Guanine Inc.
Product Data Sheet
November 18, 2022

Offering:

Guanine Dx Reader ($500)

HIGH MULTIPLEX AND VERY LOW LIMIT OF DETECTION WITH A RAPID, MOBILE AND INEXPENSIVE TEST

Guanine Dx Reader is a mobile testing system for the rapid diagnosis of complex infections. Our patented electrochemical oligonucleotide tags detect up to 24 unique low concentration nucleic acid and protein targets from a single sample with the accuracy of PCR and ease of a glucose meter. Our breakthrough technology provides signal amplification by attaching targets with millions of tags instead of replicating millions of copies and extracting more targets for higher accuracy by processing large samples with filter concentration and magnetic purification..

- Palm-size reader (2"x 3" x 5", 2 lbs)
- Automated test cartridge
- Detecting up to 24 nucleic acid or protein targets per sample
- Qualitative or quantitative results
- Transmit test results to database and smart phone

Guanine Dx Sepsis ($200)

DETECT 24 SEPSIS PATHOGENS AND DRUG RESISTANCE TARGETS IN UNDER 1 HOUR WITHOUT A CULTURE

Guanine Dx Sepsis is a rapid test for detecting low level sepsis pathogens including viral sepsis in under 1 hour without a culture. Users input a 20 mL whole blood sample into the test cartridge and connect the cartridge to the Guanine Dx Reader. Samples undergo filter concentration to capture orders of magnitude more targets than a PCR sample, magnetic separation to remove nonspecific materials that cause false outcomes, and hybridization to form magnetic microparticle-target sandwiches on sensor electrodes. 70-mer capture sequences are used for high selectivity and rapid hybridization. A voltammetry scan generates oxidation signals for each target proportional to their concentrations.

- Sample to results in 1 hour without a culture
- No culture errors
- Automated test cartridge
- Rapid signal amplification by attaching targets with millions of oligonucleotide tags

- Quantitative results for:

GRAM-NEGATIVE BACTERIA:

- *Acinetobacter calcoaceticus-baumannii* complex
- *Enterobacter cloacae* complex
- *Escherichia coli*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae* group
- *Pseudomonas aeruginosa*
- *Salmonella* spp.

GRAM-POSITIVE BACTERIA:

- *Listeria monocytogenes*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*

YEAST:

- *Candida auris*

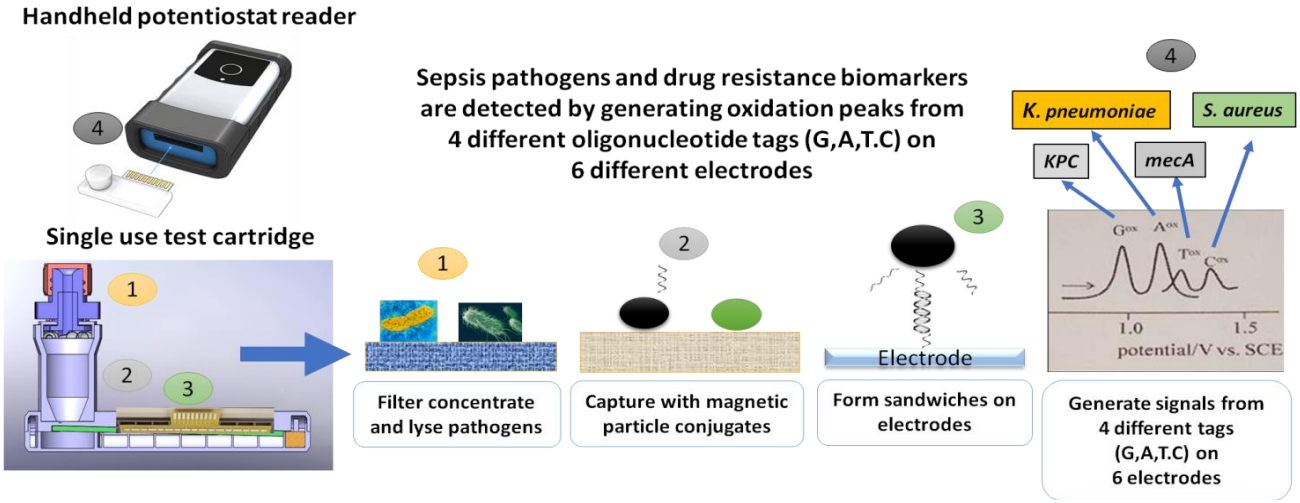
VIRUS:

- *COVID-19*

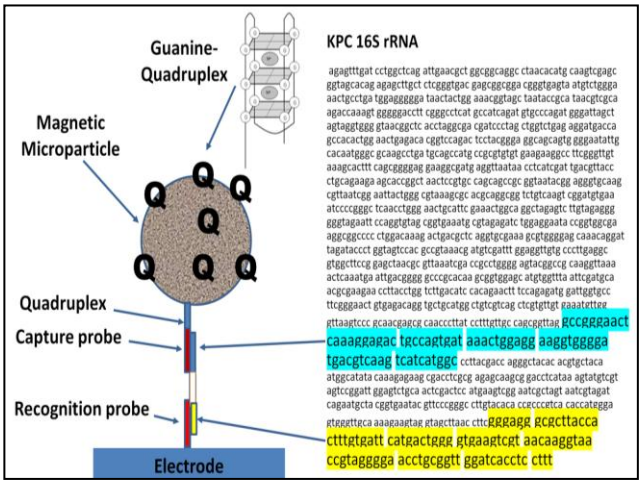
ANTIMICROBIAL RESISTANCE GENES:

- **Carbapenemases**
  - IMP
  - KPC
  - OXA-48-like
  - NDM
  - VIM
- **Colistin Resistance**
  - *mcr-1*
- **ESBL**
  - CTX-M
- **Methicillin Resistance**
  - *mec*A/C
  - MREJ (MRSA)
- **Vancomycin Resistance**
  - *van*A/B

Operation: After a user inserts a 20 mL blood sample into a cartridge the following steps are automatically performed: (1) The sample is filter concentrated and pathogens on the filter are lysed. A sub-filter is used to retain viruses and other small targets. (2) Targets are captured with magnetic microparticles conjugates. (3) A magnetic field is applied and nonspecific materials are washed away, then the magnetic microparticle-targets form sandwiches on electrodes. (4) A voltammetry scan generates oxidation current peaks associated with different targets. Each electrode can have 4 different peaks depending the tag being used.



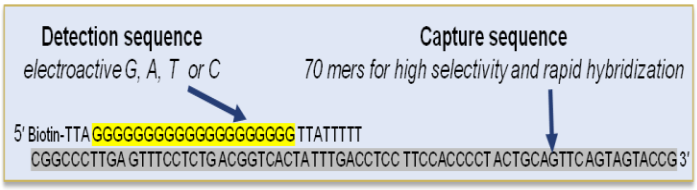
Principles of the Assay



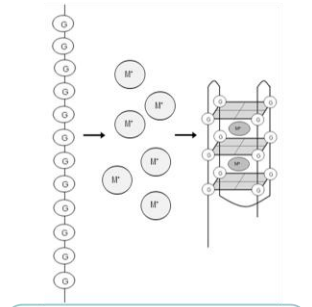
Overview: Guanine Dx Sepsis is a sandwich hybridization assay for detecting low level sepsis pathogens including viral sepsis in under 1 hour without a culture. Users input a 20 mL whole blood sample into the test cartridge and connect the cartridge to the Guanine Dx Reader. The reader employs a potentiostat that electrochemically measures tags bound to detection targets, similar to a glucose meter for sepsis. Samples undergo filter concentration to capture orders of magnitude more targets than a PCR sample, magnetic separation to remove nonspecific materials and minimize false outcomes, and then form magnetic microparticle-target sandwiches on sensor electrodes. A voltammetry scan oxidizes the tags and produces an electrical current proportional to each target's concentration.

Electrochemical oligonucleotide detection tags: The assay employs Guanine's patented oligonucleotide detection tags that make use of the electrochemical properties of nucleotides such as guanine. Unlike other electrochemical tags that bind to targets in a 1-to-1 relationship, Guanine's nucleotides can be scaled to bind 1,000,000's per target using a particle to greatly

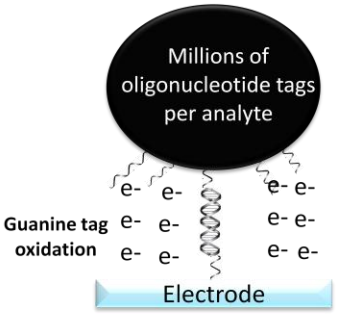
amplify the detection signal. The detection tags comprise a 20mer detection sequence of polyGuanine or other nucleotide, and a 70mer capture sequence on the same oligonucleotide. The long capture sequence provides high selectivity and rapidly hybridizes at room temperature.



