



Development and performance characteristics evaluation of a new Bioelectric Recognition Assay (BERA) method for rapid Sars-CoV-2 detection in clinical samples

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ABSTRACT

Introduction: As the second wave of COVID-19 pandemic is in progress the development of fast and cost-effective approaches for diagnosis is essential. The aim of the present study was to develop and evaluate the performance characteristics of a new Bioelectric Recognition Assay (BERA) regarding Sars-CoV-2 detection in clinical samples and its potential to be used as a point of care test.

Materials and methods: All tests were performed using a custom portable hardware device developed by EMBIO DIAGNOSTICS (EMBIO DIAGNOSTICS Ltd, Cyprus). 110 positive and 136 negative samples tested by RT-PCR were used in order to define the lower limit of detection (L.O.D.) of the system, as well as the sensitivity and the specificity of the method.

Results: The system was able to detect a viral concentration of 4 genome copies/μL. The method displayed total sensitivity of 92.7 % (95 %CI: 86.2–96.8) and 97.8 % specificity (95 %CI: 93.7–99.5). When samples were grouped according to the recorded Ct values the BERA biosensor displayed 100.00 % sensitivity (95 %CI: 84.6–100.0) for Ct values <20–30. For the aforementioned Ct values the Positive Predictive Value (PPV) of the method was estimated at 31.4 % for COVID-19 prevalence of 1% and at 70.5 % for 5% prevalence. At the same time the Negative Predictive Value (NPV) of the BERA biosensor was at 100.0 % for both prevalence rates.

Conclusions: EMBIO DIAGNOSTICS BERA for the detection of SARS-CoV-2 infection has the potential to allow rapid and cost-effective detection and subsequent isolation of confirmed cases, and therefore reduce household and community transmissions.

1. Introduction

In early January 2020, a hitherto unknown coronavirus - now called Severe Acute Respiratory Syndrome Coronavirus -2 (SARS-CoV-2), was identified as the leading cause of a group of suspected pneumonia cases in Wuhan, China (Zhu et al., 2019). Due to the rapid spread of the virus, by the end of January 2020, the World Health Organization (WHO) declared a public health emergency as an international concern (Mahase, 2020).

Until presently, the gold standard method for COVID-19 diagnosis is the detection of SARS-CoV-2 genetic material with real-time PCR (RT-

PCR) (Espy et al., 2006), and although the amplification process can be completed in a relatively short timeframe, the stages of extraction, sample processing and data management (including reporting) can be time consuming and lead to a turnaround result time 24–48 hours. In addition, special equipment and trained personnel is required in order to perform the analysis resulting in high cost of the RT-PCR test.

As the third wave of the pandemic is in progress the development of fast and cost-effective approaches in order to control the COVID-19 pandemic is essential. Furthermore, since the clinical manifestations at the onset of COVID-19 resemble to other respiratory infections such as influenza, it is vital to develop methods for rapidly confirming or

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clearing suspected cases during global outbreak scenarios.

To date the point of care (POC) tests that have been developed for rapid diagnosis of SARS-CoV-2 infection, belong in three main categories; molecular-based, antigen and biosensor technologies. In recent years, automated, single-step RT-PCR methods as well as other nucleic acid amplification methods, such as isothermal amplification that do not require the sophisticated thermo-cycling involved in RT-PCR, have been developed (Carter et al., 2020). These technological advances have allowed molecular technologies to be developed that are suitable for use in a point-of-care context (Kozel and Burnham-Marusch, 2017). In a recent evaluation of Dinnes et al., published at the Cochrane database the average sensitivity of rapid molecular-based POC tests was 95.2 % (95 %CI: 86.7–98.0) and specificity 98.9 % (95 %CI 97.3–99.5) (Dinnes et al., 2020). These methods have the potential to reduce the time to produce test results after extraction and sample processing to minutes, but the time for the whole process may still be significant and the cost of the analysis is usually higher than RT-PCR.

Rapid antigen tests are mainly based on lateral flow immunoassays. Viral antigen is captured by specific antibodies and detected by a secondary virus-specific antibody that is labelled with an enzyme, fluorophore or colloidal gold. Their use is simple, they are cheap but as reported in the previous study their sensitivity varied considerably across studies (from 0% to 94 %): the average sensitivity was 56.2 % (95 %CI: 29.5–79.8) and average specificity was 99.5 % (95 %CI: 98.1–99.9) (Dinnes et al., 2020).

