected in each measurement. Shortening the time of this lag phase, however, would be ideal if time pressure demand of diagnostics is taken into consideration.

The applied relatively high cell numbers (10^6 CFU/ml) assured the unambiguous evaluation of the metabolic activities already in 2 hours. If, however, the starting CFU of the tested microorganism in the anodic chamber was set onto $1/10^{th}$ of this cell number (10^5 CFU/ml), a longer lag phase and/or a declivous logarithmic phase extended the time of detection (Figure 4.).

3.4. Data acquisition

Practically, the signal can be presented directly as a measurable electric signal. In comparison with other existing sensors (i.e. pH, temperature), MFC biosensors work as small bioreactors with high selectivity [11]. In the platform we have developed, this selectivity is based on two pillars: one is the tested bacterium strain itself, which grows if there is no hindering compound present in the anodic chamber. On the other hand, if an efficient antibiotic is present, metabolic activity of the isolate will stop and no electric signal will be generated. Since the biological signal is not suitable for direct measurements, some analog signals and conditioning are necessary. The sampling frequency was in the KHz range. This was unavoidable for proper signal evaluation, since a relatively big averaging -1024 or larger- was necessary for a good signal/noise ratio. Without averaging only ≈10 bit is effective in +/-10 input range (resolution: 20 mV) but with the applied averaging 15 bit could be reached (resolution: 0.6mV). Other filtering for interpretable signal processing was not necessary in this set-up.

4. Conclusions

We have demonstrated the applicability of MFC-based biosensors in antibiotic susceptibility testing. The application of mediators is required since, up till now, it has assured the most reliable electron transfer between different microbes and electrodes, which is a basic requirement in this system. Printed circuit board technology provides an appropriate technology for electrode construction in miniaturized MFC systems. The application of this technology could contribute to the spread of MFC based biosensors.

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The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table:

Table 1.: Official Minimal Inhibitory Concentration (MIC) ranges of the tested beta-lactam antibiotics and their applied test-concentrations. Beta-lactams involved into the study are represented in column 1. CLSI based MICs (μg/ml) of each antibiotic for *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 are shown in columns 2 and 3, respectively, while their applied concentrations (μg/ml) in the MFC system and in the control disk diffusion tests are represented in columns 4 an 5.

Antimicrobial agent (class/subclass)	Official *Staphylococcus aureus* ATCC 29213 MIC values	Official *Escherichia coli* ATCC 25922 MIC values	Applied test concentrations	
			MFC system	Disc diffusion
Penicillin (penicillins/penicillin)	**0.25-2**	**-**	**10**	**10 IU/6**
Ampicillin (penicillins/aminopenicillin)	**0.5-2**	**2-8**	**40**	**25**
Ticarcillin (penicillins/carboxypenicillins)	**2-8**	**4-16**	**75**	**75**
Cefazolin (cephems/cephalosporin I)	**0.25-1**	**1-4**	**20**	**30**
Cefuroxime (cephems/cephalosporin II)	**0.5-2**	**2-8**	**40**	**30**
Cefoperazone (cephems/cephalosporin III)	**1-4**	**0.12-0.5**	**20**	**30**
Cefepime (cephems/cephalosporin IV)	**1-4**	**0.015-0.12**	**10**	**30**
Cefoxitin (cephems/cephamycin)	**1-4**	**2-8**	**40**	**30**
Cefaclor (cephems/cephalosporin)	**1-4**	**1-4**	**20**	**30**
Imipenem (penems/carbapenem)	**0.015-0.06**	**0.06-0.25**	**1.25**	**10**

Figure legends:

Fig. 1. Illustration the buildup of the MFC-based biosensor platform. The platform (a.) used in this study is based on a panel system in which upload of bacterial samples can be carried out by using a simple multichannel pipette (b.). During assembly, each part of the panel system is sandwiched (c.). Anodes and cathodes of the µl-scale MFCs are integrated into a PCB base, which also underlie the sides of the panel and involve the outer contact points for the instrument (d.).

Fig. 2. DAQ flow of the constructed instrumental platform.

