Prostate specific antigen (PSA)

Measurand characteristics, reference measurement procedures and reference materials for PSA

JCTLM Symposium

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PSA

- Why is standardization of PSA assays important?
- Characteristics of PSA
- Reference materials
- Reference measurement procedures
- Conclusions

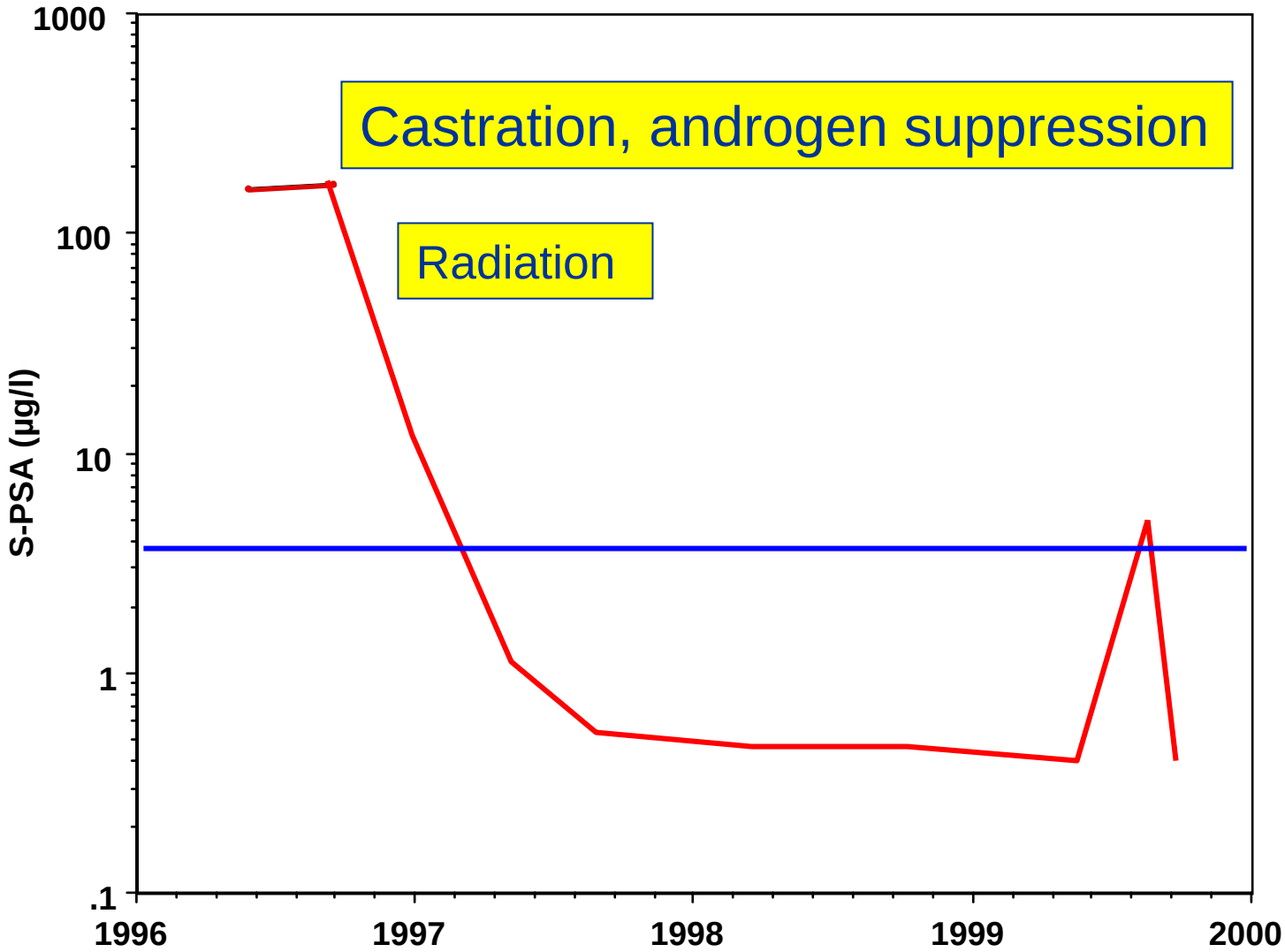
Prostate cancer, basic facts

- 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)

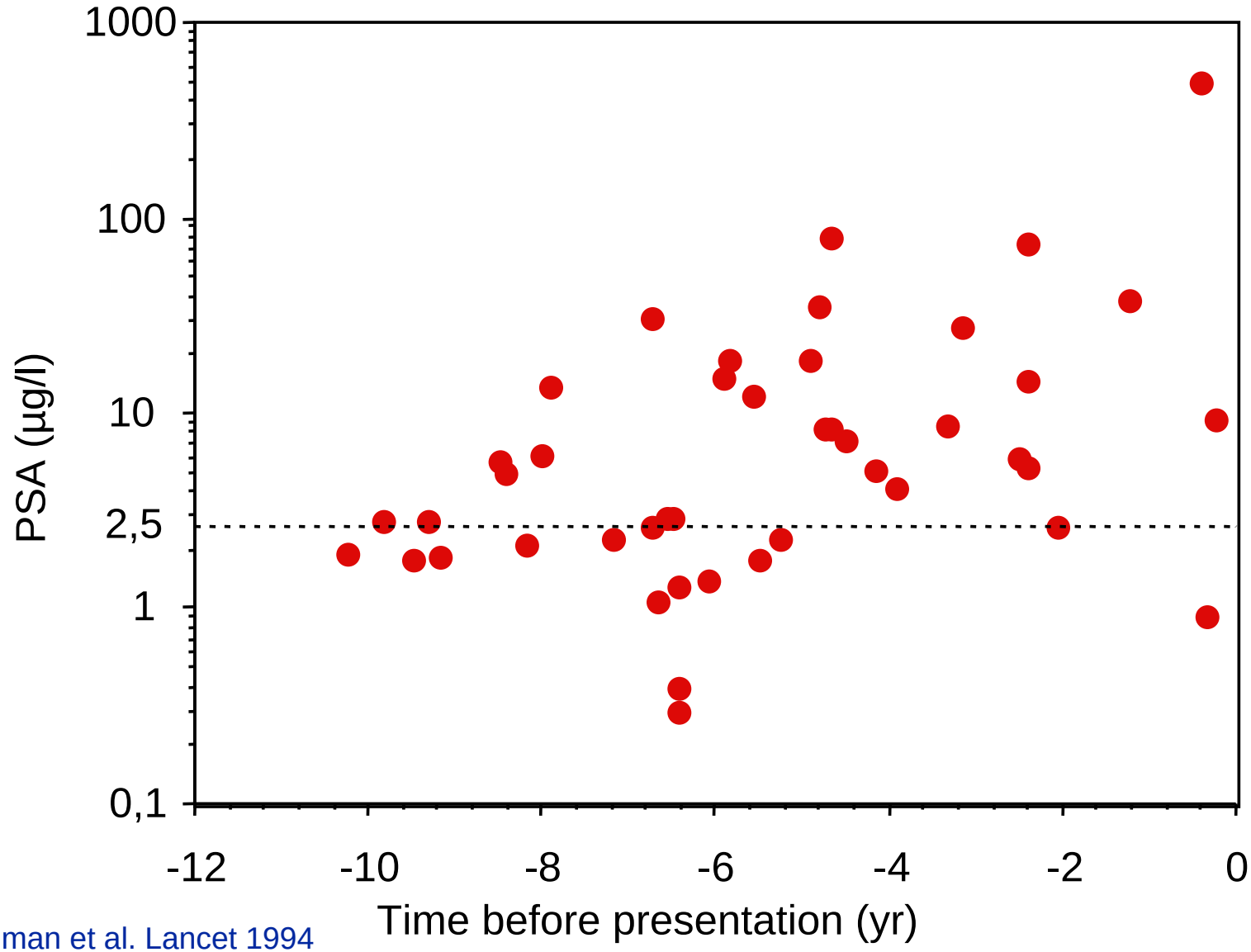
PSA during monitoring of a prostate cancer patient



