Chapter 1

Carbapenem-Resistant *Enterobacteriaceae* Testing in 45 Minutes Using an Electronic Sensor

Neil Gordon, BEng, MBA,[a] Raj Bawa, MS, PhD[a,b,c] Garry Palmateer, MSc,[a] Mehdi Rajabi, PhD,[a,d] Jesse B. Gordon,[e] Noor M. Kotb,[f] Rakshika Balasubramaniyam,[g] and Benjamin R. Gordon[h]

[a]*Guanine, Inc., Rensselaer, New York, USA*
[b]*Patent Law Department, Bawa Biotech LLC, Ashburn, Virginia, USA*
[c]*The Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Albany, New York, USA*
[d]*Kinentia Bioscience LLC, Rensselaer, New York, USA*
[e]*Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA*
[f]*Department of Biomedical Sciences, University of Albany, Albany, New York, USA*
[g]*Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, New York, USA*
[h]*Department of Microbiology and Immunology, McGill University, Montreal, Quebec, Canada*

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1.1 Introduction

Antibiotic-resistant bacterial infections are becoming a public health crisis in the United States and worldwide. In the United States alone, antibiotic-resistant bacteria are responsible for at least 2.8 million infections and 35,000 deaths annually [1]. The Centers for Disease Control and Prevention (CDC) has led a series

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of actions involving education, surveillance, and antibiotic stewardship that have reduced antibiotic-resistant infections and deaths by 18% in communities and 28% in hospitals. The breakdown of infections and deaths is supplied in the CDC report for seven antibiotic-resistant bacteria for 2012 and 2017 and is summarized in Table 1.1. The data show that the CDC actions succeeded in reducing illnesses and deaths across all antibiotic-resistant bacteria, except for *Enterobacteriaceae* such as *Klebsiella pneumoniae* and *Escherichia coli.* Of particular concern are infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) since carbapenems are considered the drug of last resort with limited new treatments on the horizon [2].

Table 1.1 Changes in infections and deaths from antibiotic resistant bacteria in 2012 and 2017

Antibiotic-resistant bacteria	2012 infections	2017 infections	% Variance	2012 deaths	2017 deaths	% Variance
Enterobacteriaceae increases in infections and deaths						
ESBL-producing *Enterobacteriaceae*	131,900	197,000	49%	6,300	9,100	44%
Carbapenem-resistant *Enterobacteriaceae*	11,800	13,100	11%	1,000	1,100	10%
Non-Enterobacteriaceae decreases in infections and deaths						
Methicillin-resistant *Staphylococcus aureus*	401,000	323,700	–19%	13,600	10,600	–22%
Drug-resistant Candida	44,800	34,800	–22%	2,200	1,700	–23%
Carbapenem-resistant Acinetobacter	11,700	8,500	–27%	1,000	700	–30%
Drug-resistant *Neisseria gonorrhoeae*	46,000	32,600	–29%	3,900	2,700	–31%
Vancomycin-resistant Enterococcus	84,800	54,500	–36%	8,500	5,400	–36%

Note: Data based on the CDC Report [1].

Abbreviation: ESBL, extended-spectrum beta-lactamases.

Carbapenems are highly effective and are often the only treatment option for severe bacterial infections. Some CREs possess genes that produce carbapenemase enzymes, including KPC, New Delhi metallo-β-lactamase (NDM), oxacillinase-48 (OXA-48), Verona integron-encoded metallo-β-lactamase (VIM), and imipenemase (IMP) [3]. These carbapenemase enzymes allow CREs to survive carbapenem treatments by hydrolyzing the carbapenem's β-lactam ring thus deactivating the molecule's antibacterial properties. Some CREs have developed additional resistance mechanisms including redundant β-lactamases, genes conferring resistance to other antimicrobial classes, chromosomal porin mutations that prevent accumulation of β-lactam agents, and over-expression of efflux pumps that extrude β-lactam agents [4–6]. Carbapenemases are commonly expressed from

mobile genetic elements such as plasmids or transposons, which can be acquired and passed on through horizontal gene transfer, making CP-CREs extremely virulent and highly resistant to any therapy.

Timely treatment of CP-CRE infections is critical and is most successful when administered at the early stage of the infection when the bacteria are at a low concentration. A study of over 50,000 patients hospitalized with complicated urinary tract infections, complicated intra-abdominal infection, hospital-associated pneumonias, or bloodstream infections determined that CRE patients whose treatment is delayed by 2 or more days have double the hospital costs and a fourfold increased risk of mortality compared with timely treatment (Table 1.2) [7–9].

Table 1.2 Impact of delayed therapy on CRE infection

Infection	Timely appropriate therapy	Delayed appropriate therapy by 2+ days
Hospital cost/patient		
CRE infection	$9,875	$25,506
Non-CRE infection	$11,539	$21,828
Risk of mortality or discharge to hospice		
CRE infection	0.9%	3.7%
Hospital stay		
CRE infection	5.1 days	8.5 days

Note: Data based on [7, 8].