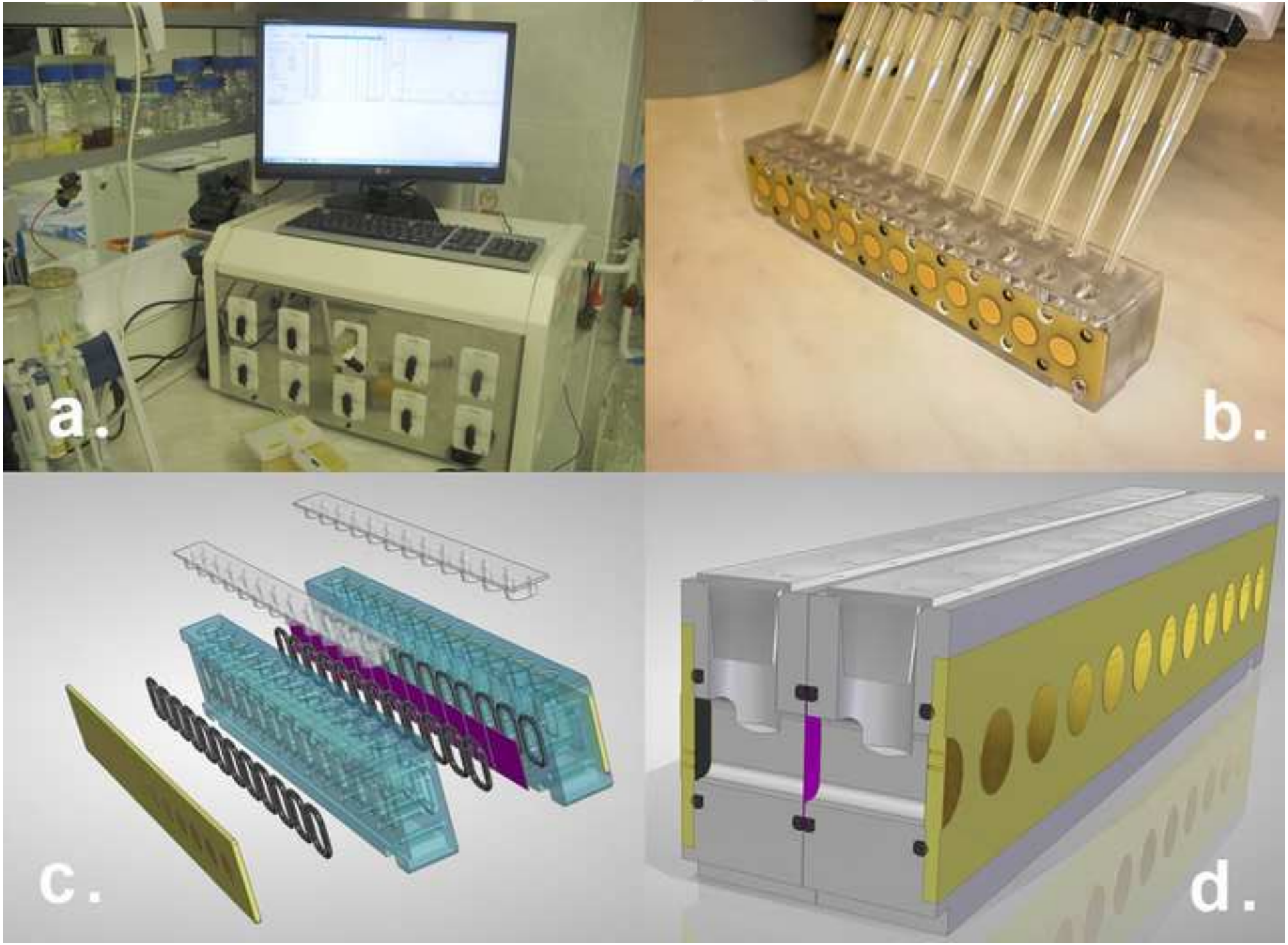
Fig. 3. Longitudinal section of the PCB-based electrode (a.) and schematic view of its internal side (b.).

Fig. 4. Comparison of the results of the MFC-based biosensor and the traditional Kirby-Bauer disk diffusion method. Results are presented in two columns: *S. aureus* ATCC 29213 in column **A.**, while *E. coli* ATCC 25922 in column **B.** Left side of each column shows results of the MFC experiments while right sides the disk diffusions'. Upper two rows (r) of both columns demonstrate the CFU detection limit of the system. 10^6 /ml CFUs were applied (**1st row**) for our experiments since lower initial cell number

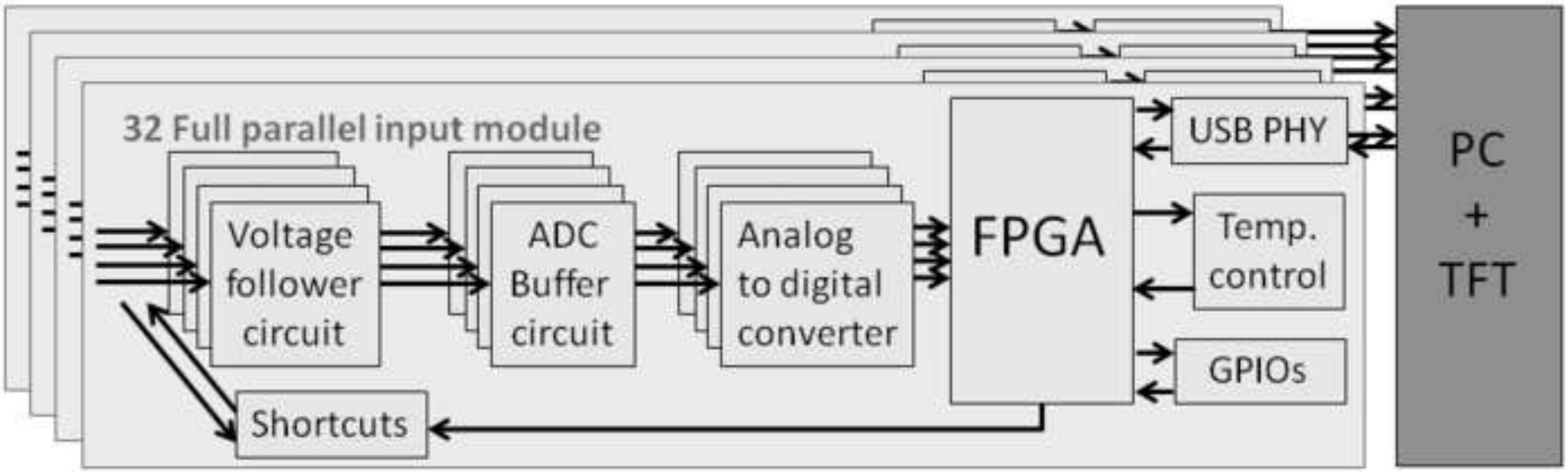
476 (10^5 /ml) in the anodic chamber resulted in a drawling curve characteristic and therefore,
477 a more uncertain detection (**2nd row**). In subsequent rows (**rows 3 to 12**) results are
478 shown in the presence of different beta-lactam antibiotics. Flat signal in the MFC detects
479 antibacterial effect and therefore, corresponds to the presence of inhibition zone in the
480 Kirby-Bauer disk diffusion method. (Used antibiotics: r3: penicillinG; r4: ampicillin; r5:
481 ticarcillin; r6: cefazolin; r7: cefuroxime; r8: cefoperazone; r9: cefepime; r10: cefoxitin;
482 r11: cefaclor; r12: imipenem.)
483