On the other hand, biosensors are considered fast, cost-effective, portable, and sensitive detectors that could be a promising diagnostic method. The principle of operation of biosensors is based on their ability to detect the target and turn this recognition into a detectable signal (Rodriguez-Mozaz et al., 2006; Scognamiglio et al., 2010). At the same time, due to their high sensitivity, low manufacturing costs (approximately 9 euros with the potential of reducing to 2 euros after mass production), and size, they are excellent candidates for the development of portable biosensors (Ronkainen et al., 2010). The reagents usually used for the detection are enzymes, antibodies, or whole cells. Immunosensors, unlike enzyme biosensors that assess overall toxicity, have the ability to be specific for a molecule. This is achieved due to the high affinity of the antibodies (Ab) or antigens (Ag) that are immobilized on the transducer surface relative to the target analyzers that are Ag or Ab respectively (Suri et al., 2009).

Live, cell-based biosensors have been shown to have high selectivity, sensitivity, and fast response times. Such detection systems, like the Bioelectric Recognition Assay (BERA), have been used in environmental, chemical and medical applications (Kintzios, 2007) which have shown unique and measurable changes on the electrical properties of the bio-recognition elements (Mavrikou et al., 2017; Moschopoulou and Kintzios, 2006; Apostolou et al., 2020) when the target molecules bind to electro-inserted antibodies.

The aim of the present study was to develop and evaluate the performance characteristics (sensitivity-specificity) of a new BERA - membrane biosensor method regarding Sars-CoV-2 detection in naso-and oro-pharyngeal swabs and its potential to be used as a reliable POC test.

2. Materials and methods

2.1. Equipment description

All tests were performed using a custom portable hardware device (Bio Electric Diagnostics – B.EL.D) developed by EMBIO DIAGNOSTICS (EMBIO DIAGNOSTICS Ltd, Cyprus). The working principle of the device is an open circuit potential (OCP). EMBIO Biosensor Tool is based on a proven, robust cell-based biosensor technology known as the Bioelectric Recognition Assay (BERA). The system uses high-precision Analog-to-digital (A/D) converters to measure electrical signals from cells used as bio-identification elements, enabling high-performance

control, parallel measurements, wireless transmissions and high-speed testing. The device is capable of measuring real-time changes in the electrical properties of cells (up to eight simultaneous measurements) from eight carbon-screen printed electrodes (working electrode: carbon, reference: Ag / AgCl) to a disposable sensor strip (iMiCROQ SL, Tarragona, Spain). In addition, the system can be connected via Bluetooth 4.0 to a smartphone, thus allowing the end user to be informed immediately of any analysis result (Apostolou et al., 2020). The device is shown in Fig. 1.

2.2. Experimental design

Materials and reagents: Monkey African green kidney (Vero) cell cultures (ATCC CCL-81) were originally provided from the LGC Promochem (Teddington, UK). Dulbecco's Modified Eagle Medium (DMEM), Fetal bovine serum (FBS), antibiotics (streptomycin-penicillin), L-glutamine & L-alanine, and trypsin/EDTA were purchased from Sigma-Aldrich (Taufkirchen, Germany). Monoclonal antibodies against SARS-CoV-2 Spike protein S1 subunit (No. ABIN6952616) were purchased from antibodies-online.com.

Cell culture and sensor fabrication: Cell culture was performed according to Apostolou et al. 2020. Briefly, Vero cells were cultured in Dulbecco medium with 10 % fetal bovine serum (FBS), 10 % antibiotics (streptomycin-penicillin) and 10 % L-glutamine, L-alanine (nutrient). The cells were removed from the culture vessel by adding 0.05 % trypsin / EDTA (1X) for 10 min at 37 °C and the cells were harvested by centrifugation (2 min / 1200 rpm) at a final density of 2.5×10^6 mL⁻¹.

Membrane-constructed cells were generated by electro-insertion of monoclonal antibodies against the S1 SARS-CoV-2 Spike protein subunit in the Vero cell membrane. Briefly, cells were detached and harvested after centrifugation (6 min / 1000 rpm / 25 °C). The cell pellet was resuspended in 400 µL PBS (pH 7.4) containing 5 µg mL⁻¹ antibody and incubated for 20 min at 4 °C. After incubation, the cell-antibody mixture was transferred to electroporator (Eppendorf Eporator, Eppendorf AG, Germany) cuvettes (4 mm) and electroinserted by applying two square electrical pulses at 1800 V / cm. The mixture was then transferred to a Petri dish (60 × 15 mm²) containing 3 mL of medium and incubated at 37 °C and 5% CO₂ for 24 h. The medium was then discarded from the Petri dish and the Vero / anti-S1 cells were mechanically removed and collected with the medium in Eppendorf tubes.

Definition of the Lower Limit of Detection (LOD): In order to define the LOD of the system we used a positive sample with a viral concentration of 4×10^{11} genome copies (gc)/mL. Subsequently, sequential 10⁻¹ dilutions (10 dilutions in total) of the sample were prepared to a final concentration of 40gc/mL.