Abbreviation: CRE, carbapenem-resistant *Enterobacteriaceae*.

Despite the need for timely treatment of CP-CRE infections, the current turnaround time for detecting CP-CRE, including all approved antimicrobial susceptibility testing (AST) assays for CRE, is 2 to 4 days as noted on Table 1.3. These include broth microdilution, matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, real-time polymerase chain reaction (RT-PCR), microarray, multidisc mechanism testing, gradient minimum inhibitory concentration (MIC) strip (Etest), and Carba NP. Virtually all CP-CRE detection methods require a time-intensive bacteria culture to isolate and produce a measurable quantity of bacteria for testing as the minimum threshold of detection is well above concentrations found in human samples. CP-CRE detection tests also vary by their availability in clinical labs, accuracy, ease of use, cost, and need for specialized equipment [10–12]. Based on the increasing prevalence of antibiotic-resistant CRE infections [1], the rise of new resistance strains and the lack of new antimicrobials planned for the market, the best defense against antibiotic-resistant CRE would be a rapid, simple, inexpensive, and accurate test that could be used in essentially any healthcare setting.

Table 1.3 Antimicrobial susceptibility testing methods for detecting CP-CRE

	Time from sample to result		
CP-CRE detection method	**Isolation culture**	**Test process**	**Total**
Quadruplex hybridization assay	**None**	**45 min**	**45 min**
Broth microdilution	1 –2 days	1–2 days	2 –4 days
MALDI-TOF	1 –2 days	< 1 day	2 –3 days
Real-time PCR	1 –2 days	< 1 day	2 –3 days
Microarray	1 –2 days	< 1 day	2 –3 days
Multidisc mechanism testing	1 –2 days	2 days	3 –4 days
Gradient MIC strip (Etest)	1 –2 days	2 days	3 –4 days
Carba NP	1 –2 days	< 1 day	2– 3 days

Note: Data based on [10–12].

Abbreviations: CP-CRE, carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; MIC, minimum inhibitory concentration; PCR, polymerase chain reaction.

1.1.1 Approach

CP-CRE detection was performed using a sandwich hybridization assay. Nucleic acid targets were captured with a magnetic microparticle containing oligonucleotides with guanine detection tags and capture probes complementary to a segment of the target. A second portion of the target hybridizes to a recognition layer of oligonucleotides on a biosensor electrode (Fig. 1.1). A voltammetry technique produces an electrical signal from the redox properties of the detection tags bound to the particle. In order to overcome the poor detection limits currently encountered with nucleic acid biosensors, guanine nucleotides are employed as 20-mer polyguanine oligonucleotide detection tags and millions of detection tags are conjugated to the microparticle to amplify the detection signal. The 20-mer polyguanine oligonucleotides are pre-fabricated into guanine-quadruplexes which generate 8-oxoguanine oxidization signals at ~0.47 V for improved signal-to-noise resolution over guanine oxidation signals. The microparticle has a magnetic core which allows the nucleic acid targets and detection tags to be magnetically separated from nonspecific materials that cause false detection signals and degrade the signal-to-noise resolution.

The detection method used in the experiments provides a new paradigm for electrochemical detection of nucleic acids [13–17]. Electrochemical detection is an appealing technique because of its rapid, simple, and inexpensive applications in measuring redox chemicals such as glucose. Electrochemical biosensors for detecting redox nucleotides such as guanine have not attained widespread appeal because of their inability to achieve low detection limits necessary for clinical applications.

