Guanine – Overview of IP and Patents

Electrochemical detection of low level pathogens

Electrochemical detection is a widely used technique to detect redox materials such as glucose and metals. A voltammetry method changes the chemical state of the redox material and produces an electrical signal that is measured with a sensor. Electrochemical detection is fast, easy to use, inexpensive, quantitative and mobile. Commercial instruments such as the Abbott iSTAT simultaneously detect multiple redox chemicals and glucose from the same sample.

Redox materials also include nucleotides such as guanine. Specific DNA, RNA and gene sequences can be detected by hybridizing targets with complementary probes bound to the electrode surface on a sensor. When voltage is applied guanine molecules oxidize and emit electrons to the electrode which produce an electrical current. The specific nucleic acid target is determined to be present when the peak current exceeds a threshold value from electrical noise.

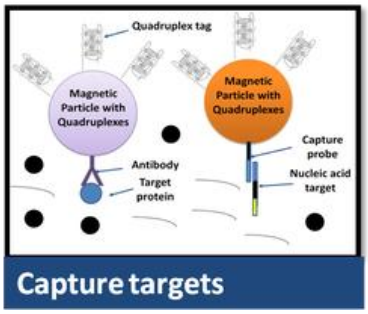
Nucleic acid sensors are rarely used commercially since their detection limits are not capable of measuring low levels needed for clinical use. Attempts have been made to improve the signal-to-noise resolution using sensor electrodes made from nanomaterials such as carbon nanotubes. However nanosensors are expensive to manufacture, have a low production yield, and their signals are highly variable from electrode-to-electrode due to manufacturing inconsistencies.

Redox tags are an alternative approach for electrochemical detection. Redox tags such as ferrocene can be bound to targets instead of conventional optical tags. Low detection limits are achieved by replicating nucleic acid targets in advance of detection. GenMark increases bacteria targets using PCR. Its blood pathogen assay employs a 5 hour multiplex PCR stage following a 12 hour culture to detect a high number of bacteria and antimicrobial resistance markers. Cue Health replicates COVID-19 targets using isothermal amplification with a redox substrate to detect COVID RNA in a 20 minutes process. Because of the high number of primers used in isothermal amplification, it may not be feasible to detect multiplex targets or to quantify target concentrations.

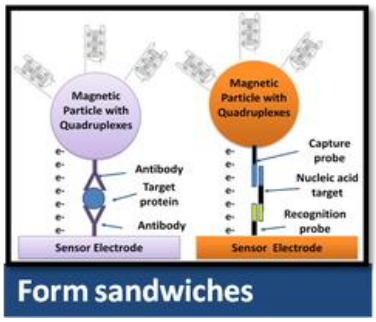
Guanine Inc. takes a different approach to detect multiple targets at low detection limits while maintaining all of the benefits achieved from electrochemical detection. Guanine invented the oligonucleotide tag (synthetic DNA) that binds specific detection targets with millions of tags on a microparticle. This amplifies detection signals without using PCR or isothermal replication. The oligonucleotide tags contain a 70 mer capture sequence for rapid and specific hybridization, and a 20 mer detection sequence from a string of G, A, T or C to produce unique oxidation peaks on a common electrode. Multiple electrodes and microparticle conjugates enable high multiplexing from a single sample.

Guanine has re-invented electrical detection using synthetic DNA tags that will revolutionize accurate, mobile detection of multiple infections

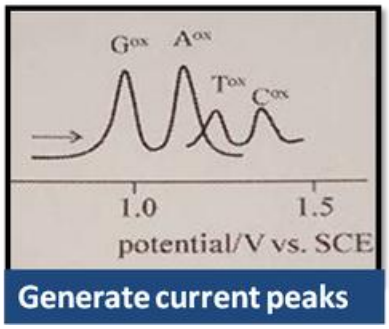
Simple test process: Detection employs simple steps that can be conducted in minutes in a test cartridge that captures targets after onboard lysing, magnetic separation and electrochemical detection.