Early detection and screening for prostate cancer with PSA

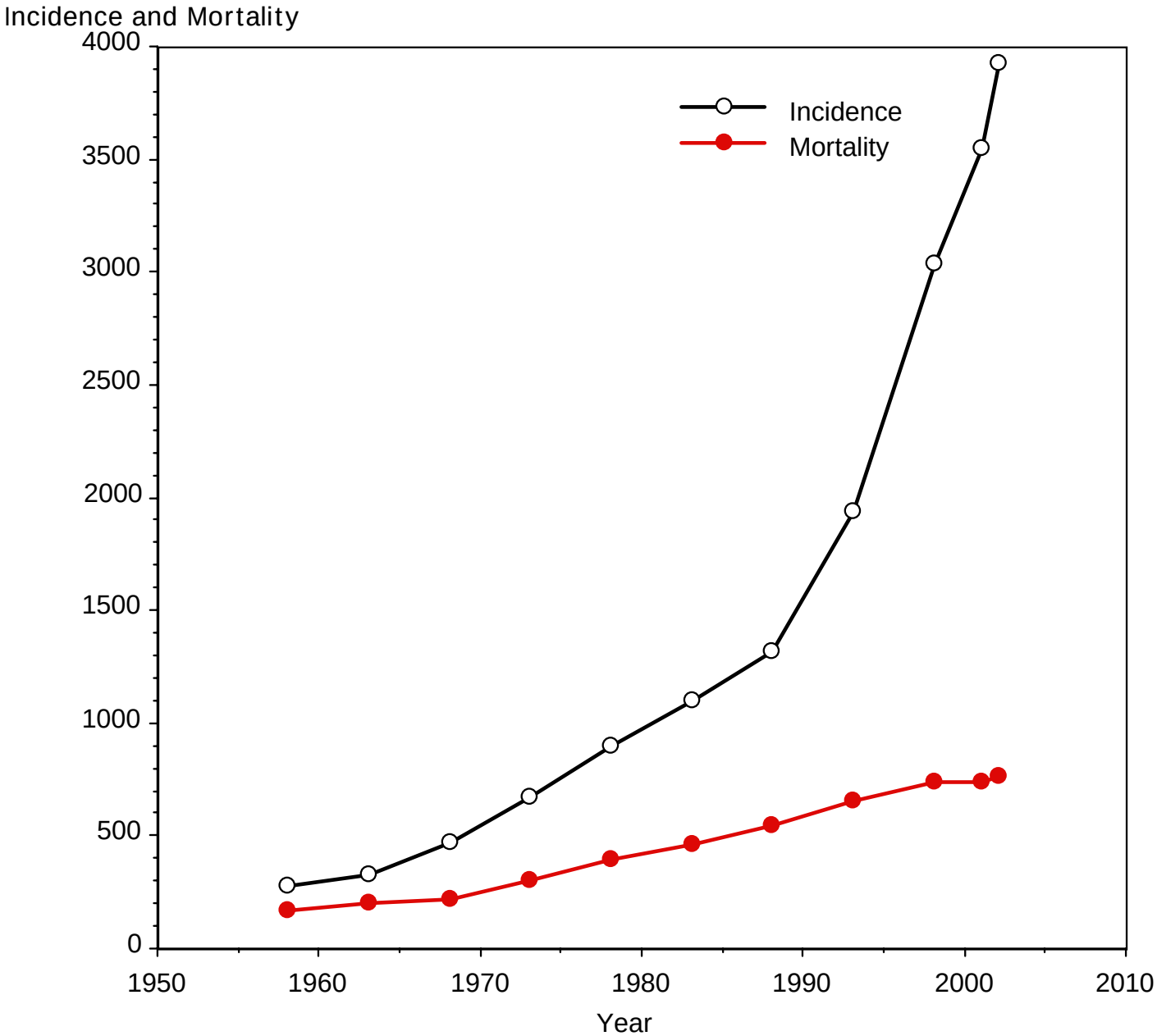
Pathophysiological basis

Serum PSA at sampling and time to presentation

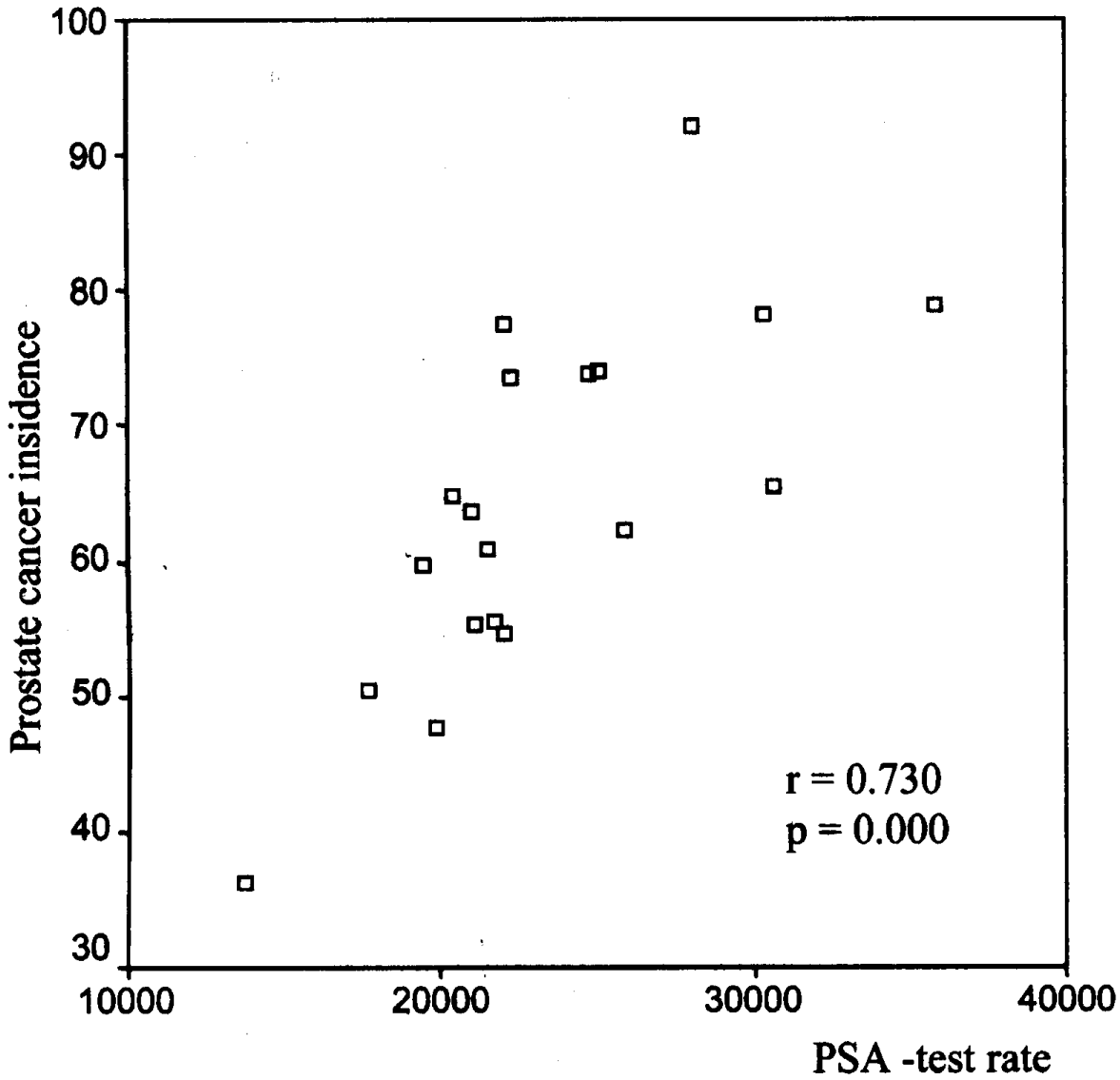


Stenman et al. Lancet 1994

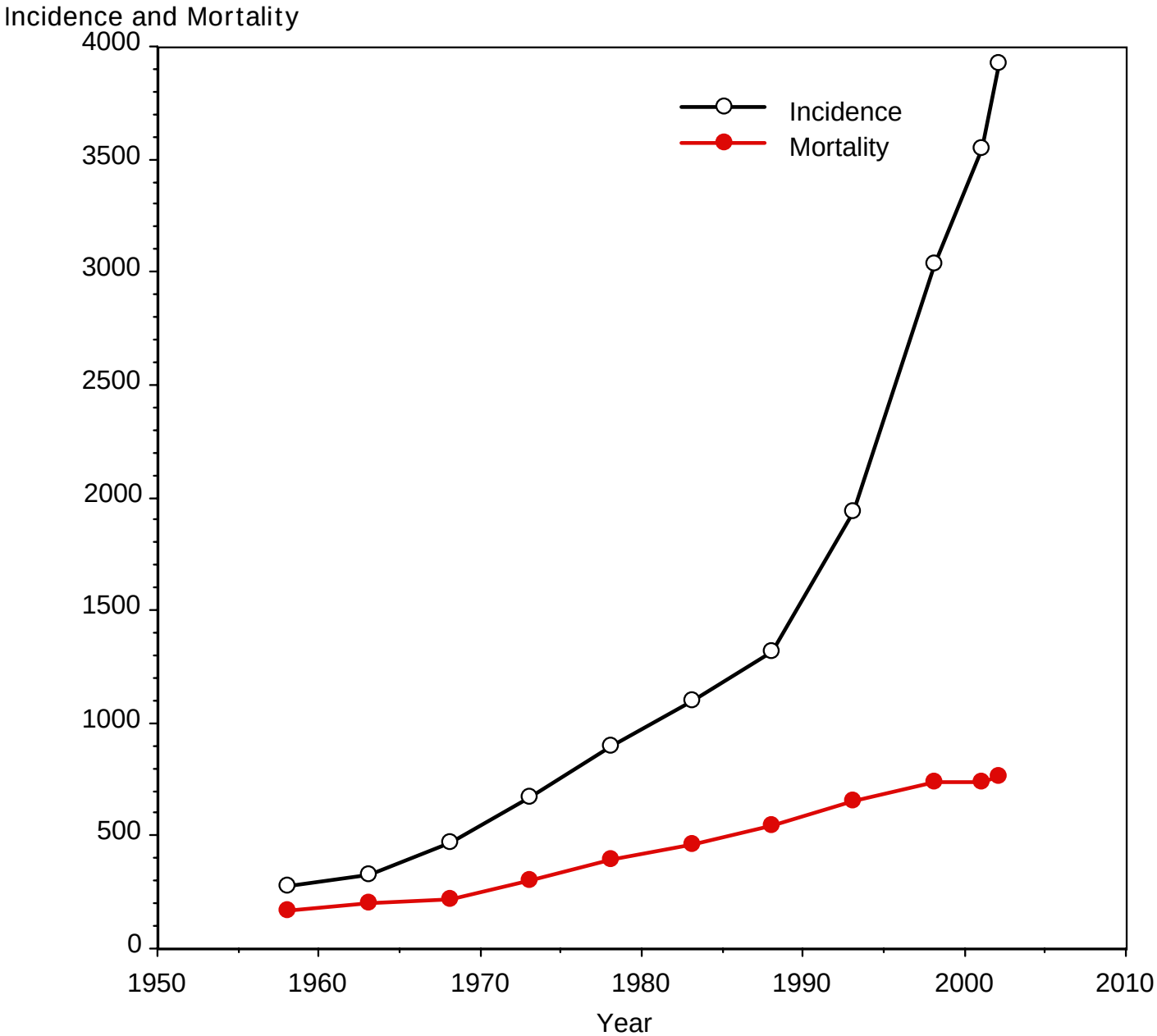
Prostate cancer incidence and mortality in Finland 1958-2001



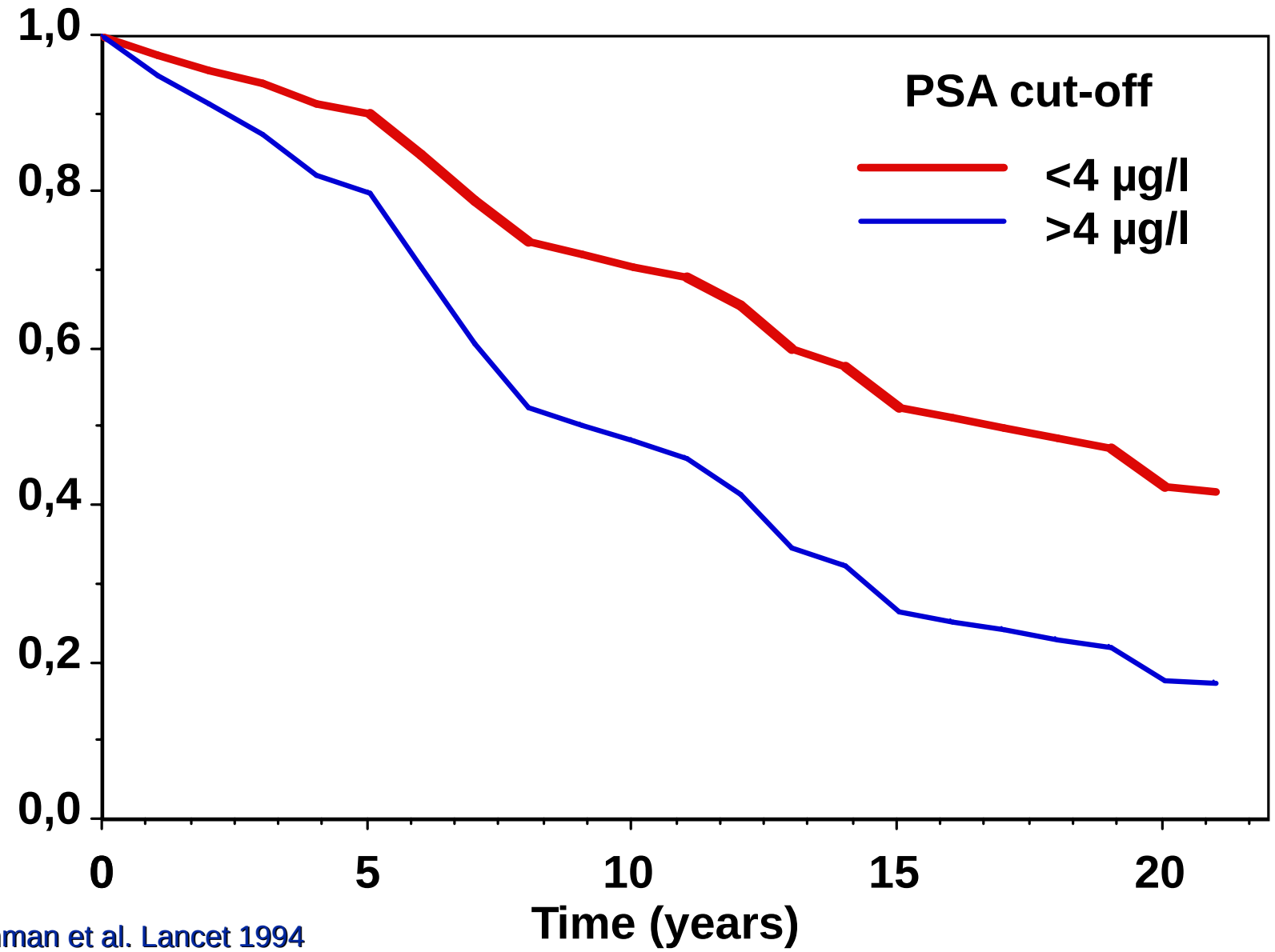
Use of PSA and prostate cancer incidence in Norway