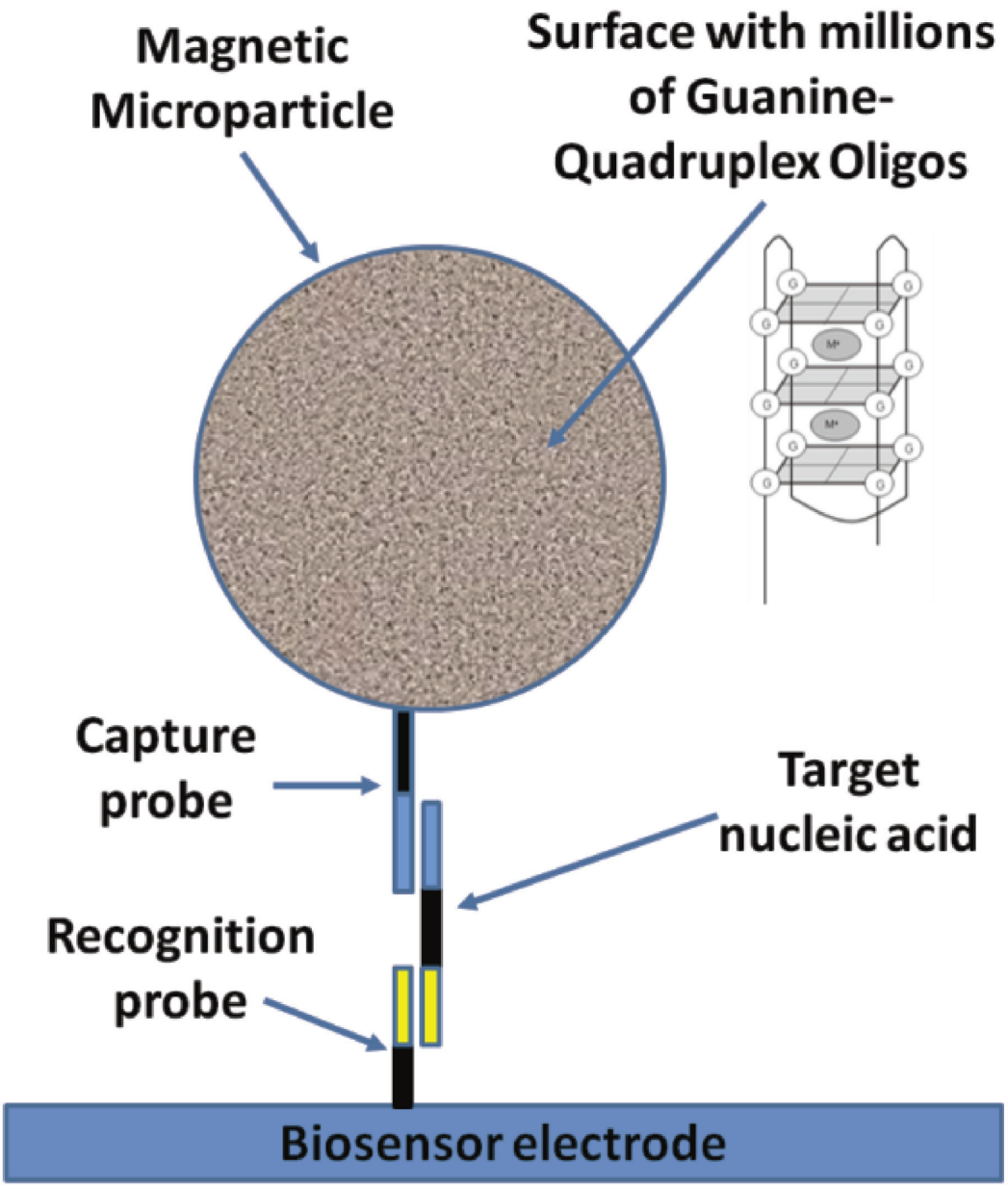

Figure 1.1 Quadruplex sandwich hybridization assay components [13, 14].

1.2 Materials and Methods

1.2.1 Isolates

Study isolates included three quality control (QC) strains of CP-CRE that are available to clinical laboratories: *Klebsiella pneumoniae* ATCC BAA 1705 (KPC-2),

Klebsiella pneumoniae ATCC BAA-2814 (KPC-3), and *E. coli* ATCC BAA 2340. Non-carbapenemase-producing *Klebsiella pneumoniae* ATCC 13883 was also evaluated. The study tested 28 samples from 22 carbapenemase-producing sample, 4 non-carbapenemase-producing samples, and 2 samples with no bacteria.

Table 1.4 Identification of CP-CRE carbapenemase tested

Bacteria	Carbapenemase	Identification
K. pneumoniae	KPC-2	ATCC BAA-1705
K. pneumoniae	KPC-3	ATCC BAA-2814
E. Coli	KPC	ATCC BAA-2340
K. pneumoniae	None	ATCC 13883

Abbreviations: CP-CRE, carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*; KPC, *Klebsiella pneumoniae* carbapenemase; KPC-2 *Klebsiella pneumoniae* ATCC BAA 1705; KPC-3, *Klebsiella pneumoniae* ATCC BAA-2814.

1.2.2 Probe Design

Detection probes were designed to hybridize with targets associated with the bla_{KPC} gene. Suitable targets were identified as the 16S rRNA gene (GenBank: X87276.1) associated with strain ATCC BAA-1705 and corresponding to bla_{KPC-2} and with strain ATCC BAA-2814 corresponding to bla_{KPC-3} (ATCC, Manassas, VA). As illustrated in Fig. 1.2, nucleotide sequences of ~50 bases were used for capture and recognition. The recognition sequence was selected at the 3′ end and the capture sequence was selected a few hundred bases from the 3′ end. This configuration produced a larger oxidation signal peak than binding at the 5′ end because the quadruplexes were closer to the biosensor. The capture probe also contained a 20-mer polyguanine sequence on the same oligonucleotide (Table 1.5). Polyguanine was transformed into guanine-quadruplexes by heating the oligonucleotides in a sodium acetate–formamide solution.

