Using real-time PCR for quantification of proteins

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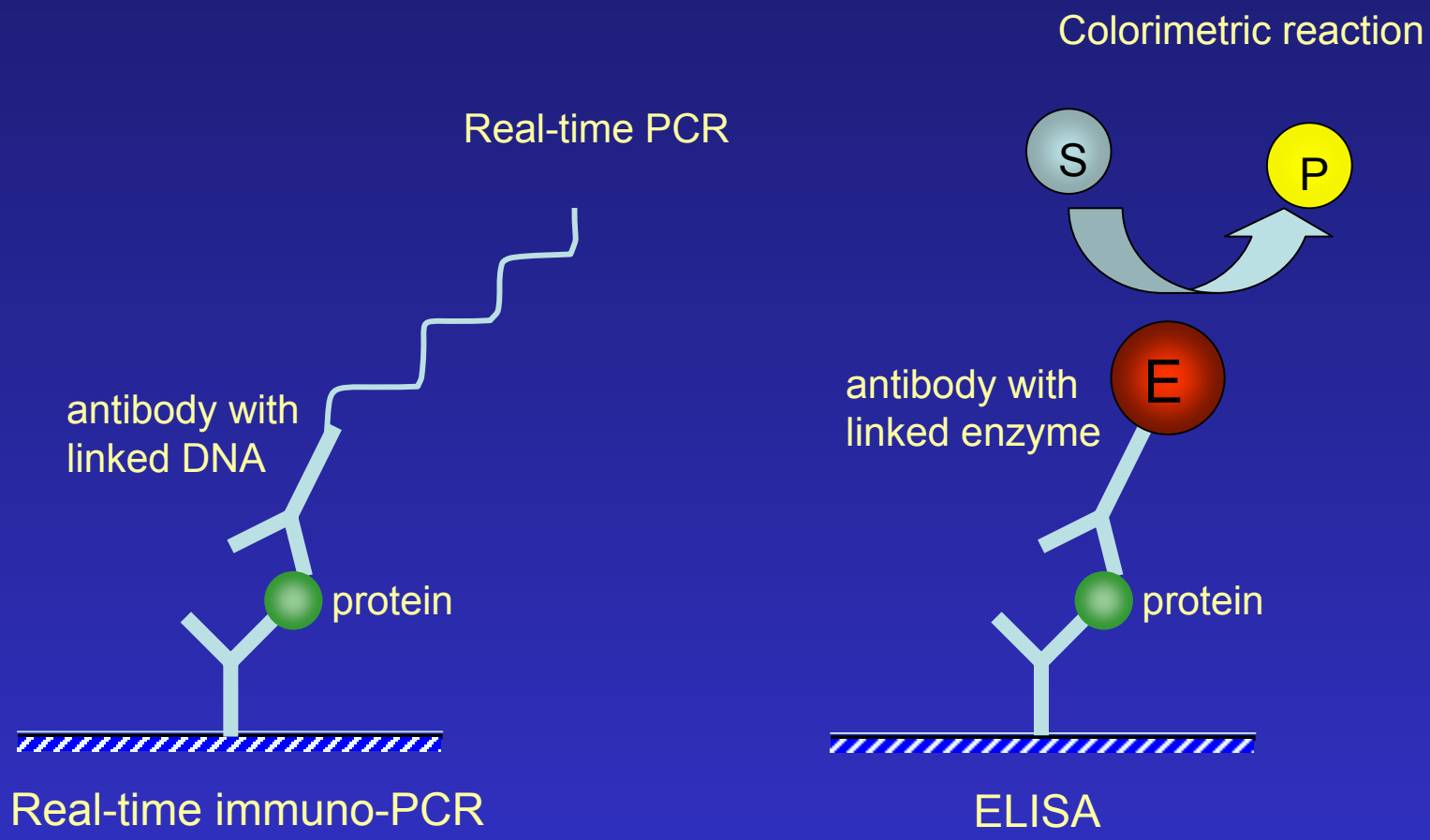
Real-time immuno-PCR

Run real-time PCR!