Prostate cancer incidence and mortality in Finland 1958-2001

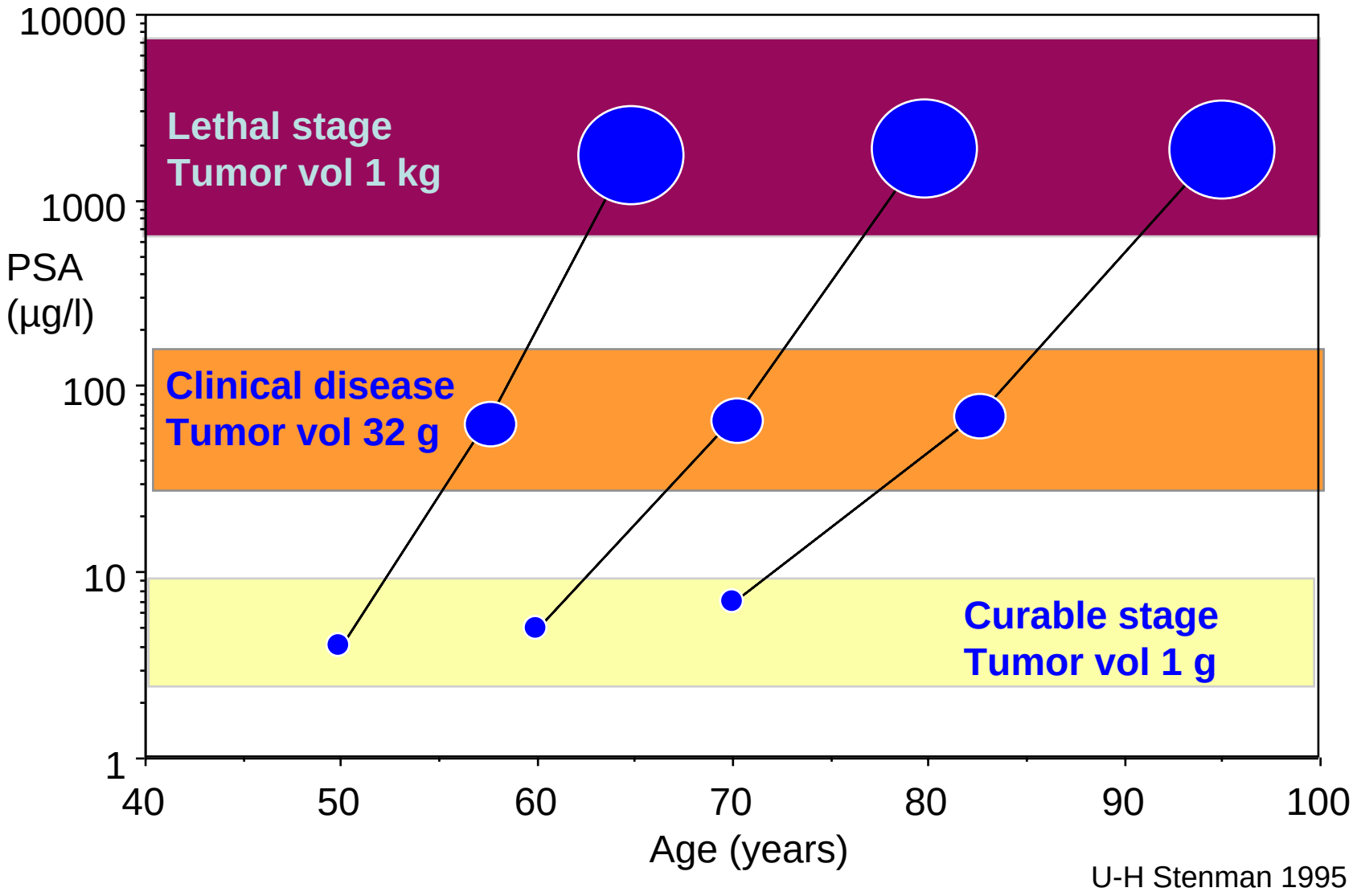


Survival of cancer patients and serum PSA at sampling



Stenman et al. Lancet 1994

Development of prostate cancer, screening and age



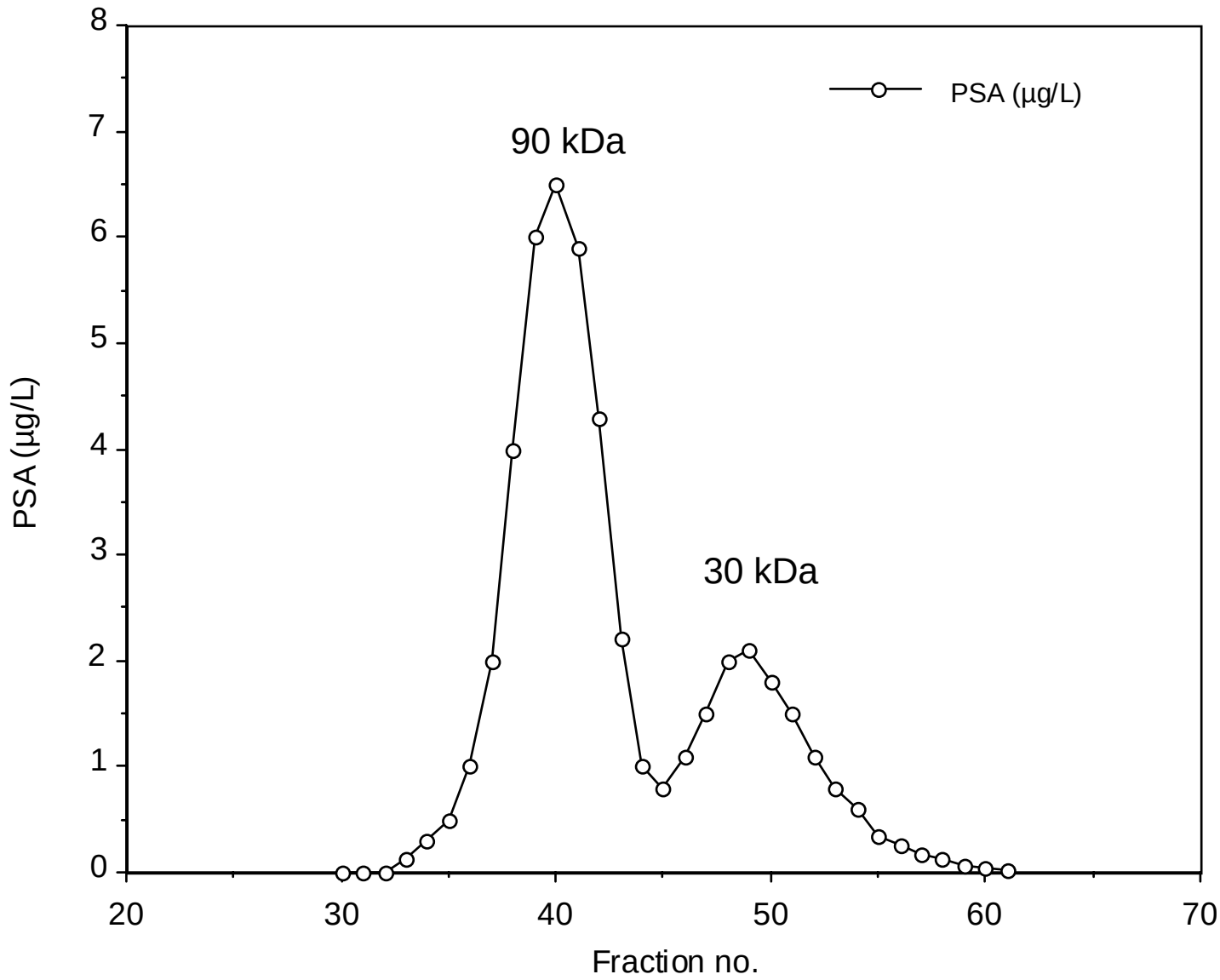
Are we doing something wrong?

- 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)

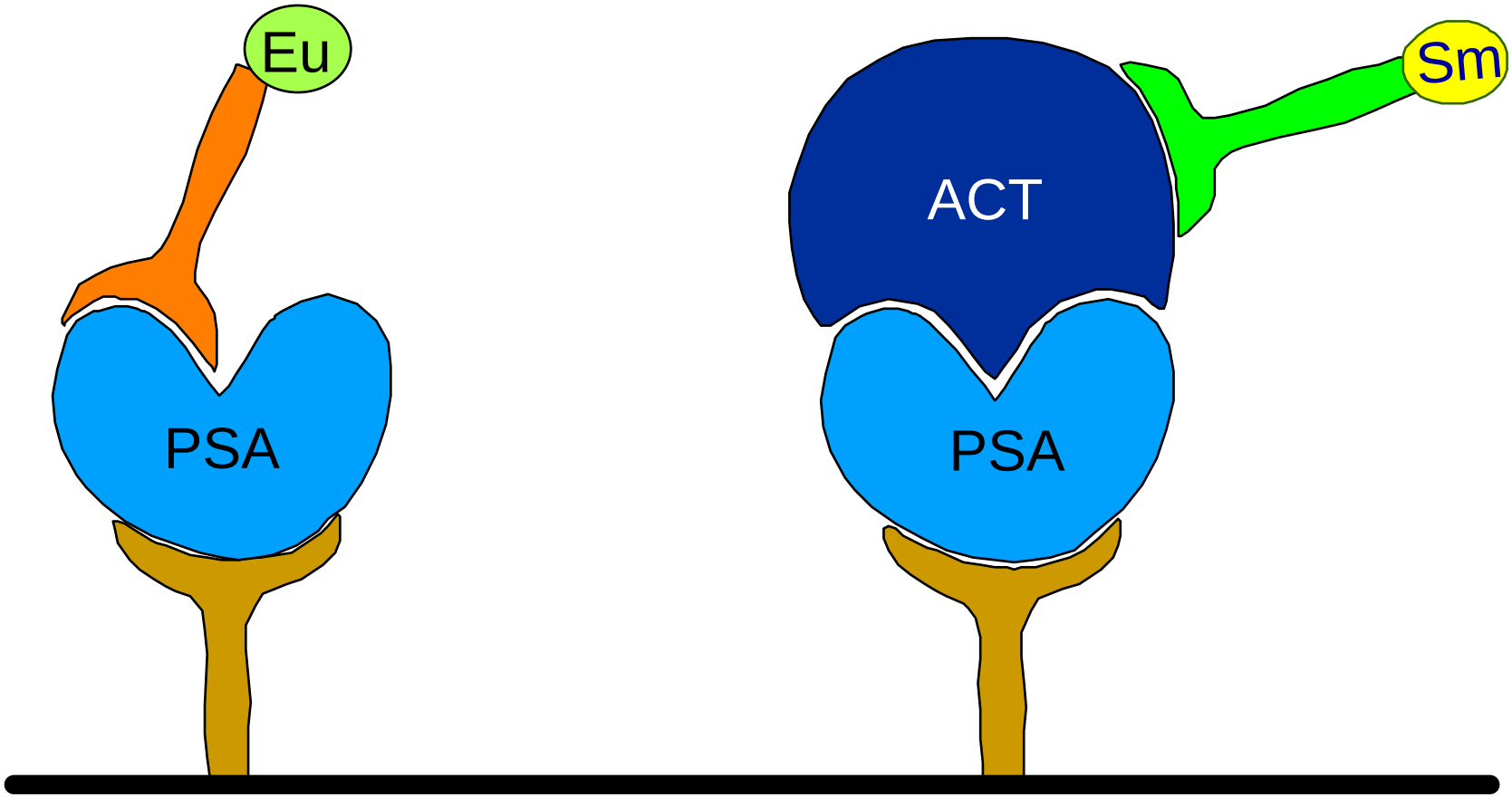
- 30 kDa chymotrypsin-like serine proteinase
- Produced by epithelial cells of the prostate
- Prostate specific, not cancer specific
- Dissolves seminal clot by degrading semenogelin
- PSA in plasma is heterogeneous
  - Complexes with protease inhibitors
  - Partially degraded forms and proforms of free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

Gel filtration of PSA in prostate cancer serum



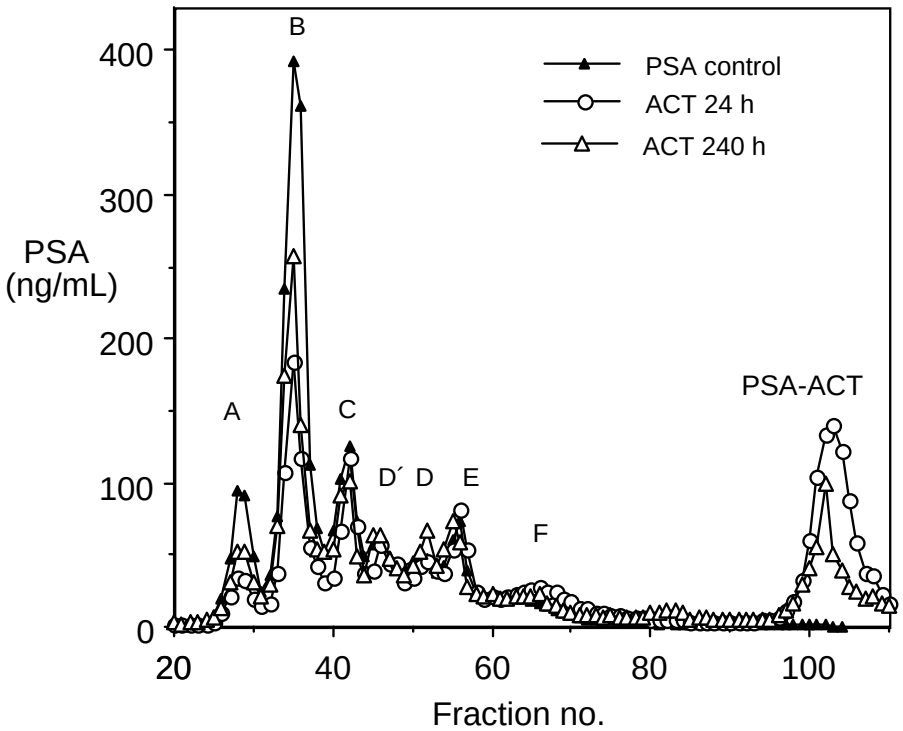
Stenman et al. J Nucl Med Allied Sci 1990

Assays for free PSA and PSA-ACT

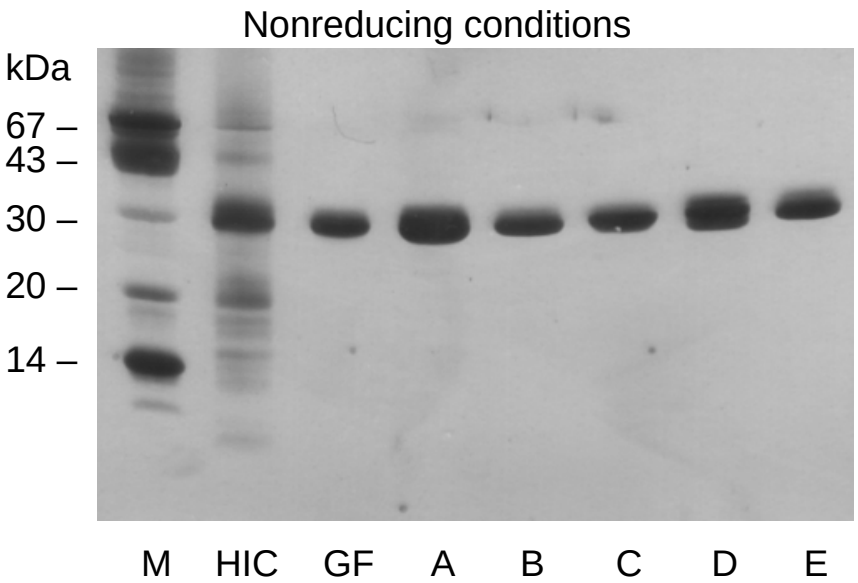


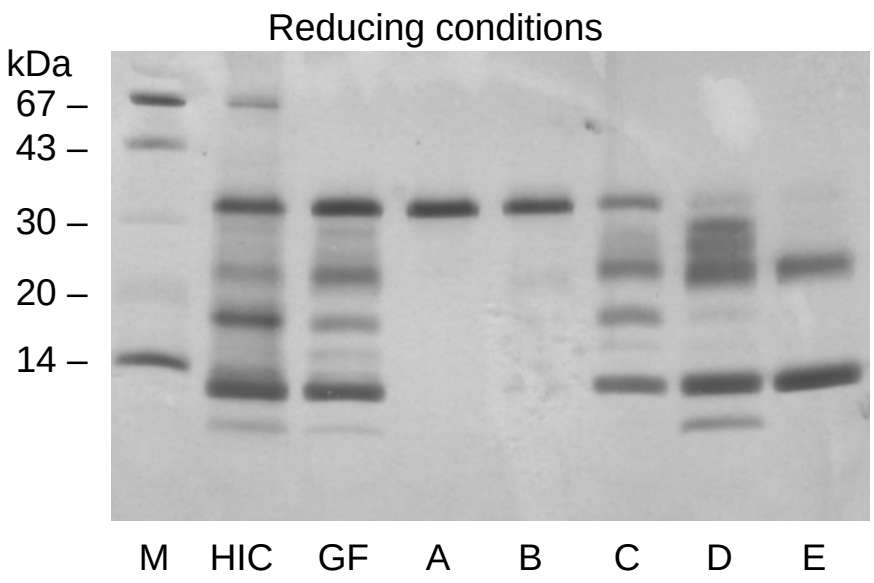
Forms of immunoreactive PSA in prostate cancer patients and men with BPH

