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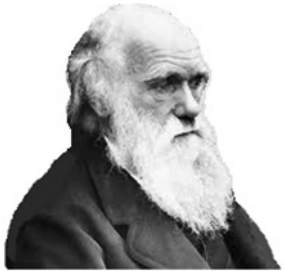

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Integrated microsystem for multiplexed genosensor detection of biowarfare agents

Sagbor Grinity Ema[1,*], Kwumasi Sylvester Mwadwo[1], Bosu P. Paul[2]

[1]CSIR/BRRI UP Box 40 Kumasi, Ghana

[2]CSIR/FORIG P.O Box 63 KNUST Kumasi, Ghana

*E-mail address: amitagbor@yahoo.co.uk

ABSTRACT

An early, rapid and definite detection for the presence of biowarfare agents, pathogens, viruses and toxins is required due to their harmful effect to human population. Those potentially encountering the aforementioned include people involved in civil rescue and security, homeland security, military operations, as well as public transportation securities such as airports, metro and railway stations. This work informs the reader of an electrochemical genosensor with an integrated microsystem array combined with microtube fluidics that allows simultaneous detection of different biowarfare agents such as *Bacillus anthracis, Brucella melitensis, Bacteriophage lambda, Francisella tularensis, Burkholderia mallei, Coxiella burnetii, Yersinia pestis,* and *Bacillus thuringiensis* var. kurstaki. The chip electrode arrays were modified via coimmobilisation of a 1:100 (mol/mol) mixture of a thiolated probe and a polyethyleneglycol terminated bipodal thiol. Herein, PCR products from relevant biowarfare agents were detected reproducibly through a sandwich assay format with the target hybridised between a surface immobilised probe into the electrode and a horseradish peroxidase-labelled secondary reporter probe, which provided an enzyme based electrochemical signal. Cross-reactivity studies over potential interfering DNA sequences have demonstrated high selectivity using the developed platform producing high-throughput.

Keywrods: Electrochemical DNA biosensor, Biowarfare agent, Multiplex detection, Self-assembled monolayer (SAM), *Bacillus anthracis, Brucella melitensis, Bacteriophage lambda, Francisella tularensis, Burkholderia mallei, Coxiella burnetii, Yersinia pestis*

1. INTRODUCTION

Considering the general availability of know-how to culture microorganisms in large quantities, there is now a global argument about the possibility of using different pathogens with high risk not only limited to public health safety but also in plants and animals for bioterroristic attacks. The threat on bioterrorism attacks has attracted attention due to the recent event that has struck Syria [1] which killed hundreds men, women, and children aside from the anthrax sporecontaining letter attack [2] that happened in United States which threatened the whole world. There are numerous pathogens which include bacteria, viruses, fungi, toxins and among others, are listed by various agencies that are potentially dangerous agents [3]. Thus, immediate detection of these potential biowarfare agents is required in different situations which include civil rescue and security units, homeland security, military operations, public transportation securities such as airports, metro and railway stations due to its harmful effect on the human population [4] as well as to environment. Therefore, there is a need to develop analytical screening tools which could be portable, rapid, costeffective and simple detection for the responders as well as specialised laboratories.

To date, plenty of techniques to detect and identify biowarfare agents like cell culture [6], molecular techniques including polymerase chain reaction (PCR) [7] as well as recombinase polymerase amplification, real time PCR [6-9], or, alternatively using enzymelinked immunosorbent assay (ELISA) [10] have been developed. Nucleic acid-based detection systems have been widely explored and it is more sensitive than antibody-based detection systems [3]. Recent advances have taken place for multiple analyte detection using microarrays for pathogenic species detection that involves nucleic acid-based detection system [11-13]. Although these standard techniques are sensitive, the use of microarrays involves many manual handling steps that rather time consuming due to long hybridisation times, requires intensive handling of the infectious agent and has no direct combination with an automated biosensor system.