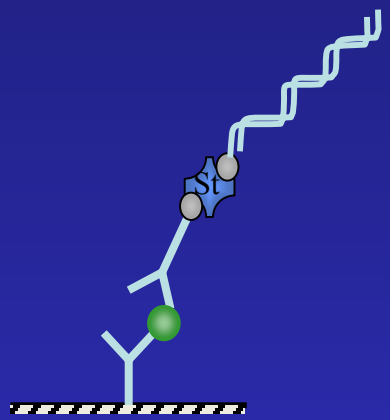
Real-time immuno-PCR vs ELISA



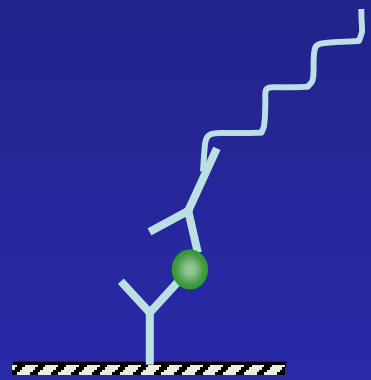
Benefits of real-time immuno-PCR

- More sensitive.
- Larger quantification range.
- Multiplexing possible with the same instrument.

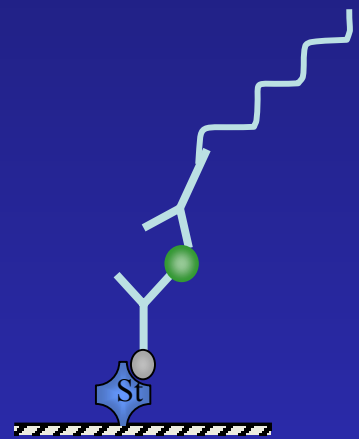
Comparison of different assemblages



Assemblage I

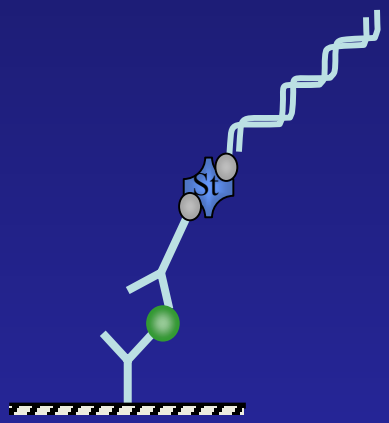


Assemblage II

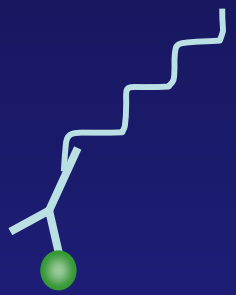


Assemblage III

Assemblage I



- Capture antibody adsorbed to the well surface.
- All components added stepwise.
- Streptavidin-biotin link between detection antibody and DNA.
- 6 incubations and washing steps.



