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# Biosensors and Bioelectronics

## Electrochemical label-free pathogen identification for bloodstream infections diagnosis: towards a machine learning based smart blood culture bottle.

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<b>Abstract:</b>	<p>Bloodstream infections are a growing public health concern. Current pathogen identification systems are based on complex and expensive devices, intended for use in centralized laboratories. Subsequent identification requires harmful chemical reagents, specialized personnel and time. Here we describe a new approach for rapid and decentralized diagnosis of positive blood cultures using electrochemical sensors. By implementing a multi-material potentiometric platform in a blood culture bottle, we have developed a portable system for pathogen identification of Gram and of genus. Bacterial growth in human blood generates a specific label-free multiplex electrochemical fingerprint according to the detected species. Analysis of these fingerprints using homemade machine learning algorithms allow for rapid identification of the pathogen after detection (14 species and 9 genus) (GRAM= 99% accuracy 5.75 hours after detection, GENUS= 85% accuracy 7.8 hours after positivity) without further handling of the contaminated sample.</p>

## Full Paper

### **Electrochemical label-free pathogen identification for bloodstream infections diagnosis: towards a machine learning based smart blood culture bottle.**

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**Keywords:** blood culture, electrochemical fingerprint, machine learning, multi-material electrode array

#### **Abstract**

Bloodstream infections are a growing public health concern. Current pathogen identification systems are based on complex and expensive devices, intended for use in centralized laboratories. Subsequent identification requires harmful chemical reagents, specialized personnel and time. Here we describe a new approach for rapid and decentralized diagnosis of positive blood cultures using electrochemical sensors. By implementing a multi-material potentiometric platform in a blood culture bottle, we have developed a portable system for pathogen identification of Gram and of genus. Bacterial growth in human blood generates a

specific label-free multiplex electrochemical fingerprint according to the detected species. Analysis of these fingerprints using homemade machine learning algorithms allow for rapid identification of the pathogen after detection (14 species and 9 genus) (GRAM= 99% accuracy 5.75 hours after detection, GENUS= 85 % accuracy 7.8 hours after positivity) without further handling of the contaminated sample.

## **1. Introduction**

Bloodstream infections (BSIs) are a global health problem: it is estimated that there are more than 30 million episodes of BSI per year worldwide, resulting in 6 million deaths.<sup>[1,2]</sup> Untreated, bacteremia can lead to sepsis. According to the World Health Organization, sepsis are responsible for almost 20% of all global death.<sup>[3]</sup> Even if an infection is detected, the appropriate antibiotic needs to be administered. Inappropriate treatment could lead to the death of the patient<sup>[4-6]</sup> and is the cause of antibiotic resistance which is “one of the biggest threats to global health, food security, and development today”.<sup>[7]</sup> A recent study reports more than 1.27 million deaths directly caused by antimicrobial resistance in 2019; this number could rise to 10 million each year by 2050,<sup>[8]</sup> more than cancer nowadays. Therefore, fast detection of BSI and identification of the pathogen are crucial. Reducing the time without appropriate antibiotics allows the best survival rate and decreases antibiotic resistance appearances. Today, two major research axes aim at providing a faster diagnosis of infections: more sensitive technologies, allowing the detection and characterization of always smaller biomasses, down to single-cell,<sup>[9,10]</sup> and point-of-care diagnostics, which greatly helps in sparing time, for instance by using the transport time to start remote incubation and analysis. This latter option is the strategy we present and discuss, in this article, through the development of an instrumented blood culture bottle.

Currently, blood culture monitoring can be performed through the detection of carbon dioxide using different transduction methods such as radiometric respirometry,<sup>[11,12]</sup> fluorescence,<sup>[13,14]</sup> infrared absorbance<sup>[15-18]</sup> or colorimetry.<sup>[19]</sup> Manometric monitoring of the bottle headspace pressure is also currently implemented, yielding sensitive sterility testing.<sup>[20,21]</sup> These methods are based on the monitoring, through diverse transduction techniques, of volatile metabolites during culture, either in a solid phase in contact to water, or in the bottle headspace. As blood culture is actually a sterility test, the targeted metabolites must provide a generic detection, without *a priori*. Carbon dioxide fulfils this requirement but is poorly volatile, as it is predominantly dissolved in the liquid phase in its anionic form in the culture conditions. This strongly slows down the phase transfer between water phase and the sensed phase. Thereby, other approaches have been proposed for a gas-phase detection of microorganisms, relying on much more hydrophobic and volatile metabolites, either endogenous<sup>[22]</sup> or enzyme-generated.<sup>[23]</sup>

Due to its generic nature, CO<sub>2</sub> provides only sterility testing without identification or orientation. Complete diagnostic imposes further isolation process through culture on agar medium followed by the identification step. One time-saving strategy is to merge the two steps of detection and identification. This can be done, through the non-invasive acquisition, all along the blood culture, of the gas fingerprint characteristic of the bacteria metabolome. This can be performed at laboratory scale using gas-chromatography coupled to mass spectrometry.<sup>[24]</sup> The development of cheaper and simpler ways of collecting and characterizing the volatile fingerprint have led to the development of colorimetric artificial noses, such as the colorimetric sensor array, that are deployable out of lab. This artificial nose, inserted in the cap of the blood culture bottle (BCB), is made of an array of specific chromophores reacting with a fraction of the microbial volatile organic compounds (VOC) emitted during blood culture,<sup>[25]</sup> yielding a colorimetric fingerprint specific to pathogen species. However, this analysis requires the phase

transfer of the VOC from aqueous to gas phase. This transfer has limitations, i. transduction delay due to the equilibration time between gas and liquid phases and ii. dilution effects of the VOCs between the two phases. Thus, a similar concept has recently been related, for the analysis in liquid phase that relies on an antibody microarray coupled to Surface Plasmon Resonance imager (SPRi).<sup>[26]</sup>

