LabKvalita, Slovakia 2009

Common Reference Interval Concept.

A need to differentiate between reference intervals and decision limits

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Reference Interval

Statistical description of a well definde population

- Reference individuals
- Reference values
- Reference distribution (of reference values)
- Reference interval
- Reference limits

Decision limit

- A value for which consequences are different for measurements below and above this limit

Note that reference limits are never decision limits

Unless you define it

Common Reference Intervals

Prerequisites for common reference intervals

Biological: Homogeneous groups

- The biology must be common (racial, environmental, gender/age, etc.)
- The state of health for the reference individuals must be defined
- The sampling conditions, materials and storage conditions must be standardised

Analytical: Transferable measurements

- The quantity (component) must be well defined
- It must be traceable to a reference preparation or method
- Analytical bias and imprecision must be within acceptable limits
- The analytical method must be specific without interfering factors
- The analytical method must be controlled by commutable control materials

Hyltoft Petersen et al. Clin Chem Lab Med 2001;39:1146-53

(Common) Decision Limits

- **Common decision limits recommended by**

International expert groups
National expert groups
Local expert groups

Biological: Homogeneous groups

- The biology must be common (racial, environmental, gender/age, etc.)

Analytical: Transferable measurements

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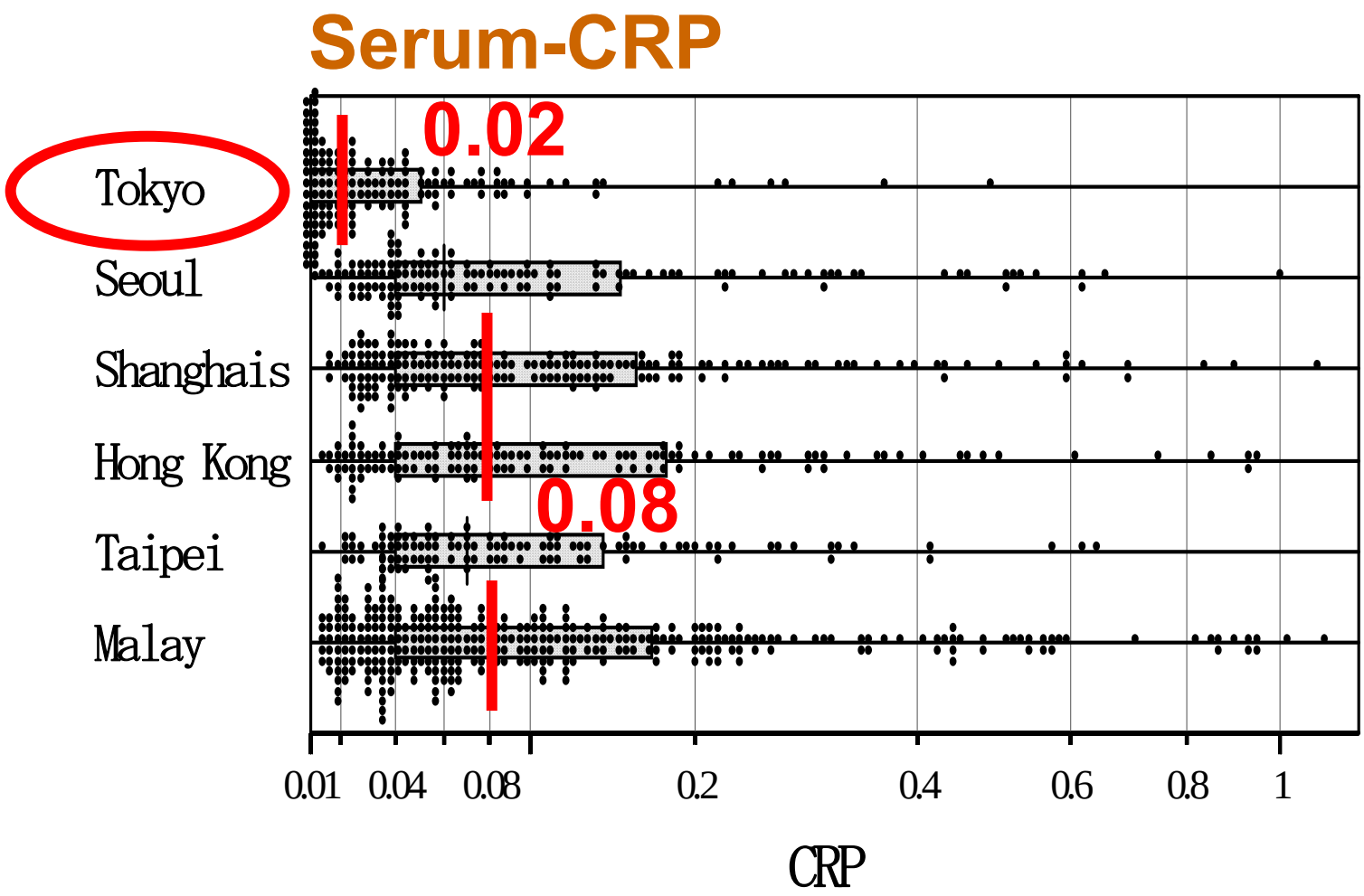
East Asian reference values