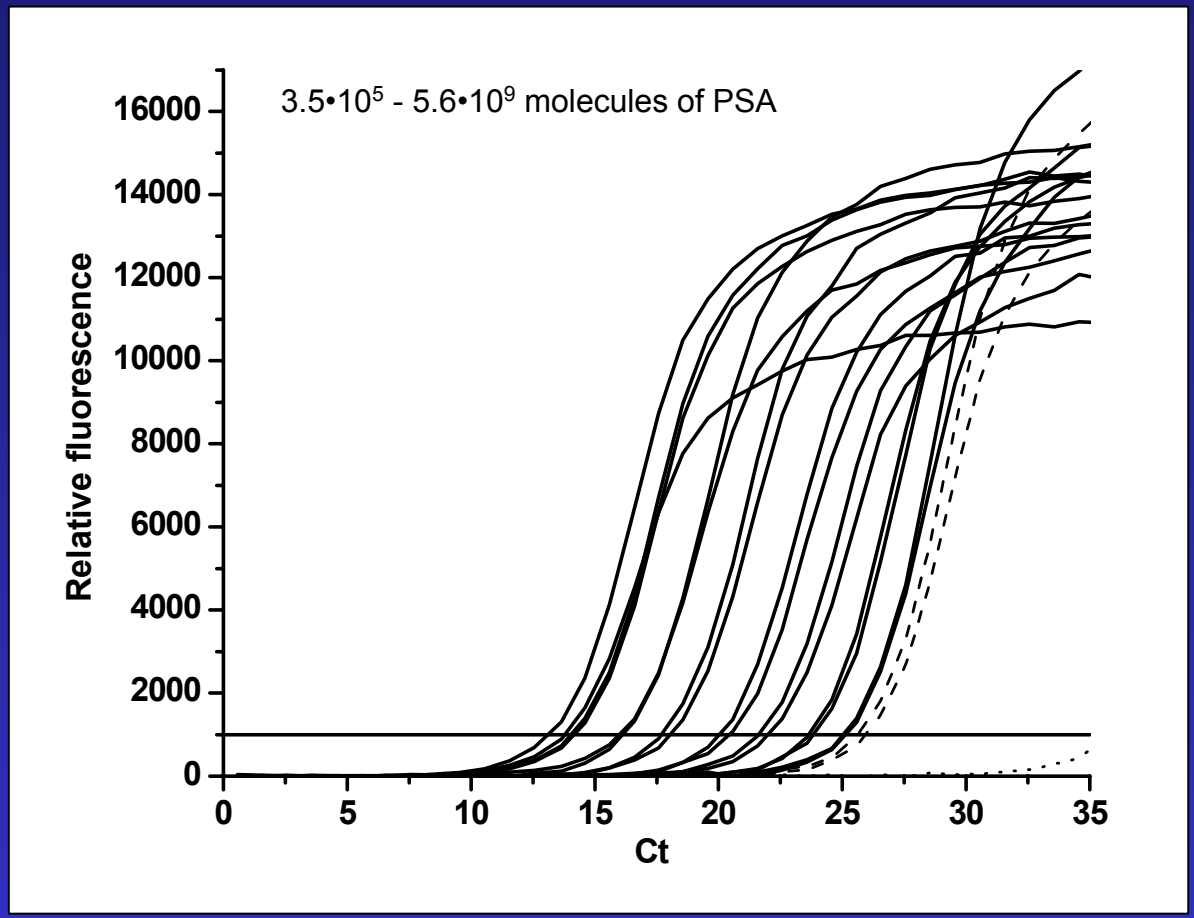
Assemblage II

- Capture antibody adsorbed to the well surface.
- Covalent conjugation between detection antibody and DNA-label.
- 3 incubations and washing steps.