Figure(s)

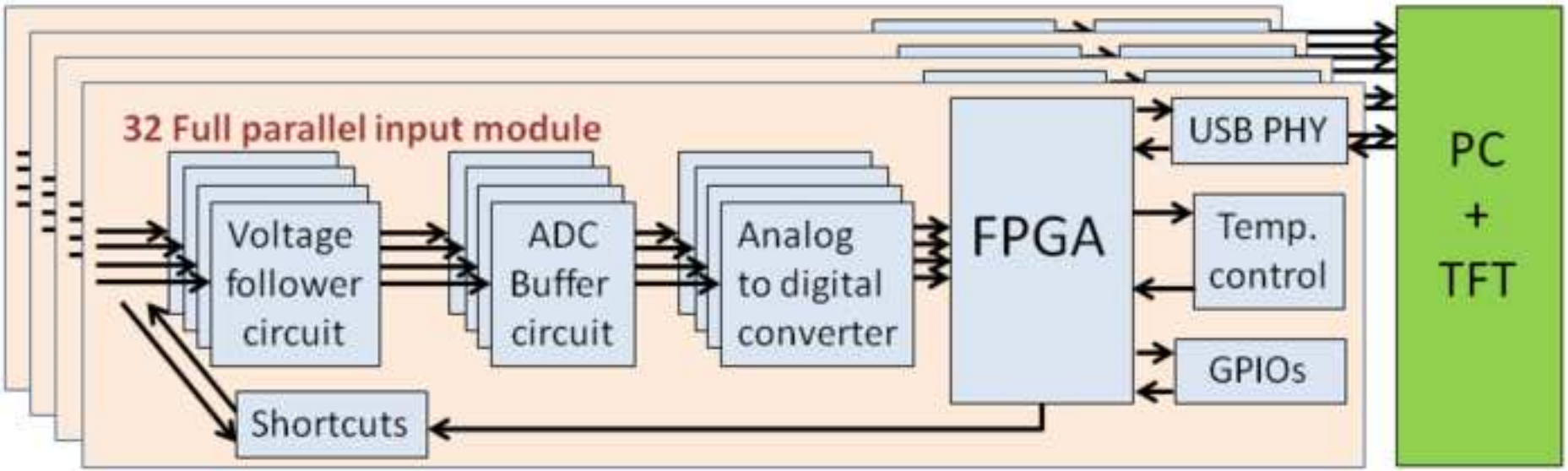
Figure(s)



Figure(s)


32 Full parallel input module
Voltage follower circuit
ADC Buffer circuit
Analog to digital converter
FPGA
USB PHY
Temp. control
GPIOs
Shortcuts
PC + TFT

Figure(s)