Samples were first added to the top of each carbon electrode (20 µL). Then, biosensors with Vero membrane cells made with monoclonal antibodies (20 µL $\approx 5 \times 10^4$ cells) were added. Cell response was recorded as a time series of potentiometric measurements (in Volts). Each measurement lasted 180 s and 360 values per sample were recorded at a sampling rate of 2 Hz. After each measurement, the cell responses were downloaded to a cloud server and calculations were performed, based on a newly developed algorithm that yielded results on the presence of SARS-CoV-2 that appeared on the smartphone screen. Each sample was tested eight times using a set of eight individual sensors and each experiment was performed in duplicate.

The sensitivity and specificity of the BERA were determined using 110 samples with a range of Ct values, and 136 negative samples all confirmed with RT-PCR. All samples were analyzed at the Laboratory of Hygiene and Epidemiology of the University of Thessaly, a Biosafety Level 2 laboratory, from July 2020–September 2020. All samples were sent for consented molecular laboratory investigation from symptomatic patients, as part of the routine diagnostic procedure. After the test, the samples were stored at -80°C. The number of the samples used as well as their Ct Values are shown in detail in Table 1.

Nucleic acids were extracted from specimens using the iPrepTM

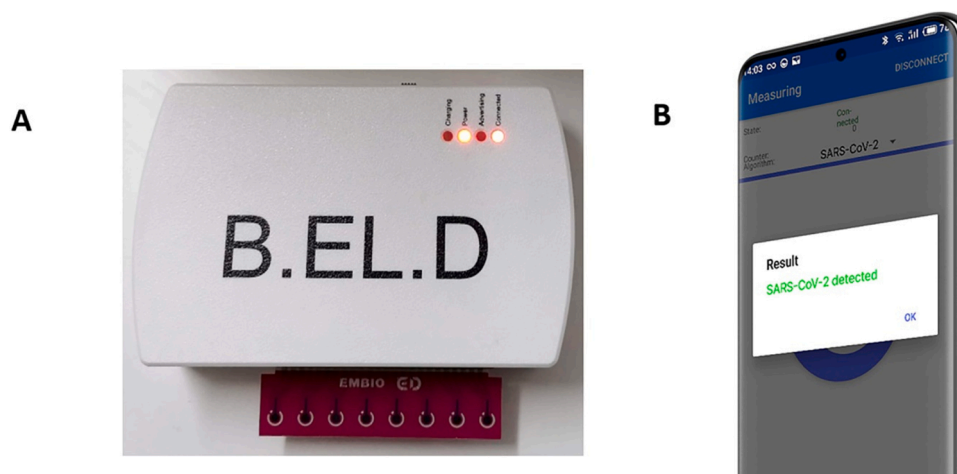


Fig. 1. Detection Device and Mobile application. An eight-channel carbon screen printed electrode connected on the potentiometer device (A). The device is connected to a smartphone for recording of the measurements immediately after the sample application and the result appears on the smartphone screen (B).

Table 1

Specificity and sensitivity of the method according to Ct values and different prevalence rates of the disease.

		Sensitivity (%)			Specificity (%)			PPV (%)		NPV (%)			
		Positive samples (n)			Negative samples (n)			Prevalence		Prevalence			
			95 % CI			95 % CI		1%	5%	1%	5%		
Total		102/110	92.7	86.2	96.8	133/136	97.8	93.7	99.5	29.8	68.9	99.9	99.6
	<20	22/22	100	84.6	100					31.4	70.5	100	100
	20–25	22/22	100	84.6	100					31.4	70.5	100	100
Ct groups	25–30	22/22	100	84.6	100					31.4	70.5	100	100
	30–35	21/22	95.5	77.2	100					30.4	69.5	100	99.8
	<20–35 (total)	87/88	98.9	93.8	100					31.2	70.2	100	100
	>35	15/22	68.2	45.1	86.1					23.8	61.9	99.7	98.3

● **Sensitivity**, that is the probability that a test result will be positive when the disease is present (true positive rate) was calculated for each group according to the following type: $a / (a + b)$.

● **Specificity**, that is the probability that a test result will be negative when the disease is not present (true negative rate) was calculated according to the following type: $d / (c + d)$.

● **Positive predictive value**, that is the probability that the disease is present when the test is positive was calculated for each group according to the following type: $PPV = Se \times Prevalence / Se \times Prevalence + (1 - Sp) \times (1 - Prevalence)$.

● **Negative predictive value**, that is the probability that the disease is not present when the test is negative was calculated for each group according to the following type: $NPV = Sp \times (1 - Prevalence) / (1 - Se) \times Prevalence + Sp \times (1 - Prevalence)$.