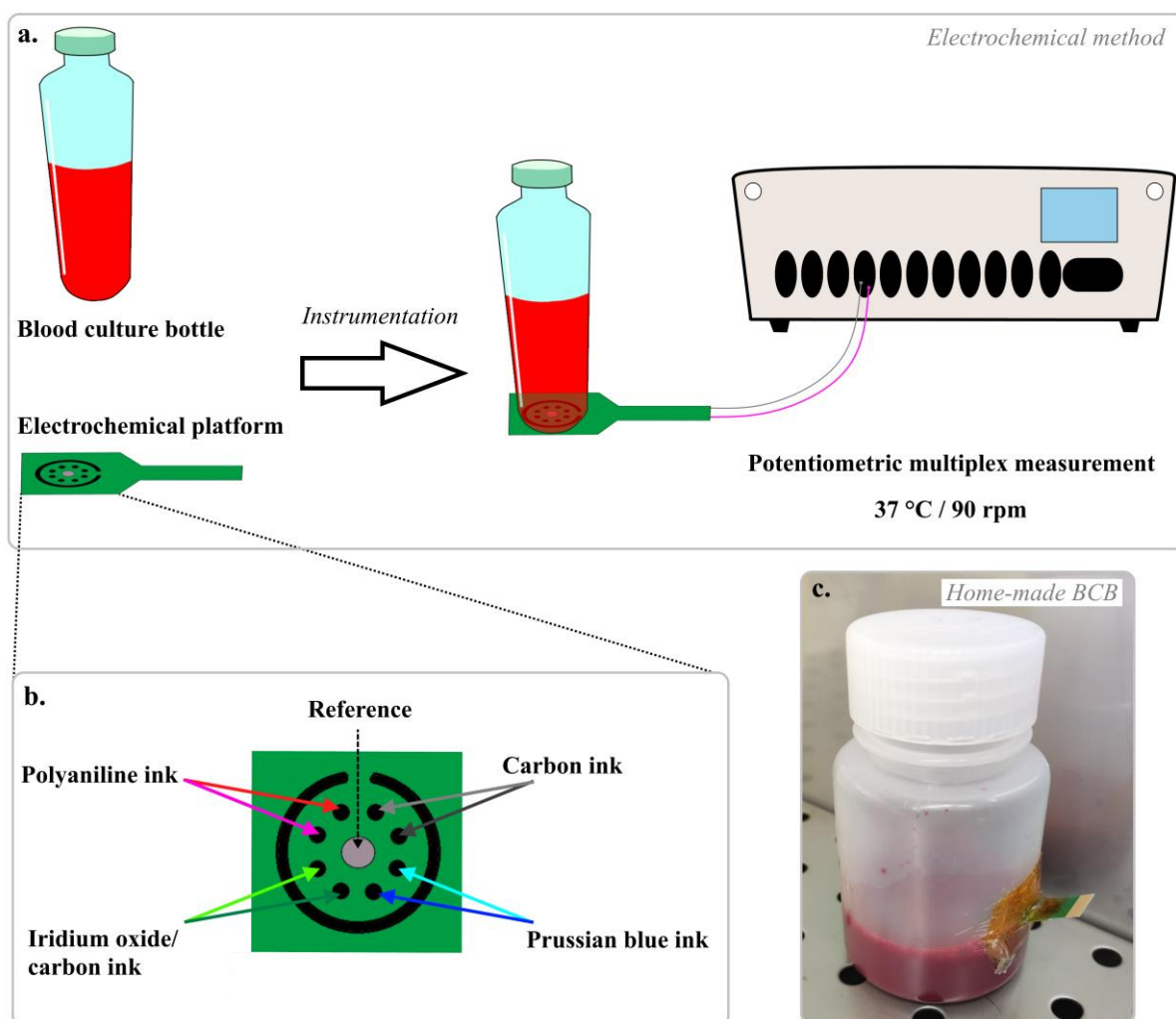
In this article, we investigate the use of potentiometric measurements in blood cultures, using a multi-material electrode array. The implementation of the electrode array into a blood culture bottle allows continuous monitoring of culture with minimal instrumentation. This concept enables valorisation of incubation time, which represents a blind period in classical bloodstream infection diagnosis. This method is moreover non-destructive and, like CO<sub>2</sub> detection, without *a priori*. Our measurements show that each tested microorganism has its own electrochemical signature with an excellent reproducibility and no false negative nor false positive responses. Thanks to machine learning, this electrochemical tongue is able to deliver the identification (of the Gram and further of the genus) few hours after positivity without any further manipulation or reagent use. By preserving standard blood culture practices, portable devices yielding both incubation and electrochemical measurement could be implemented in health facilities where larger CMBCS (continuous monitoring blood culture systems) are not accessible, such as field hospitals or health centres in developing countries.

## **2. Results**

Bacterial growth in a culture medium leads to an increase in carbon dioxide concentration and thus to acidification of the liquid. A potentiometric measurement using specific materials can be implemented to detect a change in the pH of the liquid phase. Here, we have instrumented a blood culture bottle with a multi-material sensing platform that allows us to monitor this acidification, but also the production of other bacterial metabolites (**Figure 2|a**).

We used four electrode materials based on commercially available inks that present differentiated electrochemical behaviour. Two materials were selected for their pH redox sensitivity, polyaniline and Iridium oxide/carbon inks. The third material exhibits an internal redox couple (Prussian blue) which is sensitive to redox metabolites. The latter electrode is composed of carbon and is essentially sensitive to the oxidation power of the environment.

The inks were deposited (**Figure S1**) on printed circuit boards (**Figure 2|b**) that are, integrated in blood culture bottles (**Figure 2|a**). The potentiometric signal is recorded between the different working electrodes (WE) and the common reference electrode (**Figure 2|b**) during incubation in blood cultures at 37°C under orbital motion.



**Figure 1. a.** Schematic representation of the implementation of a multi-material potentiometric electrode array into a blood culture bottle. **b.** Design of the multi-material potentiometric sensing platform. Polyaniline and Iridium oxide/carbon inks are pH sensitive and present respectively volume and surface redox behaviour. **c.** Image of the instrumented home-made blood culture bottle (BCB).