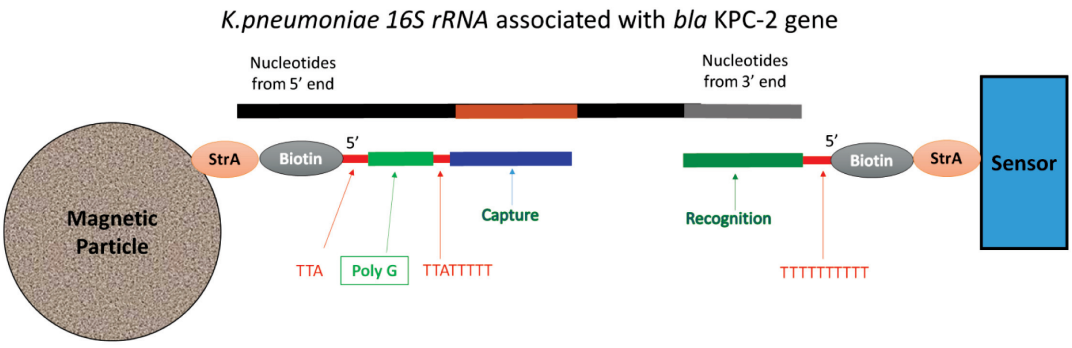

Figure 1.2 A *K. pneumoniae* 16S rRNA associated with bla_{KPC-2} forms a sandwich hybridization assay with a magnetic particle conjugated with a polyguanine capture probes and a biosensor conjugated with a recognition probe.

The recognition and capture probes are biotinylated. The capture probes were conjugated to streptavidin-coated magnetic microparticles (Bangs Laboratories, Fishers, IN) and the recognition probes were conjugated to unique working electrodes on a 96-well streptavidin-coated carbon biosensor microtiter (DropSens, Llanera, Spain) using streptavidin–biotin interactions.

Table 1.5 Sequences for detecting bla_{KPC-2} gene from *K. pneumoniae* 16S rRNA

Target	Sequence ID.	ssOligo Probe	
KPC-2 16s rRNA (ATCC BAA 1705)	GenBank: X87276.1	5′ Biotin-TTA GGGGGGGGGGGGGGGGGG TTATTTTT CGGCCCTTGA GTTTCCTCTG ACGGTCACTA TTTGACCTCC TTCCACCCCT ACTGCAGTTC AGTAGTACCG 3′	Capture
		5′ Biotin-TT TTT TT AAAG GAGGTGATCC AACCGCAGGT TCCCCTACGG TTACCTTGTT ACGACTTCAC CCCAGTCATG AATCACAAAG TGGTAAGCGC CCTCCC 3′	Recognition

Abbreviation: KPC-2 *Klebsiella pneumoniae* ATCC BAA 1705.

1.2.3 Filter Concentration and Lysis

Bacteria were prepared in tryptic soy agar medium (Becton, Dickinson (BD), Franklin Lakes, NJ) and serial diluted in broth medium and commercial urine (Sigma-Aldrich, St. Louis, MO) to 10^4 cfu/mL. Samples were concentrated with a 0.45 μm filter (Pall, Port Washington, NY) and re-suspended in biology-grade water and incubated at room temperature for 15 min. The β-lactam antibiotic meropenem was added to the resuspension to assess if meropenem caused a signal increase from the KPC-producing bacteria. Meropenem doses ranged from 0 μg/mL to 2048 μg/mL. Bacteria were then lysed in 200 μL lysis buffer consisting of 2 M guanidinium thiocyanate (GTC), 80 mM beta-mercaptoethanol (BME), 25 mM sodium citrate, 20 μ g/ml of glycogen (pH 6) with 5 μL dimethyl sulfoxide (1%) then incubated at room temperature (RT) for 5 min.

1.2.4 Magnetic Separation

The solution was mixed for 5 min with 7 μL of 1.5 μm streptavidin-coated magnetic particles conjugated with quadruplex tags and DNA detection probes (Integrated DNA Technologies (IDT), San Jose, CA), then incubated at RT for 10 min. Samples were placed in a magnetic separation microtiter (Epigentek, Farmingdale, NY) and a magnet was applied for 2 min; then the supernatant was discarded. The magnet was removed and the magnetic particle complexes were washed with 100 μL 80 mM sodium acetate (pH 9). The magnetic particle complexes were then re-suspended in sodium acetate and allowed to hybridize for 10 min

at RT on a streptavidin-coated graphite biosensor microtiter conjugated with capture probes and enable sandwich structures to form as illustrated in Fig. 1.1.

Prior to the experiments, different sized particles were evaluated for their corresponding 8-oxoguaine signals. The 100 nm and 350 nm magnetic particles (Micromod, Rostock, Germany) did not provide measurable 8-oxoguanine oxidation signals and the particles appeared to have decomposed during the test process. The 500 nm, 750 nm and 1.5 μm streptavidin-coated magnetic microparticles (Bangs Laboratories, Fishers, IN) were all successfully able to measure 8-oxoguanine oxidation signals. The 1.5 μm particles generated the strongest signals and were thus used in the experiments.