PureLink® Virus Kit and iPrep™ Purification Instrument (Invitrogen) according to the manufacturer's instructions. Four hundred µl from a commercially available viral transport medium (VTM) for the safe transfer of virus were transferred to the strip and viral RNA was eluted to a final volume of 100 µl.

Specific SARS-CoV-2 RNA (E gene) was detected with the RIDA®-GENE SARS-CoV-2 kit (R-Biopharm, Germany, CE, IVD) in an ABI STEP ONE (ThermoFisher Scientific) real time validated system (Being validated, provide reference citation). The PCR conditions were set according to the manufacturer's indications.

2.3. Algorithm for response processing and statistical analysis

The BERA method offers the opportunity for virus detection in minutes, without the use of any extraction protocol. To achieve this, the matrix effect of various VTM should be addressed (Sun, 2018). After testing a series of VTM, the different responses that were observed on their potential difference (millivolts) were analyzed. A specific algorithms were then developed for each VTM to assess the detection of SARS-CoV-2.

Data were uploaded on the online database using Google Firestore and Google Cloud Functions to run analytics. A specific algorithm which was developed according to a previously described procedure

(Hadjilouka et al., 2020) was used to produce/calculate the final results.

There are four different stages prior to result analysis:

Data set: Contained measurements from negative and positive samples. Each measurement consisted of a time series of potentiometric measurements (in Volts).

Training/test data set: The data set was divided into training and test data sets. The training data set was used to determine the algorithm limits and the test data set was used to evaluate the algorithm.

Editing / Exporting features: The data set was processed in a two-step process. In the first step, the background noise was subtracted to normalize and calibrate the signal and in the second step, the purified data was used as input for the development of an algorithm capable of detecting positive and negative samples. Each feature vector was calculated based on (a) the average values (Mean) for each cleaned data set, (b) the rolling average with rolling window size 50 (Min Sums), and (C) the rolling average with rolling window size 50 (Max Diffs), as it was described by Hadjilouka et al. (2020). This procedure was applied in each electrode channel and the overall test data set.

Algorithm: The algorithm used the feature vectors from the previous step as input for generating/calculating the final results. Three thresholds were set for the mean values, the minimum sums, and the maximum differences, and were compared with the corresponding values from each measurement. The final result was the dominant result (e.g. if 6

electrodes had a 'Positive' result and 2 had a 'Negative' result based on the thresholds, the result would be 'Positive') obtained from the above calculations.

The result is displayed on smartphone after being compared to the 3 different thresholds obtained from the data analysis. The tests offer an easy-to-use user interface (UI) with simple login option parameters such as temperature of person under testing and it runs directly. The process of the data analysis is summarized in Fig. 2.

From the experiments, we observed a biosensor response due to the enhanced matrix effect of the Viral Transport Media (VTM). Consequently, further research to identify and eradicate possible impediments due to the matrix effect is required for the optimization of the system. On the other hand, utilizing the receiver operating characteristic (ROC) analysis was possible to define the optimal model for increasing the specificity of the system, having in mind that this will decrease the sensitivity of the developed system.

A ROC curve was designed (not shown) in order to illustrate the diagnostic ability of the system as its discrimination threshold was varied. To draw a ROC curve, only the true-positive rate (TPR) and the false-positive rate (FPR) were needed. A ROC space was defined by FPR and TPR as x and y axes, respectively, which depicted relative trade-offs between true positive and false positive.

3. Results

3.1. LOD of SARS-CoV-2 of the Vero cell-based biosensor

The biosensor, based on Vero/anti-S1 cells membrane-engineered was able to give a positive signal when a positive sample with a viral concentration of approximately 4 gc/μL or 4×10^3 gc/mL (Ct value = 37) was analyzed. The biosensor measurements at each dilution, as shown in Fig. 3, were distinct and significantly different from the control solution (i.e. the solution where the dilutions were made) as well as from

the dilutions 10^{-9} and 10^{-10} which were not detected with RT-PCR.

3.2. Sensitivity and specificity of the Vero cell-based biosensor

One hundred and two (102) out of one hundred ten (110) RT-PCR confirmed positive samples were positive with the BERA biosensor. The method displayed sensitivity of 92.7 % (102/110). The specificity of the method, 133 out of 136 RT-PCR confirmed negative samples were also negative with the BERA biosensor, which corresponds to 97.8 % specificity (133/136).

When samples were grouped according to the Ct values the BERA biosensor displayed 100 % sensitivity (95 %CI: 84.6–100) for Ct values <20–30. For the aforementioned Ct values the PPV of the method was estimated at 31.4 % for 1% prevalence and at 70.5 % for 5% prevalence of COVID-19. At the same time the NPV of the BERA biosensor was at 100 % for both prevalence rates.