Samples are lysed and targets are captured by magnetic particles

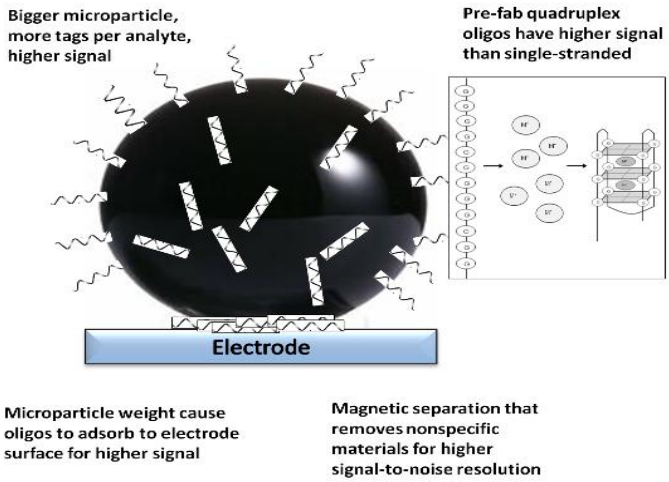


Particle-target complexes for proteins and nucleic acids are magnetically separated and form sandwiches on electrodes



Tags composed of majority guanine, adenine, thymine or cytosine generate peaks at different voltage potentials

Signal amplification: Signal amplification is achieved by binding analytes with a magnetic particle conjugated with millions of guanine-rich oligonucleotide tags instead of replicating millions of copies. Limit of detection is configured for the intended use since bigger particles bind more tags to produce a stronger signal for measuring lower limit of detection.

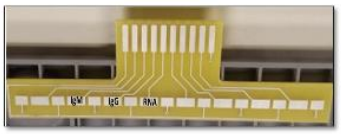


Configurable Limit of Detection: Signal amplification is achieved by binding analytes with a magnetic particle conjugated with millions of guanine-rich oligonucleotide tags instead of replicating millions of copies. Voltage generates a current peak if the pathogen is present. A bigger particle with more tags produces a stronger signal for lower limit of detection.

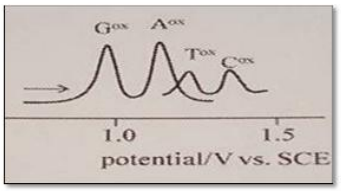
Particle Size	Signal from 10^4 targets/mL
500 nm	0.4µA
750 nm	0.6µA
1.5 µm	1.2µA
Can go > 20 µm	

Multiplexing: Signal amplification is achieved by binding analytes with a magnetic particle conjugated with millions. A sensor can detect up to 24 targets per sample using 6 working electrodes that can each detect 4 groups of unique tag signals.

