Prostate specific antigen (PSA)
Measurand characteristics, reference measurement procedures and reference materials for PSA

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PSA

• Why is standardization of PSA assays important?
• Characteristics of PSA
• Reference materials
• Reference measurement procedures
• Conclusions
Prostate cancer, basic facts

- Most common (non-skin) cancer in men
  - Life-time risk of acquiring prostate cancer 7 - 17%
- Second most common cause of cancer death
  - Life-time risk of death from prostate cancer 2 - 4%
  - 50-70% of cases advanced when giving rise to symptoms
- Preclinical stage during which early diagnosis and curative therapy are possible
- Potential target for screening
Clinical use of PSA

• Sensitive marker for prostate cancer
  – Expressed by more than 99% of all prostate cancers
  – Serum PSA increased by 1 gram tumor
  – Prostate-specific rather than cancer specific

• Used for
  – Monitoring of response to therapy
  – Detection of relapse
  – Evaluation of prognosis
  – Early diagnosis and screening (case finding)
PSA during monitoring of a prostate cancer patient

- Castration, androgen suppression
- Radiation
Early detection and screening for prostate cancer with PSA

Pathophysiological basis
Serum PSA at sampling and time to presentation

Stenman et al. Lancet 1994
Prostate cancer incidence and mortality in Finland 1958-2001

Prostate cancer incidence and mortality in Finland 1958-2001

Incidence and Mortality

Incidence
Mortality

Year


0 500 1000 1500 2000 2500 3000 3500 4000
Use of PSA and prostate cancer incidence in Norway

![Graph showing the relationship between PSA test rate and prostate cancer incidence. The Pearson correlation coefficient is r = 0.730 and p = 0.000.]
Prostate cancer incidence and mortality in Finland 1958-2001

Incidence and Mortality

Year

Incidence and Mortality

- Incidence
- Mortality
Survival of cancer patients and serum PSA at sampling

PSA cut-off

- <4 µg/l
- >4 µg/l

Time (years)

Stenman et al. Lancet 1994
Development of prostate cancer, screening and age

- **Lethal stage**
  - Tumor vol 1 kg

- **Clinical disease**
  - Tumor vol 32 g

- **Curable stage**
  - Tumor vol 1 g

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>10000</th>
<th>1000</th>
<th>100</th>
<th>10</th>
<th>1</th>
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<tbody>
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<td>40</td>
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<td>100</td>
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PSA (µg/l) vs. Age (years)

U-H Stenman 1995
Are we doing something wrong?

- Are we over-diagnosing?
- Yes, in 75% of those over 70 years of age
- Are we finding the patients that need to be cured?
- Not all, 35% of patients with clinically localized tumors experience a relapse
- We need to improve our diagnostic methods and procedures
  - Even earlier diagnosis
  - More cancer-specific methods
  - Avoid detection of clinically indolent tumors
  - Prognostic methods
Characteristics of measurand (PSA)

- 30 kDa chymotrypsin-like serine proteinase
- Produced by epithelial cells of the prostate
- Prostate specific, not cancer specific
- Dissolves seminal clot by degrading semenogelin
- PSA in plasma is heterogeneous
  - Complexes with protease inhibitors
  - Partially degraded forms and proforms of free PSA
  - Crossreacting substances
    - hK2
Gel filtration of PSA in prostate cancer serum

Assays for free PSA and PSA-ACT
<table>
<thead>
<tr>
<th>Forms of immunoreactive PSA in prostate cancer patients and men with BPH</th>
</tr>
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<tbody>
<tr>
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<tr>
<td>----------------</td>
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<tr>
<td>PSA-ACT</td>
</tr>
<tr>
<td>Free PSA</td>
</tr>
<tr>
<td>PSA- API (-AAT)</td>
</tr>
<tr>
<td>hK-2</td>
</tr>
<tr>
<td>PSA-A2M</td>
</tr>
</tbody>
</table>
SDS gel electrophoresis of PSA isoenzymes from seminal fluid separated by ion exchange chromatography.
Amino acid sequence of PSA

