Guanine Inc.
Development Plan
November 22, 2022

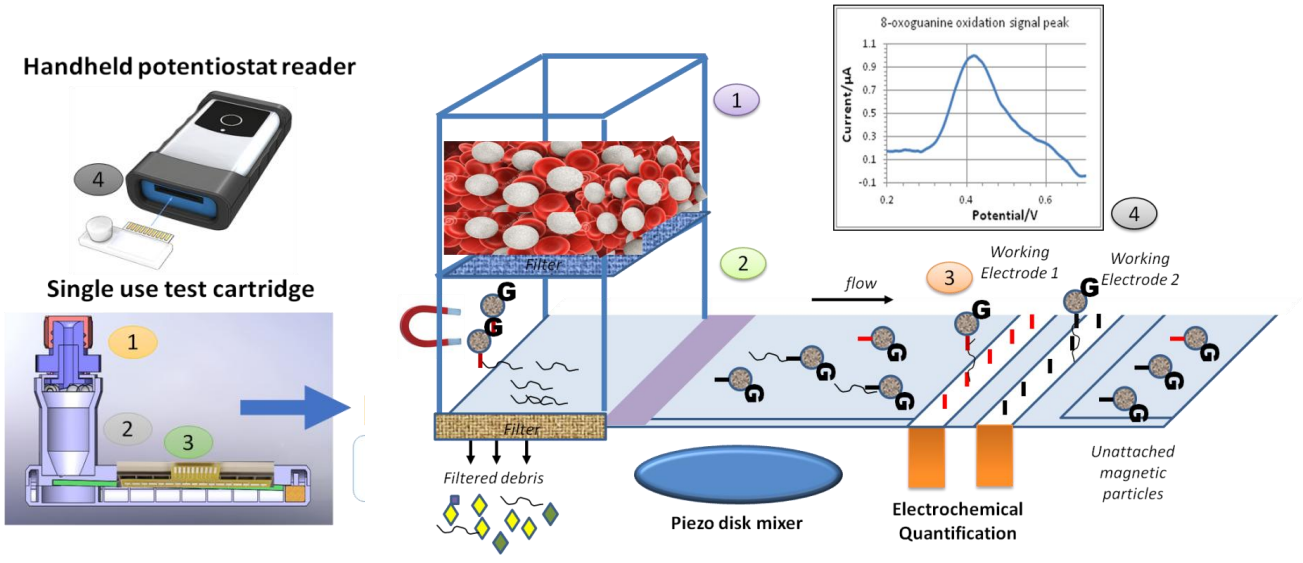
1. Overview

Deliverables: Guanine's development plan will produce the following deliverables:

- **Guanine Dx Reader - a Handheld potentiostat reader**
  - Potentiostat with multiplexer for 8 electrodes
  - Operate cartridge for sample preparation and target quantification

- **Guanine Dx Sepsis – Single use test cartridge for detecting sepsis pathogens and drug resistance genes**
  - Cartridge that processes whole blood sample and detects 24 sepsis targets
  - LOD of 10 targets per mL from 20 mL whole blood sample
  - Sensitivity >90% and specificity >f 90%
  - 1 hour sample-to-results with potential to re-test second patient sample hours later when viable targets replicate
  - Quantitative results with potential to assess treatment effectiveness by measuring the change in pathogen concentration from a second test sample

Test Operation: The cartridge will be designed to perform 4 specific steps: (1) filter concentrate and lyse sepsis pathogens, (2) capture targets with magnetic particles, (3) form sandwiches on electrodes, and (4) generate signals from the electrochemical oxidation of tags in the sandwiches. The instrument will provide systems to automatically operate the cartridge including: (a) heating elements to melt reagent seals (b) piezo disk mixer, (c) magnetic field generator , (d) potentiostat , and (e) communications card. A schematic of the test cartridge and associated steps are summarized below.



Step 1 – Filter concentration and lysis	Step 2 – Capture targets with magnetic particles	Step 3 – Form sandwiches on electrodes	Step 4 – Generate signals from tag oxidation
1.1 Filter sample 1.2 Release lysis buffers 1.3 Mix^{B}	2.1 Add magnetic particles 2.2 Mix to capture targets 2.3 Apply magnetic field 2.4 Release to sensor 2.5 Release detection buffer	3.1 Remove magnetic field 3.2 Release detection buffer 3.3 Mix to form sandwiches 3.4 Apply voltammetry scan	4.1 Measure currents 4.2 Convert to concentrations 4.3 Transmit results

Approach: Development will be undertake with the following tasks:

- **Assay development in 96 well microtiter:** The microtiter development process was optimized in the CDC grant and technician capacity is 96 experiments per daily batch. Assay development milestones will include: f (a) 4 targets on 1 electrode, (b) 8 targets on 2 electrodes and (c) 24 targets on 6 electrodes

- **Optimization of key process steps**: In parallel with microtiter development, **i**ndividual specialists will be tasked with optimizing key process steps to reach objectives for detection limit and multiplexing. These involve: (a) filter concentration of pathogens and lysing by a microbiologist (b) probe design for capture and recognition by a molecular biologist, (c) multiplex magnetic microparticle yield and sandwich formation by a mechanical engineer, and (d) multiplex voltammetry process by an electrochemist.