	PCa	Controls
• PSA-ACT	86%	79%
• Free PSA	13%	19%
• PSA- API (-AAT)	1%	2%
• hK-2	1.5%	1%
• PSA-A2M	2%	4%

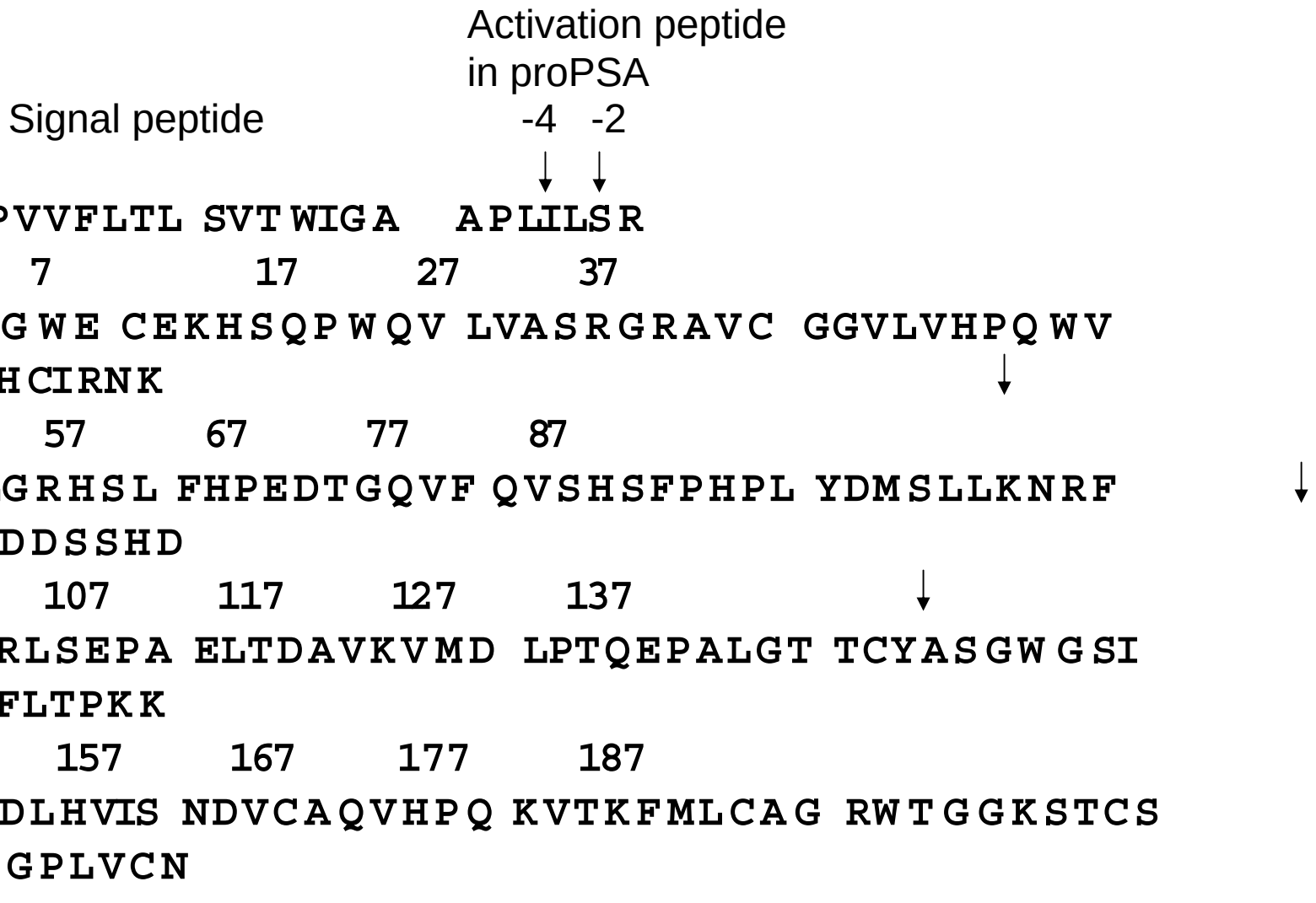


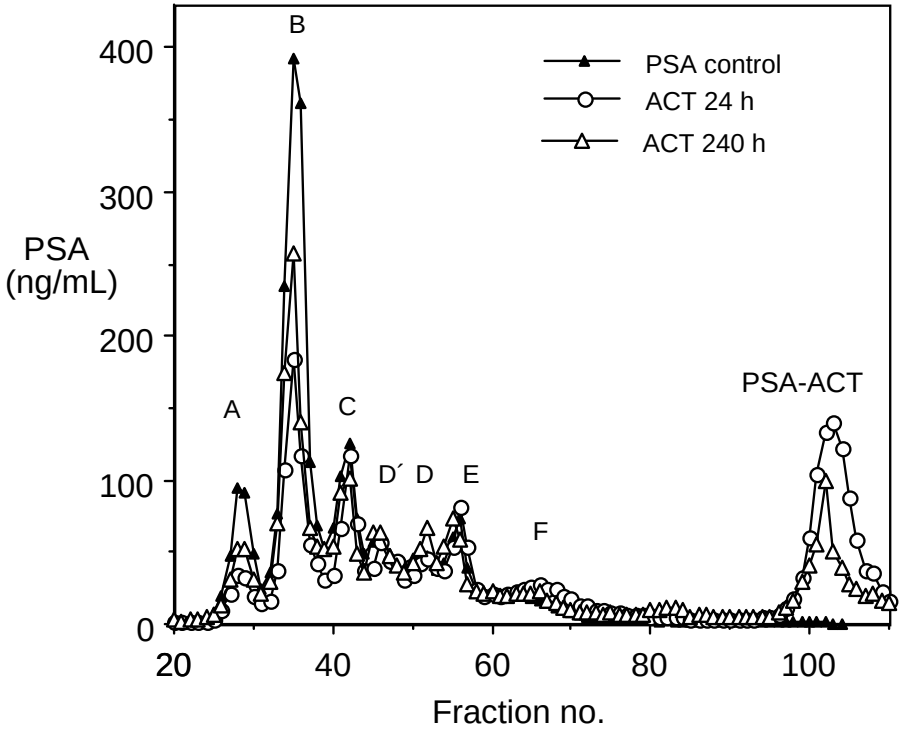
SDS gel electrophoresis of PSA isoenzymes from seminal fluid separated by ion exchange chromatography





Amino acid sequence of PSA





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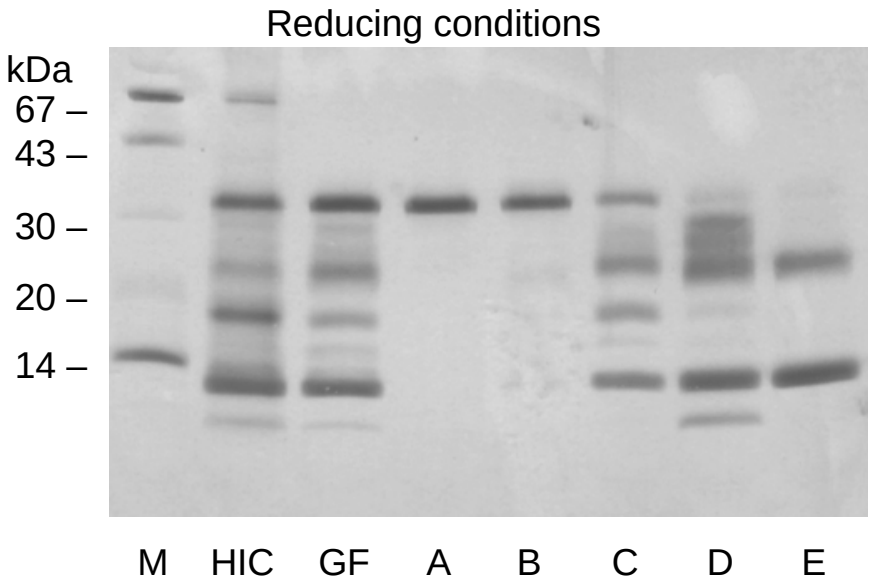
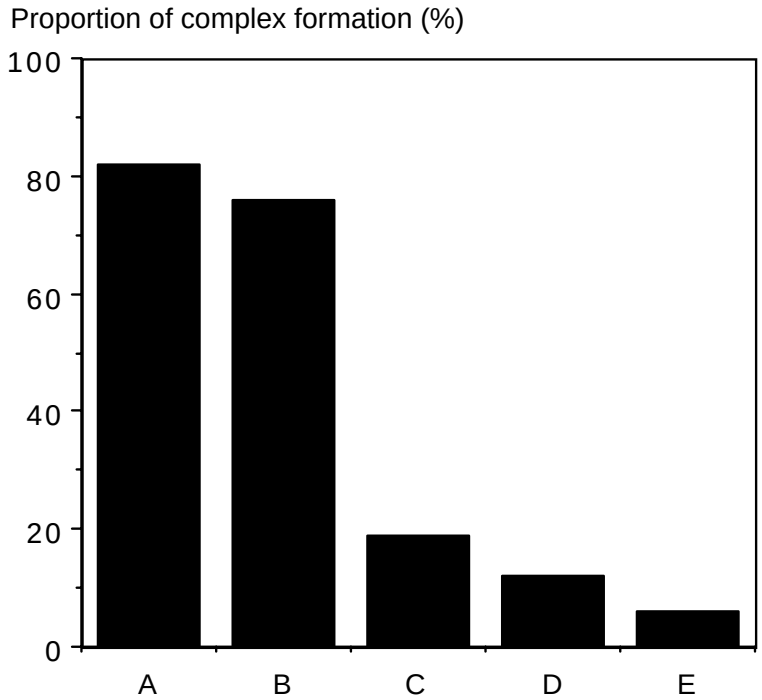
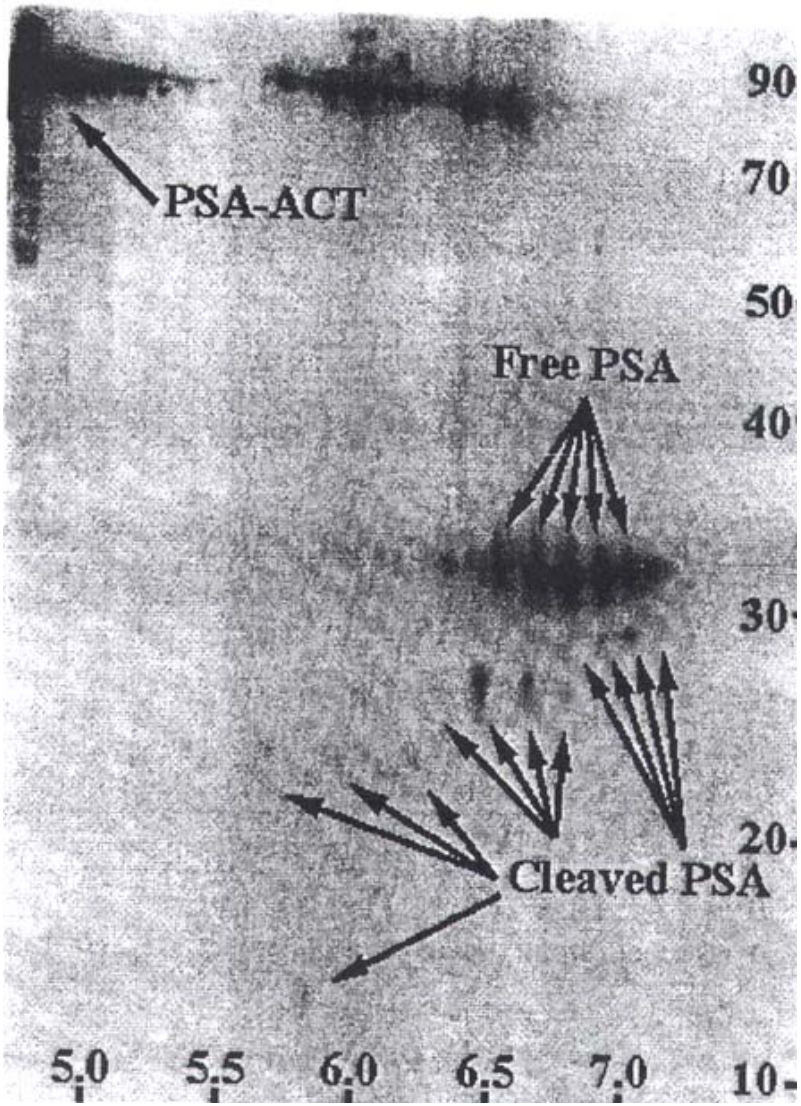




2-D electrophoresis of serum PSA

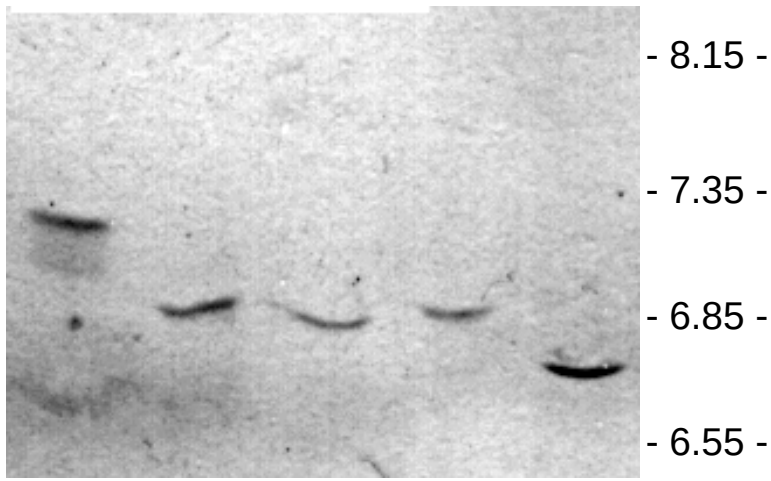


Isoelectric point

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

2-dimensional electrophoresis of free PSA in serum and isoelectric focusing of PSA isoenzymes isolated from seminal fluid

Isoelectric focusing of seminal plasma PSA



Zhang et al. Clin Chem 41:1567-73 1995

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)

Rafferty et al. Clinical Chemistry 46: 1310-1317, 2000

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 compostion = 1.0
  - Assignment of mass concentration for PSA-ACT
- Preparation of PSA-ACT-free PSA 90/10 mixture

Prestigiacomo AF, Chen Z, Stamey TA. A universal calibrator for prostate specific antigen (PSA). Scand J Clin Lab Invest Suppl 1995;221:57-9.