The number of samples, the calculations with nominator/denominator values and the results of sensitivity and specificity of each Ct group are shown in detail in Table 1.

4. Discussion

As the COVID-19 pandemic continues, smart sensors for rapid and reliable SARS-CoV-2 detection would better facilitate pandemic management and impact analysis.

In the present study we developed a robust cell-based biosensor technology known as the Bioelectric Recognition Assay (BERA) for the POC detection of SARS-CoV-2 S1 spike protein in naso- or oropharyngeal samples. The method was performed using a membrane-engineered procedure. The response was measured according to BERA principles and the results were obtained using B.EL.D, a portable multipurpose chemical analyzer developed by EMBIO Diagnostics. Test results (positive or negative) are displayed in mobile applications

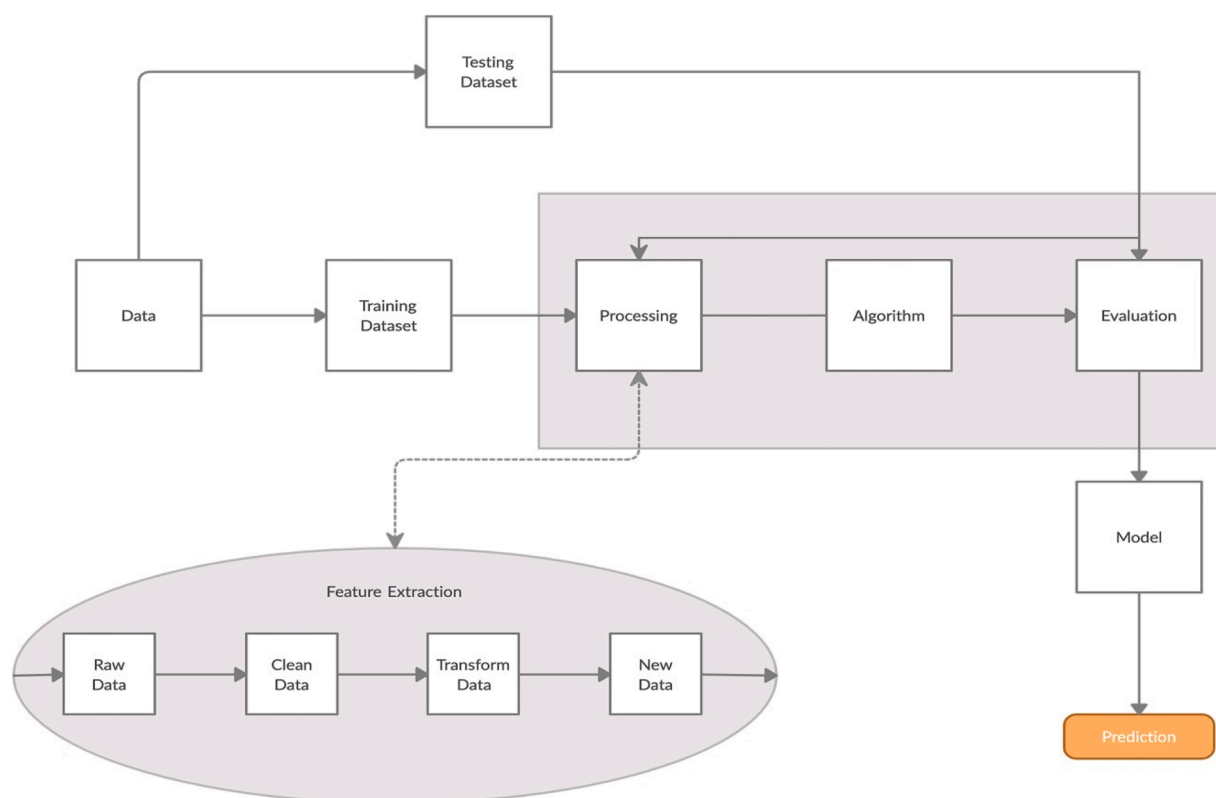


Fig. 2. Data analysis process on cloud functions. The analysis is being done in real time after the tests completion (Figure adapted from Hadjilouka et al. (2020). Newly Developed System for the Robust Detection of *Listeria monocytogenes* Based on a Bioelectric Cell Biosensor. Biosensors (Basel), Nov 17;10(11):178).