Activation peptide
in proPSA

-4 -2

MWVPVVFLTL SVTWIG APLILSR
1 7 17 27 37

IVGGWE CEKHSQPWQV LVASRGR AVC GGVLVHPQWV

Signal peptide

LTAAHCIRNK
47 57 67 77 87

SVII LGRHSL FHPE DTGQVF QVSHSFPHPL YDMSL LKNRF

LRPGDSSHD
97 107 117 127 137

LM LRLSEPA ELTD AVKVMD LPTQEPALGT TCYASGW GSI

EPEEFLTPKK
147 157 167 177 187

LQCVDLHVIS NDVCAQVHPQ KVTKFMLCAG RWTGGK STCS
GD SGGPLV CN
SDS gel electrophoresis of PSA isoenzymes from seminal fluid separated by ion exchange chromatography

Reducing conditions

Proportion of complex formation (%)
2-dimensional electrophoresis of free PSA in serum and isoelectric focusing of PSA isoenzymes isolated from seminal fluid

Charrier et al. Electrophoresis 20: 1075-81, 1999

Development of PSA standards

• Stanford conferences organized by Thomas Stamey in 1992 and 1994
• Preparation of standards for
  – Free PSA
  – PSA-ACT and free PSA 90/10 mixture
• Establishment of WG on PSA standardization
• Adoption of standards as WHO reference materials
  – WHO 96/668 (free PSA)
  – WHO 96/670 (PSA-ACT/PSA 90/10)

Development of PSA standards

- Purification of PSA from seminal plasma
- Value assignment by amino acid analysis (mol)
- Determination of MW by mass spectrometry, 28430
- Calculation of mass concentration
- Formation of PSA-ACT in vitro
- $A_{280}$ of PSA-ACT based on AA composition = 1.0
  - Assignment of mass concentration for PSA-ACT
- Preparation of PSA-ACT-free PSA 90/10 mixture

**Figure 4.** Potency of human serum sample (97/568) in individual assays calculated using IHR (A) and PSA 90:10 (96/670; B)

**Figure 5.** Potency of human serum sample (97/566) in individual assays calculated using IHR (A) and PSA 90:10 (96/670; B).
Epitope mapping of PSA

Summary Report of the TD-3 Workshop: Characterization of 83 Antibodies against Prostate-Specific Antigen.
Epitope mapping of PSA

Correlation Hybritech Tandem E - Wallac AutoDelfia before 2003

\[ y = -6.5112 \times 10^{-2} + 0.88453x \quad R^2 = 0.994 \]

Intercept: -0.084 [-0.198 to 0.000]
Slope: 0.994 [0.970 to 1.020]

Passing-Bablok agreement test N = 147

Intercept: -0.050 [-0.228 to 0.071]
Slope: 1.352 [1.315 to 1.392]
Passing-Bablok agreement test N = 147

Passing-Bablok agreement test N = 160
Slope : 0.963 [ 0.933 to 1.000 ]
Intercept : 0.033 [ 0.010 to 0.055 ]

Passing-Bablok agreement test N = 160
Slope : 0.787 [ 0.767 to 0.808 ]
Intercept : -0.022 [ -0.037 to 0.001 ]
**Correlation F/T**

**AutoDelfia**

\[ y = 8.7969 \times 10^{-3} + 0.95312x \quad R^2 = 1.000 \]

**DPC Immulite**

\[ y = -5.8281 \times 10^{-3} + 0.57812x \quad R^2 = 1.000 \]
Probability of prostate cancer in relation to total and free PSA
Excel formula available at: www.finne.info
Proportion of free PSA and tumor grade

Bangma J Urol 1997; 157: 544
Equimolarity of assays for PSA

• Ability to detect free and complexed PSA equally

• Assays based on polyclonal antibodies tend to overestimate free PSA
Effect of proportion of F-PSA on ratio between Access and Delfia total PSA

Tandem-E vs. Delfia

Beckman Access vs. Delfia
Recognition of free PSA by Delfia and Access assays

<table>
<thead>
<tr>
<th>Control containing &gt;90% F-PSA</th>
<th>Delfia T-PSA</th>
<th>Delfia F-PSA</th>
<th>Access T-PSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>2.12</td>
<td>2.00</td>
<td>4.1</td>
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<tr>
<td>Sample 2</td>
<td>19.6</td>
<td>19.1</td>
<td>28.0</td>
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</table>
## Recognition of various forms of free PSA by AutoDelfia and Beckman Access assays