Multiplexed assays can screen multiple analytes in a single assay which is significantly simpler, more rapid and requires less sample and reagent consumption in comparison to multiple single target. Several studies have been explored for the multiplex detection of target analytes through electrochemical measurement system [14-17]. Electrochemical biosensors are popular for their excellent sensitivity, selectivity, versatility, simplicity [18, 19] and are capable of detecting low concentrations of target agents without interference from background materials [20]. The development of these technologies has garnered a continual interest for application in clinical diagnostics [14], food quality control [21] and environmental monitoring [22], as promising alternatives to traditional methods in detecting pathogens.

Overall, biowarfare agents' threat has created a rapidly rising demand for new emerging sensor technologies to speed up testing. Here we describe an electrochemical sensor array for the simultaneous recognition of PCR amplified gene segments of *Bacillus anthracis, Brucella melitensis, Bacteriophage lambda, Francisella tularensis, Burkholderia mallei, Coxiella burnetii, Yersinia pestis, and Bacillus thuringiensis var. kurstaki*. These eight pathogens are among the biowarfare agents of the highest threat potential listed [23, 24]. The biosensor array was housed within a microfluidic set-up and the assay was automated via the use of a peristaltic pump, with the only required end-user intervention being sample addition. Parameters such as incubation time and temperature were optimised and applied to the detection of complementary target for each biothreats agents.

2. EXPERIMENTAL DETAILS

2. 1. Materials

All the starting materials were obtained from commercial suppliers and used without further purification. Eight thiolated ssDNA probes designed specifically for eight specific synthetic ssDNA complementary target and eight ssDNA as secondary reporter probes were purchased from biomers, Germany, (see Supplementary Information Table 4.S1). Biotynilated PCR products of *Bacillus anthracis, Brucella melitensis, Bacteriophage lambda, Francisella tularensis, Burkholderia mallei, Coxiella burnetii, Yersinia pestis, and Bacillus thuringiensis var. kurstaki* were kindly provided by the FriedrichLoeffler-Institut, Institut für bakterielle Infektionen und Zoonosen, Germany. Dithiol 16-(3,5-bis((6mercaptohexyl)oxy)phenyl)-3,6,9,12,15pentaoxahexa-decane) (DT1) was purchased from SensoPath Technologies (USA), sulfuric acid, potassium dihydrogen phosphate, phosphatebuffered saline (PBS) (dry powder), PBS-Tween-20, hydrogen peroxide 30%, acetone and ethanol (synthetic grade), 0.1 M hydrochloric acid, and acetic acid were purchased from Scharlau (Spain); Tris(hydroxymethyl) aminomethane, Sodium Hydroxide, Sodium Chloride and 3,3,5,5Tetramethylbenzidine (TMB) Liquid Substrate System for ELISA was obtained from Sigma. Aqueous solutions were prepared with Milli-Q water Millipore (18mΩ.cm) and all reagents were used as received.

2. 2. Instrumentation

Electrochemical studies were carried out using an Autolab PGSTAT 10 potentiostat with measurements performed using an array of 24 gold electrodes (1 mm-diameter) with internal reference and counter electrodes. The final format of the biosensor assay has been integrated within the microfluidic set-up. The lithographically produced gold electrodes were provided by Fraunhofer ICT-IMM (IMM), Germany, and were produced as previously reported [25]. All sonication procedures were conducted with an ultrasonic bath (Branson ultrasonic corporation, model 2510EMT, USA). Enzyme-linked oligonucleotide assay (ELONA) studies were performed using bioNOVA cientifica, S.L. (Madrid, Spain) and HydroFlex 3-in-1 well washer, TECAN (Spain).

The microfluidic set-up for incubation of analytes and flushing/washing with built in peristaltic pump was provided by IMM, Germany and the polymeric microfluidics were supplied by microfluidic ChipShop GmbH, Germany.

2. 3. Cultivation and inactivation of raw bacterial cells for DNA preparation

Bacterial cells were cultivated on cysteine heart agar (Becton Dickinson GmbH, Heidelberg, Germany) supplemented with 10 % chocolatized sheep blood. Incubation was carried out for 3 days at 37 °C in an atmosphere with 5 % CO_2. Heat assisted inactivation was carried out for 10 min at 95 °C (Thermomixer Compact, Eppendorf AG, Hamburg, Germany). To check sterility, the suspension was plated on agar plates and incubated for 7 days and no growth was observed.