32 Full parallel input module
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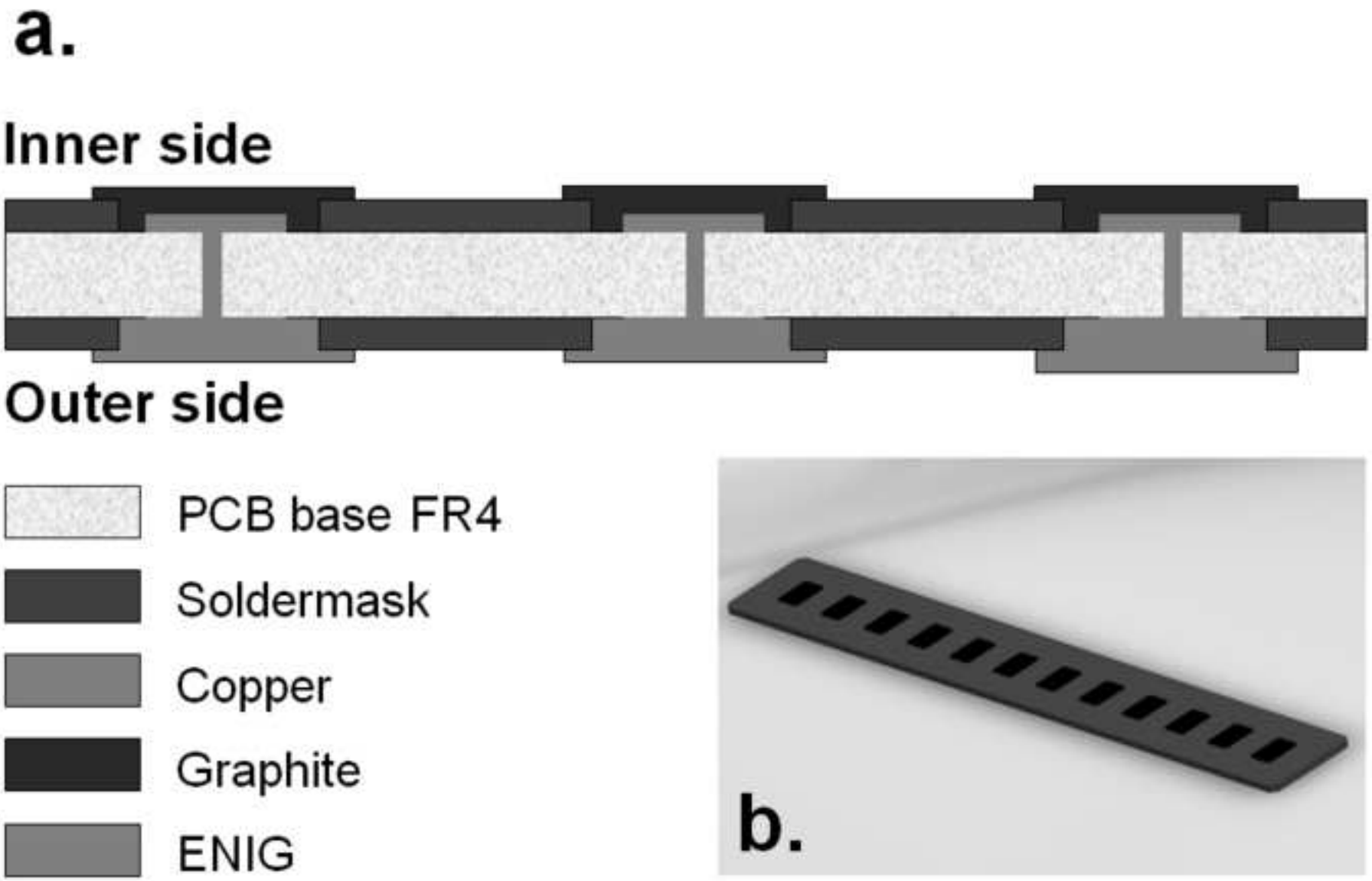