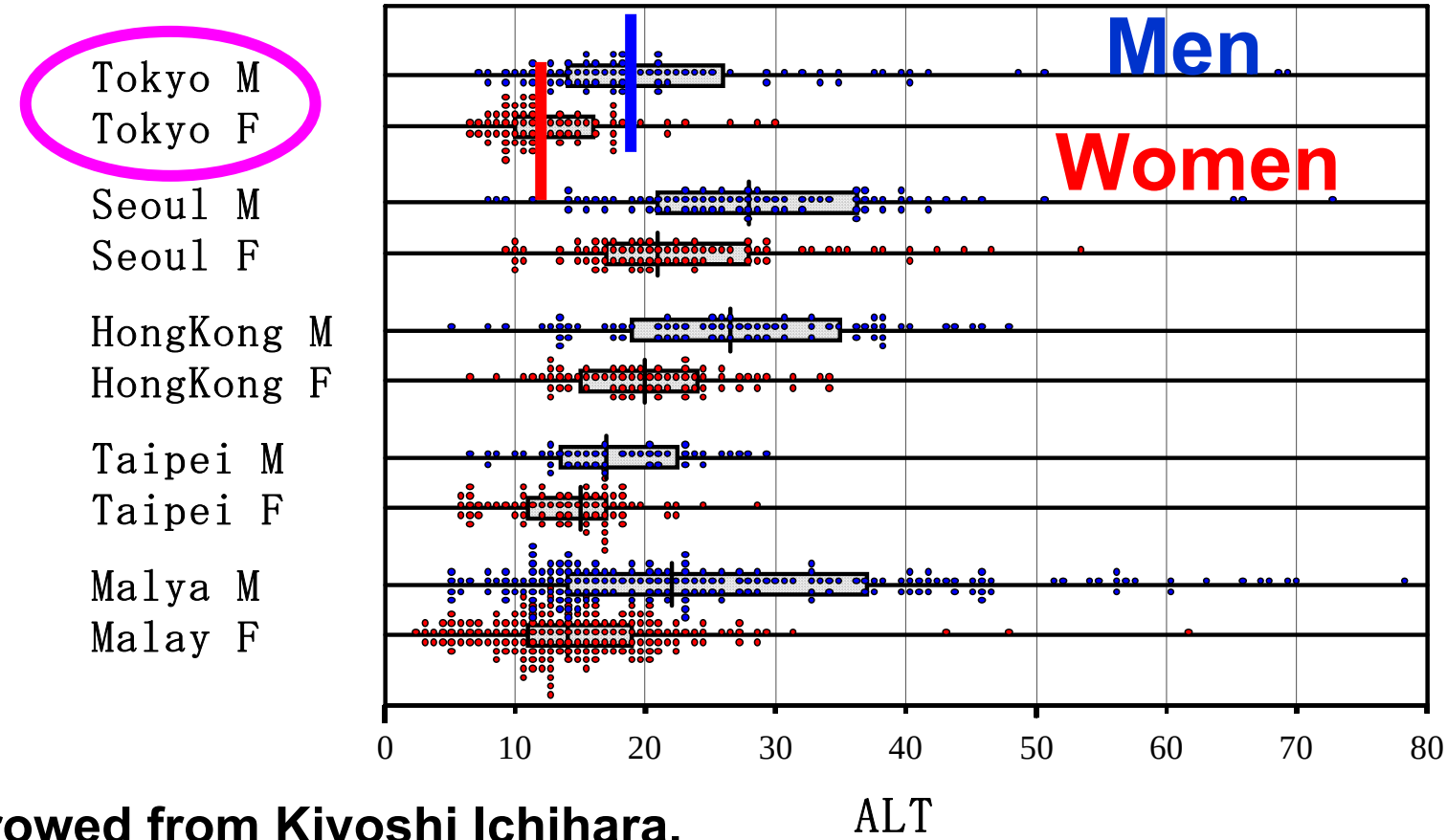


Borrowed from Kiyoshi Ichihara, MD Ph D, Yamaguchi University (Japan)

East Asian reference values

Median

Serum-Alanine Aminotransferase



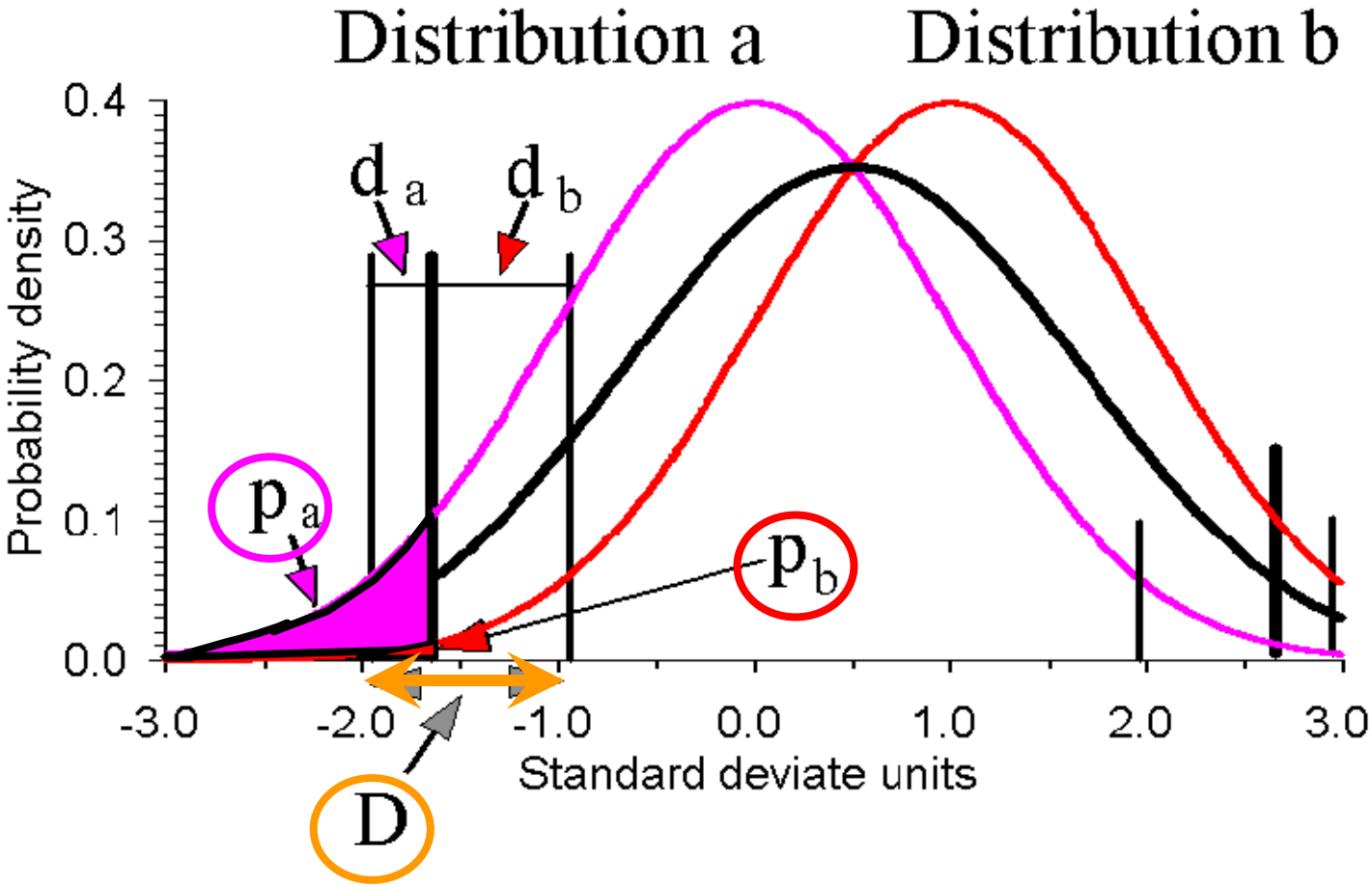
Borrowed from Kiyoshi Ichihara, Yamaguchi University (Japan)

Criteria for Partitioning

When to partition reference intervals according to subgroups, *e.g.*, gender, pregnancy, racial groups *etc.* ?