- **Cartridge development:** Cartridge prototypes with 8 working electrodes will be 3D printed to operate the test protocol initially with an end to end test for 1 target on 1 electrode. The cartridge will be modified to accommodate more targets and process improvements. Cartridge development will stagger microtiter development and provide the same milestones for test comparison of (a) 4 targets on 1 electrode, (b) 8 targets on 2 electrodes and (c) 24 targets on 6 electrodes

- **Instrument development** which will employ mostly low cost and highly reliable off-the-shelf components to for rapid development. Components will be needed to release reagents, mix solutions, temporarily apply magnetic field, apply voltage and measure electrical current, process algorithms and transmit test results with development of :(a) alpha system for internal testing, (b) beta system for external testing, (c) minimum sellable unit for pre-release and certification.

Milestones: Key development timelines include specific deliverable by the end of specific months

(a) Month 3 will have 4 targets detected on 1 microtiter electrode.
(b) Month 4 will have 8 targets detected on 2 microtiter electrodes, and 4 targets detected on 1 cartridge electrode in the Generation 1 cartridge.
(c) Month 5 will have 8 targets detected on 2 cartridge electrodes in the Generation 2 cartridge and be operated by the Alpha reader.
(d) Month 6 will have 24 targets detected on 6 microtiter electrodes with 2 control electrodes.
(e) Month 7 will have 24 targets detected on 6 cartridge with 2 control electrodes in the Generation 3 cartridge and be operated by the Beta reader. A pre-submission will be forward to the FDA.
(f) Month 9 a PMA will be submitted to the FDA.
(g) Month 12 will have the final cartridge and reader being fully tested with patient specimens and data filed with the FDA

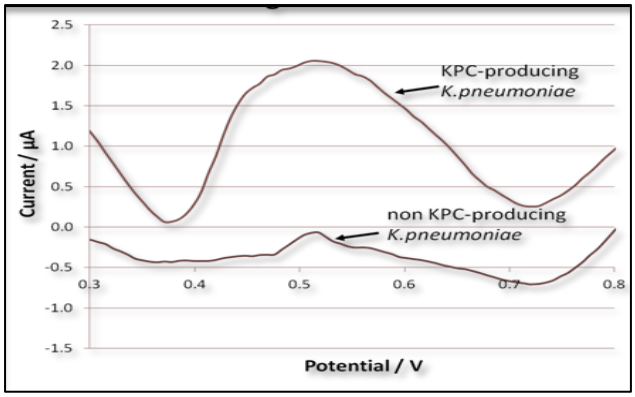
Schedule: The corresponding schedule is as follows and includes llicensing sales by Month 13 and sepsis product sales by month 16.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ramp up staff																
Microtiter test			4t/1e	8t/2e		24t/8e										
Cartridge test				4t/1e	8t/2e		24t/8e									
Cartridge dev.				Gen 1	Gen 2		Gen 3					Final				
Instrument dev.					Alpha		Beta					Final				
Certification							PreSub		PMA			Filing				
Product Sales																Sales
Licensing Sales													Sales			

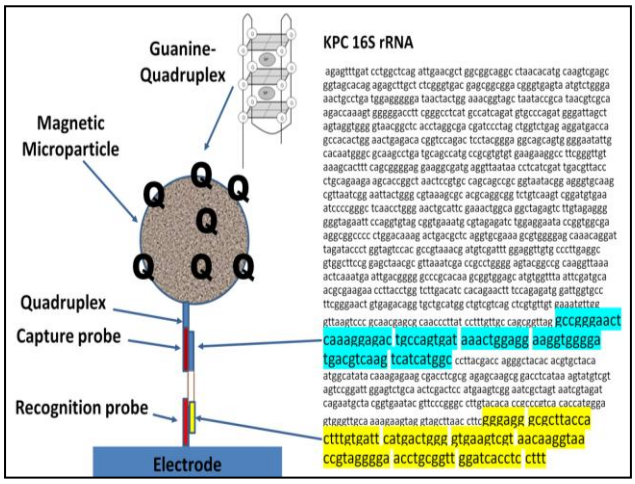
2. Current Status

Data: Data was produced for the first sepsis target measuring carbapenem resistance, *Klebsiella pneumoniae carbapenemase* (KPC) enzymes. Samples tested included two strains of KPC-producing *K. pneumoniae*, KPC-producing *E.coli* and a non-KPC producing *K. pneumoniae*. The KPC producers generated a current peak exceeding the threshold current. Samples were detected as low as 100 targets per mL from a 1 mL sample and validation testing was 22/22 true positives and 5/6 false negatives.