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

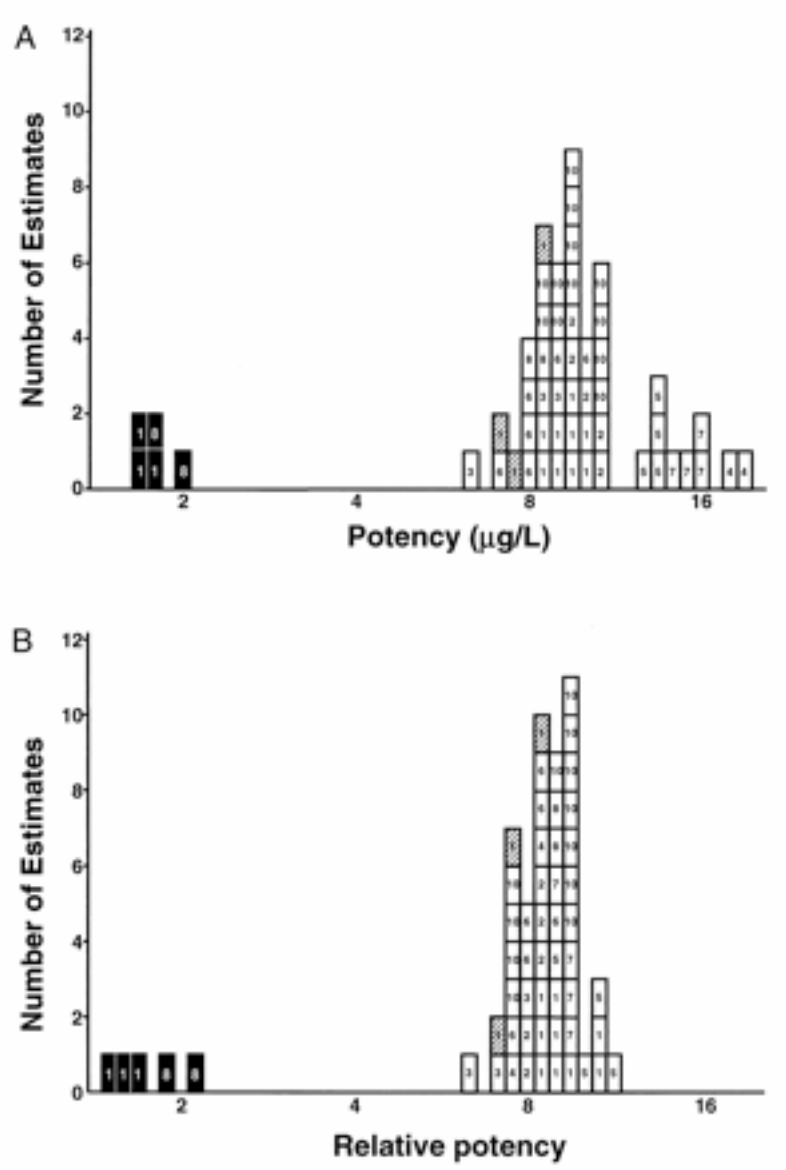
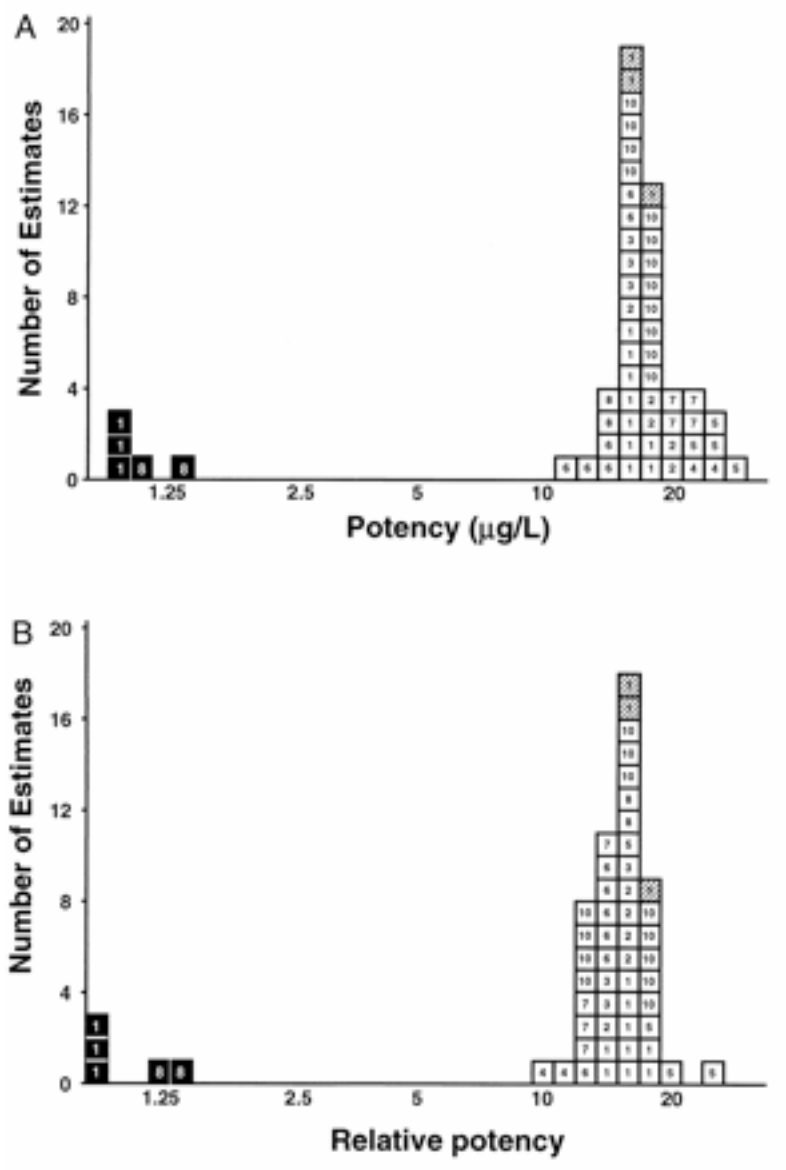


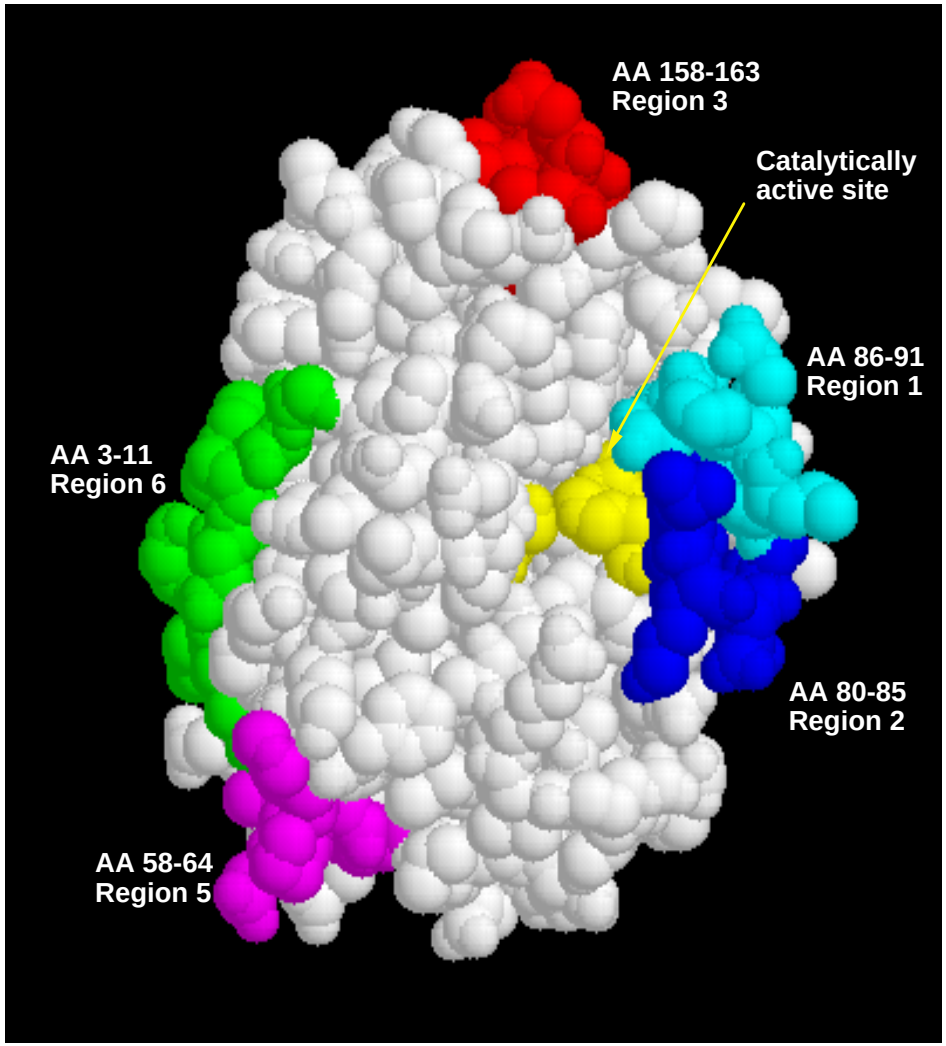
Figure 5. Potency of human serum sample (97/566) in individual assays calculated using IHR (*A*) and PSA 90:10 (96/670; *B*).

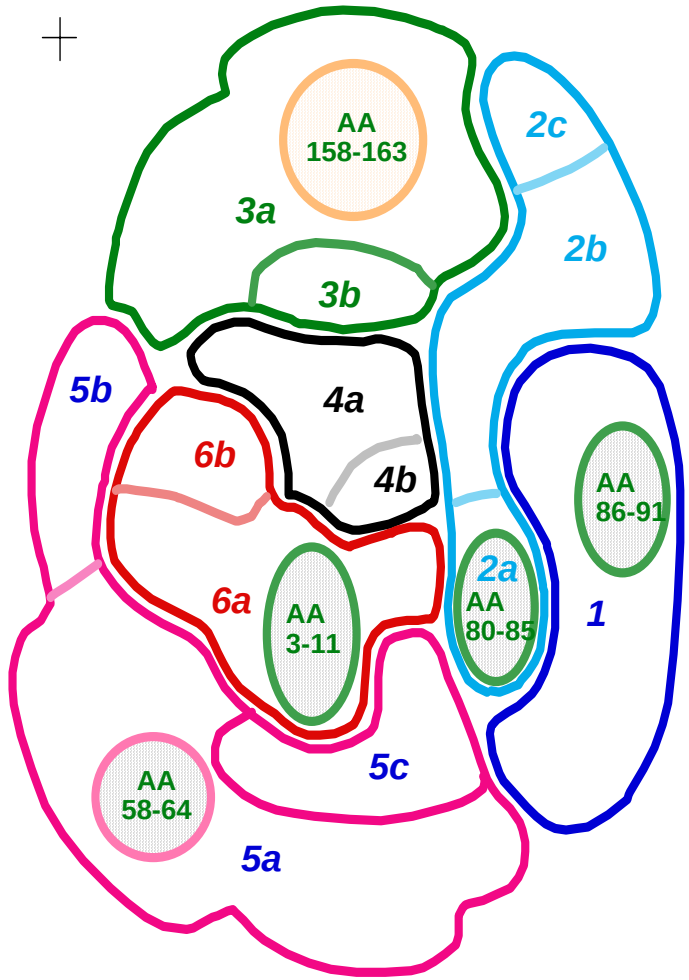


Epitope mapping of PSA

Summary Report of the TD-3 Workshop: Characterization of 83 Antibodies against Prostate-Specific Antigen.
Stenman U-H, Paus E, Allard WJ, Andersson I, Andres C, Barnett TR, Becker C, Belenky A, Bellanger L, Pellegrino CM, Börmer OP, Davis G, Dowell B, Grauer LS, Jette DC, Karlsson B, Kreutz FT, van der Kwast T, Lauren L, Leinimaa M, Leinonen J, Lilja H, Linton HJ, Nap M, Nilsson O, Ng PC, Nustad K, Peter A, Pettersson K, Piironen T, Rapp J, Rittenhouse HG, Rye PD, Seguin P, Slota J, Sokoloff RL, Suresh MR, Very DL, Wang TJ, Wigheden I, Wolfert RL, Yeung KK, Zhang W, Zhou Z, Hilgers J.
Tumour Biol 1999; 20 (Suppl 1): 1-12.