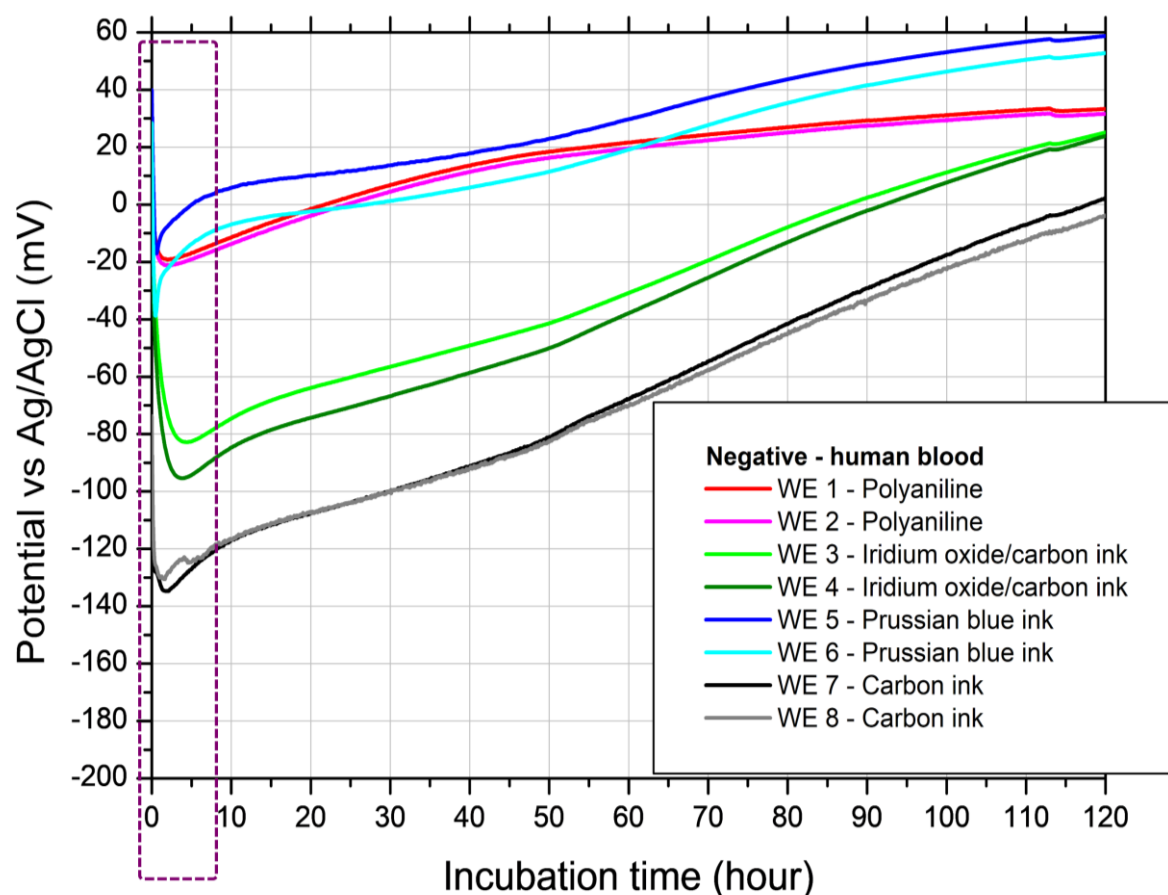
## 2.1. Electrochemical stability in human blood

First of all, it was primordial to characterize the electrochemical behaviour of the electrode array in non-infected blood (**Figure 2**). Each experiment was performed with 10 mL of sterile blood from different patients, which imitates at best the real variability encountered in clinical settings.

The first hours of measurement are associated with large variations in potential due to the adsorption of blood proteins on the surface of the electrodes.<sup>[27]</sup> Then, a slow and constant increase of the potential is observed for each material, without variation of the pH of the medium (as verified by measuring the pH with a pH glass electrode, before and after incubation). Lysis of the blood cells and oxidation of the blood, which may participate in electrodes' potential evolution, was qualitatively observed through colour change of the blood as already reported in the literature.<sup>[28-30]</sup> The potentiometric responses of the electrodes are related to the blood evolution since the materials more sensitive to oxidation power (including Iridium oxide/carbon ink electrode, which is mainly composed of carbon) exhibit similar monotonous increasing evolution whereas polyaniline, which is highly sensitive to pH, shows a stabilisation. This type of experiment was repeated several times (**Figure S2 – S5**) to obtain a baseline that will be compared to the curves recorded along bacterial growth.

The signal was recorded during the first five days of measurement since a lack of detection during this period is commonly associated to a negative blood culture.





Hydration of the electrodes  
+  
Protein adsorption

**Figure 2.** Typical potentiometric signals recorded during negative test (without bacteria) in human blood. The first few hours of measurement are associated with large variations in potential due to the equilibration of the electrodes caused by their hydration.

## 2.2. Electrochemical detection of bacterial growth with a polyaniline electrode

Reference bacterial strains (**Table S1**) were spiked into human blood at amounts consistent with the concentrations observed in patients (i.e. between 2 and 300 cfu per bottle containing 10 mL of blood). For each positive experiment (90 experiments in human whole bloods for 13 genus and 20 species), specific signals were recorded with all electrode materials without any false positive or false negative tests. To clearly demonstrate our strategy, only the behaviour of

