

# Universal Bioanalyte Signal Amplification for Electrochemical Biosensor

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## Abstract

A rapid, simple, and inexpensive electrochemical biosensor with guanine-conjugated microbead tags can achieve the detection limits of PCR and culturing. Guanine is a redox species that functions as an electrochemical tag for bioanalyte detection. Microbeads conjugated with guanine-rich oligonucleotides deliver millions of electrochemical tags per bioanalyte. When guanine-conjugated microbeads are used in a sandwich assay with magnetic beads and matched ligand pairs, extremely low levels of diverse bioanalytes can be electrochemically measured including microorganisms, proteins and nucleic acids.

**Keywords:** Universal bioanalyte signal amplification; Biosensor; Sensitivity; Limit of detection; Limit of quantification

## Introduction

While many techniques are currently employed to detect biological analytes, electrochemical detection is very appealing because of its low cost, ease of use, and rapid time for producing a quantitative result. Electrochemical signals are generated by a redox method when redox analytes are present in a sample in measureable quantities, such as  $\sim 10^{14}$  glucose molecules associated with 1.1 mmol/L glucose in blood.

However, most clinical and environmental bioanalyte applications require much lower quantities to be measured such as  $\sim 10^6$  guanine molecules associated with 5,000 copies/mL of HIV RNA. This is below the detection limit of electrochemical biosensors.

Various approaches have applied to reduce biosensor detection limit. Nanostructured biosensor working electrodes improve signal-to-noise resolution by reducing the active surface area of the electrode [1], but encountered challenges with reliably measuring low nanoamp signals, and in fabricating nanoscale structures with poor batch to batch consistency, limited throughput, low production yields, and high unit costs. PCR used in advance of detection raises signals by increasing the number of nucleic acid targets [2], but added complexity, time and cost to negate the biosensor benefits. Magnetic separation reduces noise by removing background interferences before detection [3], but the inherent background signals from water electrolysis prevents the detection of low redox signals. Nanoparticles increased signal strength by delivering multiple detection tags per analyte [4] but the number of tags was limited by the small surface area of the nanoparticles. To date, none of these approaches have attained the detection levels in commercial applications comparable to PCR and cultures.

Most commercial bioanalyte detection methods bind optical labels to bioanalytes which are measured with optical readers. In a typical colorimetric ELISA assay, detection limits measure  $\sim 10^6$  bioanalytes associated with 1 pg/mL concentration. When lower levels need to be detected, bioanalytes are artificially increased in number using amplification, so that there can be a high level of labels provided for detection. PCR and cultures typically provide a 106 fold increase in bioanalytes along with the labels linked to the bioanalytes. However, if the objective of amplification is to actually increase the number of labels, then a more efficient approach is to bind the required number of labels to the bioanalyte and avoid resource-intensive PCR or time intensive culturing.

Microbeads and oligonucleotides offer a unique solution to greatly reduce biosensor detection limits. Microbeads have a much larger

surface area than nanoparticles and can bind millions of oligonucleotide tags using biotin-streptavidin chemistry. Oligonucleotides provide long sequences of electroactive guanine. Polystyrene microbeads at least 1 micron in diameter are conjugated with the oligonucleotides (20 or more bases) and a detection antibody or other ligand depending on the bioanalyte to be detected. A 15 micron microbead and 20 guanine oligonucleotide provides about 108 guanine. Lower detection limits are attained by increasing the microbead size so the surface can bind with more oligonucleotides, and increasing the length of the oligonucleotide to deliver more guanines. The system achieved 3 cfu/mL of *E. coli* O157:H7 in environmental water samples [5].

A biodetection test cycle comprises a) purifying analytes with magnetic separation to remove nonspecific materials, b) amplifying bioanalytes with guanine oligonucleotide tags to create sandwiches, c) elute oligonucleotide tags by raising the temperature or adding chemicals, d) allow eluted guanine tags to hybridize with cytosine rich oligonucleotides bound to a biosensor working electrode, e) generate a guanine oxidation signal with an electron transport mediator, and f) convert the signal to a bioanalyte concentration with a preprogrammed calibration curve. A cocktail of magnetic beads and guanine-conjugated microbeads can be used to detect multiple bioanalytes in the same test. Oligonucleotides unique sequences of guanine and other bases if multiple bioanalytes are to be individually measured on separate working electrodes with complementary cytosine rich probes for hybridization.

In conclusion, the benefits of electrochemical biosensor can be extended to detecting low bioanalyte concentrations by replacing single optical tags with millions of electrochemical labels bound to microbeads. This can open new applications for biosensor use in ultrasensitive detection of pathogens for environmental, biosecurity, and health surveillance including point-of-care and point-of-use applications for microorganisms, proteins and nucleic acid targets.

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