	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	

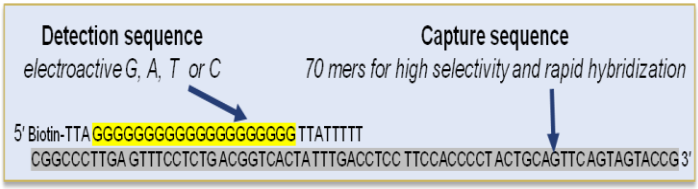


Protocol: The protocol steps were: (1) Filter 1 mL sample (1 min), (2) Add 200 µL lysis buffer and incubate at RT (5 min) then add 7 µL magnetic bead conjugates shake gently & incubate RT (10 min). (3) Apply magnet (2 min) and wash with detection buffer (4 min) (4) Resuspend with 100 µL NaOAc (pH 9) detection buffer and deliver solution to biosensor and incubate (10 min), and (5) Apply Square Wave Voltammetry Scan (1 min) to generate current peak.



Sandwich Hybridization Assay: Capture probes and recognition probes hybridized with different potions of the KPC16S rRNA target to form a sandwich between the electrode and magnetic microparticles. The polyGuanine sequences were pre-fabricated into guanine-quadruplexes with a Na+ cation by heating in a sodium acetate solution. The polyGuanine sequence is prefabricated into a quadruplex which emits 8-oxoguanine oxidation signals that has a higher signal-to-noise resolution than guanine oxidation. This also avoids cross signals from guanine on the capture sequence and target.

Probe Design: The detection tags employed a 20mer polyGuanine detection sequence and a 70mer capture sequence on the same oligonucleotide. Recognition probes also contained a 70mer sequence.



Target	Accension	Target Sequence	Oligonucleotides	Probes
KPC 16s rRNA	X87276	GCCGGGAACT CAAAGGAGAC TGCCAGTGAT AAACTGGAGG AAGGTGGGGA TGACGTCAAG TCATCATGGC	5′ Biotin-TTA GGGGGGGGGGGGGGGGGGGG TTATTTTT CGGCCCTTGA GTTTCCTCTG ACGGTCACTA TTTGACCTCC TTCCACCCCT ACTGCAGTTC AGTAGTACCG 3′	Detection and capture
		GGGAGGGCGC TTACCACTTT GTGATTCATG ACTGGGGTGA AGTCGTAACA AGGTAACCGT AGGGGAACCT GCGGTTGGAT CACCTCCTTT	5′ Biotin-TT TTT TTT TT AAAGGAGGTGATCC AACCGCAGGT TCCCCTACGG TTACCTTGTT ACGACTTCAC CCCAGTCATG AATCACAAAG TGGTAAGCGC CCTCCC 3′	Recognition

3. Technical Objectives

Assay Metrics: The criteria used to measure the effectiveness of the assay are the following:

- Performance which is measured as (1) Sensitivity = the ratio of True Negatives / (True Negative + False Positive). The goal for specificity is 90-95% for each target, and (2) Specificity = the ratio of True Positives ÷ (True Positives + False Negatives).The target for specificity is 90-95% for each target. Assay performance will be evaluated with multiple nonspecific targets that are similar to the evaluation targets.
- Limit of Detection (LOD) - as the lowest quantity of bacteria or resistance target that can be distinguished from the absence of the variant (a blank level) at a 95% confidence level. The goal for limit of detection is 10 targets/mL for each pathogen or resistance gene.
- Multiplexing – as the ability to detect multiple targets on the same electrode. The target for multiplexing is 4 targets on one electrode and use 6 or more electrodes in the next phase for 24 or more sepsis targets from a single sample in a POC cartridge.

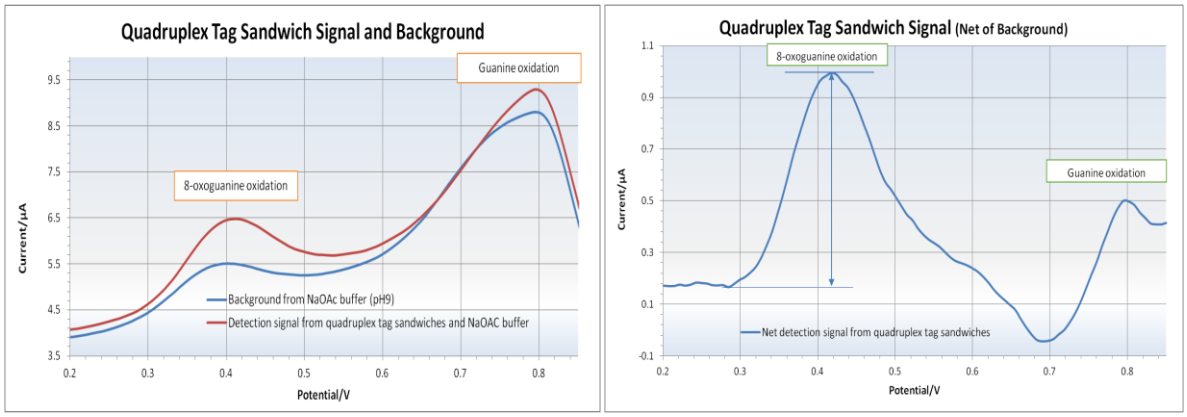
Performance: Accuracy of the proposed test can be estimated from its improvements over the direct detection of sepsis targets using PCR with and without a culture. Certain sepsis microorganisms are challenging to detect due to: low concentrations, single RNA per organism, very slow doubling time, heterogeneous concentration in blood stream, the ability to be internalized by red and white blood cells, quantities outnumbered by nonspecific materials by 5 or more logs, and species/strains have high variability. PCR is further limited by the poor sensitivity of optical labels, small sample volume of 1-100 µL, risk of sample degradation from transport to lab and freezing/thawing if stored. The use of a time-intensive and tedious culture process in advance of PCR adds to protocol complexity, human error and contamination, injured organisms unable to reproduce, dead organism from antibiotics given to patients in advance of collecting blood samples certain organisms needing different culture media and atmospheres, and inability to easily replicate viruses in a simple culture process. Guanine's proposed test will improve PCR sensitivity and specificity with the following:

- <u>Projected sensitivity improvement to 90-95% with less variability due to</u>:
  - Collecting sepsis pathogens from a 20 mL sample volume to provide 2 to 4 logs more targets for detection than PCR. (Guanine has attained >90% yield with other bacteria using filter concentration and magnetic separation from 1 mL samples)
  - Capturing sepsis pathogens as (i) circulating targets, (ii) lysed/ruptured targets from microorganisms, and (iii) lysed targets from released microorganisms internalized by red and white blood cells instead of a single target source with PCR
  - Amplifying detection signal by attaching targets with magnetic microparticles bound with millions of tags instead of replicating millions of targets with PCR or reproducing organisms with cultures
  - Conducting the test in a POC cartridge to minimize target degradation from transportation and freezing/thawing as with PCR and cultures

- <u>Projected specificity improvement to 90-95% with less variability due to</u>:
  - Capturing RNA and genetic resistance targets using 70 mer probes with higher selectivity than 20 mer probes in PCR
  - Removing nonspecific materials with magnetic separation that can inhibit capture and block the detection signal
  - Conducting tests in automated cartridges to minimize contamination and human error

Limit of Detection: The development plan will focus on reaching the lowest possible detection limits for each detection targets. The assays will then be optimized for attaining other performance metrics. The basis for a detection signal is an incremental increase in electrical current caused by oxidation from oligonucleotide tags with sodium acetate (NaOAc) detection buffer relative to the background signal caused by the sodium acetate detection buffer alone. The resulting scans are shown illustrating the calibration scan (left – lower scan) and the detection scan (left – upper scan) and the difference between

the detection scan and the calibration scan as the net detection signal. The target is present when the net detection scan peak exceeds a threshold value.

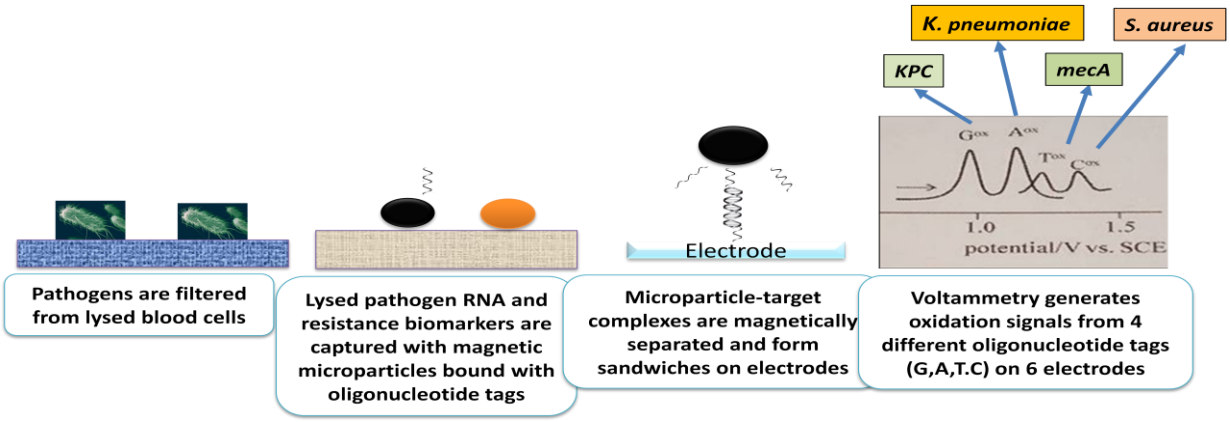


The LOD objective will be attained by:

- Maximizing the target detection signal with one or more of the following:
  - increasing the sample volume and filtration surface area to deliver more targets for detection,
  - evaluating a different lysis buffer to increase target yield,
  - increasing the microparticle contact time to increase the yield of captured targets,
  - modifying the magnetic separation protocol to improve capture yield,
  - increasing the microparticle size to deliver more quadruplexes per sandwich,
  - reducing the electrode surface area to raise signal-to-noise resolution,
  - modifying the square wave voltammetry scan rate or shape to release more electrons, and
  - adding a mediator to increase the transport of electrons,

- Minimizing the nonspecific material detection signal with one or more of the following:
  - extending the capture and recognition sequence for improved selectivity,
  - modifying the magnetic separation protocol for longer contact and magnet exposure times,
  - using a second wash,
  - reprocessing the magnetic separation waste solution for uncaptured targets, and
  - employing a different blocking solution to reduce the incidence of nonspecific binding.