Lahti *et al*. Clin Chem 2002;48:338-52

Criteria for Partitioning



Lahti *et al*. Clin Chem 2002;48:338-52

Criteria for Partitioning

Difference between two upper or lower limits

< 0.25*s: No partitioning

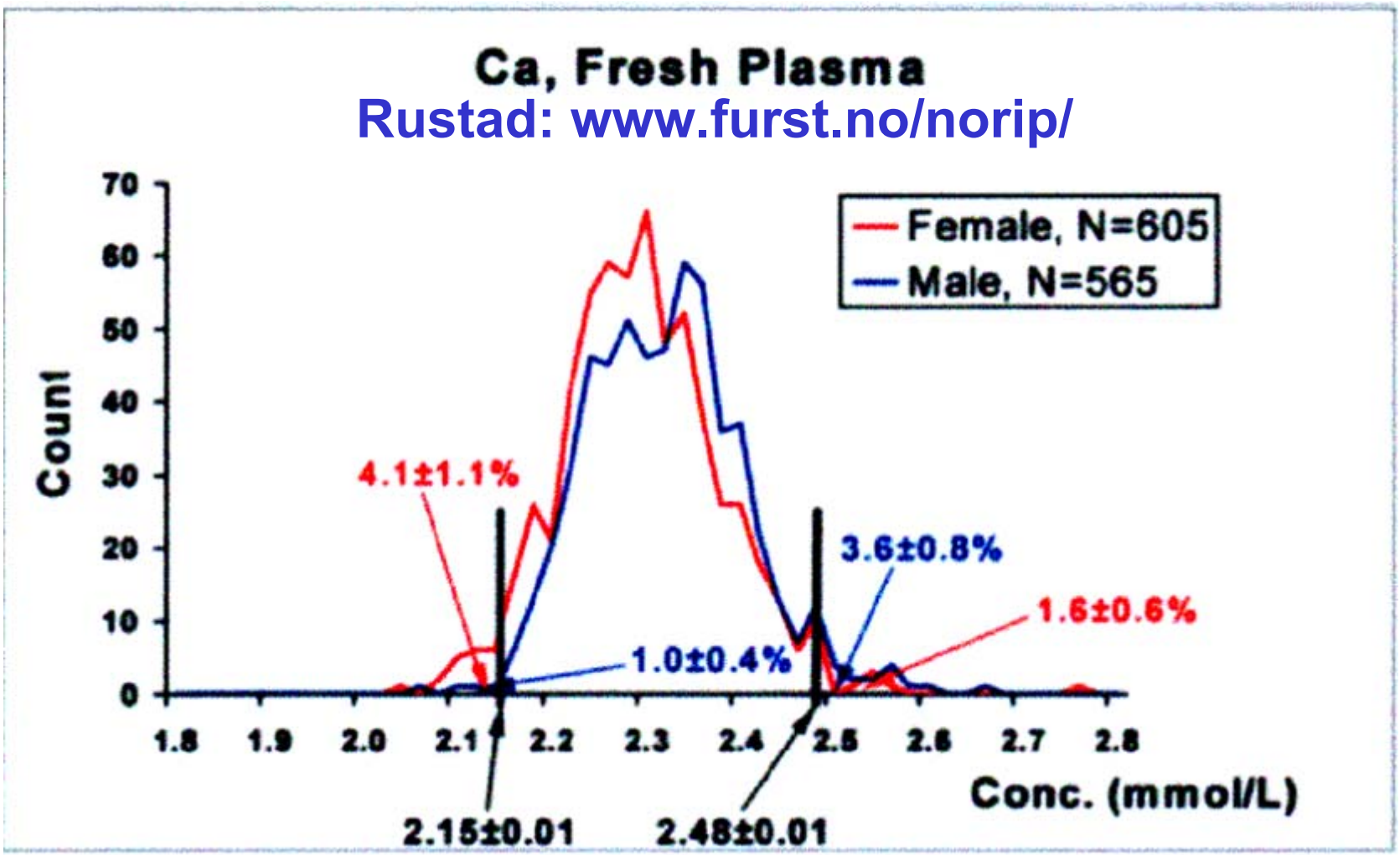
0.25 – 0.75*s: Variable

> 0.75*s: Partitioning

or percentage: 0.9 and 4.1 %

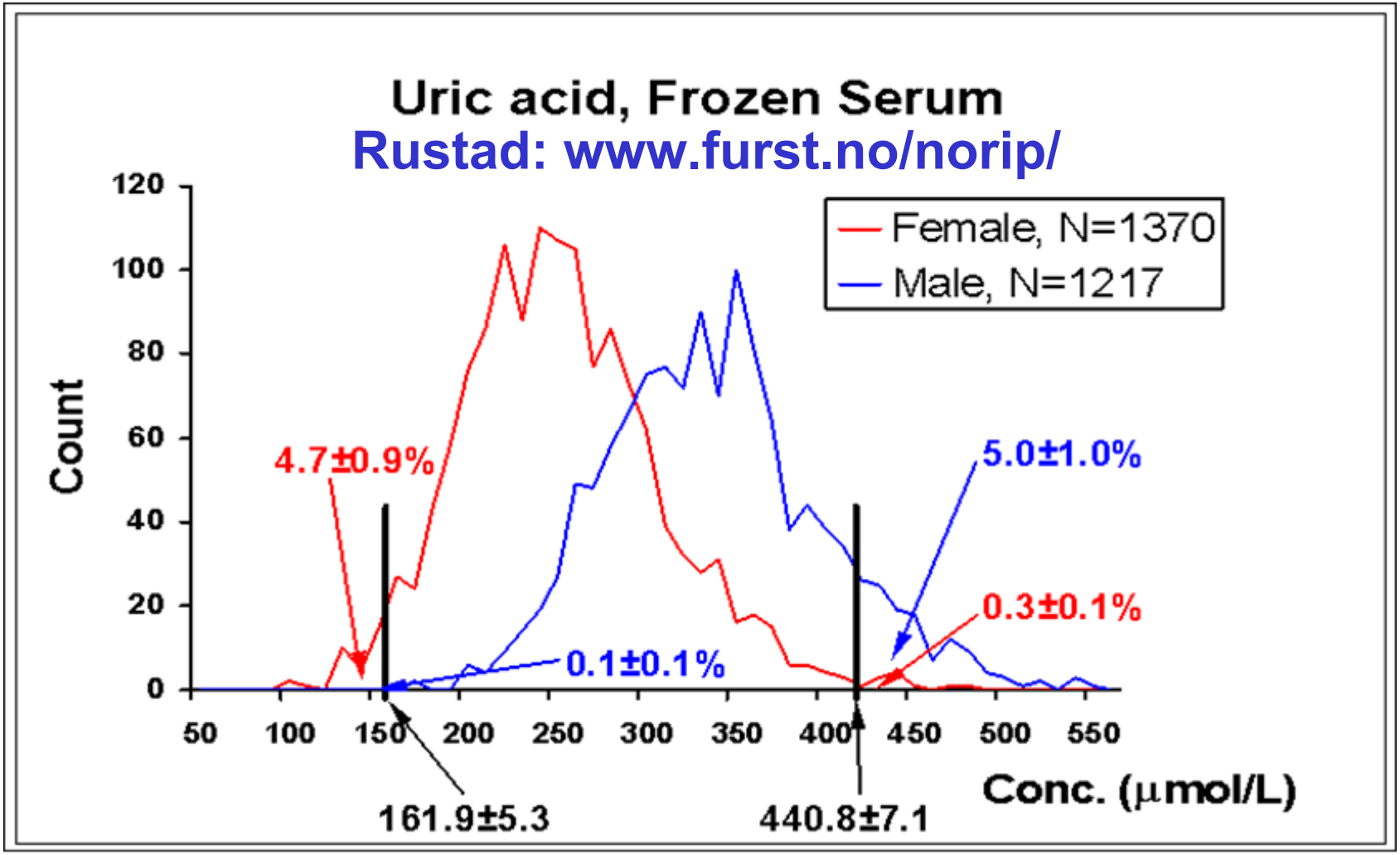
Lahti *et al*. Clin Chem 2002; 48:338-52

The Nordic project on common reference intervals



Rustad & Felding (eds) Scand J Clin Lab Invest 2004;64:263-441

The Nordic project on common reference intervals



Rustad & Felding (eds) Scand J Clin Lab Invest 2004;64:263-441

Criteria for Partitioning

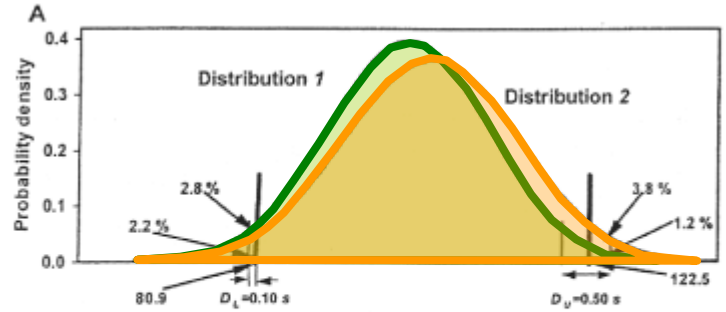
How does the number of reference individuals in each subgroup influence on the criteria ?

How does the prevalence of subgroups influence on the criteria ?