1.2.5 Amperometric Voltammetry

The biosensor microtiter was connected to a potentiostat (PalmSens). A square wave voltammetry scan produces a peak electrical current at ~0.47 V from 8-oxoguanine oxidation in sodium acetate (pH 9) (Fig. 1.3A). A voltammetry scan was made with sodium acetate (pH 9) before the magnetic particle-RNA-biosensor sandwiches were formed to provide a baseline signal. The net 8-oxoguanine oxidation signal was determined to be the difference between the 8-oxoguanine oxidation signal in sodium acetate and sodium acetate signal (Fig. 1.3B).

The assay was initially developed with the 96-well carbon microtiter sensors and was coated in the lab with streptavidin. Each sensor was evaluated for a baseline signal with 80 mM sodium acetate (pH 9) to ensure proper sensor functionality. The 8-oxoguanine oxidation signal generated for the same sample varied by 2–10% between sensors. When the development work switched to microtiter sensors pre-coated with streptavidin, the variability increased by up to 40%. Variability was reduced by subtracting the baseline sodium acetate buffer signal measured prior to hybridization, from the 8-oxoguanine oxidation signal measured on the same sensor. This reduced the same sample variation to 3–16% between sensors. The pre-coated microtiter sensor displayed a new peak at 0.15 V, which did not occur when streptavidin was coated in the lab. This new peak was likely due to oxidation from residual chemicals from the streptavidin coating process employed by DropSens. It was observed that the 0.15 V peak varied in amplitude on different sensors and in some cases 10 or more baseline scans were needed to remove the 0.15 V peak before the sensor could be used for hybridization.

Square wave voltammetry (SWV) scan parameters were optimized for 8-oxoguanine in advance of the KPC experiments by adsorbing quadruplex oligonucleotides on the sensor surface and applying SWV scans. Variations of SWV settings were evaluated to find the setting that produced the greatest 8-oxoguanine oxidation signal peak. This setting was then used for KPC assay development.

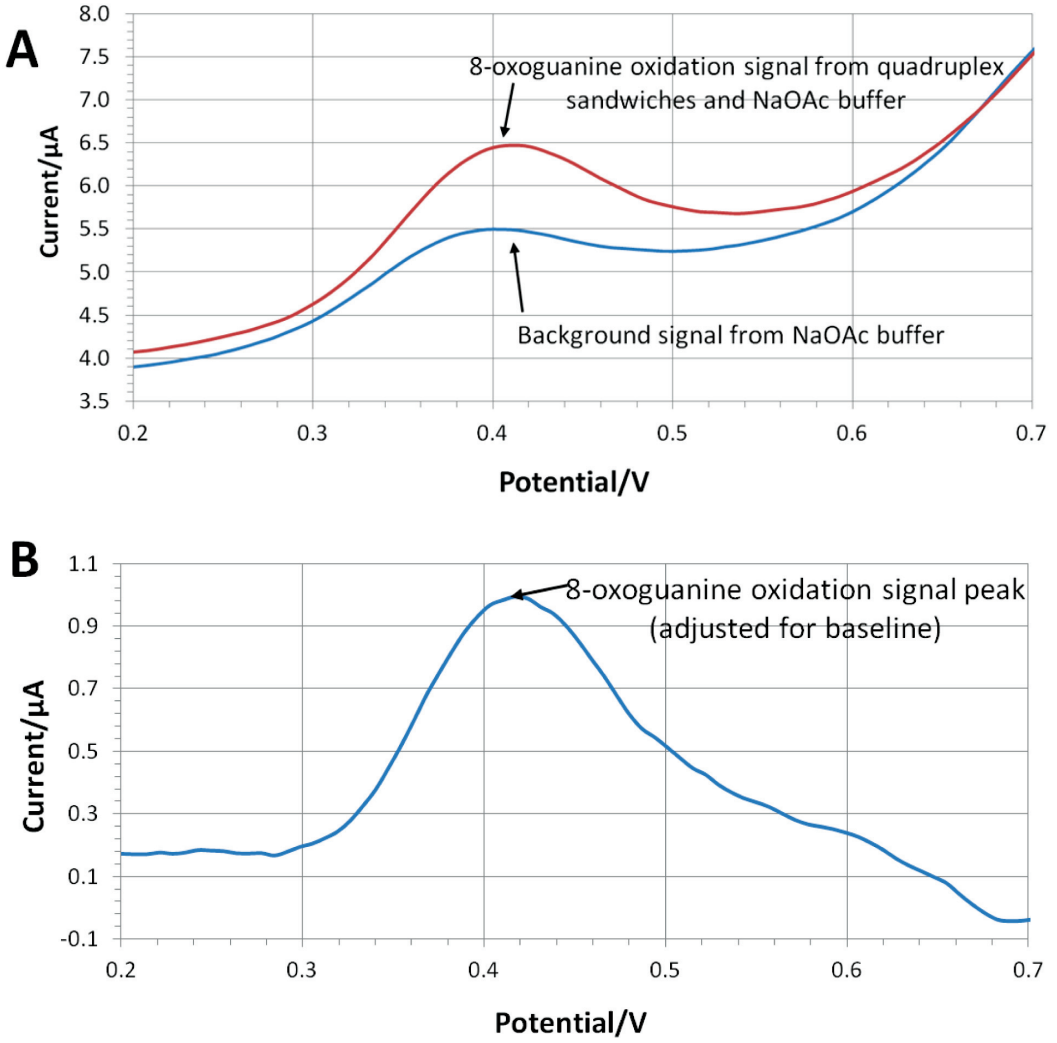

Figure 1.3 (A) Voltammetry scan of quadruplex 8-oxoguanine oxidation current peak in sodium acetate (pH 9) with a peak current signal at ~0.47 V and the background signal from sodium acetate (pH 9) on the same sensor before the sandwiches were formed, and (B) the net signal calculated from the difference of the two scans in A.