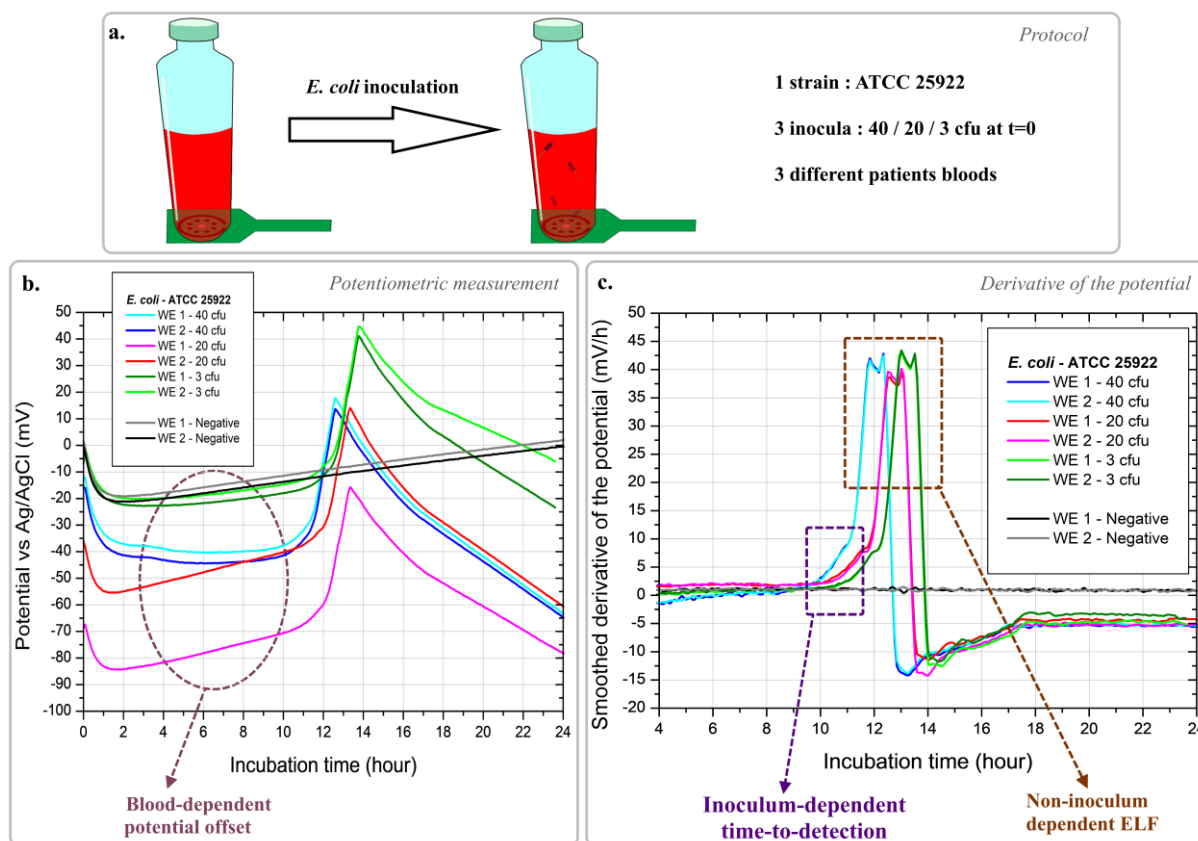
polyaniline is fully detailed in the article. Nevertheless, the whole data set was used to demonstrate the identification capabilities of the electrochemical tongue.

### *Effect of the inoculum*

**Figure 3|b** illustrates typical potentiometric responses during bacteria growth for *Escherichia coli* at different inocula for the polyaniline electrode. In the presence of the microorganism, the electrochemical potential of polyaniline increases to reach a maximum value before decreasing more slowly. Interestingly, the temporal position of the peak is dependent on the inoculated bacteria quantity. This demonstrates that the electrochemical behaviour is triggered by the exponential growth of the microorganism that generates CO<sub>2</sub> evolution. The first part of the signal, associated with an increase of the recorded potential corresponds to the change in pH of the solution (acidification from 7.4 to 6.4). However, the decrease observed in the second portion of the signal was unexpected. This behaviour is undoubtedly due to the presence of reductive species (secreted redox metabolites or redox biomolecules of the bacteria) issued from bacteria growth.

However, one can observe the presence of a potential offset, dependent on the patient's blood for each pair of curves. This offset was already observed for negative tests (**Figure S2–S5**). Moreover, as shown previously (**Figure 2**), a monotonous evolution is recorded in the first hours after inoculation. Both behaviours make the estimation of time-to-detection (TTD) difficult. Deriving potential in relation to time makes the measurements, and thus TTD, independent from both aspects. Otherwise, as already stated, the first hours of measurement are dominated by the electrode equilibration and, moreover, correspond to the minimum lag time of the micro-organisms (no growth). Thus, according to the derivate curves, we systematically exclude in further experiments the first four hours of measurement (**Figure S6**).

It appears also that the TTD decreases with increasing inocula as highlighted in **Figure 3|c**, thus giving possibilities of comparison with usual blood culture automatons. Conversely, the profiles of the potential derivatives are independent on the inoculum.



**Figure 3. a.** Protocol for each blood culture experiment and calculated quantity of bacteria inoculated in the instrumented blood culture bottles. **b.** Detection of several inocula of *E. coli* by potentiometric measurements using polyaniline electrodes in human blood. **c.** Derivatives of the potential of the polyaniline potentiometric responses in human blood for these inocula of *E. coli*.

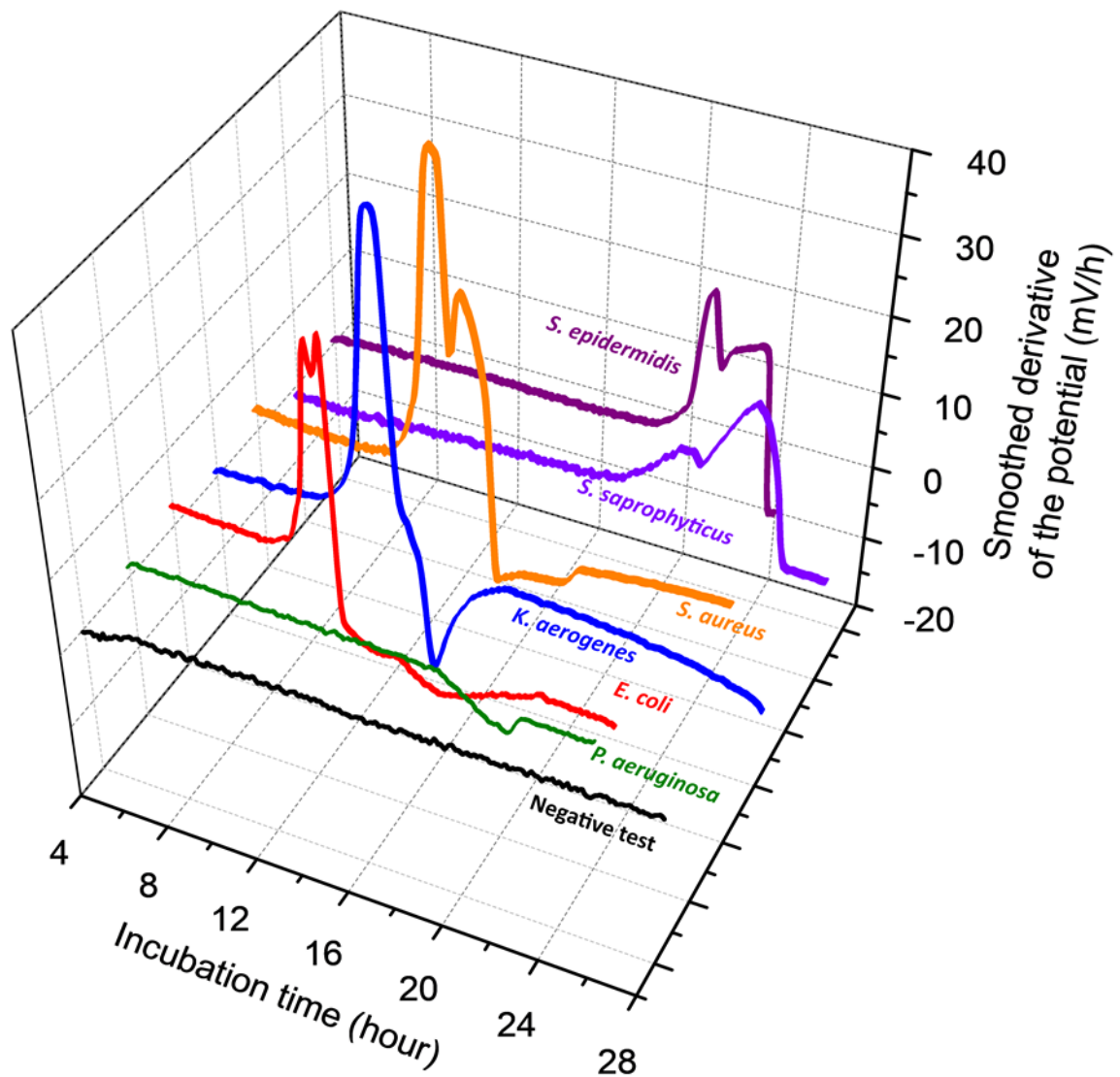
The variability of EElectrochemical Fingerprints (ELFs), measured during bacterial growth, obtained with different strains of the same bacterial species was investigated. It appears that slight variations are visible for different strains (**Figure S7**). However, these fluctuations are minimal even though each experiment is performed in bloods from different patients.