<table>
<thead>
<tr>
<th></th>
<th>AutoDelfia</th>
<th>Access</th>
<th>Ratio</th>
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<tbody>
<tr>
<td></td>
<td>F-PSA</td>
<td>T-PSA</td>
<td>F/T</td>
</tr>
<tr>
<td>PSA-A</td>
<td>96,5</td>
<td>92,2</td>
<td>104,6</td>
</tr>
<tr>
<td>PSA-B</td>
<td>59,5</td>
<td>57,4</td>
<td>103,6</td>
</tr>
<tr>
<td>PSA-C</td>
<td>41,7</td>
<td>40,3</td>
<td>103,6</td>
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<td>PSA-D</td>
<td>55,0</td>
<td>61,6</td>
<td>89,2</td>
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<tr>
<td>PSA-E</td>
<td>50,6</td>
<td>52,9</td>
<td>95,5</td>
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<tr>
<td>ProPSA</td>
<td>22,7</td>
<td>23,9</td>
<td>94,9</td>
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Mean 1,269
Equimolarity of assays for PSA

- Beckman Access uses the same antibodies as Hybritech Tandem E
- What is the difference?
- Assay kinetics
  - Small molecules react faster
Frequency of aberrant results in 20000 samples analyzed with two methods

Median ratio Access:Delfia = 1.14

<table>
<thead>
<tr>
<th>Access value comparison</th>
<th>Frequency</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Access value &gt;2-fold Delfia</td>
<td>13/2651</td>
<td>0.49%</td>
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<tr>
<td>Access value &gt;1.5-fold Delfia</td>
<td>49/2651</td>
<td>1.8%</td>
</tr>
<tr>
<td>Access value &lt;0.67-fold Delfia</td>
<td>1/2651</td>
<td>0.00%</td>
</tr>
<tr>
<td>Access value &lt;0.50-fold Delfia</td>
<td>0/2651</td>
<td>-</td>
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**Comparison of Delfia and Access on samples with aberrant results and benign biopsy**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total PSA Delfia</th>
<th>Total PSA Access</th>
<th>Free PSA (%) Delfia</th>
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<tbody>
<tr>
<td>A</td>
<td>1,55</td>
<td>18,2</td>
<td>26%</td>
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<tr>
<td>B</td>
<td>0,71</td>
<td>5,7</td>
<td>22%</td>
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<tr>
<td>C</td>
<td>0,92</td>
<td>6,1</td>
<td>24%</td>
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<tr>
<td>D</td>
<td>1,28</td>
<td>5,2</td>
<td>20%</td>
</tr>
<tr>
<td>E</td>
<td>2,15</td>
<td>4,8</td>
<td>24%</td>
</tr>
<tr>
<td>F</td>
<td>5,62</td>
<td>12,2</td>
<td>21%</td>
</tr>
<tr>
<td>G</td>
<td>5,20</td>
<td>30,7</td>
<td>31%</td>
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Effect of mouse serum on interference

<table>
<thead>
<tr>
<th></th>
<th>Beckman Access T-PSA</th>
<th>Wallac Delfia T-PSA</th>
<th>Wallac Delfia F-PSA</th>
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<tr>
<td>Routine assay</td>
<td>6.33</td>
<td>0.73</td>
<td>0.26</td>
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<tr>
<td>Addition of mouse serum</td>
<td>2.74</td>
<td>1.08</td>
<td>0.26</td>
</tr>
</tbody>
</table>
Conclusions from assay comparisons

• Assay manufacturers compete with speed, capacity and price
• Quality suffers
• Who is responsible?
  – Customer?
    – Capable of judging quality?
• Manufacturer?
• Regulatory bodies?
Reference measurement procedures
Are they necessary?

PSA assays from major manufacturers are unusually well standardized

Room for improvement

Quality of assays from minor companies varies
Stenman U-H.
Immunoassay standardization: is it possible, who is responsible, who is capable?
Is it possible?

- Identification of reference antibodies
  - Known epitope specificity
  - Generally available
- Definition of assay format
  - Sandwich assay
  - Microtitration well format, sample volume 25 ul
  - Two-step incubation, 1 + 1 h incubation time
  - Applicable to any detection method
    - ELISA, Delfia, IRMA, luminescence
- Reference laboratories
- Calibrated samples
- Mass spectrometry?
Who is capable?

- I know some
- Endangered species
- Are they willing?
- Who will pay for it?
  - Not "sexy science" that you get grants for
Who is responsible?

- WHO?
- IFCC?
- JCTLM?
- We are all responsible
- We need to justify the expenses
- Calculation of additional health care costs caused by poor assay quality
- Poor quality is expensive
- Someone has to take the lead