Preparation of DNA from bacteria suspension

2 mL of each bacteria suspension were centrifuged for 10 min at 13400 rpm (MiniSpin, Eppendorf Ag, Hamburg, Germany). The supernatant was removed and the pellet was washed with 1x PBS (Carl Roth GmbH, Karlsruhe, Germany) and 1x TE (Carl Roth GmbH,

Karlsruhe, Germany) using centrifugation steps with 11400 rpm and removing the supernatant again. For lysis, the pellet was mixed with 10 µl 1x TE 1 ml 1 % SDS (Carl Roth GmbH, Karlsruhe, Germany) and 12.5 µl RNAse A and incubated in a thermoblock (TMix, Analytik Jena AG, Jena, Germany) for 30 min at 37 °C followed by addition of 12,5 µl Proteinase K and an additional incubation step for 10 min at 72 °C. 100 µl 5 M potassium acetate were added, the solution was mixed and incubated on ice for 30 min. Centrifugation was again carried out for 10 min at 114000 rpm and the supernatant was transferred into a clean reaction vessel. One volume of phenol was added, and centrifugation was repeated for 5 min. Again, the upper phase was transferred into a clean reaction vessel and one volume of chloroform/isoamylalcohol (24:1) was added, mixed and centrifuged for 5 min. Upper phase was separated again and mixed with two volumes of ethanol. Nucleic acid precipitation was carried out for 20 min at -20 °C. Final centrifugation was carried out for 10 min and the supernatant was removed. Remaining DNA was dried, finally diluted in aqua bidest and stored at -20 °C.

2. 4. Preparation and characterisation of complementary PCR products

PCR protocol PCR was performed using 1 x MasterMix (Jena Bioscience, Jena, Germany), 1 µM primers (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany), 0.2 % BSA (Hersteller, Ort, Land), 10 µl DNA were added including 450 GE/µl. Reverse primers were applied with 5´-biotin-labelling including a 15 atomar spacer TEG. An overview about the applied templates and primer sequences is given in Supplementary Information Table 4.S2. Previously to amplification, an initial denaturation step with 95 °C for 10 min was carried out. PCR was performed with 41 cycles of 15 s at 95 °C and 60 s 60 °C for most of the targets using a Mastercycler nexus thermocycler (Eppendorf AG, Hamburg, Germany). For amplification of the bcsp31 target from *Brucella melitensis* the temperature for annealing and elongation was set to 57 °C and for amplification of the fliC target from *Burkholderia mallei* a three step protocol was applied consisting of 15 s 95 °C, 30 s 50 °C and 15 s 72 °C. Amplicons were verified via electrophoresis using 2 % agarose Gel in 1x TBE for 60 min at 200 V.

Preparation of ssDNA PCR product

Capture of the biotinylated PCR product (biotinylated forward primer) using the SiMAGstreptavidin-coated magnetic beads by chemicell^{TM} was carried out according to the manufacturer's instructions. Firstly, 150 L of magnetic beads was washed to remove any preservatives by 3 consecutive washings with 1X B&W buffer (5 mM Tris-HCl pH 7.5, 0.5 mM EDTA and 2 M NaCl). Between each washing step, Eppendorf tubes containing the solution with the magnetic beads were placed in contact with a magnet for 2 min and the supernatant was removed by aspiration with a micro-pipette. The isolated magnetic beads were subsequently resuspended with 100 µl of biotinylated PCR product and the same volume of 2X B&W buffer and incubated for 30 min at room temperature with gentle rotation. Following immobilisation of the biotinylated PCR product on the streptavidin magnetic beads, the Eppendorf tubes were again placed in contact with a magnet for 3 mins in order to discard the supernatant and the isolated beads were washed three times with 1X B&W buffer. Separation of ssDNA was performed by alkaline denaturation [26]. This procedure has been done to eight biotinylated PCR products separately. The single-stranded DNA amplicons generated were characterised using gel electrophoresis.