Thereafter, only one curve per experiment will be presented, regardless of the inoculum, as we are particularly interested in the ELF of each bacterial species.

#### *ELFs library of the most common pathogens*

We have performed experiments in human blood from 90 patients involving 20 species (10 Gram positive and 10 gram negative). Within the limits of the conducted experiments, the proposed method does not generate any false negative tests. Furthermore, the calculated TTDs are also comparable to those obtained with the BacT/Alert® Virtuo, the fastest system currently on the market (**Table S2**).<sup>[31]</sup>

For all the strains of a same species, we obtained good reproducibility of the ELF (**Figure 4 – Figure S8**). These fingerprints, species-dependent but independent of patient blood and inocula, should then be used to identify the bacteria following detection.



**Figure 4.** Different ELF's associated to the spiking of different bacterial species, obtained by derivation of the recorded potentiometric signal of polyaniline electrodes in human blood. *Pseudomonas aeruginosa* (ATCC 27853 – 70 cfu). *Escherichia coli* (ATCC 8739 – 40 cfu). *Klebsiella aerogenes* (ATCC 13048 – 100 cfu). *Staphylococcus aureus* (ATCC BAA-976 – 90 cfu). *Staphylococcus saprophyticus* (ATCC 15305 – 400 cfu). *Staphylococcus epidermidis* (ATCC 12228 – 30 cfu).

Our method therefore allows the detection of the growth of micro-organisms in complex biological environments (tests were mainly realized in human blood, but we also verified the

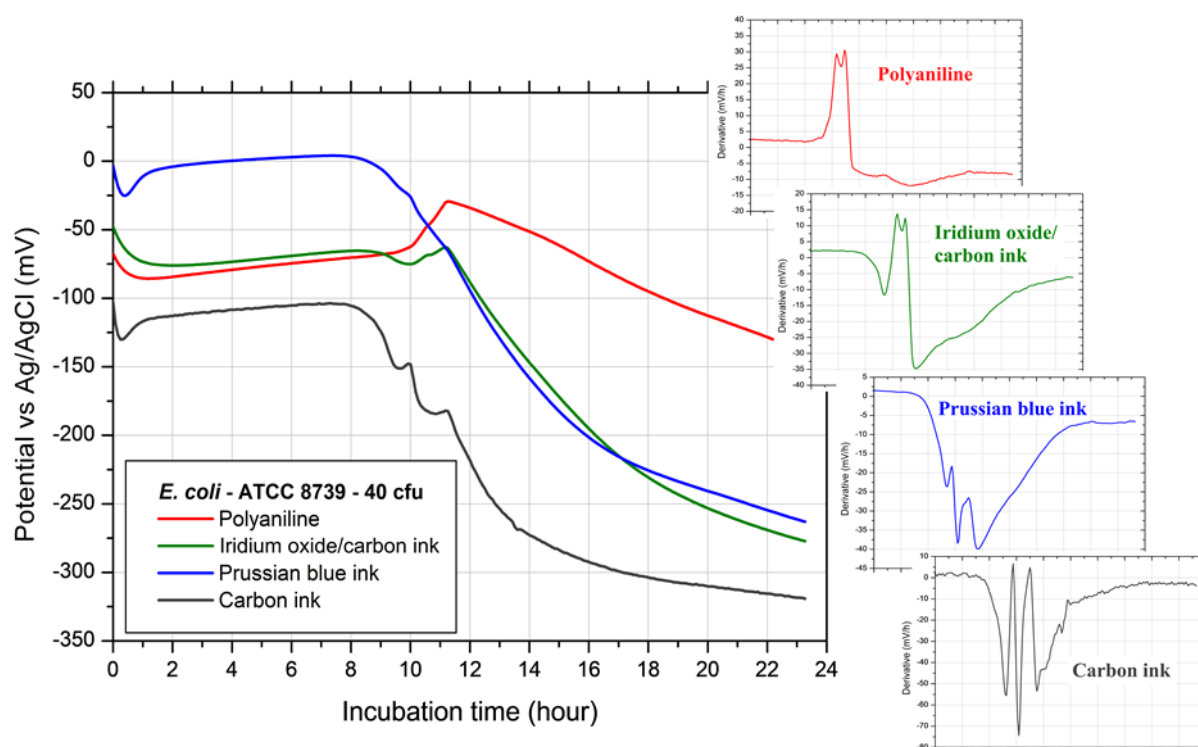
transposition of the measurements in horse defibrinated blood, blood culture media alone and in mycobacteria growth culture media) with non-significant loss of time compared to optical systems on the market. The excellent reproducibility of the results obtained, despite the use of human blood from different patients, shows the potential of this smart blood culture bottle for use in clinical settings. (**Figure S9 – S10**).