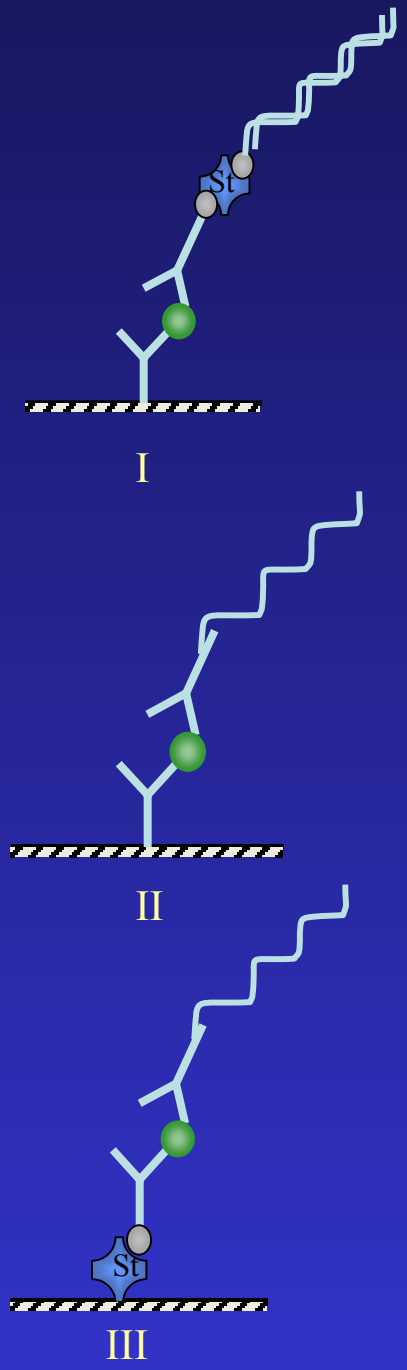
Assemblage III

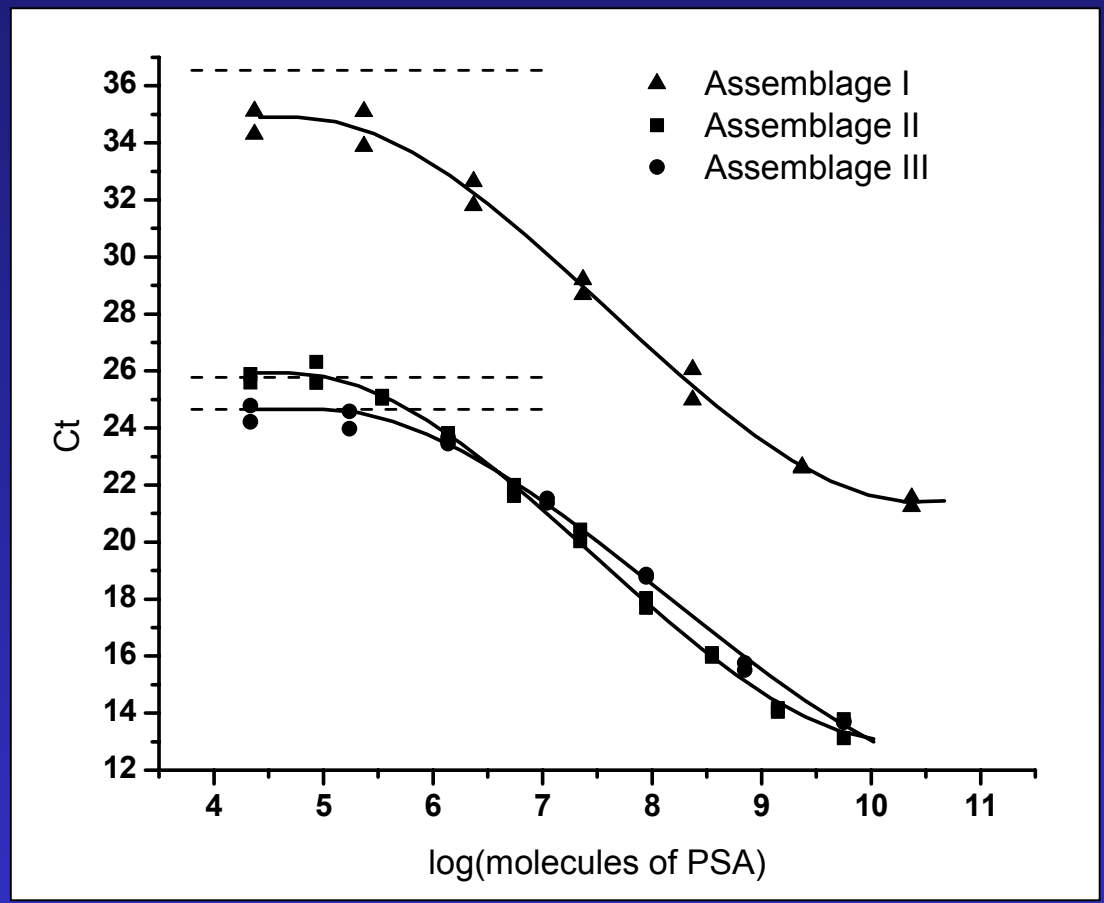
- Streptavidin-biotin link to bind the capture antibody.
- Covalent conjugation between detection antibody and DNA-label.
- 2 incubations and washing steps.