Preparation of ssDNA labelled with horseradish peroxidase as reporter probe

Eight different designed thiolated reporter probes were purified prior to conjugation experiment to eliminate any preservatives present that would affect the efficiency of conjugation. The purified thiolated reporter probes were added separately to Maleimide activated horseradish peroxidase (Maleimide HRP) to form a final concentration of 24:1 (Maleimide HRP:DNA ratio) and incubated for 90 min at 37 °C, after which 2-mercapto ethanol was added to a final concentration of 0.0015 M to stop the reaction. The final products were purified using a YM10 KDa cut-off microcon and were washed with buffer and were stored at -20 °C at 50% glycerol.

Enzyme linked oligonucleotide assay (ELONA) evaluation on cross-reactivity and specificity of designed probes

Eight (8) different designed thiolated capture probes (1 M in carbonate buffer) were prepared separately and were added to each well of a NUNC maleimide plate and incubated for 30 min at 37 °C. Following thorough washing with PBS-Tween 20 (pH 7.4, 0.01 M), the plate was then blocked by addition of 200 L of 1mM mercaptohexanol (MCH) in PBS-Tween 20 (pH 7.4, 0.01 M) and incubated for 1 h at 37 °C, followed by thorough washing of the plate. Genorecognition step was carried in three ways: 1) individual assay detection, 2) mixed complementary target where 50 L each at 5nM of each synthetic complementary target and 3) mixed HRP-labelled ssDNA reporter probe that has been prepared in PBS-Tween 20 (pH 7.4, 0.01 M) were added to each well coated with capture probes. The plate was again incubated, under shaking conditions for 30 min at 37 °C, and subsequently thoroughly washed with PBSTween 20, prior to exposure to 50 nM of DNAHRP conjugates as a secondary labelled ssDNA in individual assay detection and mixed reporter probes and again left to incubate under shaking conditions for 30 min at 37 °C. After a final wash, 50 L of TMB for ELISA substrate was added to each well and product formation were allowed to proceed for at least 15 min at room temperature. The reaction was finally stopped by addition of 1 M H_2SO_4, and the absorbance read at 450 nm. Analysis was carried out in triplicate.

2. 5. Probe immobilisation on electrode array

Prior to modification of the electrode arrays, a two-step cleaning protocol was applied. Initially in order to remove the protective resist used during storage, the arrays were sonicated for 5 min in acetone, 5 min in iso-propanol (3 times) and rinsed with water. In a second step, electrochemical cleaning was performed in 0.5 M H_2SO_4 by application of a constant potential of 1.6 V for 10 s followed by 40 voltammetric cycles in the potential range −0.2 to 1.6 V at a scan rate of 0.3 $V.s^{-1}$. Finally, the electrodes were rinsed with Milli-Q water and dried with nitrogen. Modification of the cleaned electrode arrays was carried out via co-immobilization of the specific thiolated probe (1 M) and DT1 (100 M) in 1 M KH_2PO_4 aqueous solution (pH 3.5) by deposition of 1 L of the mixture over the working electrodes for 3 h at room temperature in a humid (>90%) environment. Dithiol DT1 was co-immobilized with the thiolated probe in order to eliminate nonspecific binding of the labelled reporter probe, whilst also spacing out and orientating the probe to facilitate efficient hybridization of the target. In order to remove the non-attached molecules, the electrode arrays were washed in a stirring solution of 0.1 M PBS–Tween for 20 min, rinsed with water and dried with nitrogen.

2. 6. Electrochemical DNA detection

DNA detection of both synthetic oligonucleotides and PCR product from bacterial cell samples were performed in a sandwich hybridization format. In the developmental work, construction of a typical calibration curve of the genosensor as a model system has been done using the synthetic complementary target. A typical target of various concentrations of *F. tularensis* ranging from 0 to 10 nM (in triplicate) in 0.1 M hybridisation buffer (0.1 M Trizma buffer in 0.15 M NaCl, pH 7.4) were deposited on the oligonucleotide modified gold electrodes and incubated for 20 min at room temperature. The sensors were subsequently washed for 15 min, under stirring conditions, in 0.1 M PBS–Tween and then dried with nitrogen. A second hybridisation was performed by spotting 0.5 L of 10 nM labelled reporter in hybridisation buffer and incubating for another 20 min at room temperature with both hybridisations carried out in a humid environment. The hybridized microarray was subsequently washed with 0.1 M PBS–Tween for 15 min and dried in nitrogen. For real sample analysis, the modified electrodes were then exposed to known concentration of the ssDNA generated from PCR product quantified using Nanodrop^{TM}, in hybridisation buffer, and incubated for (2-20 min) and then incubated for a defined period of time (2-20 min) with the corresponding horseradish peroxidase labelled secondary ssDNA.