Signal Amplification: 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 with guanine on the capture sequence and target. Millions of tags are conjugated to a microparticle to eliminates the need for replicating millions of nucleic acid copies with PCR. Lower target concentrations are measured with a larger microparticle that binds more tags per target. The high density magnetic microparticle causes a portion of the oligonucleotides to adsorb to the electrode surface for high signal. Magnetic separation removes nonspecific materials in advance of detection to improve the signal-to-noise resolution. Samples undergo filter concentration to capture orders of magnitude more targets to further enhance the detection signal.



Pre-fab quadruplex oligos have higher signal than single-stranded oligos

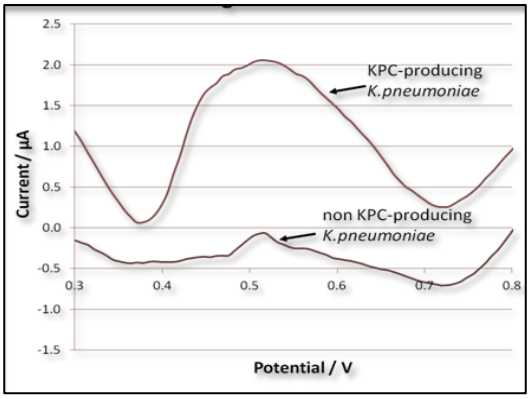


Bigger microparticle, more tags per analyte, higher signal

Microparticle weight causes oligos to adsorb to electrode surface for higher signal

Magnetic separation removes nonspecific materials for higher signal-to-noise resolution

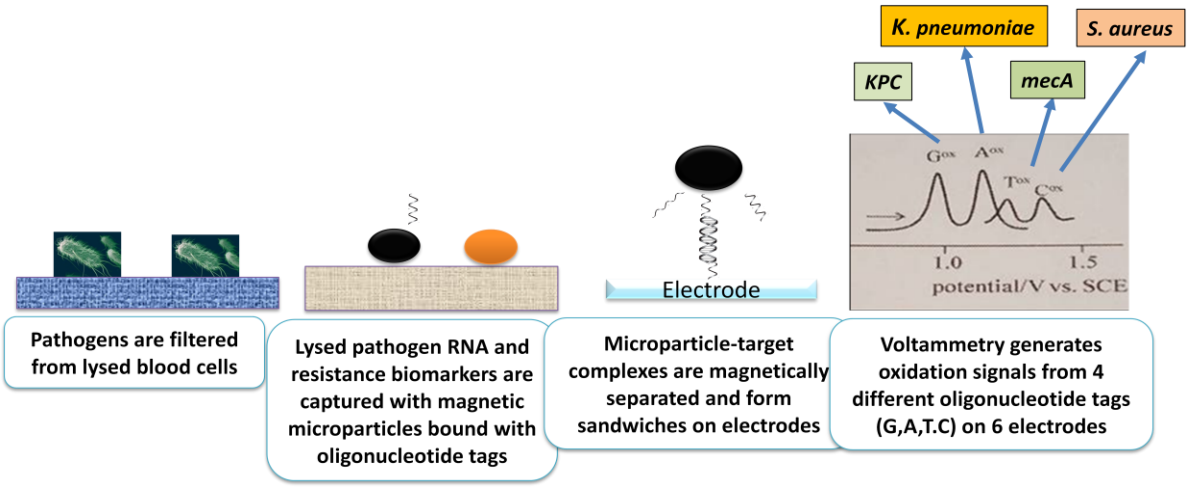
Baseline Protocol and Test Results: Data from a CDC-funded SBIR detected *Klebsiella pneumoniae carbapenemase* (KPC) enzymes from a filter concentrated 1 mL sample using a hybridization assay with electrochemical oligonucleotide tags. The protocol steps were: (1) Filter 1 mL sample (1 min), (2) Add 200 µL lysis buffer, 5 µL DMSO and incubate at RT (5 min) then Add 7 µL magnetic bead conjugates shake gently & incubate RT (10 min). (3) Apply magnet (2 min), Wash by discarding supernatant, remove magnet, add 100 µL detection buffer, shake, apply magnet (2 min), then Wash by discard supernatant, remove magnet, add 100 µL NaOAc (pH 9) detection buffer, deliver solution to biosensor and



	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	

incubate (10 min), and (4) Apply Voltammetry Scan (1 min) to generate current peak. 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 proportional to the KPC quantity. 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. The generic process can be used for any nucleic acid target or protein gene by employing a different set of probes for capture and recognition.

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. Guanine's simple process steps are readily operated in a cartridge.



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