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HIGHLY SENSITIVE CANCER BIOMARKER DETECTION IN HUMAN SERUM (75%) USING REAL-TIME LABEL-FREE BIOSENSORS

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Introduction

There is a significant difference in the limit of detection when buffer or clinical sample is used as the assay media for biosensor assays. The change in the detection limit, when clinical samples are used, is mainly due to the lower signal to noise ratio resulting from the high non-specific binding of biomolecules (exist in high concentrations in clinical sample) to the sensor surface. This is especially noticeable when label-free biosensors are employed. Therefore researchers avoid the use of high concentrations of serum in most analysis and serum concentrations of 10 to 50 % were usually reported [1-3]. Two label-free biosensor platforms, a novel, fully automated QCM biosensor (QCMA-1, Sierra Sensors GmbH, Germany) and an SPR biosensor (Biacore 3000, GE Healthcare, Sweden) for biomarker detection in human serum were used in this study. Prostate specific antigen (PSA) was chosen as the cancer biomarker to be detected in serum.
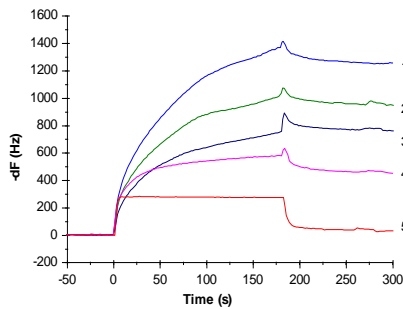
Methodology and Results

Figure 1. Effect of changing the buffer composition on the human serum signal. The injection of 10 % human serum in PBS/T buffer (1) and PBS/T buffers containing additives at varying concentrations to mouse IgG immobilised sensor chip. Buffer (5) was chosen as it minimises 98% of the non-specific binding of sera proteins to the sensor surface [4].
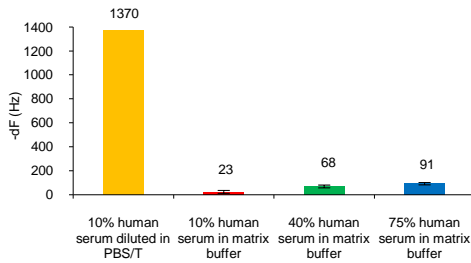

Figure 2. Effect of increasing human serum concentration on the sensor signal. Human serum was diluted in matrix buffer at varying concentrations and injected (3 minutes) on mouse IgG immobilised surface.
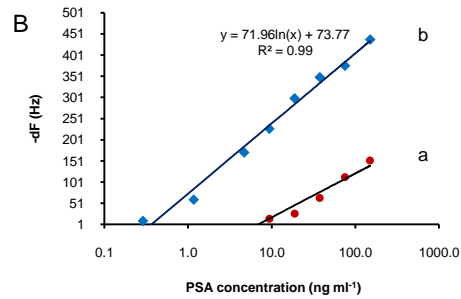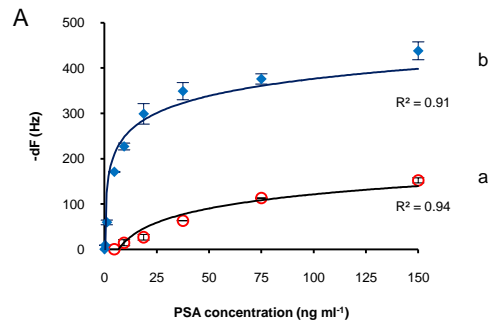

Figure 3. tPSA at varying concentrations (in 75% human serum) were injected to tPSA capture antibody immobilised QCM sensor surface. Later, as a sandwich assay tPSA detection antibody (a) or antibody modified 40 nm Au nanoparticles (b) were injected and the frequency changes due to binding of nanoparticles were recorded and calibration curves (A - linear scale; B - log scale) were obtained.

A: $R^2 = 0.91$ (b), $R^2 = 0.94$ (a)
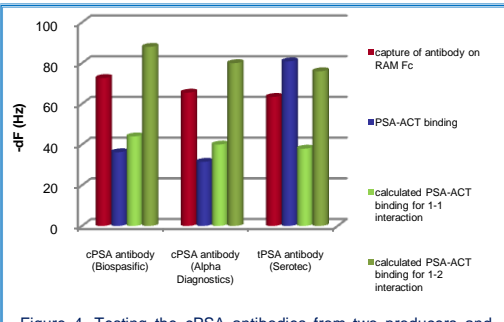
B: $y = 71.96\ln(x) + 73.77$, $R^2 = 0.99$

Figure 4. Testing the cPSA antibodies from two producers and comparison with the tPSA antibody from Serotec using QCMA-1 instrument.
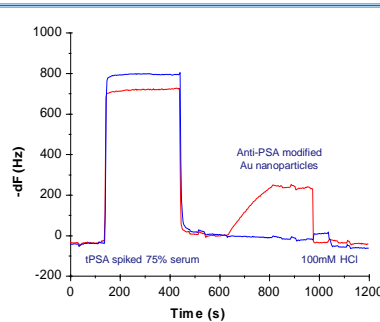

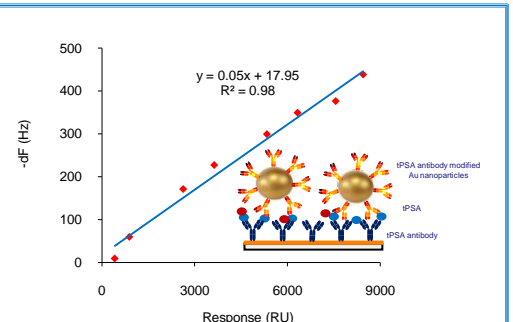Figure 5. QCMA-1 trace for tPSA sandwich assay in 75% human serum.

$y = 0.05x + 17.95$, $R^2 = 0.98$

Figure 6. The comparison of QCMA-1 and Biacore 3000 results for tPSA sandwich assay in 75% human serum.

Conclusion

Initially a new buffer was formulated to eliminate 98% of the non-specific human serum protein binding to the sensor surface. Using this new formulated 'matrix buffer', the detection of PSA concentrations down to 0.29 ng ml⁻¹ in 75% serum was achieved with a linear detection range of 0.29 -150 ng ml⁻¹ using the QCM and the SPR sensor chips with Au nanoparticles employed for sensitivity enhancement [4, 5]. The achieved results indicate that data obtained from both QCMA-1 and Biacore 3000 instruments were comparable and additionally both sensor chips can be used for the fast and easy detection of PSA in patient samples.

References

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