The detection process was carried out in the microfluidic channels in the presence of TMB substrate where the HRP-catalysed reduction of TMB [27-32] and was detected by steps and sweeps technique by applying two consecutive potential steps of 0 V for 1 ms and −0.2 V for 0.5 s. All the electrochemical measurements were performed at room temperature. The overall immobilization process and detection mechanism can be seen in Figure 1.

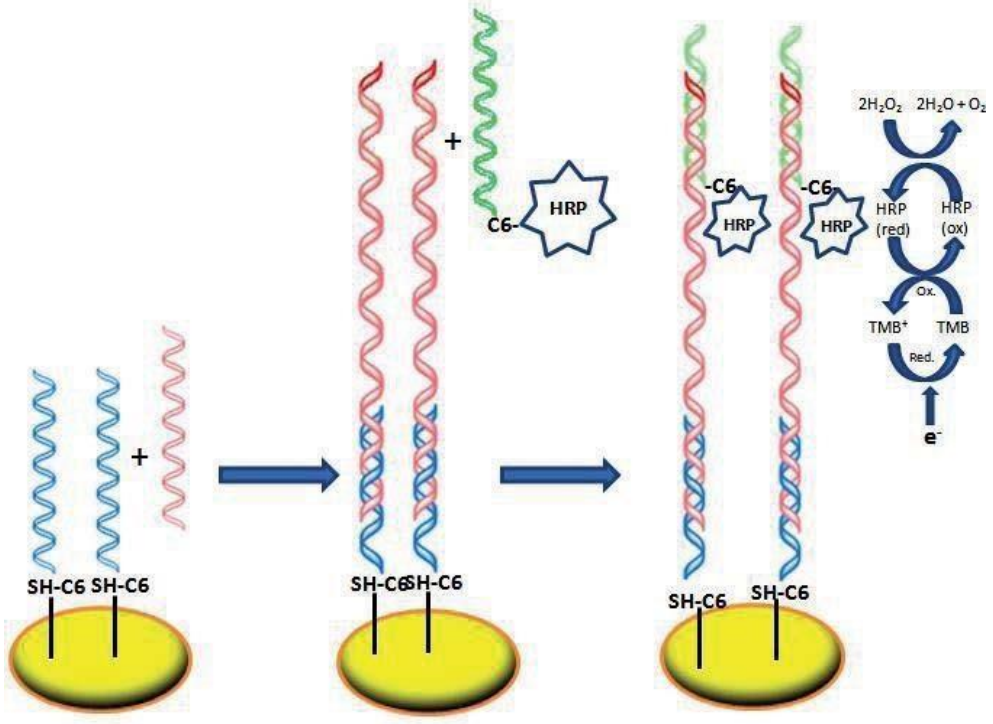

Figure 1. Schematic representation on the immobilization of thiolated ssDNA and its hybridization process to complete the sandwich assay format illustrating how the electroactive species detected into electrode surface.