Lahti *et al*. Clin Chem 2002;48:1987-99

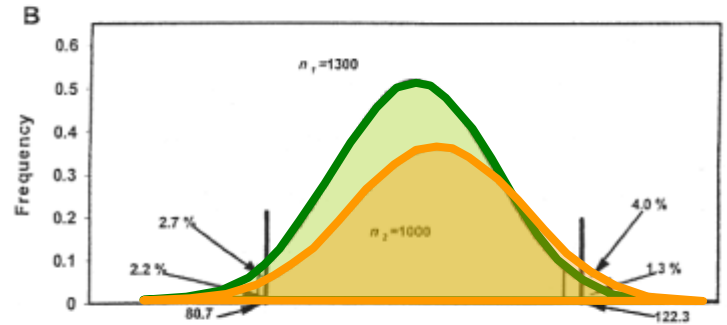
Criteria for Partitioning - prevalence

Probability density



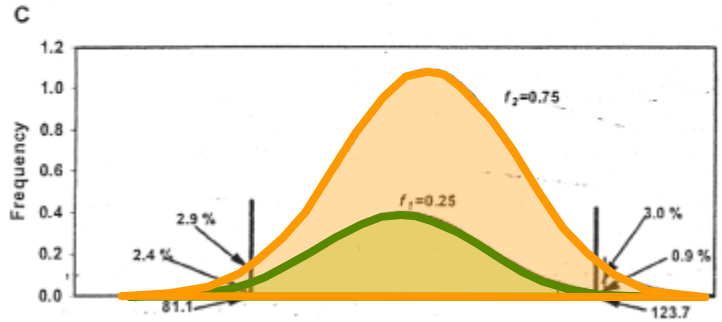
Equal numbers in the two groups

Frequency distribution



Study group:
Group 1: 1300
Group 2: 1000

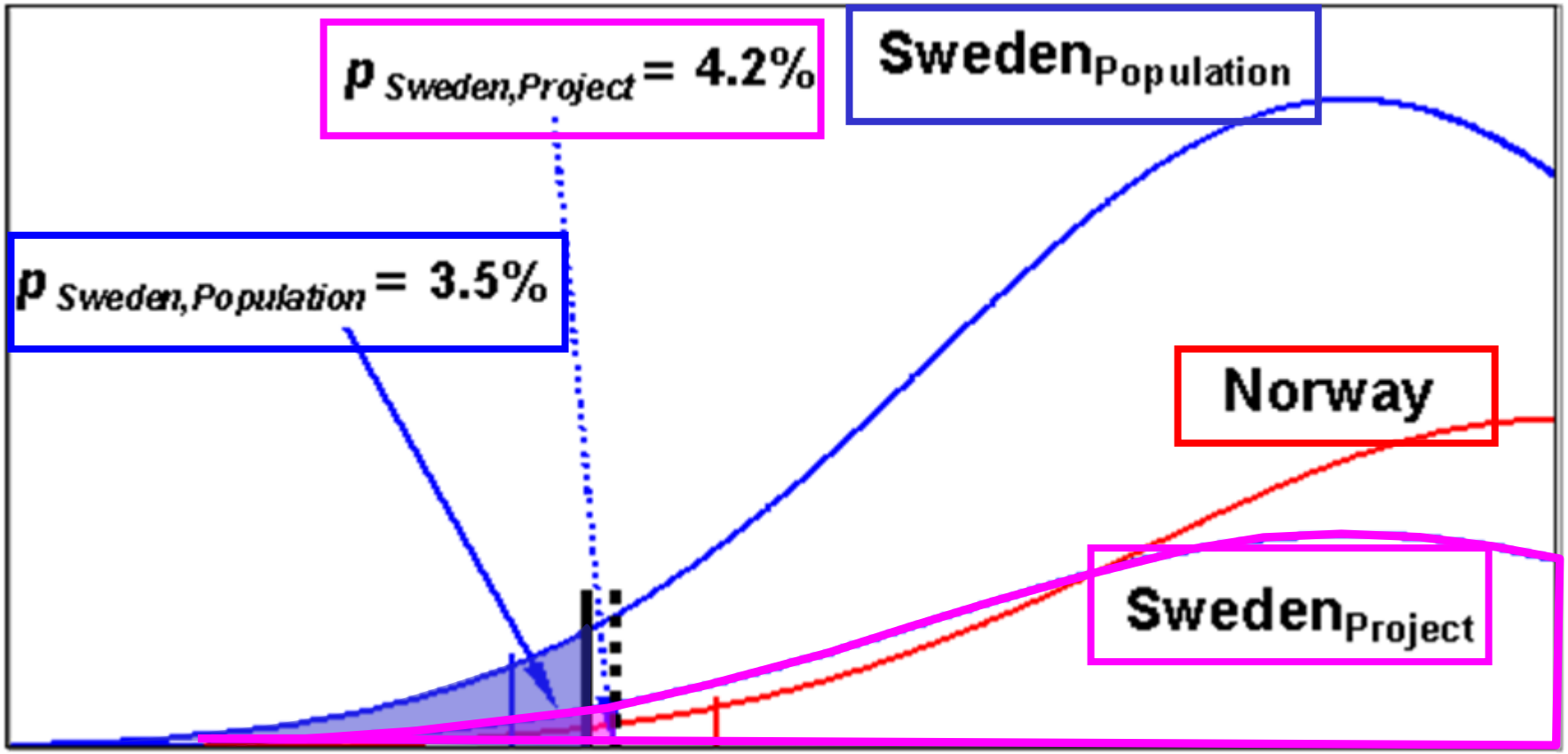
Frequency distribution



Prevalence of Group 1 = 0.25 at application

Lahti *et al*. Clin Chem 2002;48:1987-99

Criteria for Partitioning - prevalence



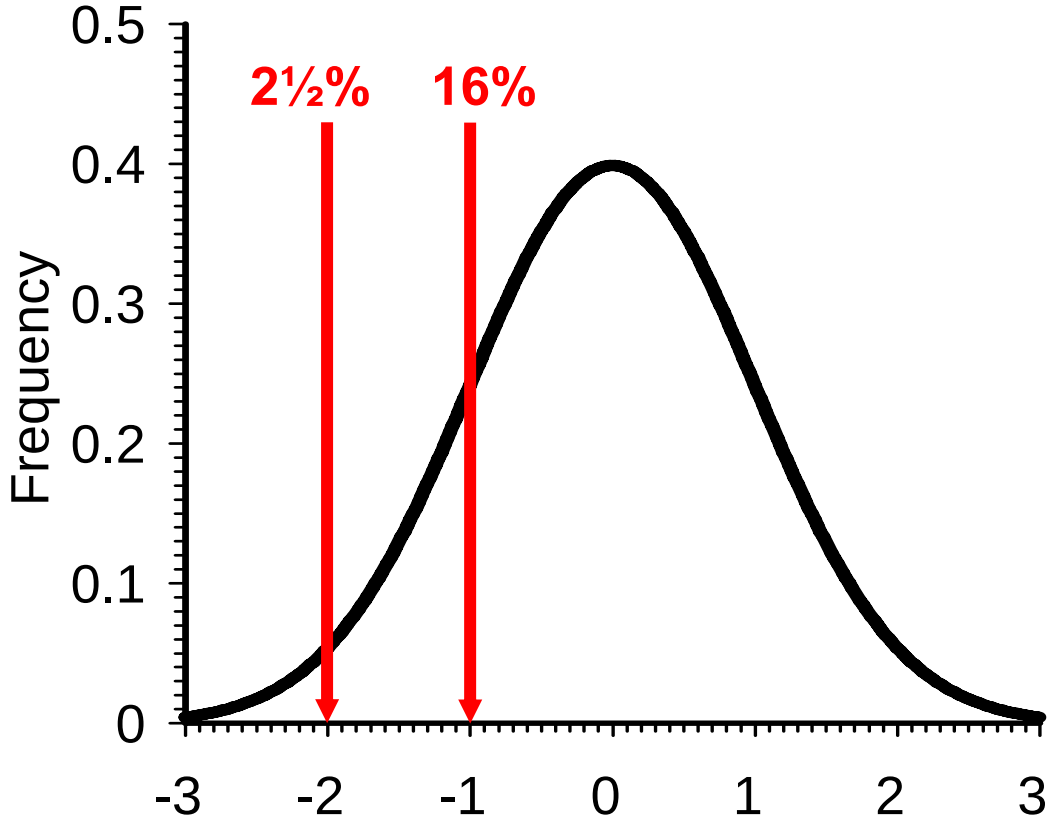
Lahti *et al*. Clin Chem 2004;50:891-900