1.3 Results

Twenty-eight samples were tested for the presence of KPC enzymes from CP-CRE and non-CP-CRE. The results are summarized in Table 1.6. All 22 samples containing CP-CRE generated a positive signal which was greater than the 0.2 µA background baseline. Of the 6 samples containing the susceptible *K. pneumoniae* or no bacteria, 5 reported true negative with the signal below the threshold.

The normalized 8-oxoguanine oxidation signal peak at ~0.47 V was used to detect the presence of carbapenemase KPC enzymes as illustrated in Fig. 1.4. One false positive occurred with a non-KPC-producing *K. pneumoniae* and was likely caused by poor sensor calibration.

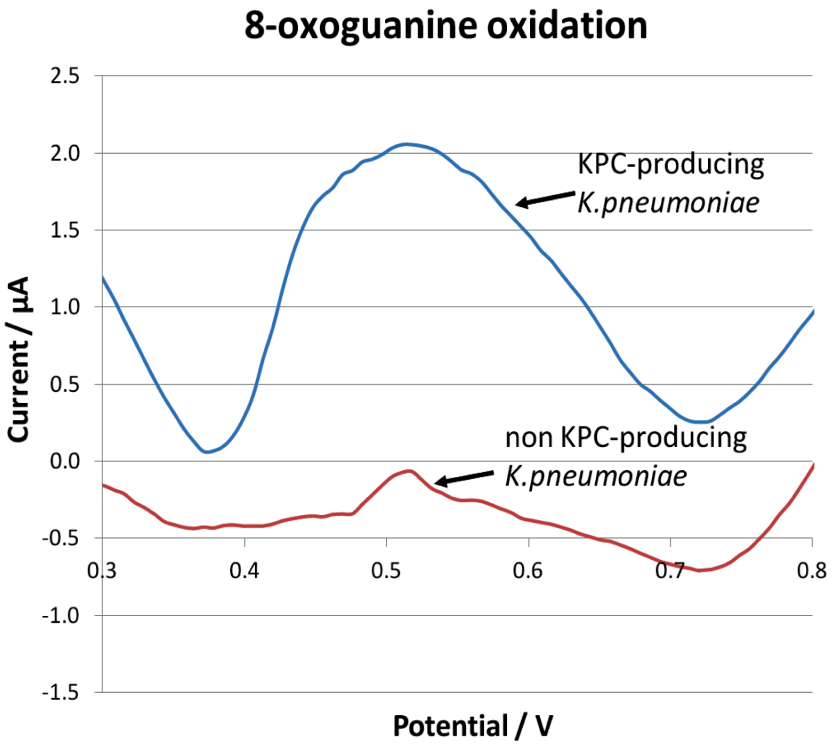

Figure 1.4 Voltammetry scan of quadruplex 8-oxoguanine oxidation signal from KPC-producing and non-KPC-producing *K. pneumoniae* after baseline scan subtraction.

The magnitude of the KPC signal was measured for each CP-CRE species. As shown in Table 1.7, all three species reported an average signal greater than the threshold but with different average signal magnitudes: 1.2 µA for the KPC-2 species, 1.1 µA for the KPC-3 species, and 0.4 µA for the *E. coli* KPC species. The strain that did not produce KPC had an average signal of 0.1 µA, which was below the threshold detection value. The magnitude of the KPC signal was also measured for samples resuspended in meropenem for the ATCC BAA 1705 (KPC-2) and ATCC BAA-2814 (KPC-3) species. High dose exposure between 512 µg/mL and 2048 µg/mL meropenem produced an average signal of 1.2 µA, while low dose exposure between 0 µg/mL and 128 µg/mL (Table 1.8) meropenem produced an average signal of 1.1 µA. Based on the test results, there appeared to be no relationship between meropenem exposure and the KPC signal. Testing with bacteria samples in broth and urine provided near identical results suggesting that filtered urine followed by magnetic separation had no impact on hybridization or 8-oxoguanine oxidation signal generation.

Table 1.6 Summary of samples tested for KPC

	True outcome	False outcome	
Positive test prediction	22	1	PPV = 22/23
Negative test prediction	0	5	NPV = 5/5
	Sen. = 22/22	Spec. = 5/6	

Abbreviation: KPC, *Klebsiella pneumoniae* carbapenemase.