3. MATERIALS AND METHODS

DNA Sequences

Table 1. S1. ssDNA sequences

Specie	Capture probe, (5'-3')	Complementary target, (5'-3')	Reporter probe, (5'- 3')
Francisella tulrensis Holarctica	**CTTAGTAATT GGG AAGCTTGTAT CAT GGCACTTAGA A**	AAGGAAGTGTAAG*ATTACAATGG CAGGCTCCA GA*AGG**TTCTAAGTGCCATGATA CAAGCTTCCC AATTACTAAG**TATGCTGAGAAG AACGATAAAA CTTGGGCAACTGTAACAGTT	*TCTGGAGCCTGCCATT GTAAT*
Bacillus thuringiensis berliner *Kurstaki*	**AGCGGAAACG TGA ATTCTGG**	AGGGCATCAAATA*ATGGCTTCTC CTGTCGGTTTT TC*GGGG**CCAGAATTCACGTTTC CGCT**ATATGG AACCATGGGAAATGCAGCTCCAC AACAACGTAT TGTTGCTCAACTAGGTC	*GAAAAACCGACAGG AGAAGCCAT*
Yersinia pestis	**ACTGGCCTGC AAG TCCAATATAT GGC AT**	CCCGAAAGGAGTGCGG*GTAATAG GTTATAACC AGCGCTT*TTCT**ATGCCATATATT GGACTTGCAG GCCAGT**ATCGCATTAATGATTTT GAGTTAAATG CATTATTTAAATTCAGCGACTGG GTTCGGGCAC ATGATAATGATGAGCACTATATG AGAGATCTTA CTTTCCGTGAGAAGACATCCGGC TCACGTTATT ATGGTACCGTAATTAACGCTGGA TATTATGTCA CACCTAATGCCAAAGTCTTTGCG GAATTTACAT ACAGTAAATATGATGAGGGCAA AGGAGGTACT C	*AAGCGCTGGTTATAA CCTATTAC*
Bacteriophage Lambda	**TTATAAATCTG CT CTTTCGCGGT**	CCCCATTAAAGGGGCATCCGTCT ACGGAAAGC CGGTGGCCAGC*ATGCCACGTAAG CGAAACA*AA AACGGGGTTTACCTTACCGAAAT CGGTACGGA **TACCGCGAAAGAGCAGATTTAT AA**CCGCTTCAC ACTGACGCCGGAAGGGGATGAA CCGCTTCCCG	*TGTTTCGCTTACGTGG CAT*

		GTGCCGTTCACTTCCCGAATAAC CCGGATATTT TTGATCTGACCGAAGCGCAGCAG CTGACTGCT GAAGAGCAGGTCGAAAAATGGG TGGATGGCA GGAAAAAAATACTGTGGGACAG CAAAAAGCGA CGCAATGAGGCACTCGACTGCTT CGTTTATGCG	
		CTGGCGGCGCTGCGCATCAGTAT TTCCCGCTG GC	
Coxiella burnetii	**AACGTCCGAT ACC AATGGTTCGC T**	GCTCAGTATGTATCCACCGTAGC CA*GTCTTAAG GTGGGCTGCGTG*GTGATGGAAGC GTGTGGAGG **AGCGAACCATTGGTATCGGAC GTT**TATGGGGA TGGGTATCCCAACGCAGTTGATC AGTCCGCAG CACGTCAAACCGTATGTCAAAAG TAACAAGAAT GATCGTAACGATGCGCAGGCGAT AGCTGAAGC GGCTTCCCGCGCCTCGATGCGGT TTGTGCAGG GTAAAACGGTGGAACAACAAGA CGTTCAAGCG CTGTTAAAGATACGCGATCGTTT AGTCAAAAGC CGCACGGCGCTGATCAATGAGAT TCGGGGGTT GTTGCAAGAATACGGACTCACGA TGGCGCGTGG	*CACGCAGCCCACCTT AAGAC*
Bacillus anthracis	**ATTTGCGGTA ACA CTTCACTCCA GTT CGAG**	CAATTAAGATTAGATA*CGGATCA AGTATATGG GAATATAGCAA*CATACAATTTTGA AAATGGAA GAGTGAGGGTGGATACAGG**CTC GAACTGGAG TGAAGTGTTACCGCAAAT**TCAA GAAACAACTG CACGTATCATTTTTAATGGAAAA GATTTAAATC TGGTAGAAAGGCGGATAGCGGC GGTTAATCCT AGTGATCCATTAGAAACGACTAA ACCGGATAT GACATTAAAAGA	*TTGCTATATTCCCATA TACTTGATCCG*