Rankit-transformation 1

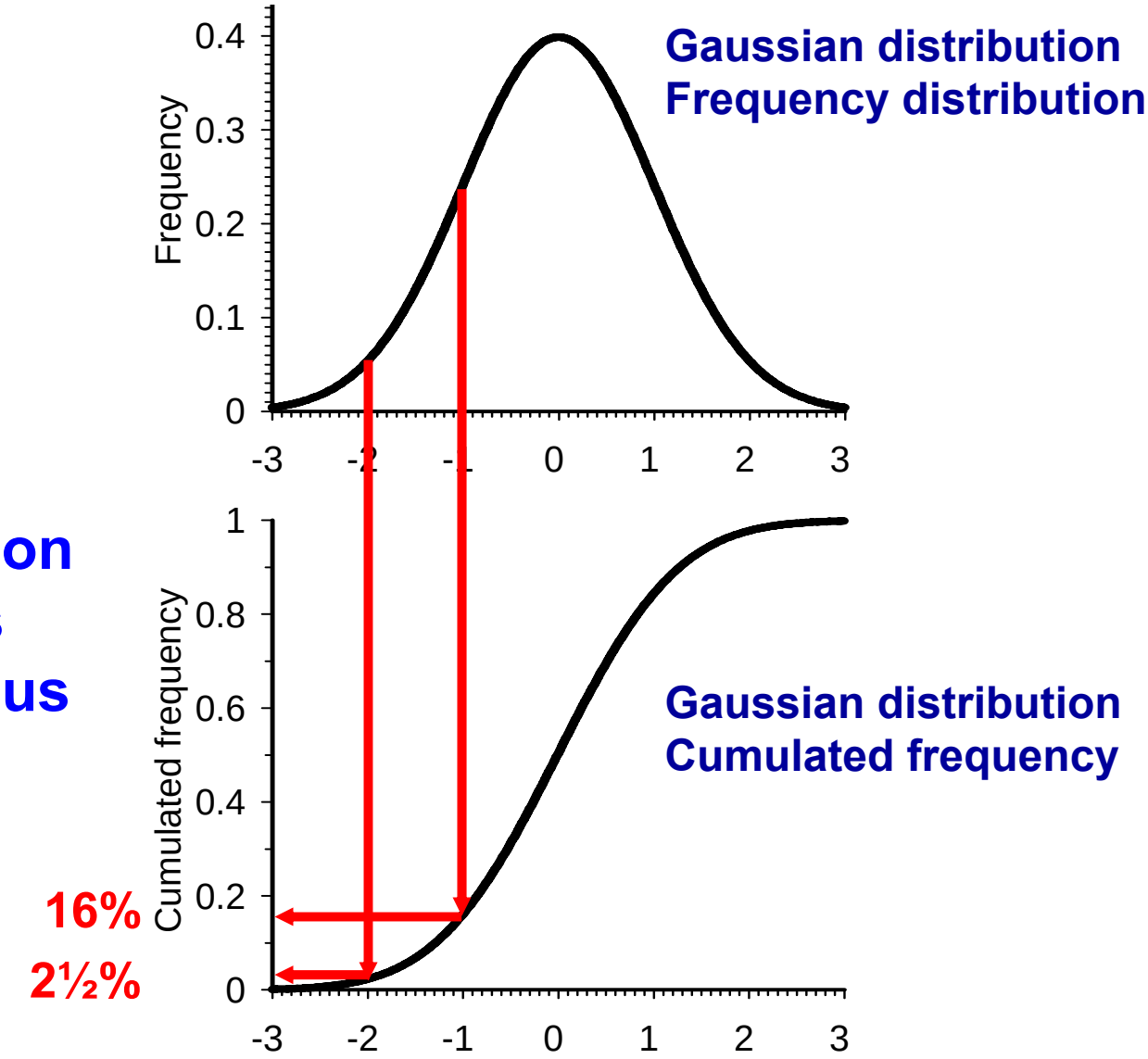
Reference population: Gauss-distribution