It is important to notice here that the results presented above are those obtained with polyaniline electrodes only. The use of electrochemical platforms containing four different materials (in pairs of electrodes) can provide multi-dimensional fingerprints.

### **2.3. Multi-dimensional ELFs**

We also performed the bacterial growth detection with the three other electrode materials (Iridium oxide/carbon ink, Prussian blue ink and carbon ink) and each material yields specific ELF for each bacterial species (database is available on demand). To exemplify the diversity and multi-dimensional aspect of the responses, **Figure 5** shows the different signals recorded with each electrode material for *E. coli* growth in human blood. It appears that Iridium oxide/carbon ink does not specifically detect the pH evolution of the environment as expected. The difference in behaviour between the polyaniline and the Iridium oxide/carbon ink electrodes is presumably related to the difference in structure and reactivity of both materials. Thus, polyaniline is 3D electroactive and an electronically conductive polymer whereas Iridium oxide shows only a surface pH response.

Otherwise, conversely than polyaniline that exhibits potential increase during the first part of the response, the signals recorded for the three other materials are dominated by large and rapid drops in potential that slightly differ in shape. Moreover, carbon ink seems to detect bacterial growth faster than the other materials.



**Figure 5.** Detection of *E. coli* thanks to potentiometric measurement in human blood using 4 different electrode materials (polyaniline, Iridium oxide/carbon ink, Prussian blue ink and carbon ink) and related ELF's for each material.

#### 2.4. Identification by machine learning

As aforementioned, we obtained multi-dimensional ELF's for each bacteria species that could be used for identification. Thereby, we worked with a machine-learning program to establish an ELF descriptor specific to the pathogens. We accurately performed identifications of the Gram and then of the genus to validate our approach. A more precise identification of the species seems to be accessible (according to our first results) but requires a bigger database that would be the object of a further clinical trial.

In order to work only on these ELF's and not on the temporal data (which depends on the inoculum), it was decided to work in the spectral domain. For each potential curve, two types of data processing were applied.

First, the potential curve was derived and then smoothed over 5 slip points (convolution) to eliminate measurement noise. A Fast Fourier Transform (FFT) algorithm was then applied. In a second step, the potential curve was directly smoothed and then an FFT was applied. In this way, the whole electrochemical fingerprint, both in terms of potential and derivative of potential, was taken into account. All these working data become a vector, the descriptor of the blood culture experiment, featuring the signal magnitudes in the frequency domain (**Figure 6|a**).

The most relevant frequencies for identification, for each material and for each data type (from potential or from derivative), were chosen by iterative testing. The details of the technical choices for the design of this algorithm are presented in **Supplementary Information 1**.

Gram identification was performed by classical cross-validation. However, for the genus classification, we used the LOBO (Leave One Bottle Out) cross-validation to evaluate our accuracies. For every training of the algorithm, the database is split into two fractions: the training set that gathers the whole data set, minus one experiment (one bottle), and the validation set including the only remaining experiment. The latter is then classified by the trained algorithm. The process was repeated as many times as necessary, so that every experiment has been used at least once as a validation set. In this way, final averaged identification accuracy yields a more realistic insight into the performances in clinical settings, where an algorithm is trained on a database that does not include the blood culture to be tested. Contribution of each material is shown in **Figure S11**.

**Figure 6|b**, shows the accuracies of Gram and genus identification plotted versus time after detection. Our methodology provides the bacterial Gram with 95% accuracy only 4.8 hours after positivity without need of any manipulation of the contaminated blood nor use of noxious chemical reagents (contrary to the gold standard Gram staining).

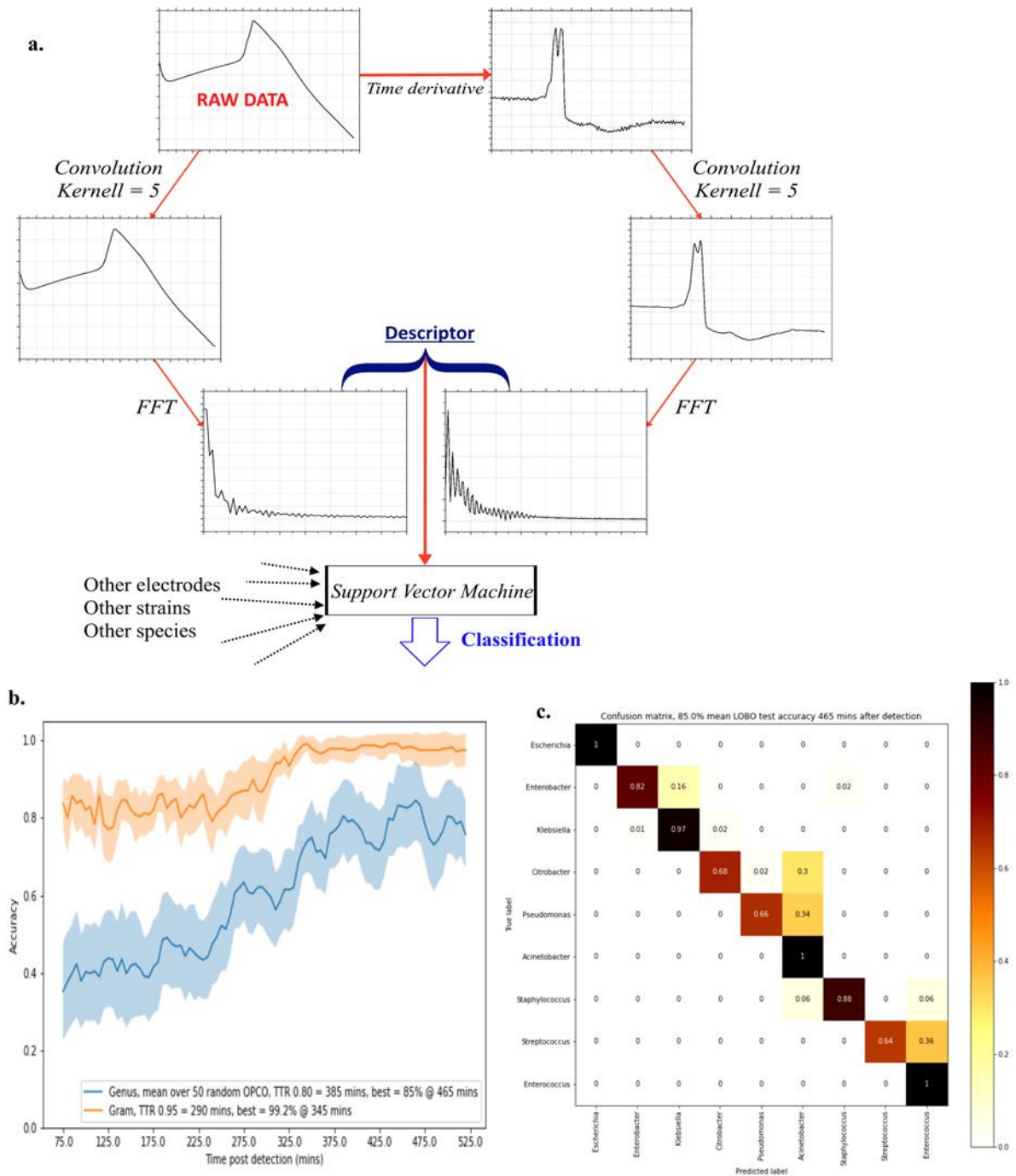
The genus is identified with 80% accuracy 6.4 hours after positivity, and with 85% accuracy 7.8 hours after positivity. Furthermore, we have also tested another type of classification: when