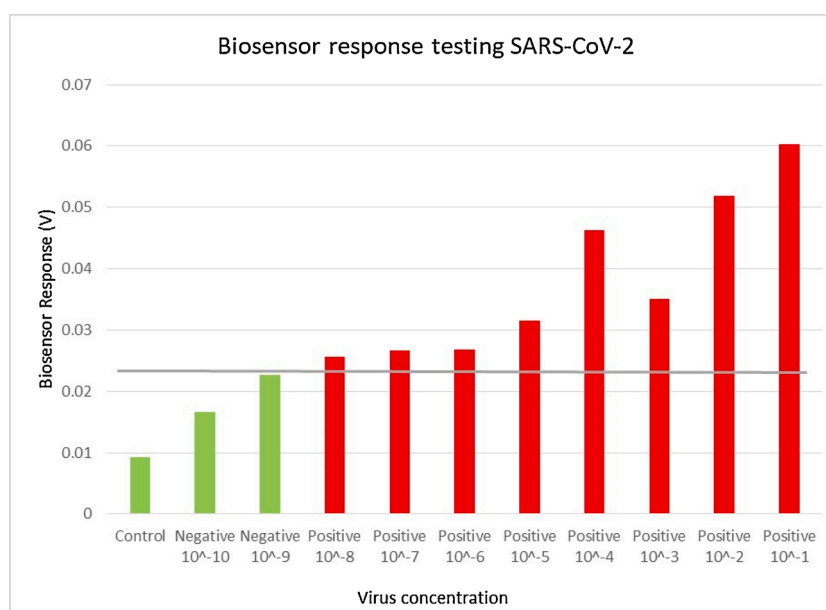


Fig. 3. Concentration-dependent biosensor responses against the SARS-CoV-2 spike S1 protein. A concentration-dependent response was observed during the analysis of increasing concentrations of isolated SARS-CoV-2 virus with the new biosensor. The samples were categorized into positive and negative based on the comparative results obtained through RT-PCR.

(Android and iOS) regardless of the location of the test subject according to EU regulations.

The technology was then evaluated and the LOD, sensitivity and specificity were defined. The method proved to be extremely fast (up to 180 s) and with a low limit for virus detection (4 gc/μL).

The results in this study support the BERA method for detection of SARS-CoV-2 virus and are in accordance with a previous study conducted by Mavrikou et al. (2020). The method was able to detect low viral loads and moreover proved to be a robust technology for SARS-CoV-2 detection since it demonstrated excellent reproducibility. The BERA method showed a sensitivity of 92.7 % (102/110) and specificity 97.8 % (133/136) against RT-PCR with clinical samples.

In contrast, other promising sensing systems that have been developed for rapid Sars-CoV-2 detection as the CRISPR-based test (Broughton et al., 2020), the dual functional SARS-CoV-2 plasma gene sensor (Qiu et al., 2020), a field effect transistor (FET) -sensitizer (Seo et al., 2020), and a lateral flow immunoassay (LFIA) (Chen et al., 2020), lack supportive studies and need further validation before their application as potential COVID-19 diagnostic tools.

Recently, many studies have been conducted in order to evaluate the performance characteristics of various commercially available POC antigen tests. The study of Fourati et al. (2020) compared the six following antigen tests: SARS-CoV-2 COVID-19 Respi-Strip (Coris BioConcept, Gembloux, Belgique), Standard Q COVID-19 Ag (SD BIOSENSOR, Inc., Coree), PanBio COVID-19 Antigen Rapid Test (Abbott, Chicago, Illinois, USA), Biosynex COVID-19 Ag BSS (Biosynex, Strasbourg, France), NG Test SARS-CoV-2 Ag (NG Biotech, Guipry, France) and COVID-VIRO Antigen Rapid Test (AAZ, Boulogne-Billancourt, France). According to the study of Fourati et al., CORIS and NG BIOTECH demonstrated poor sensitivity (42.6 % and 38.9 % respectively, for Ct values <33), while BIOSENSOR, ABBOTT, BIOSYNEX and AAZ tests showed a relatively satisfactory total sensitivity (55–62 %) that reached 87–96 % for Ct values <25 (Fourati et al., 2020). The EMBIO BERA test demonstrated superior sensitivity, from the tests mentioned above, that is 98.9 % for Ct values <35, and 100 % for Ct values <25 (Table 1).

Concerning specificity, CORIS, ABBOTT, NG BIOTECH and AAZ reached 100 %, BIOSENSOR and BIOSYNEX 93.2 % and 98.5 % respectively (Fourati et al., 2020), while the sensitivity of EMBIO BERA test was 97.8 %. Even though our method demonstrated a slightly lower

specificity, it is worth mentioning that the Negative Predictive Value of the method, when Ct values were <20–30, was 100 % (for prevalence rates both 1% and 5%). The advantages/disadvantages of the BERA method as well as the comparison to commercially available POC assays are summarized at Table 2.

Concerning the issue of the results, our approach of a SARS-CoV-2 detection test connected to a smartphone, is in accordance with the study of Song et al. (2020). In this study, the Internet of Things (IoT) -based combinatorial approaches to sensor participation, IT sharing, AI and dynamic networks were very useful for healthcare professionals in assessing COVID-19 full-spectrum perception, reliable transmission and smart processing.