Amplification curves

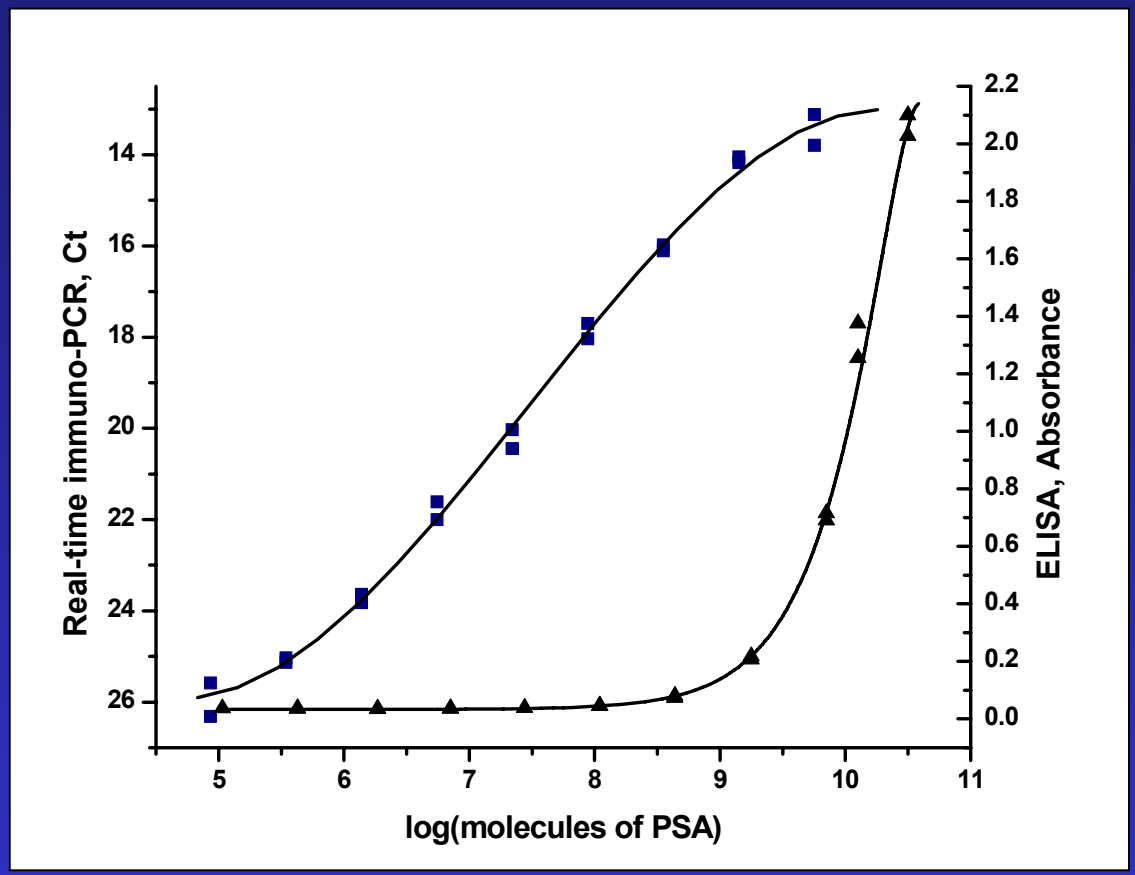


Standard curves



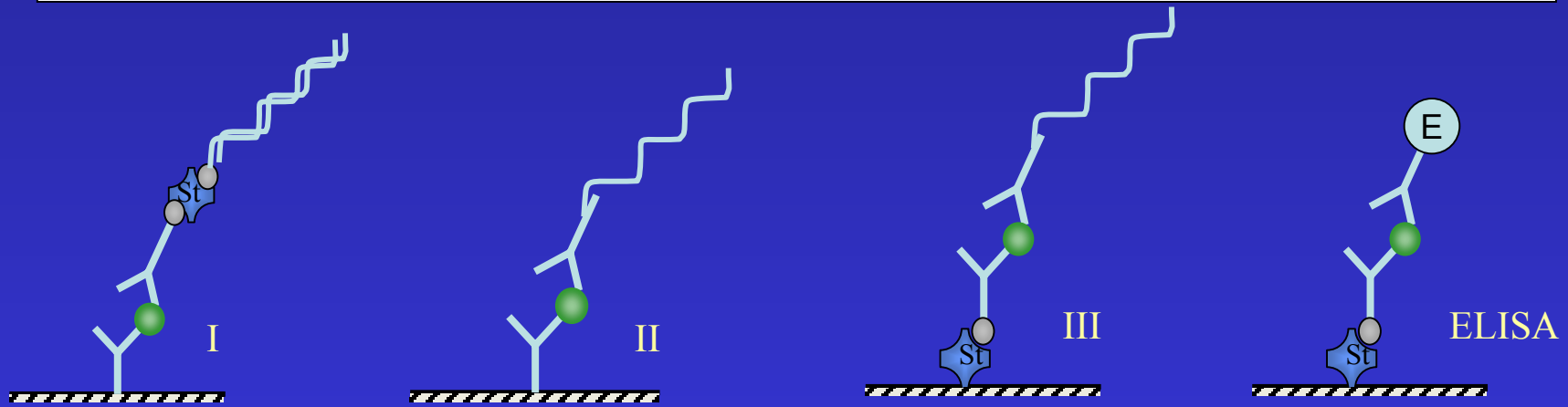


Comparison with ELISA



Summary of results

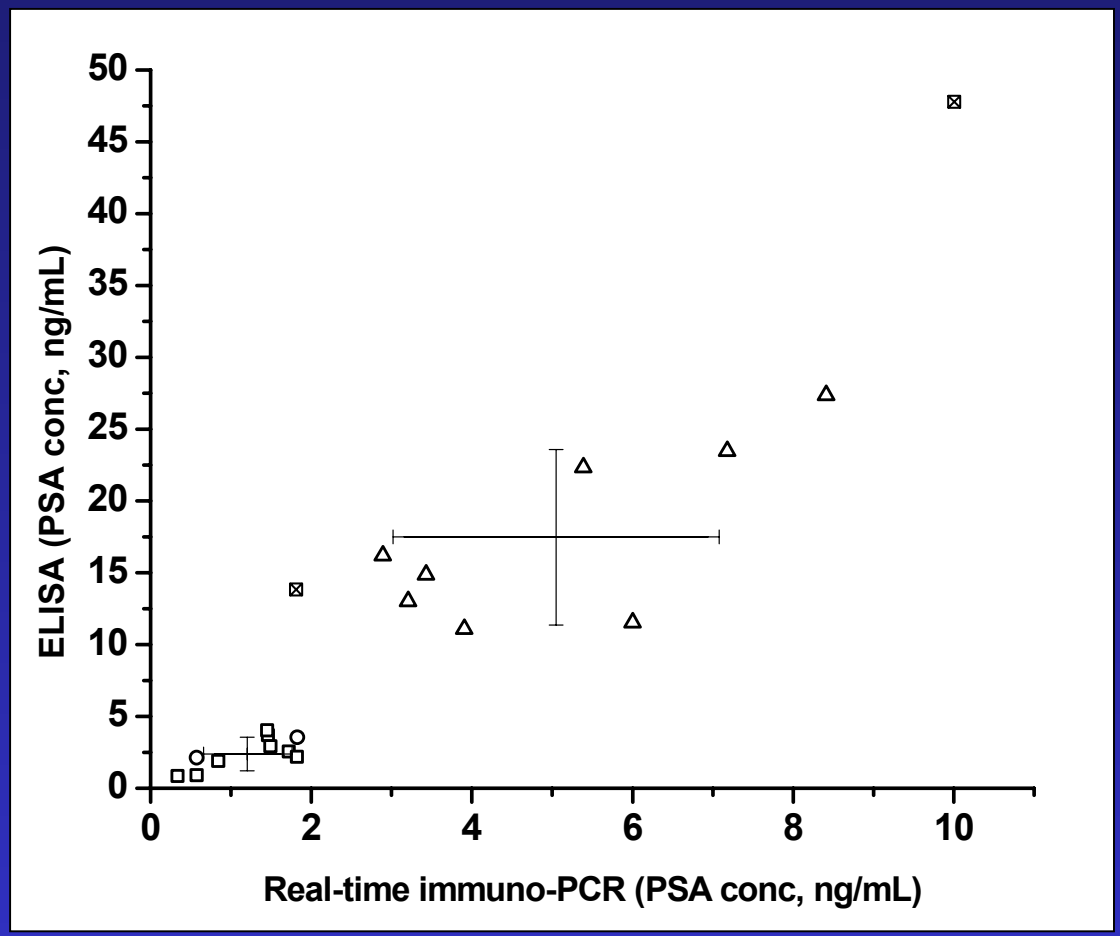
	Dynamic range (molecules)	SD (cycles)
Assemblage I	$1.7 \cdot 10^6$-$1.0 \cdot 10^{10}$	0.45
Assemblage II	$4.8 \cdot 10^5$-$5.6 \cdot 10^9$	0.25
Assemblage III	$1.8 \cdot 10^6$-$5.6 \cdot 10^9$	0.21
ELISA	$5.7 \cdot 10^7$-$2.8 \cdot 10^{10}$	-



Analysis of serum samples

- PSA, Prostate Specific Antigen.
- Marker for diseases in the prostate.
- Increased level in blood serum indicates disease.
- Analysed samples from:
  - 10 healthy male
  - 10 female
  - 8 male with prostate cancer
  - 2 male with Benign Prostatic Hyperplasia, BPH

Analysis of serum samples



Conclusions

- 100 times more sensitive than ELISA.
- Larger quantification range.
- Less incubation steps gives higher reproducibility.
- Compatible with serum samples.

Reference

Lind, K. and Kubista, M. 2005. J Immunol Methods, 304 (1-2): 107-116.

Acknowledgement

Mikael Kubista

Anders Stålberg

Tzachi Bar

CanAg DIAGNOSTICS

TATAA Biocenter

Alicia