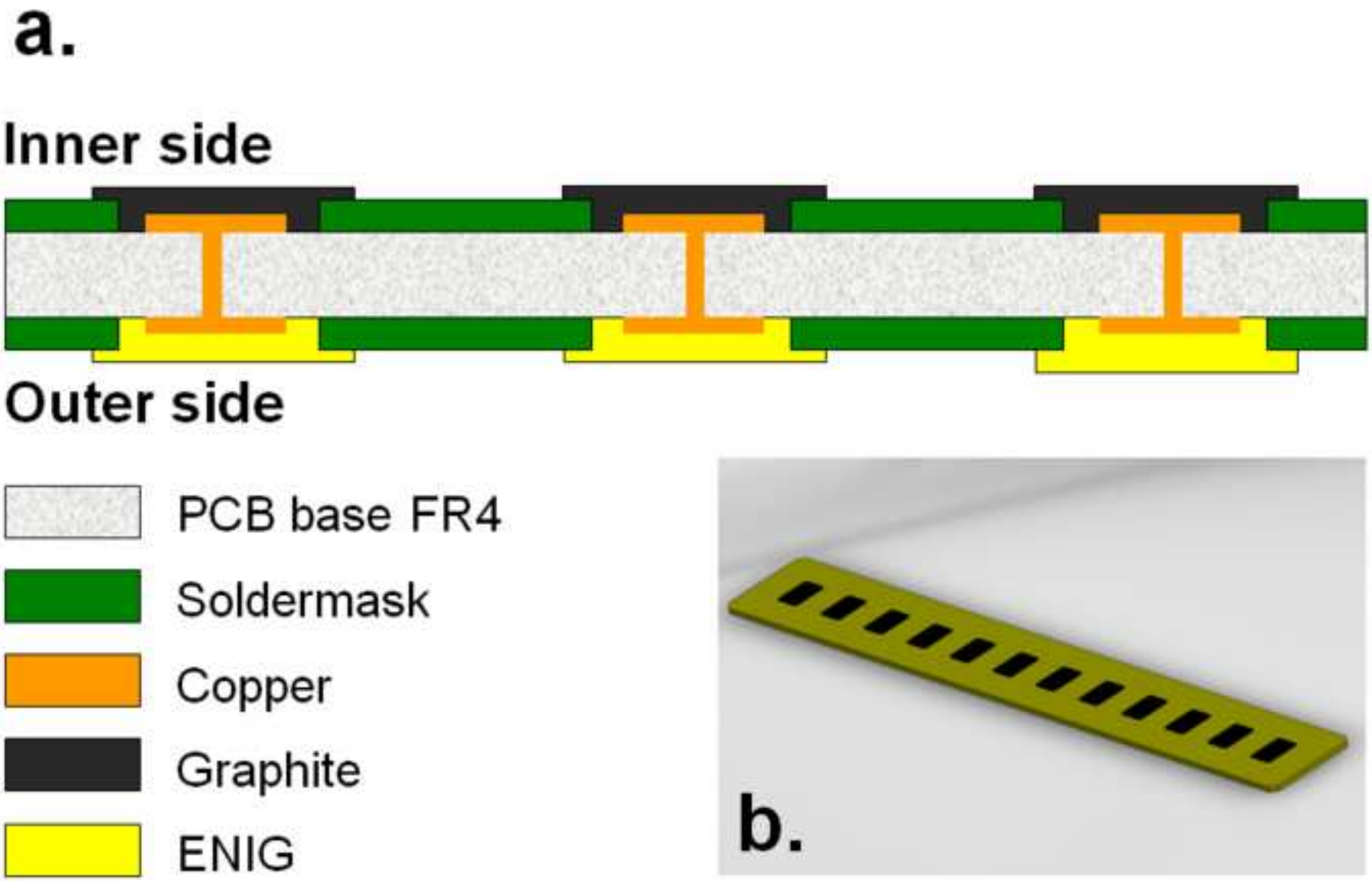
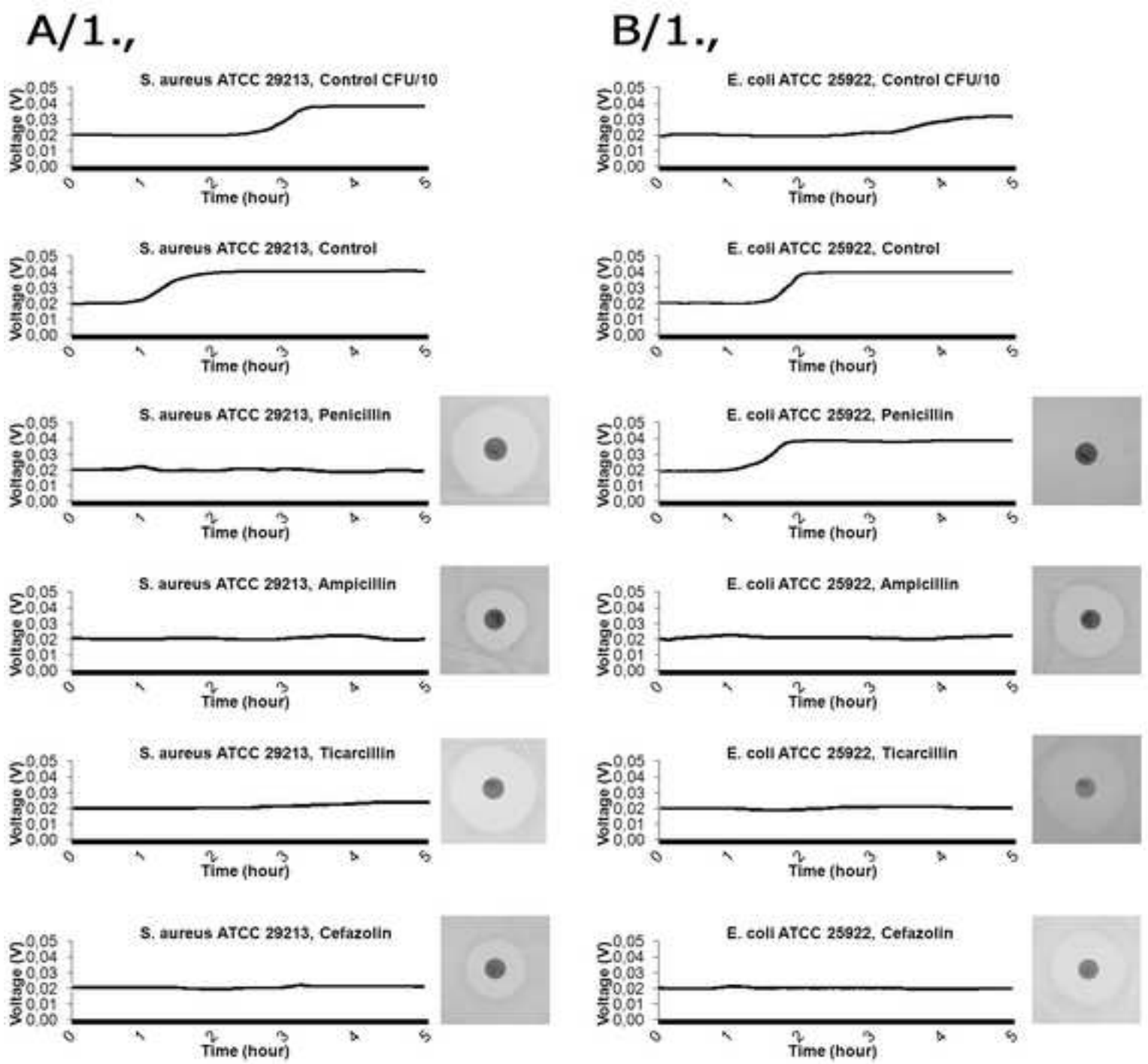