Gaussian distribution

Frequency distribution

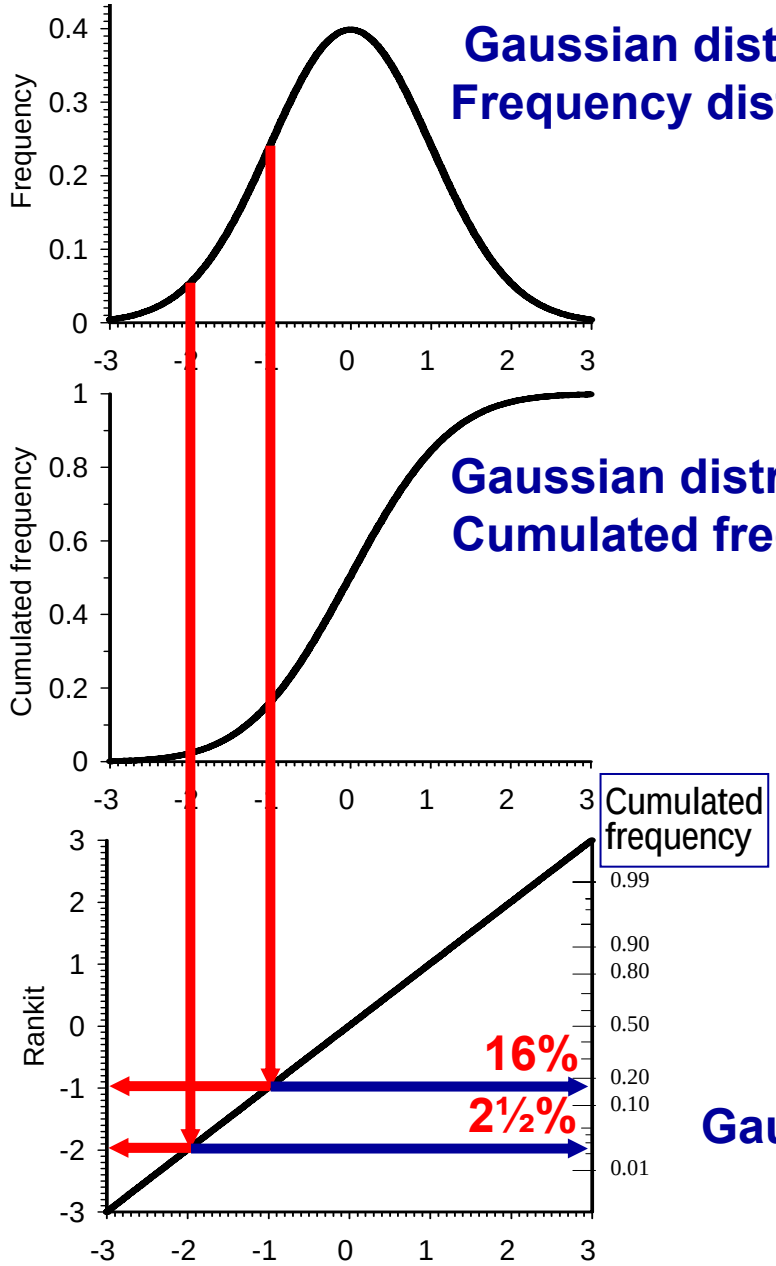


Rankit-transformation 2



Cumulation of values from minus infinite

Rankit-transformation 3

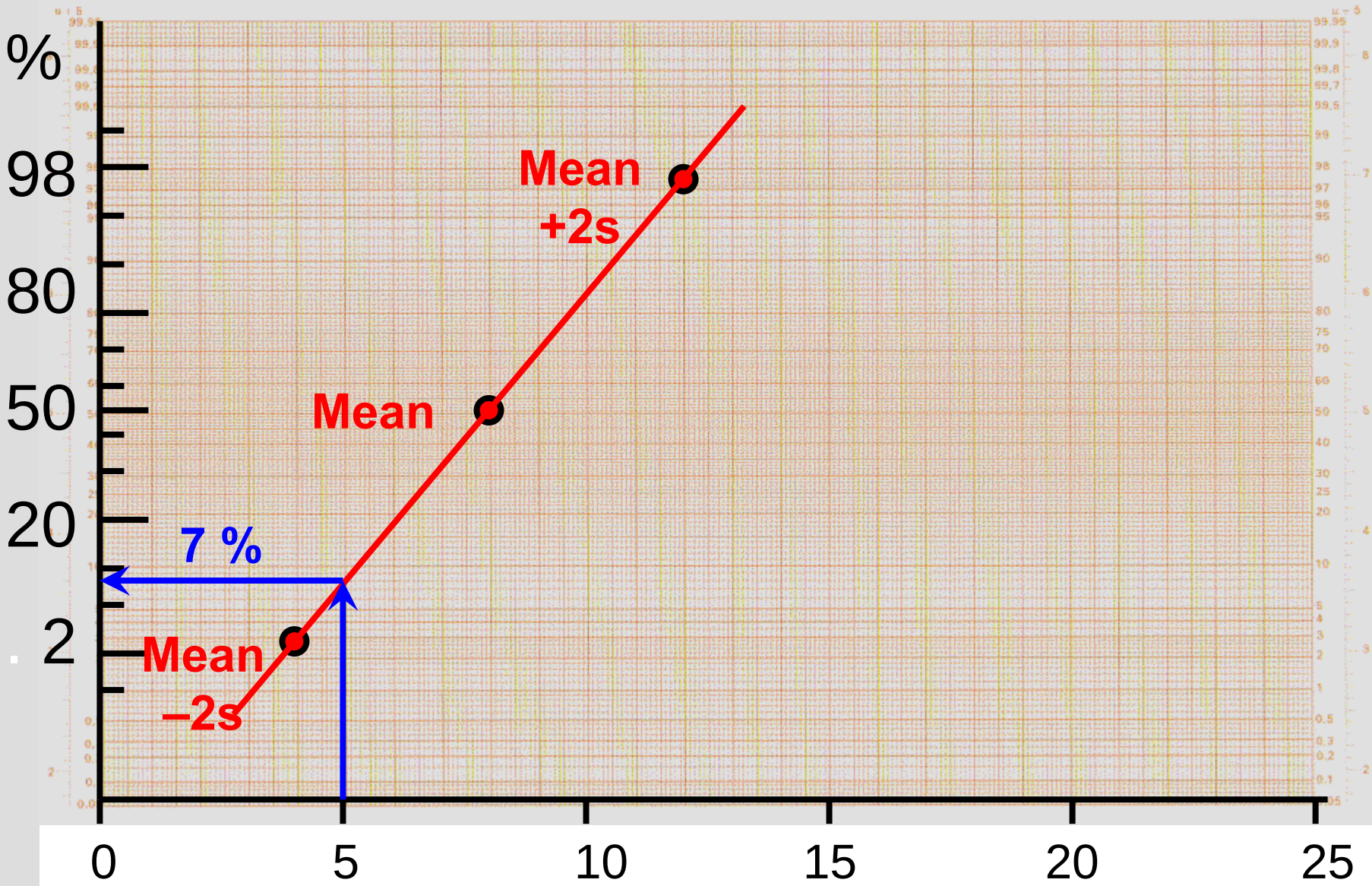


Transformation of the ordinate so that the S-shaped curve becomes a straight line

When the cumulated frequency shows up as a straight line, the distribution can be considered Gaussian

Gaussian distribution
RANKIT

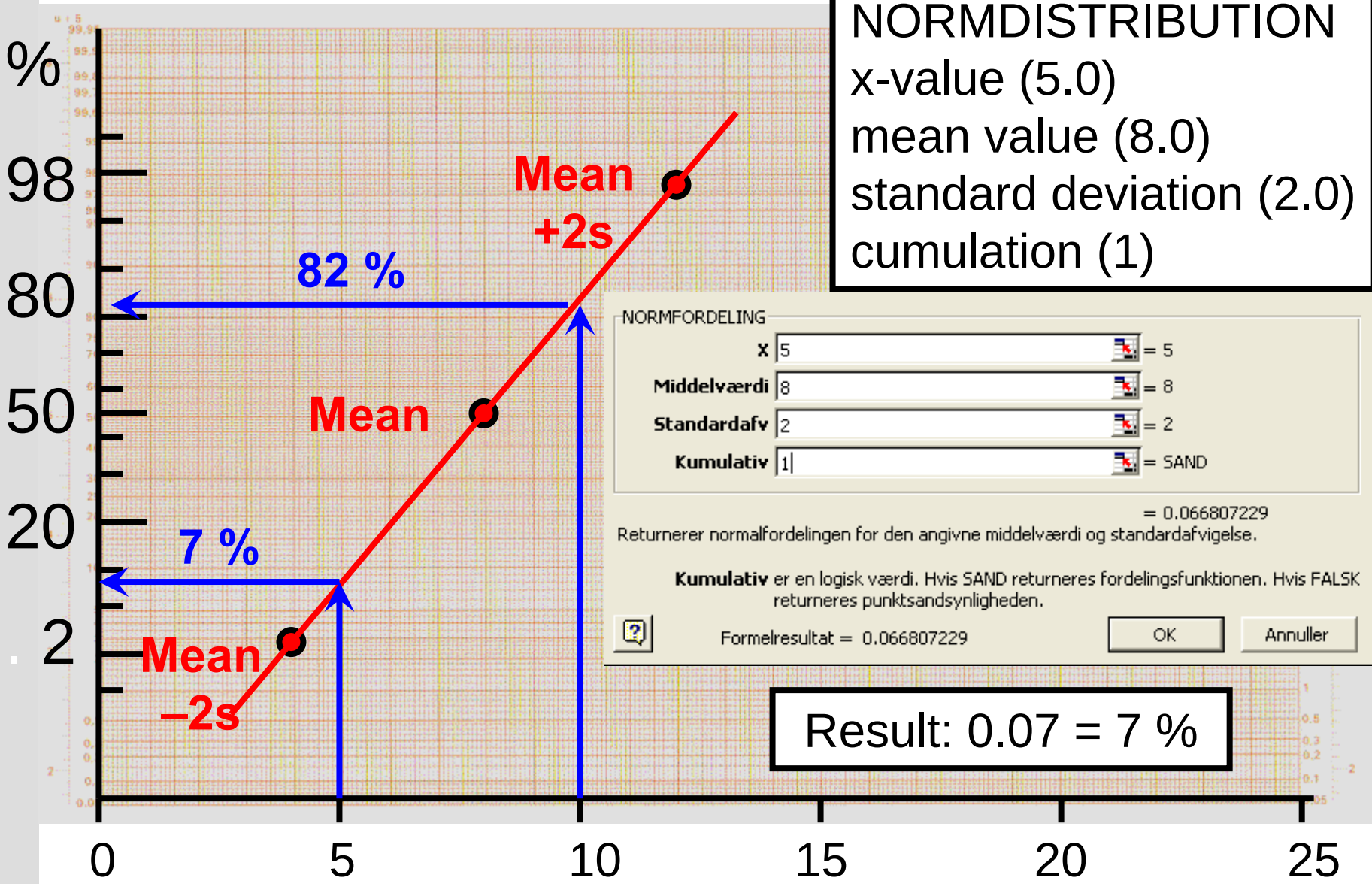
Probability paper



Probability paper (exercise)

Parameter	Concentration	Probability	Rankit
Mean μ	8	50 %	0
Standard deviation σ	2		
$\mu - 1.96*\sigma$	ca. 4	2½%	- 1.96
$\mu + 1.96*\sigma$	ca. 12	97½ %	+ 1.96
Estimate the probabilities for the concentrations			
	5		
	10		

