Using real-time PCR for quantification of proteins

Kristina Lind

Department of Chemistry and Bioscience,
Chalmers University of Technology and TATAA Biocenter
Real-time immuno-PCR

protein antibodies DNA

Run real-time PCR!
Real-time immuno-PCR vs ELISA

Real-time PCR

antibody with linked DNA

protein

Real-time immuno-PCR

Colorimetric reaction

antibody with linked enzyme

protein

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

3.5\times10^5 - 5.6\times10^9 molecules of PSA
Standard curves

- Assemblage I
- Assemblage II
- Assemblage III

[Graph showing standard curves with log(molecules of PSA) vs. Ct]
Comparison with ELISA
### Summary of results

<table>
<thead>
<tr>
<th></th>
<th>Dynamic range (molecules)</th>
<th>SD (cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assemblage I</td>
<td>$1.7 \cdot 10^6$-1.0$ \cdot 10^{10}$</td>
<td>0.45</td>
</tr>
<tr>
<td>Assemblage II</td>
<td>$4.8 \cdot 10^5$-5.6$ \cdot 10^9$</td>
<td>0.25</td>
</tr>
<tr>
<td>Assemblage III</td>
<td>$1.8 \cdot 10^6$-5.6$ \cdot 10^9$</td>
<td>0.21</td>
</tr>
<tr>
<td>ELISA</td>
<td>$5.7 \cdot 10^7$-2.8$ \cdot 10^{10}$</td>
<td>-</td>
</tr>
</tbody>
</table>
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

ELISA (PSA conc, ng/mL) vs. Real-time immuno-PCR (PSA conc, ng/mL)
Conclusions

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

Reference

Acknowledgement

Mikael Kubista
Anders Stålberg
Tzachi Bar

TATAA Biocenter

Alicia