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## Introduction

Real-time immuno-PCR is a new sensitive technique to quantify specific proteins. Antibodies are used to identify the protein and real-time PCR is used for quantification. We have developed a real-time immuno-PCR assay for quantification of Prostate Specific Antigen, PSA, which is a serum marker for disease in the prostate. In this study we have compared different ways to assemble the real-time immuno-PCR detection system.

## Methods

The three assays tested are assembled as sandwich immuno assays in polypropylene PCR-tubes, using two antibodies to identify the protein. The detection system consist of a DNA molecule that is linked to the second antibody. In assay I the DNA is linked via a biotin-streptavidin-biotin link, while in assay II and III it is covalent linked (Fig 1). Assay I has the capture antibody adsorbed to the tube surface and the assay is then assembled stepwise with washing in between. In assay II the capture antibody is also adsorbed to the tube surface but the PSA and the antibody/DNA-conjugate is premixed in a separate tube before addition. For assay III streptavidin coated PCR tubes are used. A biotinylated capture antibody, the PSA sample and the detection antibody/DNA conjugate were premixed in a separate tube before addition. After final washing of all three assays the amount of immobilized DNA was quantified with real-time PCR. This amount should correspond to the amount of captured protein target.

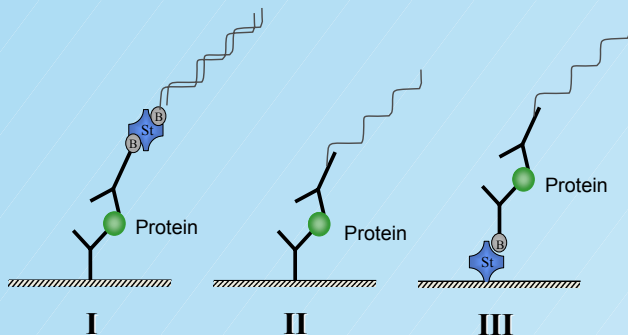


Figure 1. Schematic pictures of the three different assay setups.

## Results

The reproducibility of the assay was evaluated as the standard deviation of duplicate samples. The assays based on chemically conjugated DNA (II and III) is higher than that of the assay based on a streptavidin-biotin link and stepwise assembling (I) (table 1). This difference is most likely due to the larger number of incubation and washing steps in assay I.

Table 1

Assay setup	I	II	III
SD (Ct) <sup>1</sup>	0.45	0.25	0.19
SD (%) <sup>2</sup>	37	19	14
Sensitivity (molecules) <sup>3</sup>	$1.4 \cdot 10^6$	$9.4 \cdot 10^5$	$2.8 \cdot 10^6$

1. Standard deviation of Ct calculated from 16 duplicate samples of different concentrations.

2. Standard deviation expressed in percentage estimated as  $2^{SD}$  (assuming 100% PCR efficiency).

3. Sensitivity was calculated as the mean value + 2 SD of 10 negative samples.

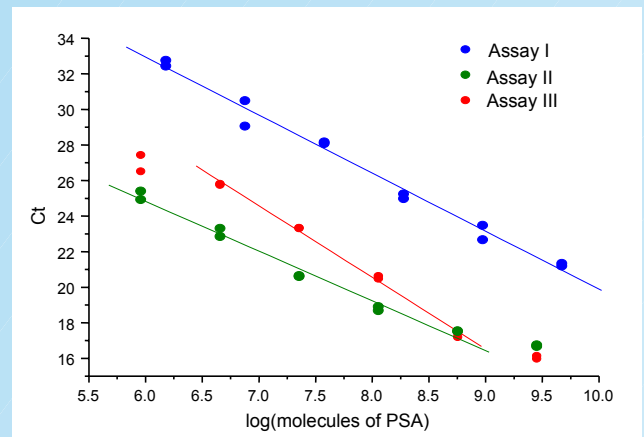


Figure 2. Standard curves based on the three real-time immuno-PCR assays

We found that all three real-time immuno-PCR assays have about the same respons range. From about  $3 \cdot 10^9$  molecules to about  $10^6$  molecules (table 1). This corresponds to about 0.01 ng/ml of PSA using a 5  $\mu$ l samples.

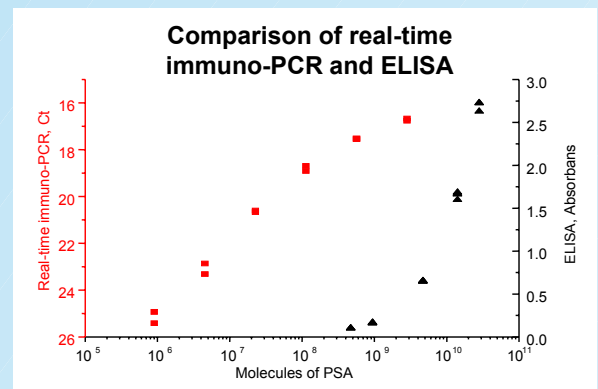


Figure 3. Real-time immuno-PCR versus ELISA

Comparison of standard curves for real-time immuno-PCR (assay II) and ELISA shows the different behavior of the two assays. While PCR is an exponential reaction ELISA respons is linear. In ELISA 25  $\mu$ l samples were used while 5  $\mu$ l was used in the real-time immuno-PCR.

## Conclusions

- Assays based on chemical coupling of the DNA-label to the detection antibody yielded higher experimental reproducibility.
- The sensitivity of the three assays tested was similar.
- The exponential behavior of PCR results in a much larger dynamic range compared to ELISA.