Development of AccuPSATM, a novel digital immunoassay for sub-femtomolar measurement of PSA in post radical prostatectomy patients


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Introduction

The ability to detect PSA in clinical prostate specific antigen (PSA) assays with sensitivity and specificity that matches or exceeds the traditional RIA has been the goal of many research groups. Digital immunoassay technology provides an alternative approach to the traditional sandwich immunoassay. AccuPSATM is a novel digital immunoassay designed to allow for the measurement of PSA in a concentration range of 0.000005 ng/mL (0.005 pg/mL, or 5 fg/mL). The signal CV from triplicate replicates of positive control standards was less than 20% over a three year period of study. A linear dynamic range was established from 0.000005 ng/mL to 50 ng/mL.

Methodology

Single Molecule Array (SMA) Methodology

SMA technology is based on including single, immobilized antibody, enzyme, and signaling molecule in an immunoassay format. The assay is initiated in a presence of enzyme substrate where fluorescence is generated. In contrast to traditional ELISAs in which fluorescent product of the signal enzyme off target in a bulk solution and emitted fluorescent photons. SMA technology allows for fluorescent product to be captured, immobilized, and trapped in the same nanoliter-sized volume, resulting in the buildup of a high concentration of fluorescent signals. SMA technology has been used in many applications. AccuPSATM uses fluorescent signals to measure PSA.

Assay Components

- Streptavidin-enzyme conjugate
- PSA,  pg/mL

Assay Sequence

1. Spot PSA onto 96 well plate (100 µL/assay)
2. Add blocking agent to the exposed plate
3. Incubate with biotinylated anti-PSA monoclonal antibody
4. Add buffer to unexposed wells
5. Rinse plate with PBS
6. Add streptavidin-enzyme conjugate
7. Rinse plate with PBS
8. Read plate on SMA reader

Linearity

Linearity was measured with guidance from NIST-SPA. The relationship of a "high" calibrator point to a "low" calibrator point and a very low tertiary serum sample is shown in the following figure. The assay demonstrated a linear dynamic range of 0.000005 ng/mL to 50 ng/mL.

Results

A characteristics of a digital assay is a step-response that results in a flat concentration CV profile across the dynamic range of the assay. In the following graph, the predicted concentration CV profile calculated from an assumed 0.01% measurement error for AccuPSATM is shown along with the actual measured concentration CV (CV) profile of the test data. The calculated values were shown to be a good match for the actual measured values.

Preclinical/In Vitro Test

Assay was tested in vitro using a panel of PSA positive cell lines and a panel of PSA negative cell lines. Assay was also tested in vitro using a panel of PSA positive cell lines and a panel of PSA negative cell lines. Assay was also tested in vitro using a panel of PSA positive cell lines and a panel of PSA negative cell lines. Assay was also tested in vitro using a panel of PSA positive cell lines and a panel of PSA negative cell lines. Assay was also tested in vitro using a panel of PSA positive cell lines and a panel of PSA negative cell lines.