the microorganism is identified at 99% of accuracy as a Gram negative, our algorithms are able to find the right genus (in this class) with 85% of precision in 5.75 hours after positivity (**Figure S11**).

The smart blood culture bottle could therefore allow identification of genus in a short period of time. Diagnosis during the night or during periods when no staff is present in the laboratory is therefore possible by automating this identification.

The confusion matrix (**Figure 6|c**), obtained after the time required for a maximum accuracy genus classification (465 mins), allows us to observe that the identification errors are often between bacterial genus of the same family or of the same order. Indeed, the contribution of electrode materials is not implicitly shown in the confusion matrix since the multivariate response of the multi-material platform is considered as input data (**Figure S12**).



**Figure 6. a.** Example, with a polyaniline curve obtained for an *E. coli* growth, of data treatment applied for each curve. **b.** Classification accuracy, of the Gram and of the genus, of the samples and error made through classification using the standard deviation of the prediction, calculated as a function of time after detection. This model uses polyaniline, Iridium oxide/carbon ink and Prussian blue ink electrode signals. Gram classification was performed by a classical cross

validation (5 folds, 20% test size); genus classification was performed by a LOBO method. **c.** Confusion matrix of the prediction for each genus, 465 minutes, 5.75 hours, after positivity (maximum accuracy of classification).

### **3. Conclusion**

We have designed an instrumented blood culture bottle with a multi-material potentiometric sensor. Initially based on polyaniline for the detection of pH variation induced by carbon dioxide production, all electrode materials exhibit changes in open circuit potential that are not coherent with simple pH sensitivity. These variations are presumably due to redox species associated to bacterial growth either through secretion or presence in the bacterial membrane. This second hypothesis is currently privileged according to recent experiments on the study of bacterial biofilm growth on the surface of our electrode array. The dependency of the ELF's on the bacterial species seems to corroborate this hypothesis.

With classic data processing, electrochemical fingerprints can be used to identify the pathogen involved, in terms of Gram and genus at least, only a few hours after detection. This identification does not need any manipulation of the infected blood. No harmful reagents or personnel are required to obtain this crucial information.

Moreover, this instrumented blood culture bottle eliminates the need for a costly and cumbersome automated system due to the simplicity and the cost effective electronics for measuring open circuit potentials. Blood cultures could therefore be performed at the patient's bedside, at the doctor's office or during transport (and incubation) time to centralized laboratories.

This new blood culture method could therefore be a game changer for microbiological diagnostics and the management of septicemia. A faster (to obtain identification) and less expensive diagnostic system would be a considerable advantage for blood stream infections

diagnostics in low and middle income countries, where antimicrobial resistance is most devastating and in low density regions where the transport time could be important.

However, several points remain to be investigated. First, the nature of the redox species involved in the ELFs needs to be determined. Experiments on defibrinated horse blood have shown electrochemical fingerprints similar to those reported in this paper, on human whole blood (**Figure S13**). The recorded behaviour is thus independent of the culture media, as verified with a *Mycobacterium tuberculosis* culture performed in Myco/F Lytic culture media (data not shown).

Secondly, our approach has to be implemented in a clinical trial to enhance the database, the artificial intelligence algorithms and thus the identification accuracy. This change in experimental scale will allow testing of real infected bloods to definitely confirm the relevance of our method.

Thirdly, other electrode materials should be considered, in order to improve the TTD or the identification accuracy. The four materials used led to very promising results, but other materials may be implemented to obtain better differentiation between two bacterial species. It would also be interesting to work on the structure of the electrodes themselves (2D or 3D).

Finally, several protocols are currently being tested to extend the principle of this technology. The detection and identification in a polymicrobial blood culture, as well as the recognition of some antibiotic resistance mechanisms, are being investigated.

## **4. Experimental Section**

### **4.1. Chemicals and reagents**

All the chemicals were purchased from Sigma-Aldrich with the highest purity grade existing and used without any further purification.

Polyaniline carbon ink was prepared by dilution (1:1 in distilled water) of the commercial polyaniline ink (*Rescol*). The mixture was vortexed during 1 min before each use. Iridium oxide/carbon ink was prepared by adding Iridium oxide (Premion 99.99% – *Alfa Aesar*) to carbon ink BQ242 (Dupont) and Ethylene Glycol Diacetate as previously described.<sup>[32]</sup> Two carbon commercial inks were also used without any modification: a carbon graphite ink with Prussian blue (SunChemical Gwent C2070424P2) called P2 in this paper, and the carbon ink BQ242 (Dupont) alone, already used in the Iridium oxide/carbon ink blend.

The reference electrode material used is an Ag/AgCl ink (Dupont 5874). To prevent from silver leakage (which is bactericidal) and increase stability of the reference electrodes, it was protected with a polyurethane membrane.