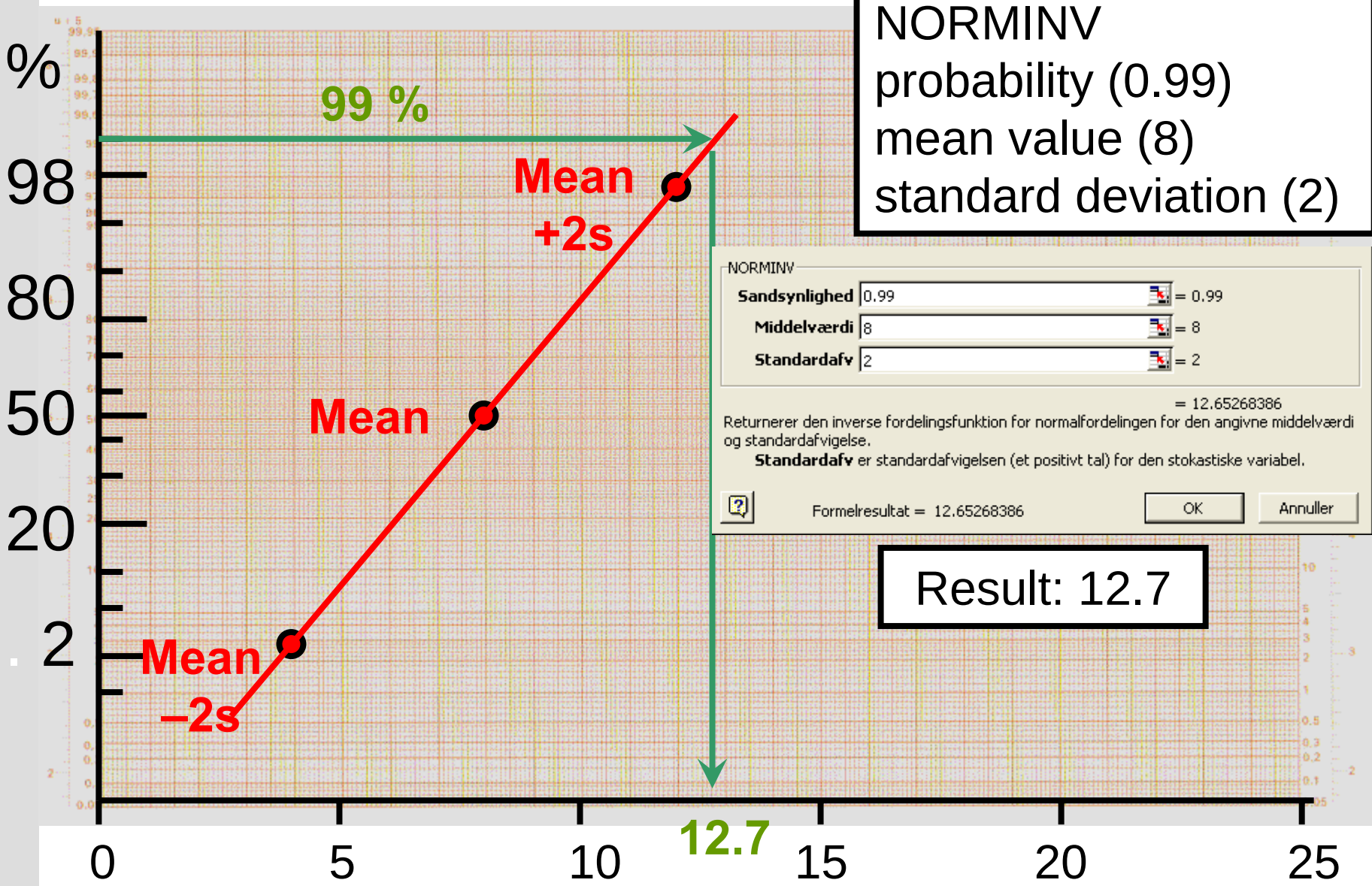
Probability paper



NORMDISTRIBUTION
x-value (5.0)
mean value (8.0)
standard deviation (2.0)
cumulation (1)

Result: 0.07 = 7 %

Probability paper



NORMINV
probability (0.99)
mean value (8)
standard deviation (2)

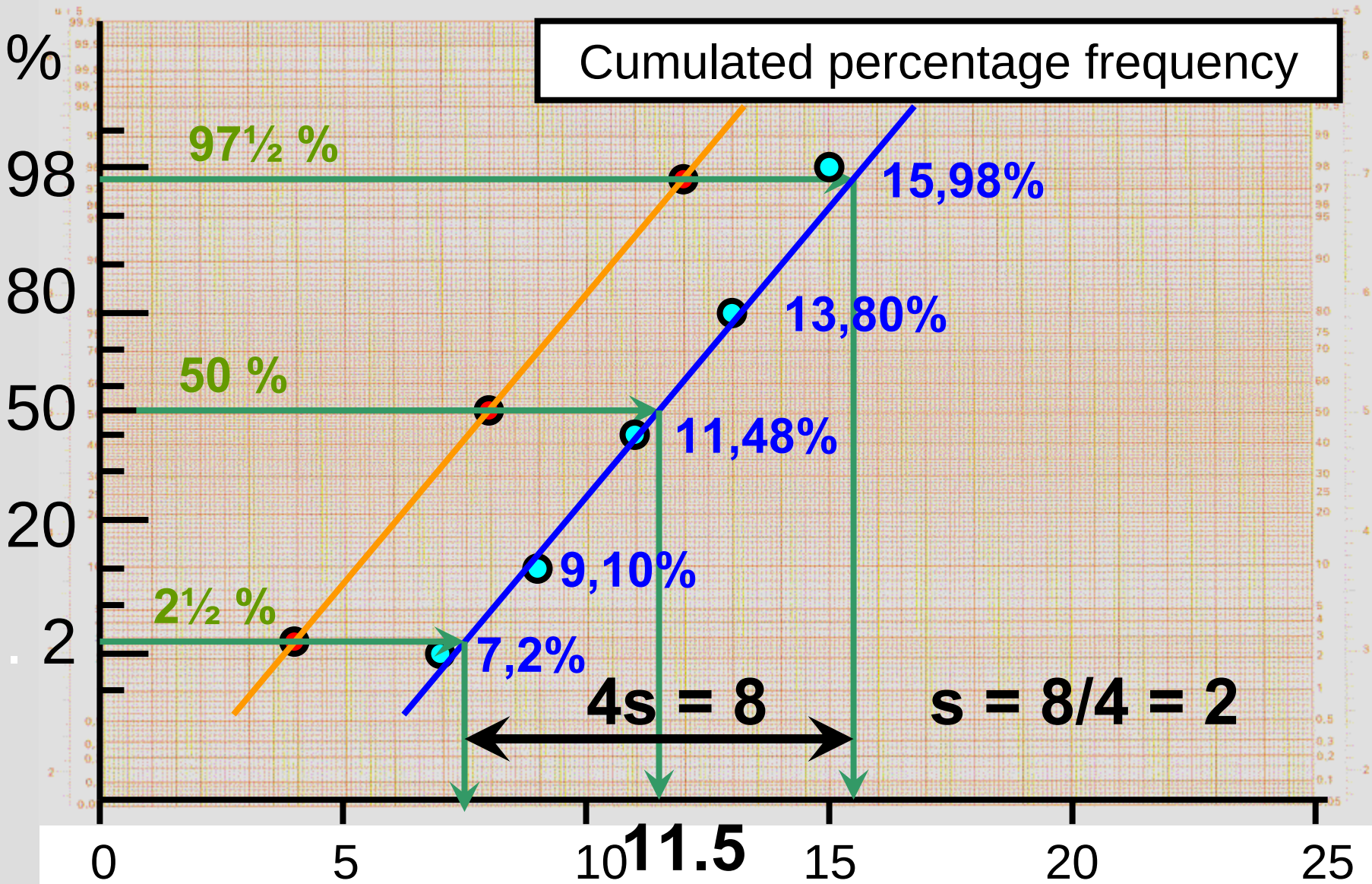
Result: 12.7

Probability paper (exercise)