Multiplexing: Multiplexing is achieved using a cocktail of microparticles with different sets of oligonucleotide tags and electrodes. Guanine detects sepsis pathogens and drug resistance biomarkers by generating oxidation peaks from 4 different oligonucleotide tags (G,A,T.C) on 6 different electrodes.



To enable 4 targets per electrode sets of targets will employ detection sequences with different nucleotides as follows:

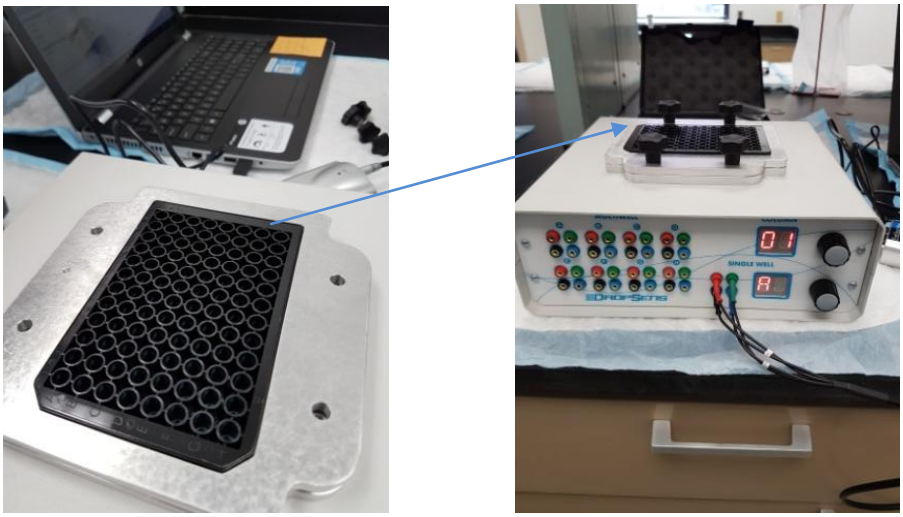
Target	Accension	Target Sequence	Oligonucleotides	Probes
KPC 16s rRNA	X87276	GCCGGGAACT CAAAGGAGAC TGCCAGTGAT AAACTGGAGG AAGGTGGGGA TGACGTCAAG TCATCATGGC	5′ Biotin-TTA GGGGGGGGGGGGGGGGGGGG TTATTTTT CGGCCCTTGA GTTTCCTCTG ACGGTCACTA TTTGACCTCC TTCCACCCCT ACTGCAGTTC AGTAGTACCG 3′	Detection and capture
		GGGAGGGCGC TTACCACTTT GTGATTCATG ACTGGGGTGA AGTCGTAACA AGGTAACCGT AGGGGAACCT GCGGTTGGAT CACCTCCTTT	5′ Biotin-TT TTT TTT TT AAAGGAGGTGATCC AACCGCAGGT TCCCCTACGG TTACCTTGTT ACGACTTCAC CCCAGTCATG AATCACAAAG TGGTAAGCGC CCTCCC 3′	Recognition
S. aureus mecA gene	X52593	TGAATTATTA ATAAGTGCTGT TACTTCTCCC TTAAATACAA TTTCTTCATT TTCATTGTAT GTTGAAAGTG	5′ Biotin-TTA AAAAAAAAAAAAAAAAAAAA TTATTTTT ACTTAATAAT TATTCACGACA ATGAAGAGGG AATTTATGTT AAAGAAGTAA AAGTAACATA CAACTTTCAC 3′	Detection and capture
		GCTACAACTT CTTCTCCGTA TTTACCTTCT TCTACCCATA ATTTAAATGA TATTGAAAGT GTATGCATGC	5′ Biotin-TT TTT TTT TT GCATGCATAC ACTTTCAATA TCATTTAAAT TATGGGTAGA AGAAGGTAAA TACGGAGAAG AAGTTGTAGC 3′	Recognition
K.pneumoniae 16S rRNA	X93214	TAAAGTATGT CGTAGTCCGG ATTGGAGTCT GCAACTCGAC TCCATGAAGT CGGAATCGCT AGTAATCGTA	5′ Biotin-TTA TTTTTTTTTTTTTTTTTTTT TTATTTTT ATTTCATACA GCATCAGGCC TAACCTCAGA CGTTGAGCTG AGGTACTTCA GCCTTAGCGA TCATTAGCAT 3′	Detection and capture
		AGTGGGTTGC AAAAGAAGTA GGTAGCTTAA CCTTCGGGAG GGCGCTTACC ACTTTGTGAT TCATGACTGG	5′ Biotin-TT TTT TTT TT CCAGTCATGA ATCACAAAGT GGTAAGCGCC CTCCCGAAGG TTAAGCTACC TACTTCTTTT GCAACCCACT 3′	Recognition
S.aureus 16S rRNA	X68417	AATGGACAAT ACAAAGGGCA GCGAAACCGC GAGGTCAAGC AAATCCCATA AAGTTGTTCT CAGTTCGGAT	5′ Biotin-TTA CCCCCCCCCCCCCCCCCCCC TTATTTTT TTACCTGTTA TGTTTCCCGT CGCTTTGGCG CTCCAGTTCG TTTAGGGTAT TTCAACAAGA GTCAAGCCTA 3′	Detection and capture
		GACAAATGAT TGGGGTGAAG TCGTAACAAG GTAGCCGTAT CGGAAGGTGC GGCTGGATCA CCTCCTTTCT	5′ Biotin-TT TTT TTT TT AGAAAGGAGG TGATCCAGCC GCACCTTCCG ATACGGCTAC CTTGTTACGA CTTCACCCCA ATCATTTGTT 3′	Recognition