Burkholderia mallei	**GCCGTCGACG ACA GCGCCTGGTT**	TGTCGGACGGC*AAGGGCGGCTTC ACGTTCA*CC GATCAGAAC**AACCAGGCGCTGT CGTCGACGGC** CGTGACCGCCGTGTTCGGCTCGT CGACCGCCG GCACGGGCACGGCGGCCTCGCC GTCGTTCCAG ACGCTGGCGCTGTCGACTTCGGC AACCAGCGC GCTGTCCGCGACGGACCAGGCG AACGCCACGG CGATGGTTGCGCAGATCAACGCG GTCAACAAG CCGCAAACGGTCTCGAACCTCGA CATCAGCACG CAGACGGGCGCGTACCAGGCGA TGGTATCGAT CGACAAC	*TGAACGTGAAGCCGC CCTT*
Brucella melitensis	**AAATCTTCCA CCT TGCCCTTGCC ATC A**	GTCTCGTCGCGACGGCCGTTTCG TCGAATG*GCT CGGTTGCCAATATCAATGC*GATCA AGTCGGGC GCTCTGGAGTCCGGCTTTACGCA GTCAGACGT TGCCTATTGGGCCTATAACGGCA CCGGCCTTTA **TGATGGCAAGGGCAAGGTGGA AGATTT**GCGCC TTCTGGCGACGCTTTACCCGGAA ACGATCCATA TCGTTGCGCGTAAGGATGCAAAC ATCAAATCG GTCGCAGAC	*GCATTGATATTGGCA ACCGAGC*

Table 2. S2. Overview about applied bacteria strains and PCR assays

Specie	Target	Primers (5´-3´) forward, reverse	Amplicon size	Reference
Francisella tulrensis Holarctica	Tul4	ATTACAATGGCAGGCTCCAGA TGCCCAAGTTTTATCGTTCTTCT	101 bp	Versage et al 2003[1]
Bacillus thuringiensis berliner Kurstaki	cryT	ATGGCTTCTCCTGTAGGGTTTTC GCTGCATTTCCCATGGTTCCA	71 bp	Matero et al. 2011[2]
Yersinia pestis	pla	GTAATAGGTTATAACCAGCGCTT AGACTTTGGCATTAGGTGTG	232 bp	Riehm et al. 2011[3]

Bacteriophage Lambda	gp17	ATGCCACGTAAGCGAAACA GCATAAACGAAGCAGTCGAGT	278 bp	Riehm et al. 2011[3]
Coxiella burnetii	IS1111	GTCTTAAGGTGGGCTGCGTG CCCCGAATCTCATTGATCAGC	295 bp	Klee et al 2006 [4]
Bacillus anthracis	pag	CGGATCAAGTATATGGGAATATAGCAA CCGGTTTAGTCGTTTCTAATGGAT	204 bp	Ellerbrok et al. 2002 [5]
Burkholderia mallei	fliC	AAGGGCGGCTTCACGTTCA GTGCTGATGTCGAGGTTCGAGA	141 bp	Tomaso et al. 2004[6]
Brucella melitensis	bcsp31	GCTCGGTTGCCAATATCAATGC GGGTAAAGCGTCGCCAGAAG	151 bp	Probert et al. 2004[7]

4. CONCLUSIONS

In this work, an electrochemical genosensor array that allows simultaneous detection of different biowarfare agents with integrated microsystem that provides an easy handling of the technology which combines with microtube fluidics setup has been developed and optimised for the following specific genoassay: *Bacillus anthracis, Brucella melitensis, Bacteriophage lambda, Francisella tularensis, Burkholderia mallei, Coxiella burnetii, Yersinia pestis,* and *Bacillus thuringiensis var. kurstaki*. The chip electrodes arrays were modified via coimmobilisation of a 1:100 (mol/mol) mixture of a thiolated probe and a polyethyleneglycolterminated bipodal thiol. PCR products from these relevant biowarfare agents were detected reproducibly through a sandwich assay format with the target hybridised between a surface immobilised probe into the electrode and a horseradish peroxidase-labelled secondary reporter probe, which provided an enzyme based electrochemical signal. The potential of the designed microsystem for multiplexed genosensor detection and crossreactivity studies over potential interfering DNA sequences has demonstrated high selectivity using the developed platform producing high-throughput.

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