a.
Inner side
Outer side
PCB base FR4
Soldermask
Copper
Graphite
ENIG
b.

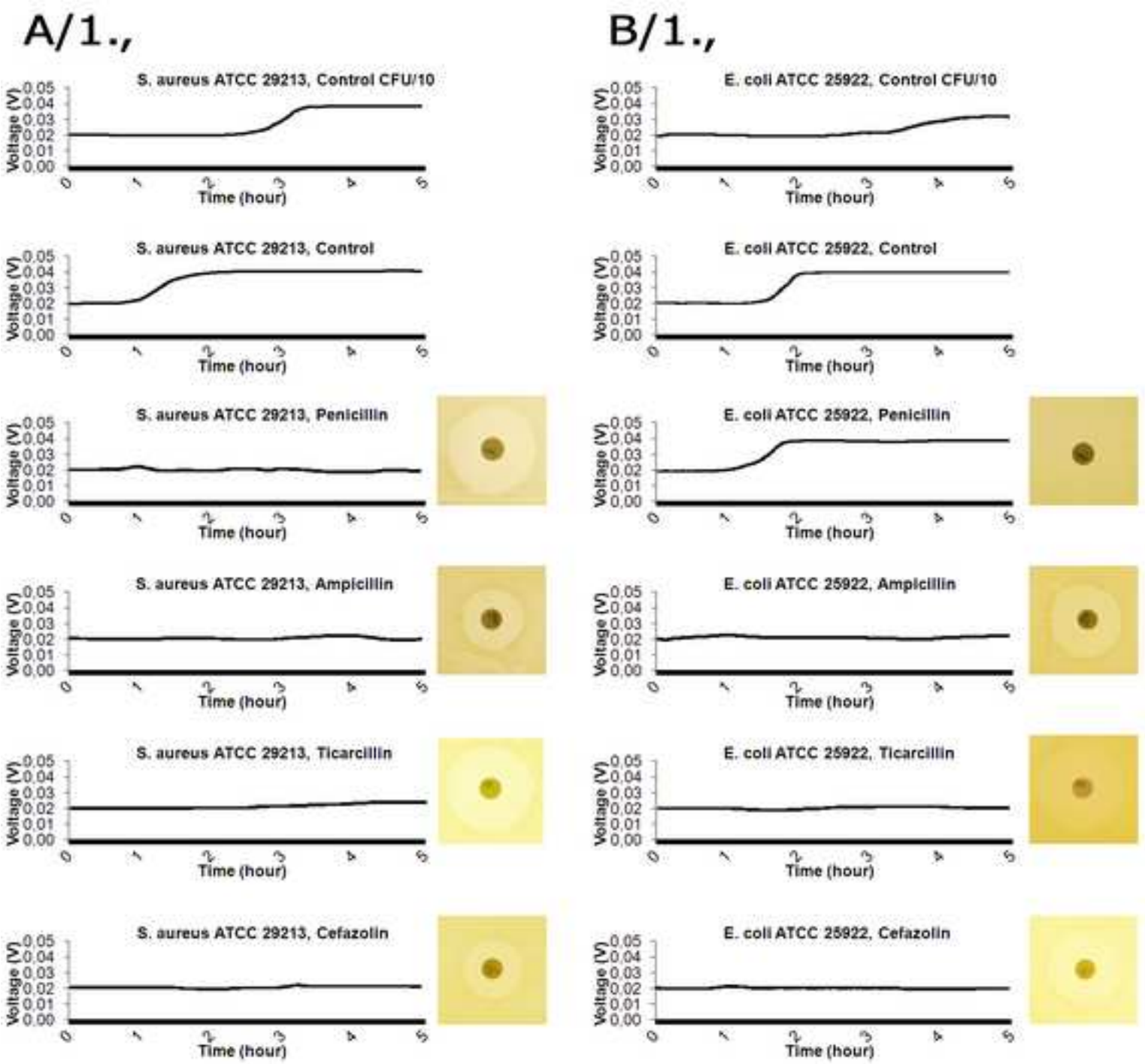
Figure(s)


a.
Inner side
Outer side
PCB base FR4
Soldermask
Copper
Graphite
ENIG
b.