The present study has some limitations. First of all, no samples of patients infected with other coronaviruses were used in order to check for cross reactivity. However, in the study of Mavrikou et al. (2020) using antibodies, enzymes and other receptor-like molecules against distinct domains within the S1 subunit, the novel biosensor assay could detect different coronaviruses or even other SARS-CoV-2 variants.

At the time the experiments were performed in Greece, the burden of the disease was low so the number of positive samples tested was also relatively low. Further experiments with more samples are required and will be performed in the near future.

The issue of cell viability remains of crucial importance. Various

Table 2
Comparison of BERA System with commercially available POC assays.

	BERA System	Immunochromatographic assays
Time of analysis	3 min	10–15 min
Cost of analysis	- Now 9 euros - After mass production estimated 2 euros	4.5 euros
Advantages	- Digitalization - DGPR compliance	- High portability - No specialize training necessary
Disadvantages	- Small training required - Invalid results due to matrix effect - Low consumables life	- No digitalization - No DGPR compliance

approaches have been proposed to overcome this limitation (microfluidic/organ-on-chip circuits in biosensing platforms or specific cell types), but so far none of these approaches have proven to be suitable and cost efficient for routine mass-screening applications (Sun, 2018). The production rate of the assay consumables and reagents for mass-scale testing remains a challenge.

The estimated sensitivity and specificity of the device provides an opportunity to be used as a point of care device at in primary health care settings or at points of entry for travelers, since requires minimal sample preparation, minimal biosafety requirements and the interpretation of the results is automated. Furthermore, one user (Android Version) can operate up to 5 B.E.L.D devices in parallel, raising thus the throughput and performing a large number of tests. In conclusion, the Bioelectric Recognition Assay (BERA) is ideal for mass screening for COVID-19 and has the potential to provide rapid and cost-effective detection of SARS-CoV-2 infection for effective public health management.

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Declaration of Competing Interest

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.