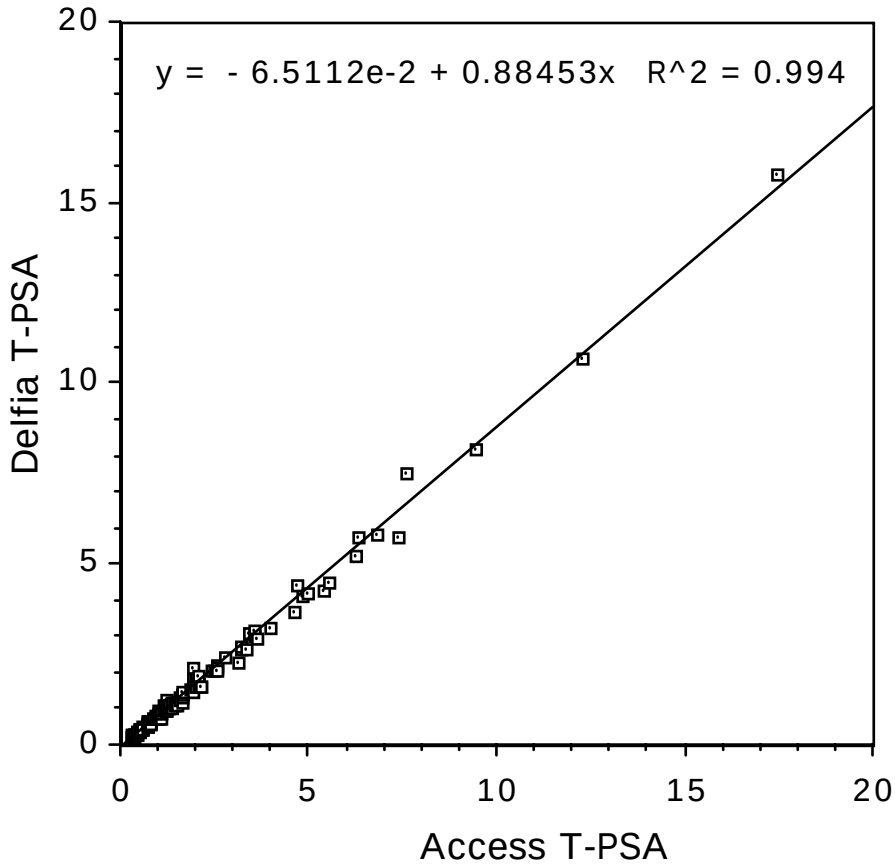
Epitope mapping of PSA

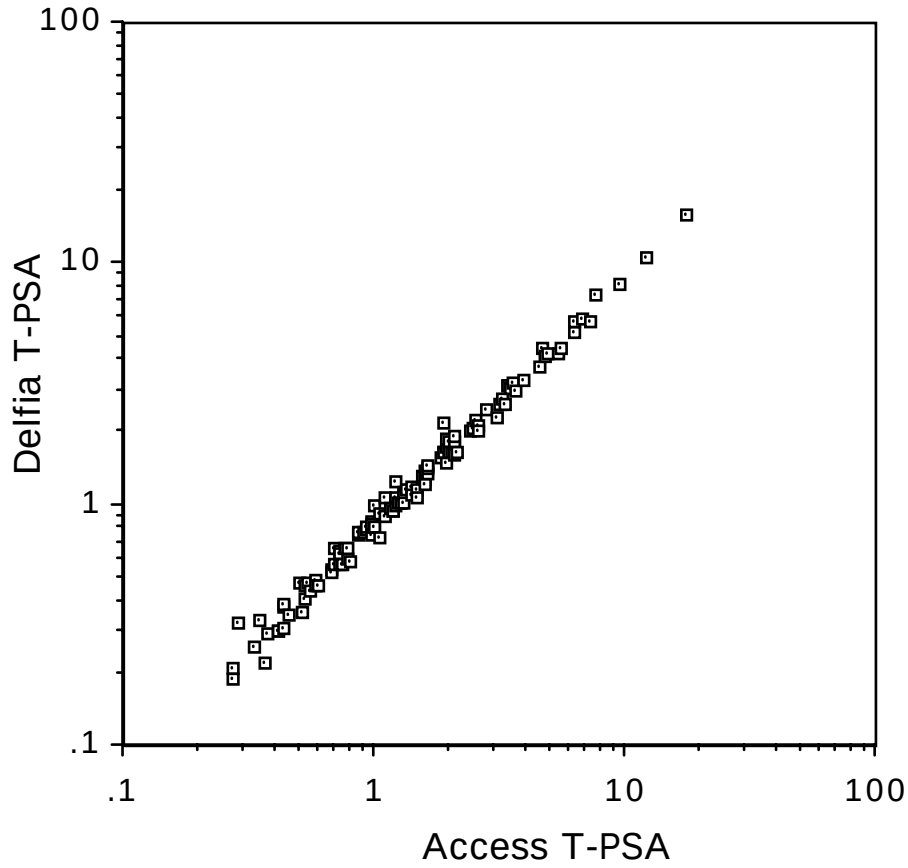




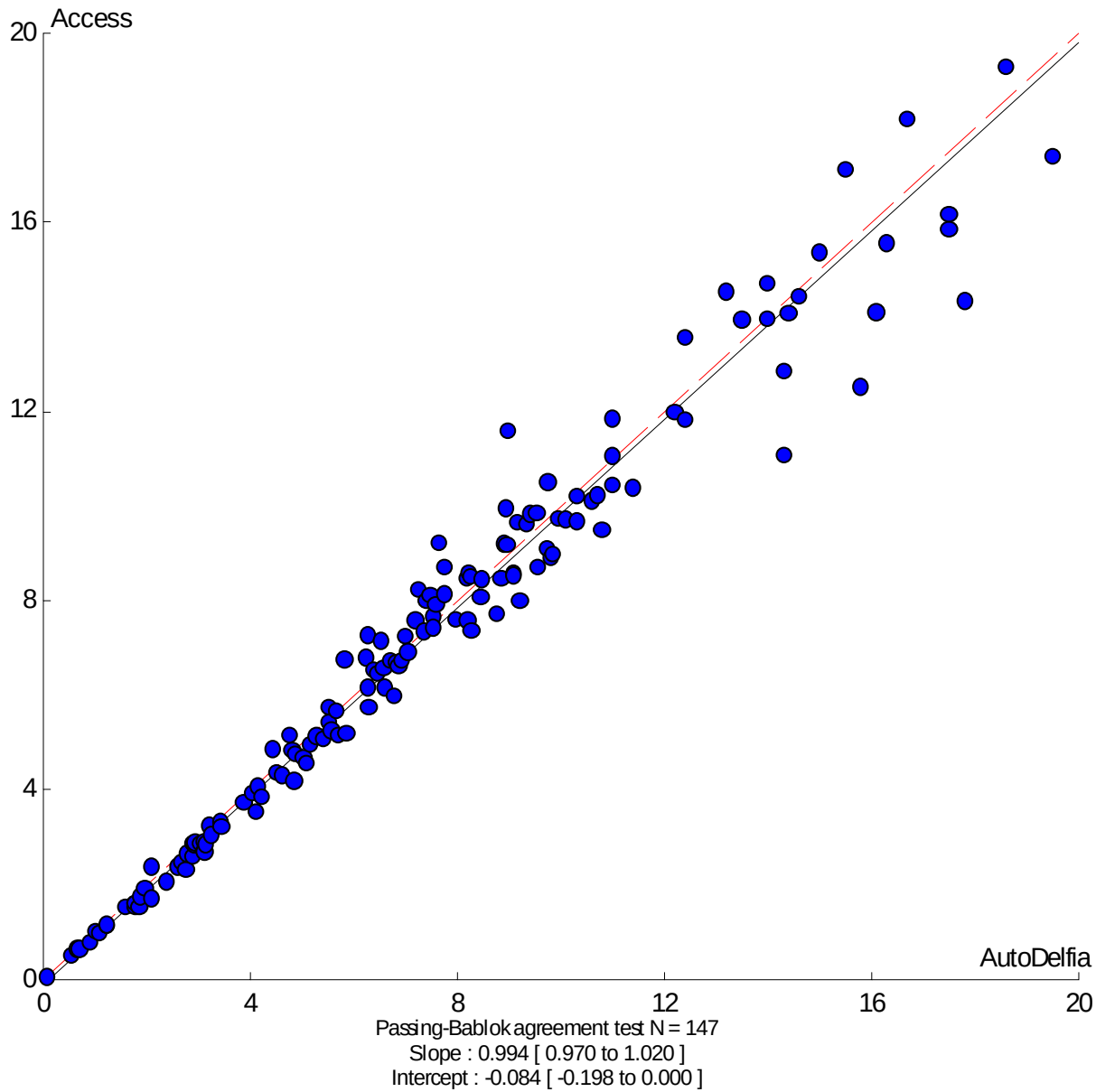
Stenman et al. Tumour Biol 1999;20 (Suppl 1):1-12.