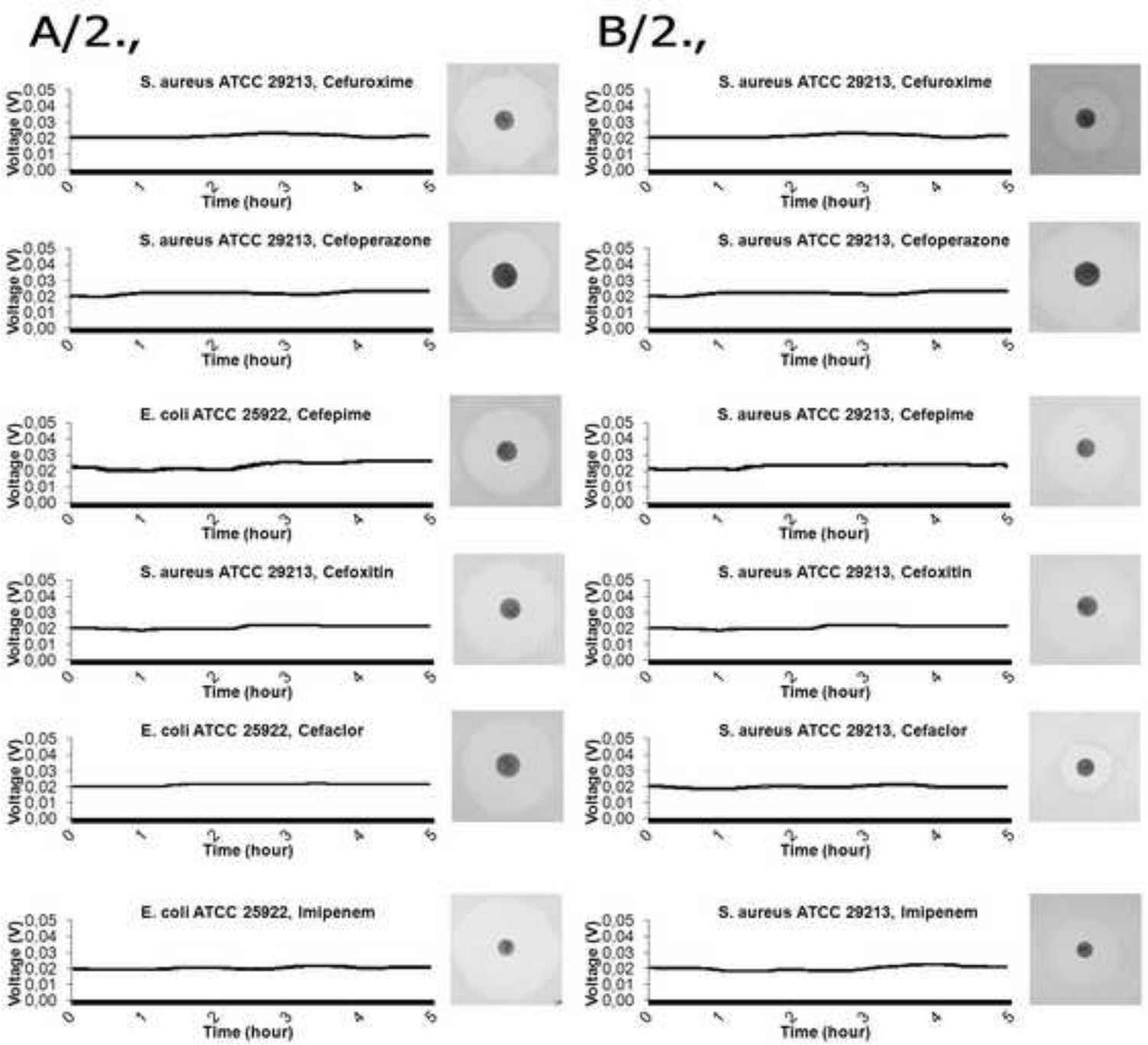
Figure(s)


A/1.,
B/1.,
S. aureus ATCC 29213, Control CFU/10
E. coli ATCC 25922, Control CFU/10
S. aureus ATCC 29213, Control
E. coli ATCC 25922, Control
S. aureus ATCC 29213, Penicillin
E. coli ATCC 25922, Penicillin
S. aureus ATCC 29213, Ampicillin
E. coli ATCC 25922, Ampicillin
S. aureus ATCC 29213, Ticarcillin
E. coli ATCC 25922, Ticarcillin
S. aureus ATCC 29213, Cefazolin
E. coli ATCC 25922, Cefazolin
Voltage (V)
0,05
0,04
0,03
0,02
0,01
0,00
0
1
2
3
4
5
Time (hour)