References

- Apostolou, T., Loizou, K., Hadjilouka, A., Inglezakis, A., Kintzios, S., 2020. Newly developed system for acetamidiprid residue screening in the lettuce samples based on a bioelectric cell biosensor. *Biosensors* 10, 8. <https://doi.org/10.3390/bios10020008>.
- Broughton, J.P., Deng, X., Yu, G., Fasching, C.L., Servellita, V., Singh, J., Miao, X., Streithorst, J.A., Granados, A., Sotomayor Gonzalez, A., Zorn, K., Gopez, A., Hsu, E., Gu, W., Miller, S., Pan, C.-Y., Guevara, H., Wadford, D.A., Chen, J.S., Chiu, C.Y., 2020. CRISPR–Cas12-Based detection of SARS-CoV-2. *Nat. Biotechnol.* 38, 870–874.
- Carter, L.J., Garner, L.V., Smoot, W., Li, Y., Zhou, Q., Catherine, J., Sasso, J.M., Gregg, A. C., Soares, D.J., Beskid, R., 2020. Assay techniques and test development for COVID-19 diagnosis. *ACS Cent. Sci.* 6, 591–605. <https://doi.org/10.1021/acscentsci.0c00501>.
- Chen, Z., Zhang, Z., Zhai, X., Li, Y., Lin, L., Zhao, H., Bian, L., Li, P., Yu, L., Wu, Y., Lin, G., 2020. Rapid and sensitive detection of AntiSARS-CoV-2 IgG, using lanthanide-doped nanoparticles-based lateral flow immunoassay. *Anal. Chem.* 92 (10), 7226–7231.
- Dinnes, J., Deeks, J.J., Adriano, A., Berhane, S., Davenport, C., Dittich, S., Emperador, D., Takwoingi, Y., Cunningham, J., Beese, S., Dretzke, J., Ferrante di Ruffano, L., Harris, I.M., Price, M.J., Taylor-Phillips, S., Hooft, L., Leeflang, M.M.G., Spijker, R., Van den Bruel, A., 2020. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database Syst. Rev.* (8), CD013705 <https://doi.org/10.1002/14651858.CD013705>.
- Espy, M.J., Uhl, J.R., Sloan, L.M., Buckwalter, S.P., Jones, M.F., Vetter, E.A., Yao, J.D.C., Wengenack, N.L., Rosenblatt, J.E., Cockerill, F.R., Smith, T.F., 2006. Real-time PCR in clinical microbiology: applications for routine laboratory testing. *Clin. Microbiol. Rev.* 19 (1), 165–256.
- Fourati, S., Audureau, E., Chevaliez, S., Pawlotsky, J.M., 2020. Evaluation de la performance diagnostique des tests rapides d'orientation diagnostique antigeniques COVID-19. *Laboratoire de Virologie et le service de santé publique des Hôpitaux Universitaires Henri-Mondor (AP-HP, Université Paris-est-Créteil, et INSERM)*. <https://www.sfm-microbiologie.org/covid-19-reactifs-evaluations/>.
- Hadjilouka, A., Loizou, K., Apostolou, T., Dougiakis, L., Inglezakis, A., Tsaltas, D., 2020. Newly developed system for the robust detection of *Listeria monocytogenes* based on a bioelectric cell biosensor. *Biosensors (Basel)* 10 (November (11)), 178. <https://doi.org/10.3390/bios10110178>. PMID: 33212801; PMCID: PMC7698059.
- Kintzios, S., 2007. Cell-based sensors in clinical chemistry. *Mini-Rev. Clin. Chem.* 7, 1019–1026.
- Kozel, T.R., Burnham-Marusch, A.R., 2017. Point-of-care testing for infectious diseases: past, present, and future. *J. Clin. Microbiol.* 55 (August (8)), 2313–2320. <https://doi.org/10.1128/JCM.00476-17>. Epub 2017 May 24. PMID: 28539345; PMCID: PMC5527409.
- Mahase, E., 2020. China coronavirus: WHO declares international emergency as death toll exceeds 200. *BMJ* 368, m408. <https://doi.org/10.1136/bmj.m408>, 2020. PMID: 32005727.
- Mavrikou, S., Flampouri, E.V., Iconomou, D., Kintzios, S., 2017. Development of a cellular biosensor for the detection of aflatoxin B1, based on the interaction of membrane engineered Vero cells with anti-AFB1 antibodies on the surface of gold nanoparticle screen printed electrodes. *Food Control* 73, 64–70. <https://doi.org/10.1016/j.foodcont.2016.06.002>.
- Mavrikou, S., Moschopoulou, G., Tsekouras, V., Kintzios, S., 2020. Development of a portable, ultra-rapid and ultra-sensitive cell-based biosensor for the direct detection of the SARS-CoV-2 S1 Spike protein antigen. *Sensors* 20 (11), 3121.
- Moschopoulou, G., Kintzios, S., 2006. Application of “membrane-engineering” to bioelectric recognition cell sensors for the detection of picomole concentrations of superoxide radical: a novel biosensor principle. *Anal. Chim. Acta.* 573–574, 90–96.
- Qiu, G., Gai, Z., Tao, Y., Schmitt, J., Kullak-Ublick, G.A., Wang, J., 2020. Dual-functional plasmonic photothermal biosensors for highly accurate severe acute respiratory syndrome coronavirus 2 detection. *ACS Nano* 14 (5), 5268–5277.
- Rodriguez-Mozaz, S., Lopez de Alda, M.J., Barceló, D., 2006. Biosensors as useful tools for environmental analysis and monitoring. *Anal. Bioanal. Chem.* 386, 1025–1041.
- Ronkainen, N.J., Halsall, H.B., Heineman, W.R., 2010. Electrochemical biosensors. *Chem. Soc. Rev.* 39, 1747–1763. <https://doi.org/10.1039/b714449k>.
- Scognamiglio, V., Pezzotti, G., Pezzotti, I., Cano, J., Buonasera, K., Giannini, D., Giardi, M.T., 2010. Biosensors for effective environmental and agrifood protection and commercialization: from research to market. *Microchim. Acta.* 170, 215–225.
- Seo, G., Lee, G., Kim, M.J., Baek, S.H., Choi, M., Ku, K.B., Lee, C.S., Jun, S., Park, D., Kim, H.G., Kim, S.J., Lee, J.O., Kim, B.T., Park, E.C., Kim, S.I., 2020. Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. *ACS Nano* 14, 5135–5142. <https://doi.org/10.1021/acsnano.0c02823>.
- Song, Y., Jiang, J., Wang, X., Yang, D., Bai, C., 2020. Prospect and application of internet of things technology for prevention of SARIs. *Clin. Ehealth* 3, 1–4.
- Sun, J.A., 2018. Matrix effect study and immunoassay detection using electrolyte-gated graphene biosensor. *Micromachines* 142.
- Suri, C.R., Boro, R., Nangia, Y., Gandhi, S., Sharma, P., Wangoo, N., Rajesh, K., Shekhawat, G.S., 2009. Immunoanalytical techniques for analyzing pesticides in the environment. *Trends Anal. Chem.* 28, 29–39. <https://doi.org/10.1016/j.trac.2008.09.017>.
- Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., 2019. China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* 382 (8), 727–733. <https://doi.org/10.1056/NEJMoa2001017>, 2020. PMID: 31978945.