Table 1.7 KPC signal amplitude by CP-CRE species

CP-CRE species	Average signal
ATCC BAA 1705 (KPC-2) (8)	1.2 μA
ATCC BAA-2814 (KPC-3) (12)	1.1 μA
ATCC BAA-2340 (*E. coli* KPC) (2)	0.4 μA
ATCC 13883 (Non-KPC) (4)	0.1 μA
No Bacteria (2)	0.1 μA

Abbreviations: CP-CRE, carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*; KPC, *Klebsiella pneumoniae* carbapenemase.

Table 1.8 KPC signal amplitude by meropenem dose

Meropenem dose	Average signal
512–2048 μg/mL Meropenem (13)	1.2 μA
0–128 μg/mL Meropenem (7)	1.1 μA

Abbreviation: KPC, *Klebsiella pneumoniae* carbapenemase.

1.4 Discussion

The 2014 US National Strategy for Combating Antibiotic-Resistant Bacteria [18] identified a critical goal for improved diagnostics to detect antibiotic resistance profiles in 30 min or less for 18 bacteria of highest concern. This was intended to allow physicians to make optimal treatment decisions and help public health officials prevent the transmission of disease and slow the development of resistance. Despite this goal, most diagnostic tests still take several days from specimen collection to results and can be extended by days or weeks if cultures are required. As a consequence, treatment decisions are typically made before laboratory results are available. Patients may be initially treated with antibiotics when none are needed, prescribed an inappropriate antibiotic, or treated with multiple antibiotics when a single antibiotic would have been effective [18].

A rapid test for antibiotic-resistant *Enterobacteriaceae* that can identify the resistance mechanism will greatly improve current outcomes. The CP-CRE test

will identify if KPC is produced and therefore enable appropriate treatment days earlier than current tests. Not only will this improve patient outcomes, but the associated healthcare costs can be reduced with the faster detection results.

This was the first use of quadruplex tags in a hybridization assay. The quadruplex CP-CRE assay requires only five steps and is intended to be used in a point-of-care cartridge. This provides the opportunity to fast track testing for CP-CREs by identifying patients who are at high risk of encountering a CP-CRE infection. A POC test can also be administered at a physician's office.

Another unmet need is a 49% increase in infections and 44% increase in deaths from extended-spectrum beta-lactamases (ESBL)-producing *Enterobacteriaceae*. The incidence of ESBL-producing *Enterobacteriaceae* is over 10 times higher than carbapenem-resistant *Enterobacteriaceae*. The KPC test can be expanded to detect other enzymes produced from *Enterobacteriaceae* to determine if carbapenem or other treatment is more appropriate.

The study encountered some limitations, which will be addressed in further development activities. The assay was tested with spiked broth and urine samples instead of patient specimens. Spiked bacteria concentrations were 10^4 cfu/mL. Lower concentrations have been previously been attained and further work will employ optimized assays. Future work will test a wider range of KPC strains, CP-CRE organisms, and carbapenemases and will optimize the assays for lower limits of detection which have been achieved with larger microparticles and bigger sample volumes. The quadruplex assay can also determine the concentration of KPC in the samples based on the amplitude of the oxidation signal. Current CP-CRE detection tests begin with an isolation culture that increases concentrations to 10^7 cfu/mL before testing and do not consider the bacteria concentration in the patient sample. This could miss valuable insight about therapy effectiveness and treatment decisions in early and late stage of diagnosis, which could potentially be known with a quantitative quadruplex test from the amplitude of the peak signal.

1.5 Conclusion

The study demonstrated that KPC from CP-CRE could be detected within 45 min without bacteria isolation, culture, or PCR. While the experiments were done with 10^4 cfu/mL spikes, acceptable accuracy will be required at lower detection limits for clinical use. The current protocol employs magnetic particles with a 1.5 μm diameter. A larger microparticle will be able to deliver more quadruplex tags per KPC RNA complex, which will provide a measurable signal at lower concentrations. Samples with 10–100 cfu/mL were successfully measured. Since the protocol employed filter concentration of a 1 mL sample, a larger sample volume of 10 mL can be used to deliver 10-fold more bacteria and help achieve lower detection limits. Further samples processing steps such as a pre-filter to remove large cells and additional reagents are expected to be required for whole blood and other complex sample media. All process steps are conducted at room

temperature, which will enable the test steps to be conducted in a point-of-care cartridge with a portable potentiostat.

The study also demonstrated that signal amplification using quadruplex tags could be an alternative to PCR and cultures for detecting bacteria and viruses since it is faster, easier, and less expensive to attach millions of tags than to replicate millions of copies.

The core components of the quadruplex detection approach are mostly generic. A new assay can be rapidly adapted to detect a nucleic acid target from a genome sequence of a RNA, DNA, or gene. The sensor and magnetic microparticle conjugates can be customized for a new target by conjugating the required probes. This allows a baseline assay to be rapidly created and subsequently optimized for the required sample type and assay performance. Protein targets can use antibodies or aptamers in a sandwich immunoassay along with the quadruplex tags on the magnetic particle conjugates.