Correlation HybritechTandem E - Wallac AutoDelfia before 2003

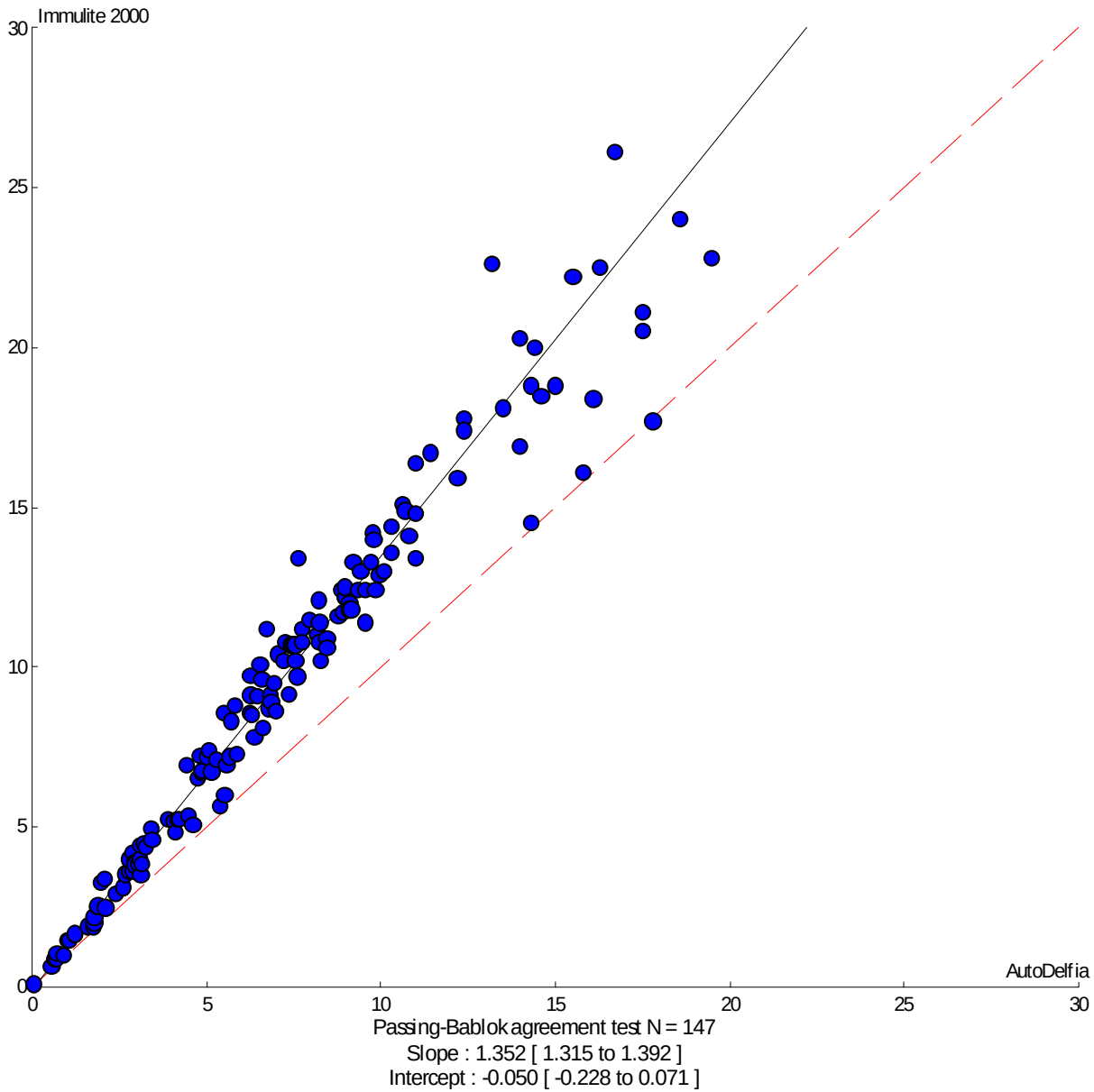




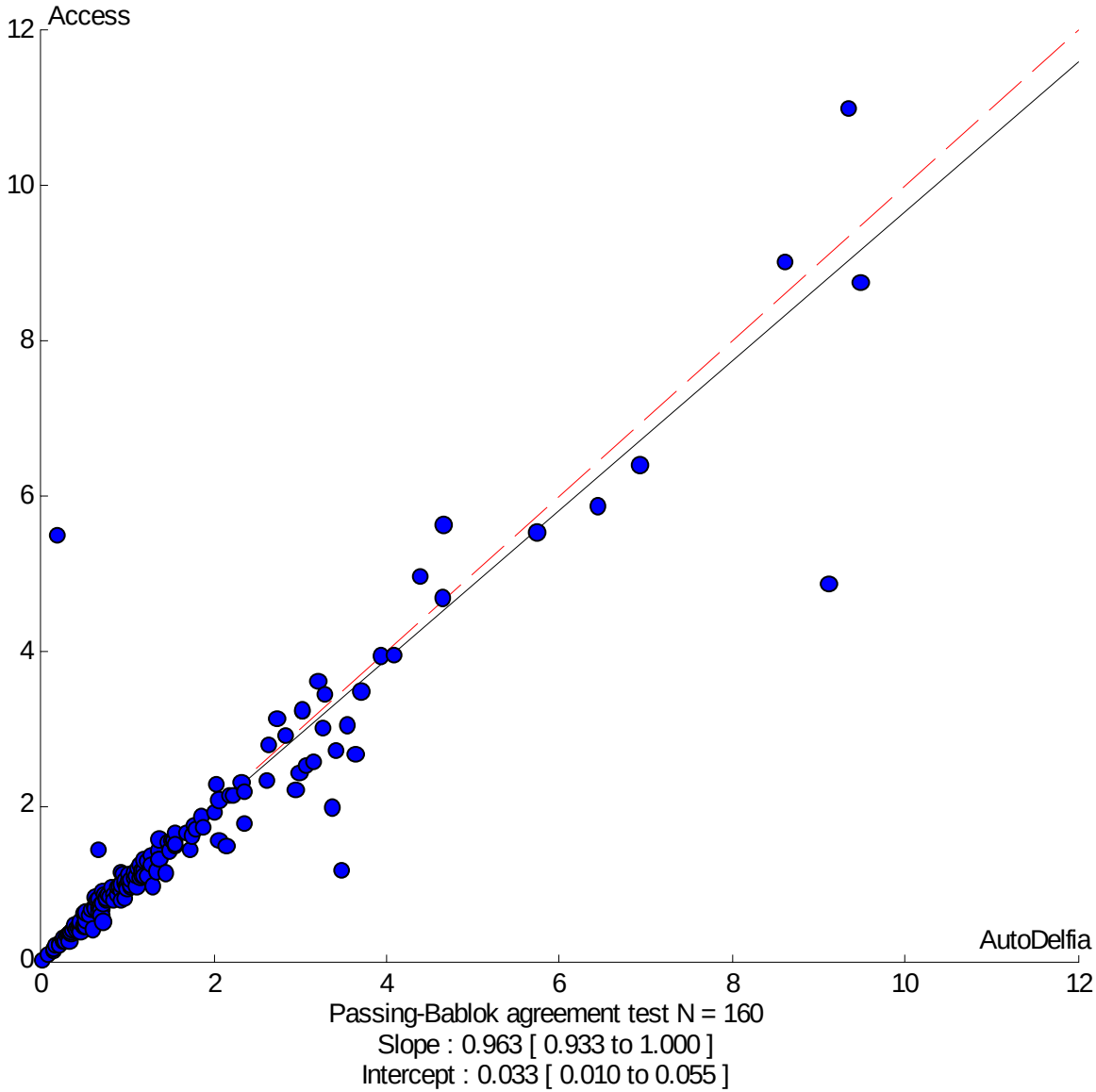
Recalibrated AutoDelfia vs. Access T-PSA (2004)



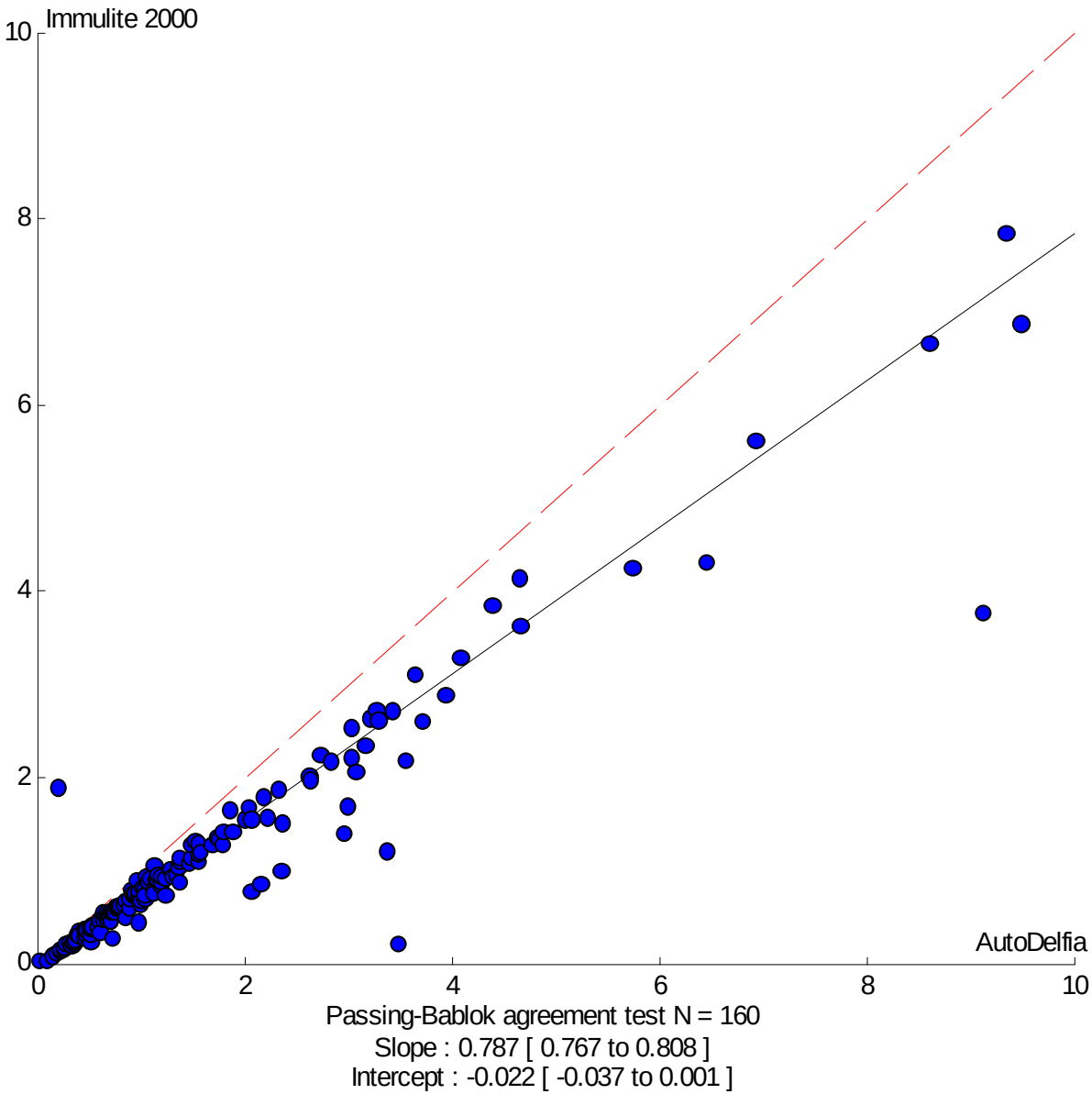
Recalibrated AutoDelfia vs. DPC Immulite 2000 T-PSA (2004)



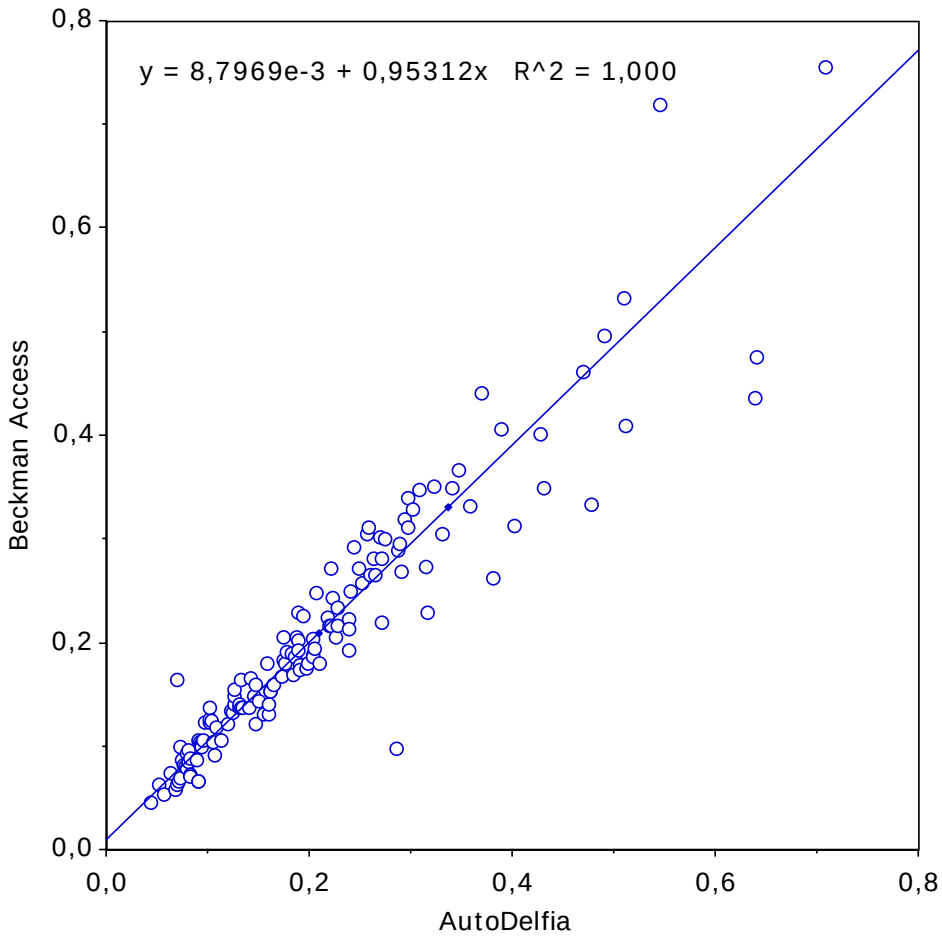
Recalibrated AutoDelfia vs. Access F-PSA (2004)

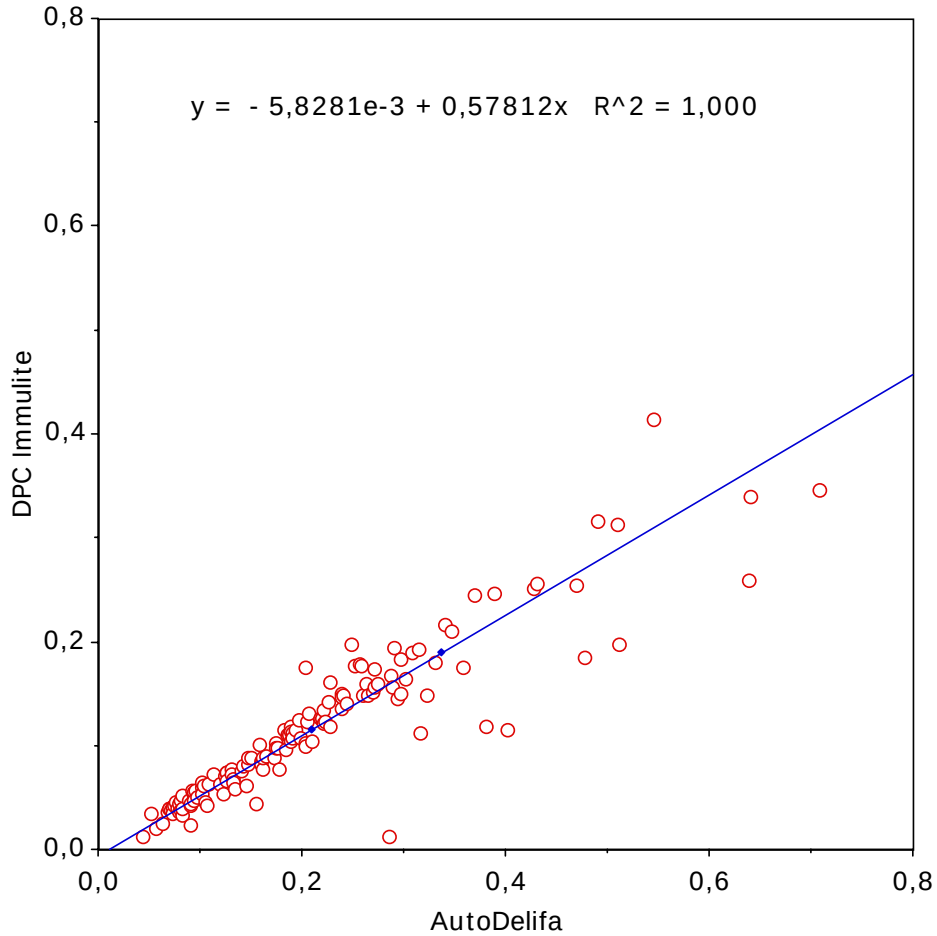


Recalibrated AutoDelfia vs. DPC Immulite 2000 F-PSA (2004)