4. Development Steps

Assay development in 96 well microtiter: Assay development milestones will include: (a) 4 targets on 4 electrodes, (b) 4 targets on 1 electrode, (c) 8 targets on 2 electrodes and (d) 24 targets on 6 electrodes. In parallel with microtiter development, individual specialists will be tasked with optimizing key process steps to reach objectives for detection limit and multiplexing. These involve: (a) filter concentration of pathogens and lysing by a microbiologist (b) probe design for capture and recognition by a molecular biologist, (c) multiplex magnetic microparticle yield and sandwich formation by a mechanical engineer, and (d) multiplex voltammetry process by an electrochemist. Once the assays are optimized for 4 targets on 1 electrode subsequent assays will be completed using a cookie cutter approach. Key development steps include:

- **Prepare magnetic microparticle conjugates and sensor probes.** Biotinylated detection oligonucleotides (Integrated DNA Technologies, Coralville, IA) will be prepared with a capture sequence and a detection sequence comprising a 20-mer polyGuanine or other nucleotide. Linkers will separate the sequences. The 20-mer polyGuanine sequence will be prefabricated into quadruplexes containing 5 guanine tetrads stacked on top of each other and separated by Na^+ ions by heating the oligonucleotides in a sodium acetate/formamide solution. The oligonucleotides will then immobilized onto streptavidin-coated 3 μm magnetic particles (MP, 3 μm) (Bangs Laboratories,

Fisher, IN) to form oligonucleotide MP conjugates. The process will be repeated for the other targets using other nucleotide detection sequences instead of guanine. Unique working electrodes on a 96-well, streptavidin-coated carbon biosensor microtiter (DropSens, Llanera, Spain) will be conjugated with biotinylated recognition probes using streptavidin-biotin interactions.



- **Measure four oligonucleotide RNA targets on separate electrodes.** Experiments will evaluate the effectiveness of the baseline protocol for 4 oligonucleotide targets. Targets will be mixed with 3 µL of each magnetic particle conjugates with different nucleotide tags to capture RNA targets and incubated for 5 min to form complexes. The complexes are then placed in a magnetic separation microtiter (Epigentek, Farmingdale, NY). Under a magnetic field, nonspecific materials, excess cellular debris, and unbound nucleic acids will be washed away. The magnetic field will be removed and the conjugates will be washed with 100 µL 80 mM sodium acetate detection buffer (pH 9) and then delivered to the sensor to form RNA sandwiches on separate electrodes. After a wash, a square wave voltammetry scan will be generated using a potentiostat, and produce a quantitative electrical current peak signal. The normalized 8-oxo peak will measure the detection signal relative to a negative control threshold current. Experiments will then determine the LOD and LOQ. At least 5 non-targets will be tested for sensitivity and specificity. Adjustments will be made to the protocol from the process specialists.

- **Develop blood filtration and lysing protocol.** Standards (ATCC, Manassas, VA) will be spiked in pooled human whole blood (Innovative Research, Novi, MI). Filters will be procured with varying pore sizes (Millipore, Burlington, MA and Pall, Port Washington, NY). Experiments will evaluate the recovery of target spikes expected to be approaching 100% with filtration time of under 5 minutes. Each target will be evaluated separately and then collectively. Additives such as a surfactant and the use of mixing can be evaluated if low yield is caused by the pores being blocked by large cells. The filter surface area can be extended to reduce blockage. A second filter is expected to be used to extract viruses. Targets will be suspended in a baseline lysis buffer (5 M guanidine isothiocyanate, 1% Triton X-100, 50 mM Tris HCl (pH=6.4), 20 mM EDTA) at room temperature and incubated for 2 min. It may be necessary to first lyse white blood cells and subsequently lyse red blood cells. Each target will be evaluated separately and then collectively. The lysis protocol effectiveness will be compared with a commercial kit (PureLink Viral RNA/DNA, ThermoFisher). Protocol adjustments can be made such as lysing time and the use of additives.