Multiple MicroParticles with ligands & tags

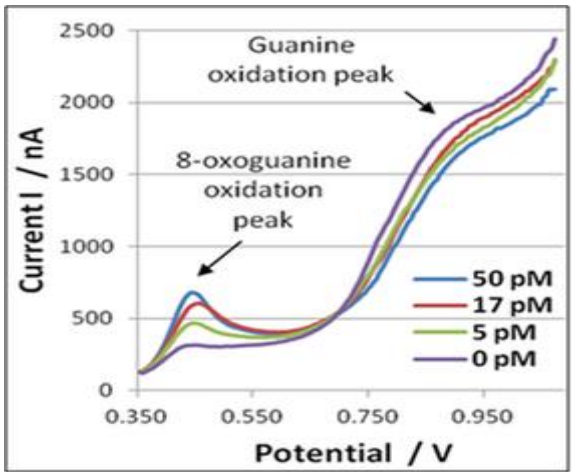


Multiple Electrodes for sandwiches

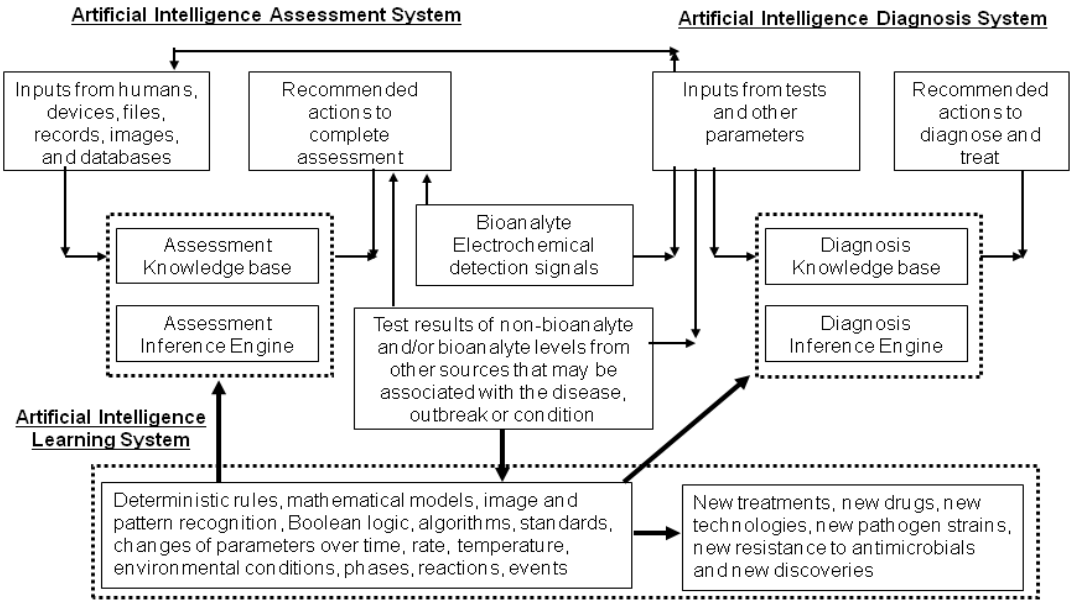


Multiple Nucleotide oxidation peaks on same electrode (G,T,A,C and/or oxo-derivatives)

Quantification: Detection tags bind to targets and produce an electrical current peak proportional to the quantity of targets in the sample. The burst is bigger when tags comprise a 20 Guanine sequence that is prefabricated into quadruplexes (planes of 4 guanine separated by Na+ cations) that generate 8-oxoguanine oxidation peaks.



Artificial Intelligence: Guanine's detection platform can be integrated with AI modules to provide cost and time savings in medical diagnosis. Modules can assist by assessing symptoms and recommending what tests are appropriate, enabling patients to supply their symptoms and history by answering specific queries, providing a preliminary diagnosis that can link symptoms with the test results, and learning new diagnostic rules by assessing multiple patient factors.



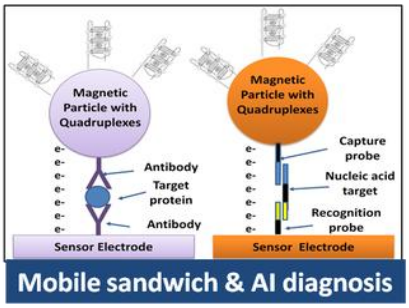
Guanine's oligonucleotide detection tags are protected in three approved patents for detection structures, devices, methods and artificial intelligence

US 11,105,801

US, CA, EP (pending)
Expiry 2039

Mobile detection:

- **Structures**
- **Devices**
- **Methods**
- **Artificial intelligence**



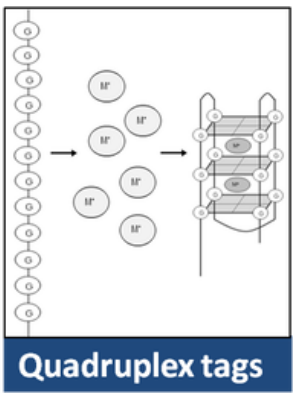
Bioanalyte signal amplification and detection with artificial intelligence diagnosis. This invention discloses a signal amplification sandwich structure for amplifying detection signals from proteins, nucleic acids and microbes using a plurality of an electrochemically detectable oligonucleotide tag bound to a multifunctional particle. The invention further discloses a method and device that uses the signal amplification sandwich structure to detect and/or quantify low levels of one or more biological analytes using an off-the-shelf point-of-care electrochemical potentiostat, like a glucose meter for virtually any biological analyte. The invention further discloses a method and device that applies an artificial intelligence (AI) system to recommend actions for assessment and diagnosis of a disease, outbreak or condition with an artificial intelligence learning system to incorporate improvements, additions and modifications to the artificial intelligence systems and its constituents.
download US 11,105,801

US 11,175,285
US, CA
Expiry 2038

Stronger signal than single-strand tags:

- **Structures**
- **Devices**
- **Methods**

Ultra-sensitive bioanalyte quantification from self-assembled quadruplex tags



Abstract

This invention allows ultra-low levels of virtually any biological analyte to be detected and quantified rapidly, simply and inexpensively with an electrochemical biosensor using a novel electrochemically detectable tag that replaces optical labels. The tag binds to an analyte directly, or indirectly using one or more ligands and particles, and consists of a quadruplex electrochemically detectable oligonucleotide rich in guanine, or a single-stranded electrochemically detectable oligonucleotide rich in guanine that self-assembles into a quadruplex electrochemically detectable oligonucleotide when exposed to cations that enable quadruplex self-assembly. Quadruplex electrochemically detectable oligonucleotide tags are exposed, adsorbed or hybridized at the surface of a biosensor working electrode. An electrochemical technique facilitates the quadruplex tags to produce 8-oxoguanine oxidation signals proportional to the analyte level in the samples. The resulting analyte levels measured from 8-oxoguanine oxidation signals are 1,000 times lower than analyte levels measured from guanine oxidation signals.

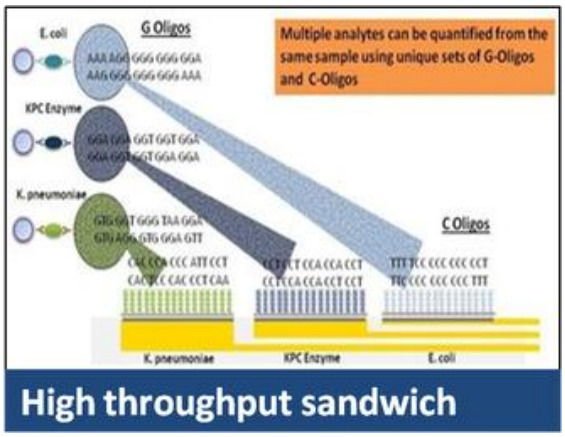
download us 11,175,285

US 9.624,532
US, CA, DE, FR, GB
Expiry 2035

High throughput detection:

- **Structures**
- **Devices**
- **Methods**

Ultra-sensitive detection of extremely low level biological analytes using electrochemical signal amplification and biosensor



Abstract

This invention allows ultra-low levels of virtually any biological analyte to be detected and quantified rapidly, simply and inexpensively with an electrochemical biosensor using a novel electrochemical signal amplification technique. The invention amplifies detection signals from low level analytes using an innovative sandwich ELISA structure that

replaces optical labels with a massive amount of electrochemically detectable guanine rich oligonucleotide tags. Selective binding is achieved with matched pairs of either commercial or custom analyte binding materials such as monoclonal antibodies or single strand DNA. The guanine tags are eluted from the sandwich structures and hybridize with complementary cytosine rich oligonucleotide recognition probes attached to the surface of a biosensor working electrode. An electrochemical technique generates a signal in proportion to the guanine level on the working electrode which is also proportional to the analyte level in the sample. Magnetic separation and a nanosensor are used to improve the signal-to-noise ratio for measuring analyte levels 1,000,000 times lower than enzyme-linked immunosorbent assay (ELISA).

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