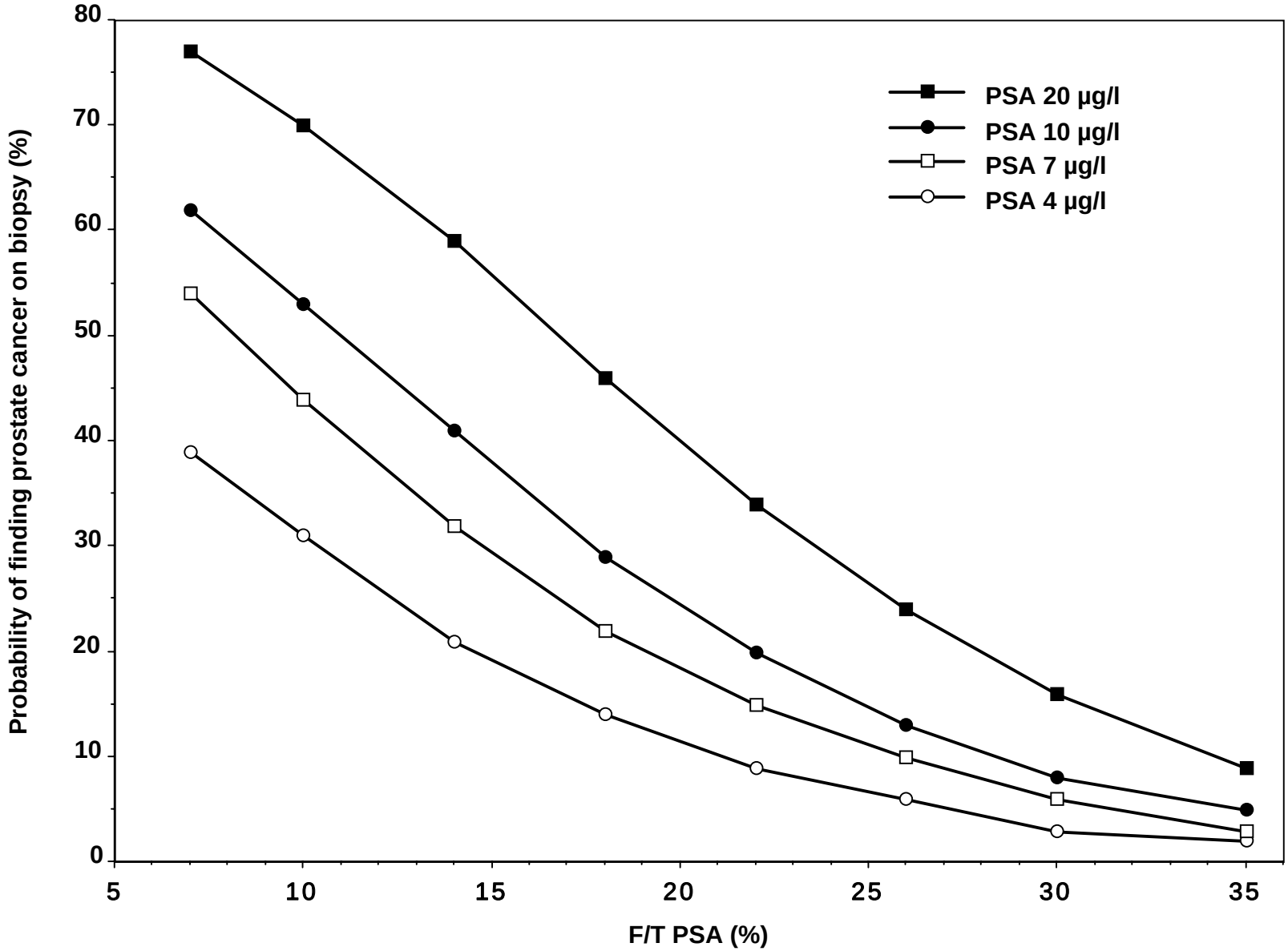
Correlation F/T



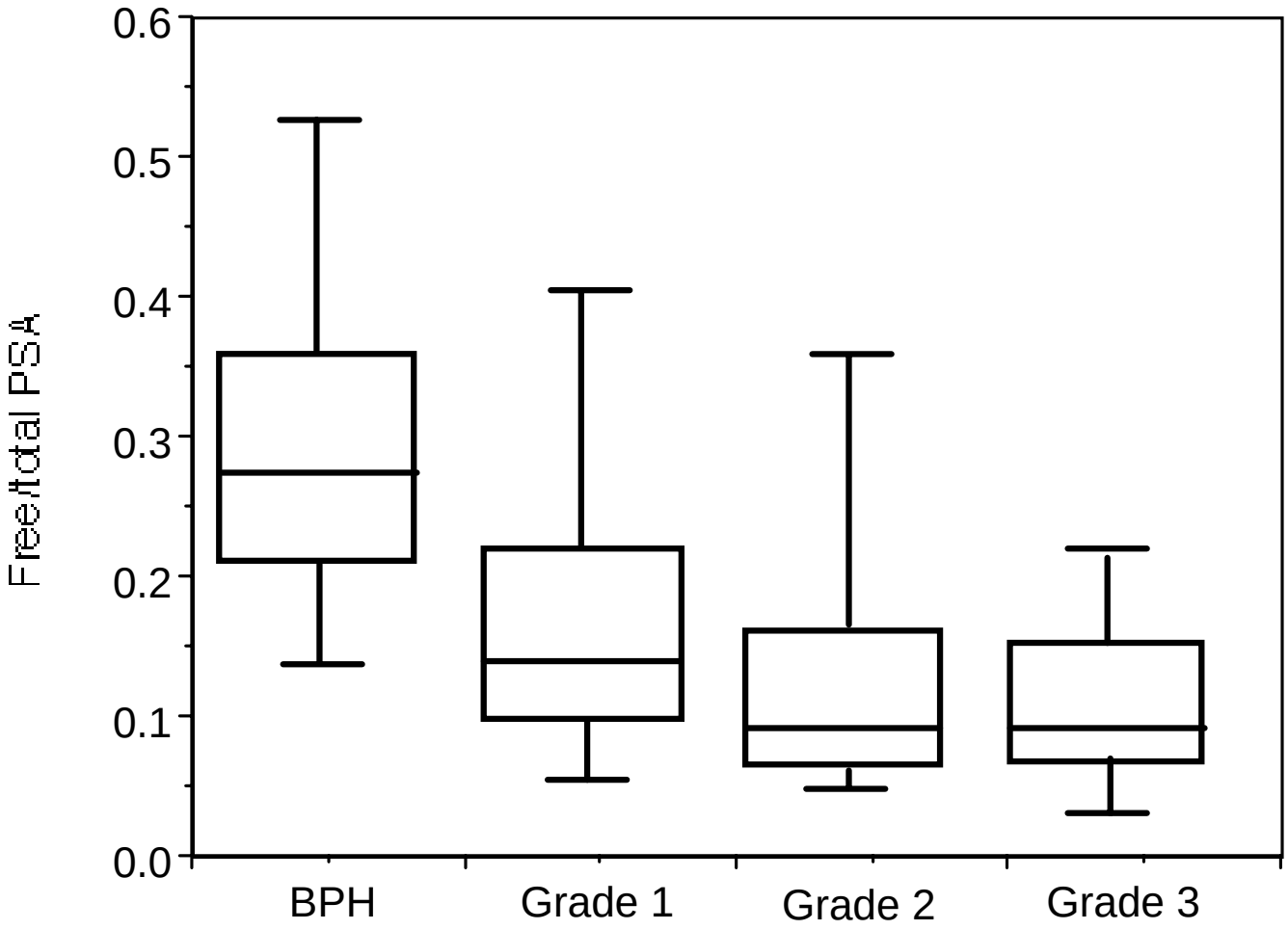


Probability of prostate cancer in relation to total and free PSA

Excel formula avavailable at: www.finne.info



Proportion of free PSA and tumor grade



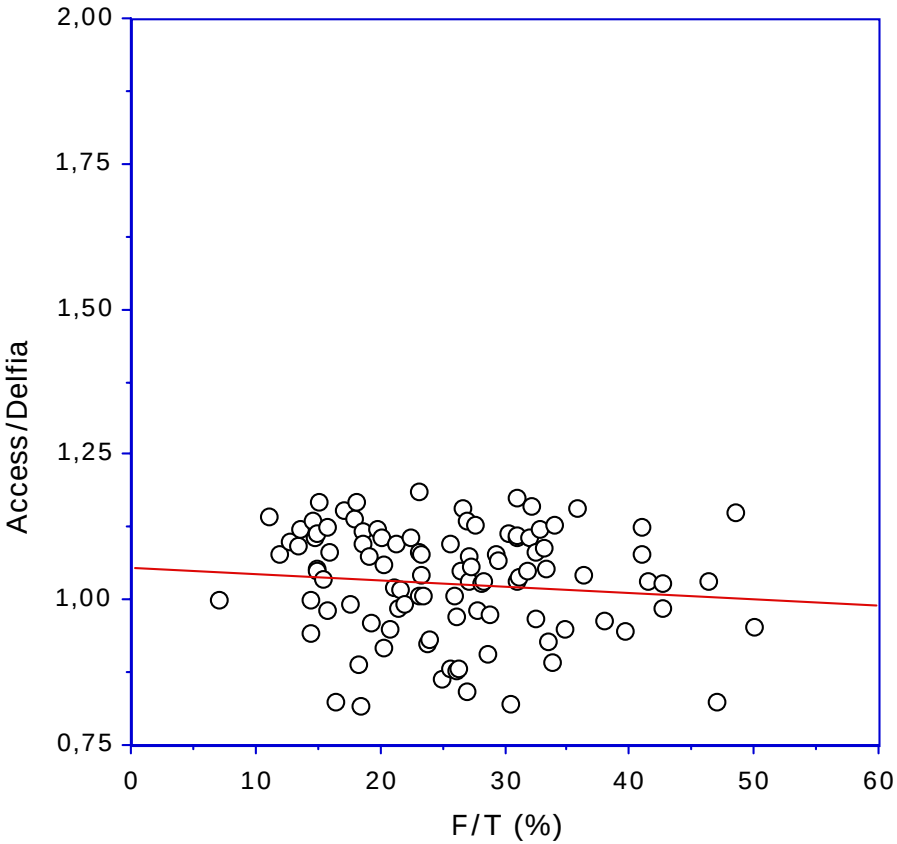
Bangma J Urol 1997; 157: 544

Equimolarity of assays for PSA

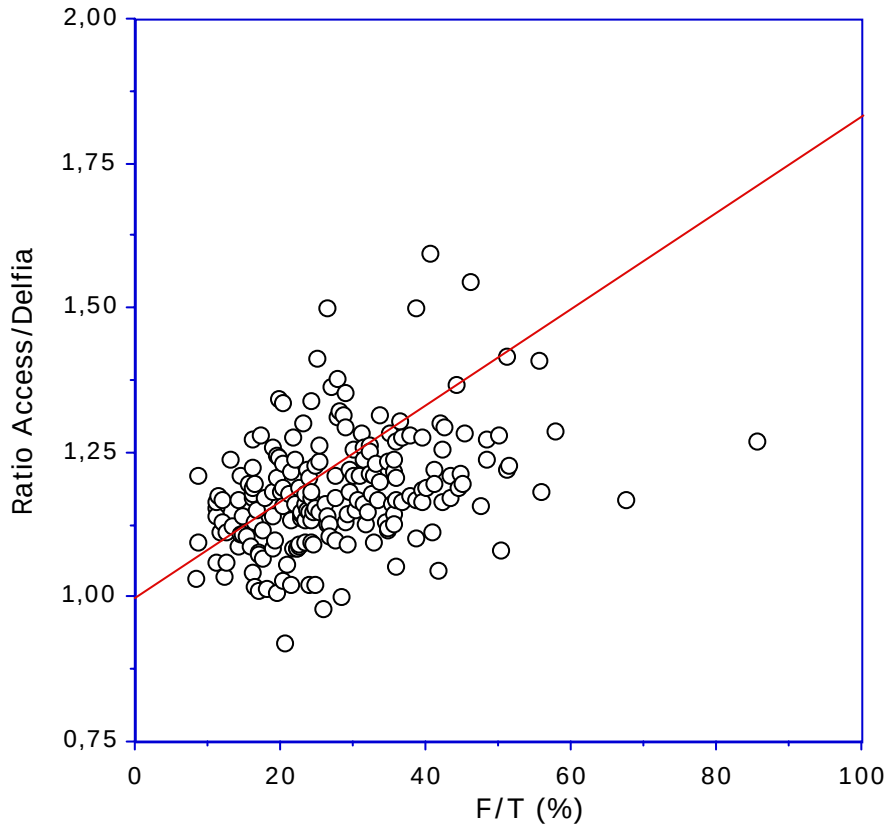
- Ability to detect free and complexed PSA equally
- Assays based on polyclonal antibodies tend to overestimate free PSA

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

Control containing >90% F-PSA	Delfia T-PSA	Delfia F-PSA	Access T-PSA
Sample 1	2.12	2.00	4.1
Sample 2	19.6	19.1	28.0

Recognition of various forms of free PSA by AutoDelfia and Beckman Access assays

	AutoDelfia			Access	Ratio
	F-PSA	T-PSA	F/T	T-PSA	Access/AD
PSA-A	96,5	92,2	104,6	114,9	1,25
PSA-B	59,5	57,4	103,6	72,7	1,27
PSA-C	41,7	40,3	103,6	53,9	1,34
PSA-D	55,0	61,6	89,2	77,9	1,26
PSA-E	50,6	52,9	95,5	63,8	1,21
ProPSA	22,7	23,9	94,9	30,9	1,29
				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

Access value >2-fold Delfia	13/2651	0.49%
Access value >1.5-fold Delfia	49/2651	1.8%
Access value <0.67-fold Delfia	1/2651	0.00%
Access value <0.50-fold Delfia	0/2651	-

Comparison of Delfia and Access on samples with aberrant results and benign biopsy

Sample	Total PSA	Total PSA	Free PSA (%)
	Delfia	Access	Delfia
A	1,55	18,2	26%
B	0,71	5,7	22%
C	0,92	6,1	24%
D	1,28	5,2	20%
E	2,15	4,8	24%
F	5,62	12,2	21%
G	5,20	30,7	31%

Effect of mouse serum on interference

	Beckman Access T-PSA	Wallac Delfia T-PSA	Wallac Delfia F-PSA
Routine assay	6.33	0.73	0.26
Addition of mouse serum	2.74	1.08	0.26

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?
Clin Chem 2001;47(5):815-20.

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
- Endagered 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