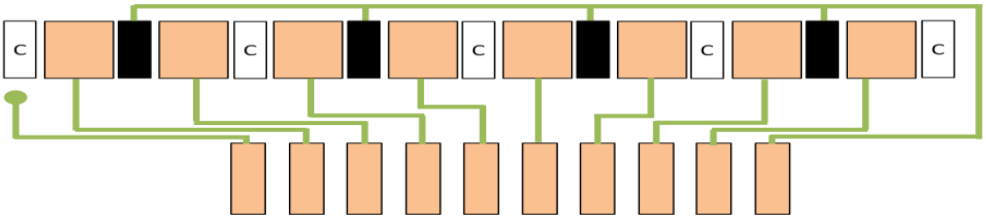
- **Measure four pathogen targets on separate electrodes**. The baseline protocol will be enhanced with filtration and lysis. Experiments will be made to optimize the recovery of targets at each process step. A wide range of concentrations will be used to access target yield, with particular emphasis on low concentrations which are critical for attaining the LOD goals. Experiments will evaluate the recovery rates of different filter pore sizes, filtration additives such as Tween, extending the time for re-suspension, lysing the concentrated bacteria before and during magnetic microparticle mixing,

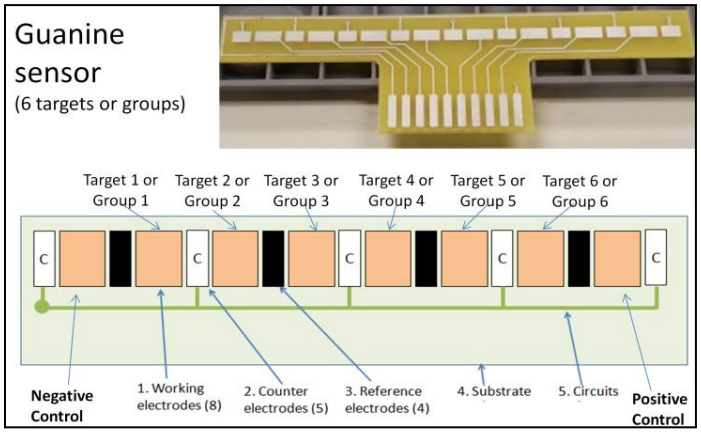
extending the times for mixing and incubation, as well as exposing the magnetic separation waste solution to a second set of magnetic microparticles. The team will also evaluate the impact of using a different volumes and different sizes of magnetic microparticles which could impact the target capture and sandwich formation. Experiments will then determine the LOD and LOQ. At least 5 non-targets will be tested for sensitivity and specificity. Adjustments will be made to the protocol from the process specialists.

- **Measure four pathogen targets on a common electrode**: Experiments will demonstrate the detection of all 4 targets on a common with oxidation peaks at different voltages to indicate the specific target being detected, then determine the LODs in spiked blood samples for all four targets from the same sample. Experiments will initially be made to detect two targets at the same time. Once successful additional targets be added until all 4 can be detected simultaneously from the same sample using different tags (G, A, T or C). The different nucleotides will form peak currents at different voltages making is possible to determine which pathogens are present in the sample and have formed sandwiches based on the associated voltageThe initial concentration will be 1000 targets/mL and be subsequently dropped to 10 targets/mL. Experiments will be made to optimize the detection signal for each target relative to background noise by increasing the detection signal and reducing the background noise. Experiments will then determine the LOD. At least 5 non-targets will be tested for specificity. Assay performance will be evaluated against commercial tests with suitable specimens.
- **Repeat the process until 24 targets are measured on 6 common electrodes**.

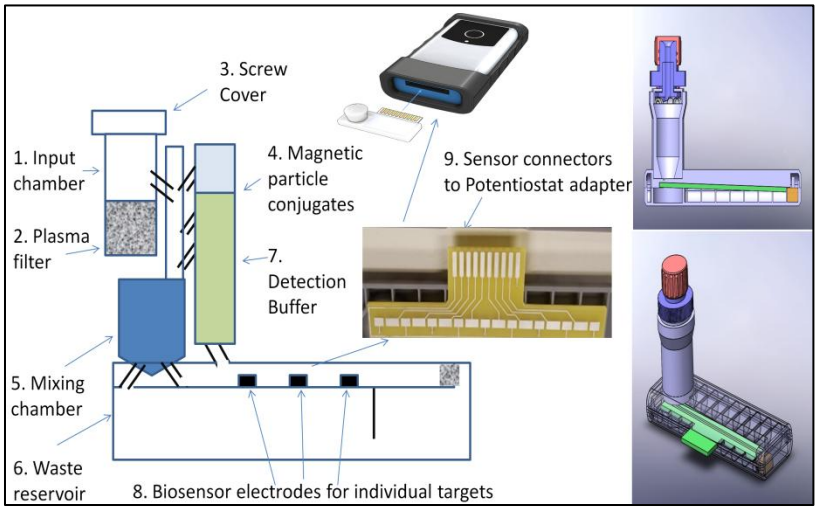
Assay migration to a cartridge with automated operation. Assay migration milestones will include: (a) Generation 1 cartridge that processes a whole blood sample and detects 1 target on 1 electrode, (b) Generation 2 cartridge that processes a whole blood sample and detects 4 targets on 4 electrode which is operated by the Alpha Reader, (c) Generation 3 cartridge that processes a whole blood sample and detects 24 targets on 6 electrodes with 2 control electrodes which is operated by the Beta Reader. Key development steps include:

- **Source and fabricate key components.** Key components will include whole blood and bacteria spikes, filters of varying pore sizes, lysis buffer, magnetic particle conjugates, and sodium acetate buffer. The prototype sensor comprised 8 carbon working electrodes with corresponding counter electrodes and reference electrodes that combine to 10 external leads.





- **Configure the cartridges.** The Generation 1 cartridge will be built with layers with the central layer for fitting the sensor and delivering solutions to form sandwiches. The appropriate polymer will enable 3D prototyping to evaluate different geometries for delivering fluids, storing and releasing reagents, and housing the filters. External device components will be sources to operate the cartridge with the intent of incorpating the components in the Alpha reader including a potentiostat and related hardware and software from PalmSens/Analog Devices, heating elements to melt reagent seals, piezo disk mixer, magnetic field generator , (d) potentiostat , and (e) communications card. Required fluid volumes will be used from the microtiter assay to determine the geometries for reservoirs, chambers and capillaries and incorporated in existing CAD designs. An indexable mechanism will sequentially release magnetic microparticle conjugates and buffers from pre-loaded chambers that may need to be manually operated for the proof of concept demonstration. Initial prototypes will be fabricated by 3D printing, thermoforming, and laser cutting and evaluated for fluid flows and sandwich formation on the electrodes. Colored rheological analogs to proposed reagents will initially will be used to visualize flow rates and retention times. Prototypes will be made at RPI's Manufacturing Innovation Center and evaluated for test metrics including volume of onboard solutions actually released, process times, magnetic particle yield, binding yield, and amplitude of the electrochemical peak currents relative to the DropSens sensors. Several design iterations are planned to achieve the functionality. Subsequent cartridge generations will incorporate modifications developed during the microtiter assay developments.
- **Measure one pathogen target on one electrode.** The Generation 1 cartridge will be prototyped to comprise an insert chamber (1) which houses a 20 mL blood sample, (2) a filter that separates blood cells from bacteria a cover (3) is screwed to clamp the input chamber with released lysis solution (not shown). A mechanism will release preloaded magnetic microparticle conjugates (4) and the lysed solution into a mixing chamber (5). A mechanism (not shown) activates a magnetic field and nonspecific materials are drained into a waste reservoir (6) then washes the magnetic microparticle conjugates with detection buffer (7). A mechanism deactivates the magnet then delivers the conjugates to the sensor (8) to form sandwiches. Sensor contacts (9) insert to a potentiostat connector that measures the incremental current signal associated with targets and tags bound in the sandwiches on one of the eight working electrodes relative to a baseline signal. Sensor electrodes will be preconjugated with recognition probes and specific electrodes will be allocated for negative and positive controls. Success will be attained by demonstrating an 8-oxoguanine signal within 45 minutes of adding a whole blood sample. A schematic of the test cartridge and associated steps are summarized below.



- **Configure the reader**. The reader will incorporate a proven electrochemical potentiostat chip from Analog Devices. The potentiostat chip was designed to operate as smart phone adapter and a Bluetooth-enabled mobile potentiostat from PalmSens that was successfully used for laboratory work

at Guanine. The COVID-19 testing instrument will measure RNA levels by connecting to biosensor electrodes in single use test cartridges. The instrument will contain processing, communications, electronics, vibration capability for sample/reagent mixing, heating elements reagent release, and an electromagnet situated adjacent to the cartridge mixing chamber. The instrument will contain software to validate the cartridge integrity, operate the appropriate test protocol, convert the sensor current measurements to determine RNA presence and concentration, transmit test results to a cloud database containing a user file which can be displayed on a smart device, and download software upgrades. The system will be enclosed with a ruggedized housing that integrates EMI shielding, and necessary insulation to facilitate operation.

- **Repeat the process until 24 targets are measured with the reader**. Experiments will optimize the yield of targets and minimize electrical crosstalk on the electrode to match the outcomes. Assay performance will be evaluated against commercial tests with suitable specimens. Experiments will be done for getting the same results from batch to batch, and the ability for the test cartridge to work effectively after being stored in a refrigerator for 6 months.