Ultrasensitive Digital Immunoassay for PSA by Single Molecule Array (Simoa™) Technology in Prostatectomy Patients

**Significance** Measurement of prostate-specific antigen (PSA) following radical prostatectomy (RP) has become standard practice for monitoring prostate cancer recurrence. Plasma PSA levels following surgery are typically undetectable by most assay methods, and it is generally agreed that undetectable postsurgical PSA over time indicates a good prognosis. To date, assessment of surgical and secondary treatment effectiveness has relied on monitoring for PSA rise using assays that are unable to measure PSA at very low concentrations. As long as PSA remains undetectable, the patient assumes that there is no biochemical evidence of cancer recurrence. The period of undetectable PSA can be brief or on the order of years, depending on the assay used. The less sensitive the assay, the longer this assurance is offered, although PSA could be rising but simply not detected. Physician response to reemergence of rising PSA after RP has depended on a number of factors, including the time to biochemical recurrence (BCR), clinicopathological factors, and patient life expectancy. BCR is generally defined as a confirmed PSA concentration of 0.2 ng/mL (200 pg/mL). BCR occurs in up to 40% of patients after RP, and a third of these patients ultimately develop metastatic disease. Importantly, although clinical data is increasingly showing that early adjuvant and salvage radiation therapies after surgery have significantly improved patient outcomes, current assays cannot measure PSA at concentrations that could help stratify patients and thus allow physicians to identify patients who would benefit from early adjuvant treatment.

Monitoring of postsurgical PSA using more sensitive assays was initially examined in the 1990s with the development of the first ultrasensitive immunoassays, which could measure PSA levels down to 10 pg/mL. A number of reports established the prognostic significance of serial ultrasensitive PSA measurements, showing that an ultrasensitive method could detect PSA rises well before a first-generation assay could, providing potentially years of early warning. Recently, even more sensitive assays having a detection cutoff of 1 pg/mL have been used to explore the prognostic significance of the lowest postsurgical PSA (nadir PSA) for biochemical recurrence. Whereas PSA was undetectable in approximately half the patients, recurrence risk could nonetheless be stratified depending on nadir PSA concentration, indicating the clinical value of ultra-low PSA measurement for predicting long-term BCR-free survival. The ability to accurately measure PSA values for all RP patients could improve assessment of patient prognosis and response to treatment, and better target secondary therapy to those who may benefit most.

**Quanterix Solution** Quanterix™ has developed an ultra-sensitive platform that can measure individual proteins at concentrations 1000 times lower than current immunoassays available today. The Single Molecule Array (Simoa™) technology at the heart of this platform enables the detection and quantification of biomarkers previously difficult or impossible to measure. The ultrasensitivity of Simoa assays sets it apart from all other immunoassays available today, offering PCR-like limits of detection for both existing and novel protein biomarkers. Briefly, this novel technology detects single protein molecules in blood or other body fluids by capturing the proteins on microscopic beads that are coated with specific antibodies and labeling the immunocomplexes with a reporter enzyme that can generate a fluorescent product. After isolating the beads in 50-fl reaction chambers designed to hold a single bead, fluorescence imaging detects the single protein molecules.

Quanterix has validated a PSA assay for the fully automated Simoa HD-1 Analyzer. Having a limit of detection (LOD) of 0.028 pg/mL, this assay exhibits a 3-log improvement in sensitivity compared to the most sensitive commercially available PSA assays. The assay is robust, with a functional

![Figure 1. LOQ of Simoa PSA Assay. LOQ was estimated by sample replicate CVs across 6 weeks of testing. Female serum samples are highlighted in pink.](image-url)
sensitivity < 0.05 pg/mL (Fig. 1), total imprecision < 10% from 1–50 pg/mL, a linear response over 4 logs of dynamic range down to sub-pg/mL levels, and excellent agreement with a comparator method. The analytical sensitivity of this assay enabled reliable quantification of PSA in all post-RP samples tested.

To further assess the clinical utility of the Simoa PSA assay, a total of 31 frozen serum specimens were obtained from men who had undergone RP. Overall, 11 (35.5%) developed BCR within a mean of 2.1 years. Men without evidence of BCR had a minimum of 5 years of PSA follow-up. The distribution of nadir PSA levels for the BCR and non-BCR groups is highlighted in Figure 2. The mean PSA levels in the non-BCR and BCR groups were 2.3 pg/mL and 47 pg/mL, respectively (P < 0.001). A value of 3.0 pg/mL was used as a threshold for defining 2 risk groups (high vs. low) for BCR. The Kaplan-Meier survival curves for the risk groups defined by the bifurcated nadir value is highlighted in Figure 3 (p = 0.00024). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the nadir threshold of 3 pg/mL for predicting BCR within 5 years were 100%, 75%, 69%, and 100%, respectively.

The Simoa PSA assay demonstrates a greater than 1000-fold improvement in analytical sensitivity and day-to-day assay reproducibility that can reliably quantify PSA in prostatectomy patients. In this case, 5-year BCR-free survival was accurately predicted after surgery. Identification of a reliable predictor of BCR soon after RP has important implications for PSA testing frequency, selecting candidates for adjuvant therapy, and reassuring a large subset of men that they are not at risk of recurrence. The Simoa technology and HD-1 platform represents an opportunity to use high-sensitivity assays for applications beyond monitoring cancer recurrence, including the assessment of neurological, inflammatory, cardiovascular, and infectious diseases.

**Conclusion** The Quanterix Simoa PSA test offers a 1000-fold improvement in sensitivity over currently available assays. This sensitivity enables reliable measurement of PSA concentrations in plasma from all human subjects who have undergone radical prostatectomy, and predicts 5-year BCR-free survival after surgery.

**References**


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**Figure 2.** Dot plot of Simoa PSA nadir (pg/mL) for non-BCR and BCR groups.

**Figure 3.** Kaplan-Meier time-to-BCR curves. Nadir 3.0 pg/mL threshold.