### **4.2. Bacterial strains and growth conditions**

In each experiment, bacterial colonies were derived from a working culture on Columbia agar (5% v/v sheep blood, VWR) seeded 24 hours earlier and incubated at 37°C.

Colonies were taken from this working culture and placed in 3 mL of pharmaceutical diluent (VWR) to obtain 0.5 McF (between 0.8 and 0.12 absorbance). This amount generally corresponds to a bacterial concentration of  $2 \cdot 10^8$  cfu/mL. As this concentration is too high for our experiments, a cascade dilution protocol was used to decrease the concentration of the sample vial to a target value. These dilutions were performed in pharmaceutical diluent (VWR).

A volume of 0.4 mL of a bacterial suspension, ranging in concentration from  $2 \cdot 10^2$  to  $2 \cdot 10^3$  cfu/mL (depending on the experiment), was added to a mixture of 10 mL of blood and 30 mL of culture medium, in our homemade blood culture bottle.

The human blood used comes from *Etablissement Français du sang* (EFS). For legal reasons, each blood sample comes from only one patient. No experiments were performed with the same blood, which allows us to confirm the versatility of our device to different blood conditions. Defibrinated horse blood was provided by Thermo Scientific Oxoid™ (OXSR0050C).

Culture media used in our instrumented blood culture bottles come from commercial blood culture bottles (BACT/ALERT® FA Plus – Ref 410851 - bioMérieux).

The blood culture bottles were incubated at 37°C with shaking at 90 rpm in an Infors HT Ecotron incubator.

In Supplementary Table 1, all references of the bacteria species used in our experiments have been listed.

To count colonies, numerating cultures in triplicate were grown in Columbia agar (5% v/v sheep blood, VWR) for 24h after inoculation. This method provides the quantity of bacteria inoculated at  $t=0$ .

### **4.3. Electrochemical platform and detection**

We used a print circuit board manufactured by the company Cibel (Bellême, France). The top layers of all electrodes and connectors were made of gold. The electrode array manufacturing process has been described previously.<sup>[32]</sup>

The platforms were embedded in polypropylene flasks. The bottles were filled with 30mL of Bact/ALERT® medium (i.e the entire volume of a blood culture bottle). These blood culture bottles were then autoclaved to ensure the absence of contamination.

A few minutes before starting the experiment, 10 mL of human blood was added to the vial with a known amount of bacteria (depending on the purpose of the experiment).

Potentiometric electrochemical measurements were performed with an AUTOLAB potentiostat (Autolab Bv, The Netherlands) controlled by Nova 2.11 software. A multiplexer was used in order to record one measurement every 5 minutes on each of the 8 working electrodes for several hours or days.

#### **4.4. Data analysis**

Initial data analysis were performed using Origin software (Version 6.0). Supervised machine learning was executed by a home-made Python program.

#### **Acknowledgements**

We thank Amelia Whiteley for critical reading of the manuscript.

#### **Conflict of Interest**

The authors declare no conflict of interest.

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Grenoble, 8<sup>th</sup> November 2022

Dear Editor

Please find enclosed the manuscript entitled "*Electrochemical label-free pathogen identification for bloodstream infections diagnosis: the combination of electrodes materials and machine learning for a smart blood culture bottle.*", by Thibaut Babin, Pierre Bouvet, Tommy Dedole, Pierre R. Marcoux, Maxime Gougis and Pascal Mailley, which we wish to submit for publication as a *Full Paper* in *Biosensors and Bioelectronics*.

On January 20<sup>th</sup> of this year, an article in *the Lancet* reported an overwhelming burden of 1.27 million deaths in 2019 attributable to bacterial antimicrobial resistance. Within this context of worldwide crisis of widespread antibiotic resistance, there is rapidly growing interest in fast diagnostics, especially for life-threatening diseases such as bloodstream infections. De-escalation is indeed the cornerstone of antibiotic stewardship programmes. This tailoring of empirical antibiotic treatment to a personalized care requires some knowledge about the pathogen to be targeted, and the sooner the information arises, the narrower will be the spectrum of the adapted antibiotic.

That is why collecting key phenotypic features of the unknown pathogen during blood culture, prior isolation on agar plate, is of crucial importance: it brings guidance in the choice of antimicrobial chemotherapy much earlier, which limits or even avoids the use of broad-spectrum molecules. In the paper we submit, we report the concept of *smart blood culture bottle (BCB)*, which relies on the multiparametric electrochemical monitoring of the liquid phase. Combined with a machine learning analysis of the resulting fingerprint, this new paradigm allows a go with the flow identification as information about electroactive metabolites is cumulated.

For instance, our smart BCB provides Gram-typing with 99% of accuracy, identification down to genus with 85% of accuracy, in a non-invasive and label-free way, with no human operation, using a low-cost set of 8 electrodes made of four different materials. A thorough database has been collected in whole human blood with reference strains from the most frequent pathogens.

For all these reasons, we are convinced that the present article will attract a wide audience, from microbiologists and clinicians to electrochemists. We confirm that this manuscript has not been published elsewhere and is not under consideration in another journal. It has been posted as a preprint on Research Square on October 17<sup>th</sup> and is available at <https://doi.org/10.21203/rs.3.rs-2153621/v1> All authors have approved the manuscript and agree with submission to *Biosensors and Bioelectronics*. The authors have no conflicts of interest to declare.

Yours Sincerely,

Pierre R. Marcoux, Ph. D. on behalf of the authors

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Pierre R. Marcoux, Ph. D. on behalf of the authors



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Pierre R. Marcoux, Ph. D. on behalf of the authors



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**Electrochemical monitoring of blood culture bottle is a breakthrough innovation in bacteremia diagnosis.** It relies on the hypothesis that potentiometric fingerprints observed during the culture can bring an in-situ label-free identification of pathogen. Thanks to supervised machine learning, identification down to genus is obtained during incubation with 85% of accuracy.

**Keywords:** Blood culture, electrochemical fingerprint, machine learning, multi-material electrode array

Thibaut C. Babin\*, Tommy Dedole, Pierre Bouvet, Pierre R. Marcoux\*, Maxime Gougis, Pascal Mailley

**Electrochemical label-free pathogen identification for bloodstream infections diagnosis: towards a machine learning based smart blood culture bottle.**

### Detection & identification of bacteria by machine-learning