Many applications can benefit by detecting nucleic acid targets at the POC or in a resource-limited setting where PCR equipment, laboratory, and skilled operators are not available. For example, quadruplex nucleic acid hybridization assays conducted at the POC can avoid false-negative test outcomes due to RNA degradation from transporting samples to a laboratory, and from RNA freezing and thawing when temporarily stored before sample preparation. The simplified test process can also reduce the incidence of false-positive test outcomes by avoiding complex sample process steps for PCR, reverse transcription, materials that interfere with optical detection, and materials that inhibit interference with optical detection. The quadruplex process also employs magnetic separation which can reduce the incidence of non-specific binding and false outcome.

As an example, the use of a rapid, mobile and accurate test could have potentially reduced the impact of the Coronavirus disease 2019 (COVID-19) pandemic. Demand for COVID-19 testing had greatly overwhelmed supply due to a delayed rollout of tests, a limited capacity at testing laboratories, and a massive backlog of untested people. The principle test for COVID-19 detects SARS-CoV-2 virus in respiratory samples from viral RNA. Because of the low concentrations of virus particles relative to surrounding nonspecific materials, and the poor sensitivity of optical detection labels, viral targets had to be replicated prior to detection to produce a measurable number of copies. High throughput sample preparation, replication, and testing typically required a Biosafety Level 3 (BSL3) laboratory, RT-PCR equipment, and specially trained personnel who can conduct the tests with high reliability and minimal cross contamination. Because of these constraints and potential shortage of RT-PCR reagents, countless people remained untested for up to 2 weeks after their samples were collected, and risked infecting others.

The Abbott ID NOW COVID test offers viral RNA testing in a POC Clinical Laboratory Improvement Amendments (CLIA) lab within minutes of sample collection. Because the test uses isothermal amplification instead of PCR, the detection limits and test performance are inferior and can lead to false outcomes.

Antigen tests are much less effective. While the Abbott platform is rapid and accurate with high viral loads, patients need to travel to a CLIA testing center and risk exposure to a community infection. In addition, there are multiple segments of the population who are suspected of being exposed and may not be willing or able to travel to a testing center such as travelers, nursing home residents, prisoners, etc., or who are asymptomatic and do not meet criteria for being tested. A Guanine COVID test could be conducted at virtually any site wherever samples are collected to address the testing requirements for businesses, universities, customs agents and residents who live in close proximity.

Another limitation of nucleic acid amplification tests, including PCR, is the inability for the test to distinguish viable organisms from dead organisms. Pathogenic nucleic acids can remain in the body for weeks after the organisms are dead and produce a positive test outcome. This can incorrectly indicate that the patient requires treatment which can be harmful, or can infect others and needs to be quarantined. In many cases a culture is the recommended test to determine if microorganisms are viable but cultures can take several days or weeks for the microorganisms to be isolated and then cultured to produce a measurable optical signal or color change. The quadruplex test is quantitative and generates an electrical signal that is proportional to the number of analytes in the sample. A portion of the sample can undergo a rapid test to determine if the target microorganism is present. A second portion of the sample can be incubated at an elevated temperature with nutrients to allow a few reproduction cycles and then tested for a higher signal if the target microorganism are viable. Because of the sensitivity of the tags, as few as 4–6 reproduction cycles could be needed which is a fraction of the time of a traditional culture using insensitive dye or optical labels.

Abbreviations

AST:	antimicrobial susceptibility testing
BME:	beta-mercaptoethanol
BSL3:	Biosafety Level 3
CDC:	Centers for Disease Control and Prevention
CLIA:	Clinical Laboratory Improvement Amendments
COVID-19:	Coronavirus disease 2019
CP-CRE:	carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*
CRE:	Carbapenem-resistant *Enterobacteriaceae*
ESBL:	extended-spectrum beta-lactamases
GTC:	guanidinium thiocyanate
IMP:	imipenemase
KPC:	*Klebsiella pneumoniae* carbapenemase
KPC-2	*Klebsiella pneumoniae* ATCC BAA 1705
KPC-3	*Klebsiella pneumoniae* ATCC BAA-2814
MALDI-TOF:	matrix-assisted laser desorption/ionization-time of flight mass spectrometry

MIC:	minimum inhibitory concentration
NDM:	New Delhi metallo-β-lactamase
OXA-48:	oxacillinase-48
PCR:	polymerase chain reaction
POC:	point of care
QC:	quality control
RT:	room temperature
RT-PCR:	real-time polymerase chain reaction
SWV:	square wave voltammetry
VIM:	Verona integron-encoded metallo-β-lactamase

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Corresponding Authors

Neil Gordon
Guanine, Inc.
7 University Place, Suite B210
Rensselaer, NY 12144-3463, USA
Email: neil.gordon@guanineinc.com

Dr. Raj Bawa
Bawa Biotech LLC
21005 Starflower Way
Ashburn, VA 20147, USA
Email: bawa@bawabiotech.com

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