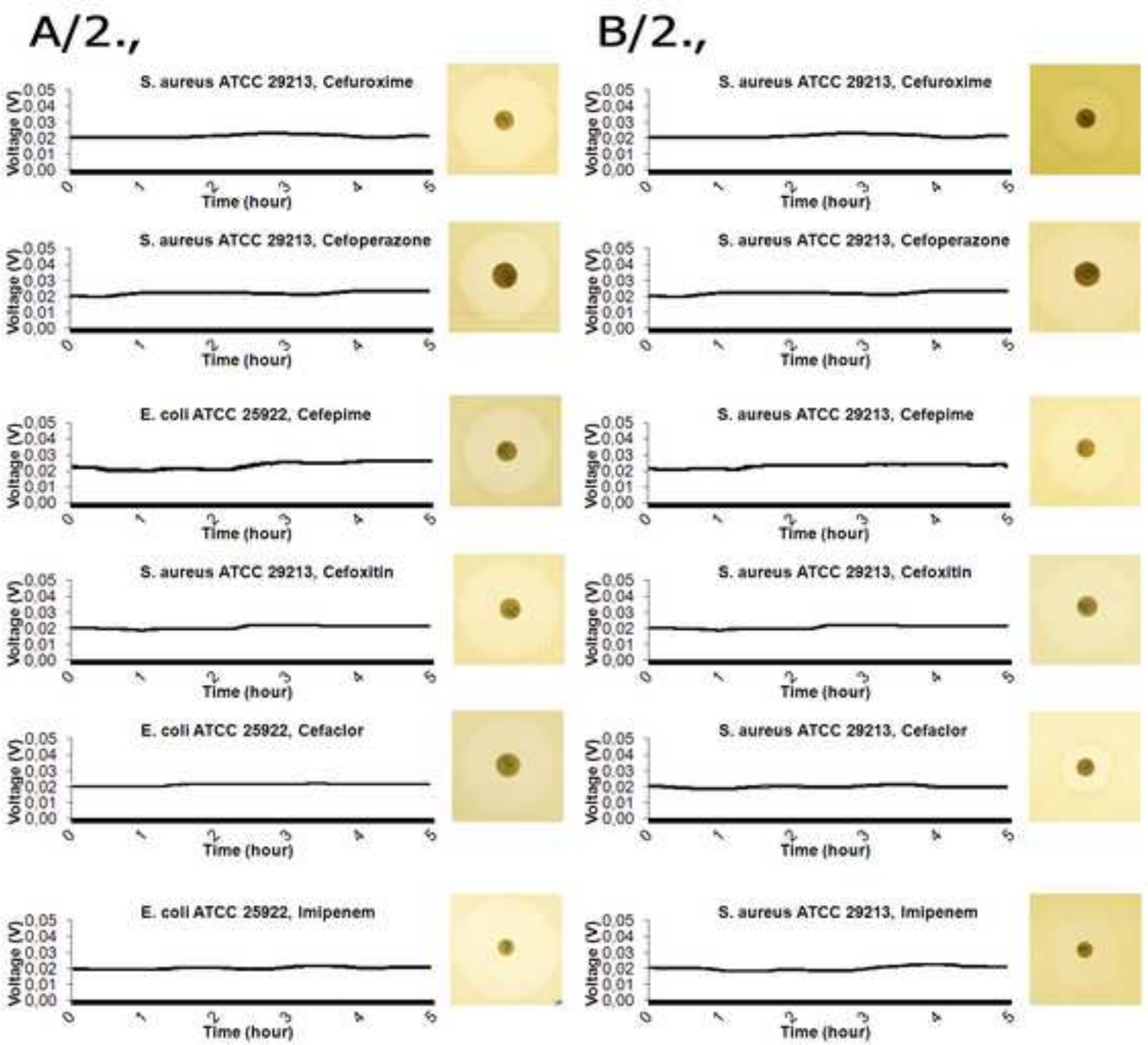
Figure(s)


A/1.,
B/1.,
S. aureus ATCC 29213, Control CFU/10
E. coli ATCC 25922, Control CFU/10
S. aureus ATCC 29213, Control
E. coli ATCC 25922, Control
S. aureus ATCC 29213, Penicillin
E. coli ATCC 25922, Penicillin
S. aureus ATCC 29213, Ampicillin
E. coli ATCC 25922, Ampicillin
S. aureus ATCC 29213, Ticarcillin
E. coli ATCC 25922, Ticarcillin
S. aureus ATCC 29213, Cefazolin
E. coli ATCC 25922, Cefazolin
Voltage (V)
0,05
0,04
0,03
0,02
0,01
0,00
0
1
2
3
4
5
Time (hour)

Figure(s)


A/2.,
B/2.,
S. aureus ATCC 29213, Cefuroxime
S. aureus ATCC 29213, Cefoperazone
E. coli ATCC 25922, Cefepime
S. aureus ATCC 29213, Cefepime
S. aureus ATCC 29213, Cefoxitin
E. coli ATCC 25922, Cefaclor
S. aureus ATCC 29213, Cefaclor
E. coli ATCC 25922, Imipenem
S. aureus ATCC 29213, Imipenem
Voltage (V)
Time (hour)

Figure(s)


A/2.,
B/2.,
S. aureus ATCC 29213, Cefuroxime
S. aureus ATCC 29213, Cefoperazone
E. coli ATCC 25922, Cefepime
S. aureus ATCC 29213, Cefepime
S. aureus ATCC 29213, Cefoxitin
E. coli ATCC 25922, Cefaclor
S. aureus ATCC 29213, Cefaclor
E. coli ATCC 25922, Imipenem
S. aureus ATCC 29213, Imipenem
Voltage (V)
Time (hour)