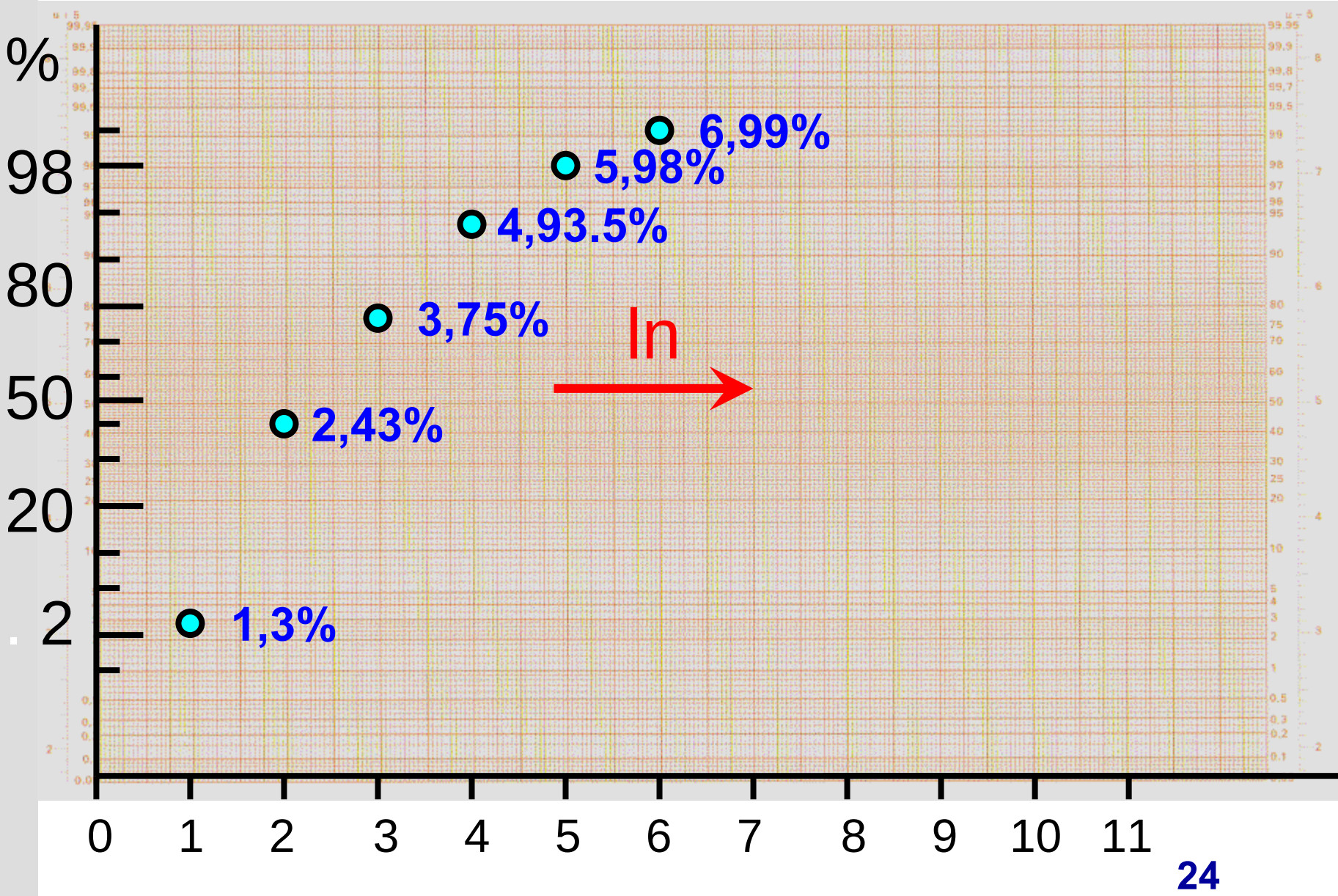
Plot the points	
X-value (concentration)	Percent (%)
7	2 %
9	10 %
11	48 %
13	80 %
15	98 %

Estimate	
Mean value	
$\mu - 1.96*\sigma$	
$\mu + 1.96*\sigma$	
Standard deviation	

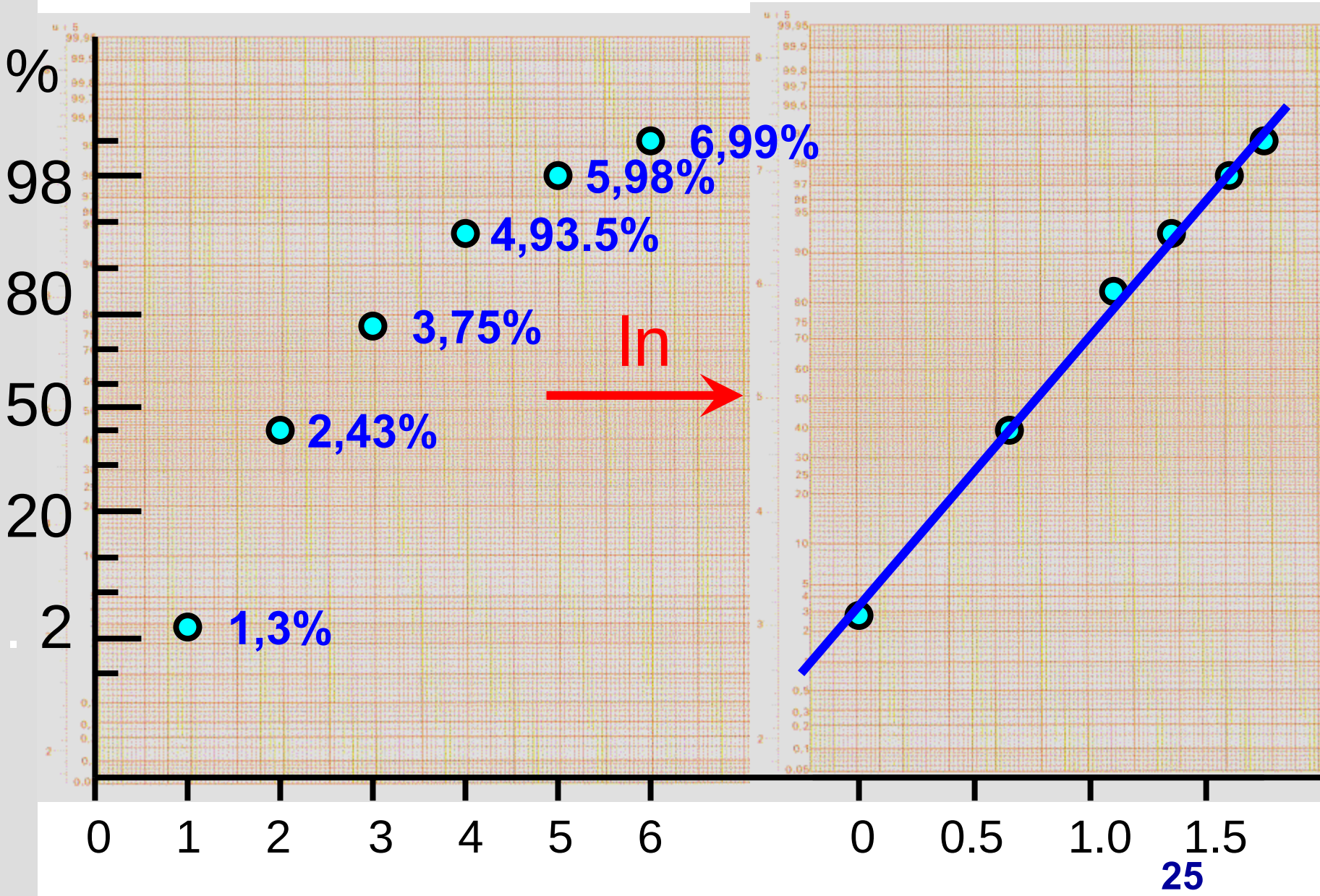
Probability paper



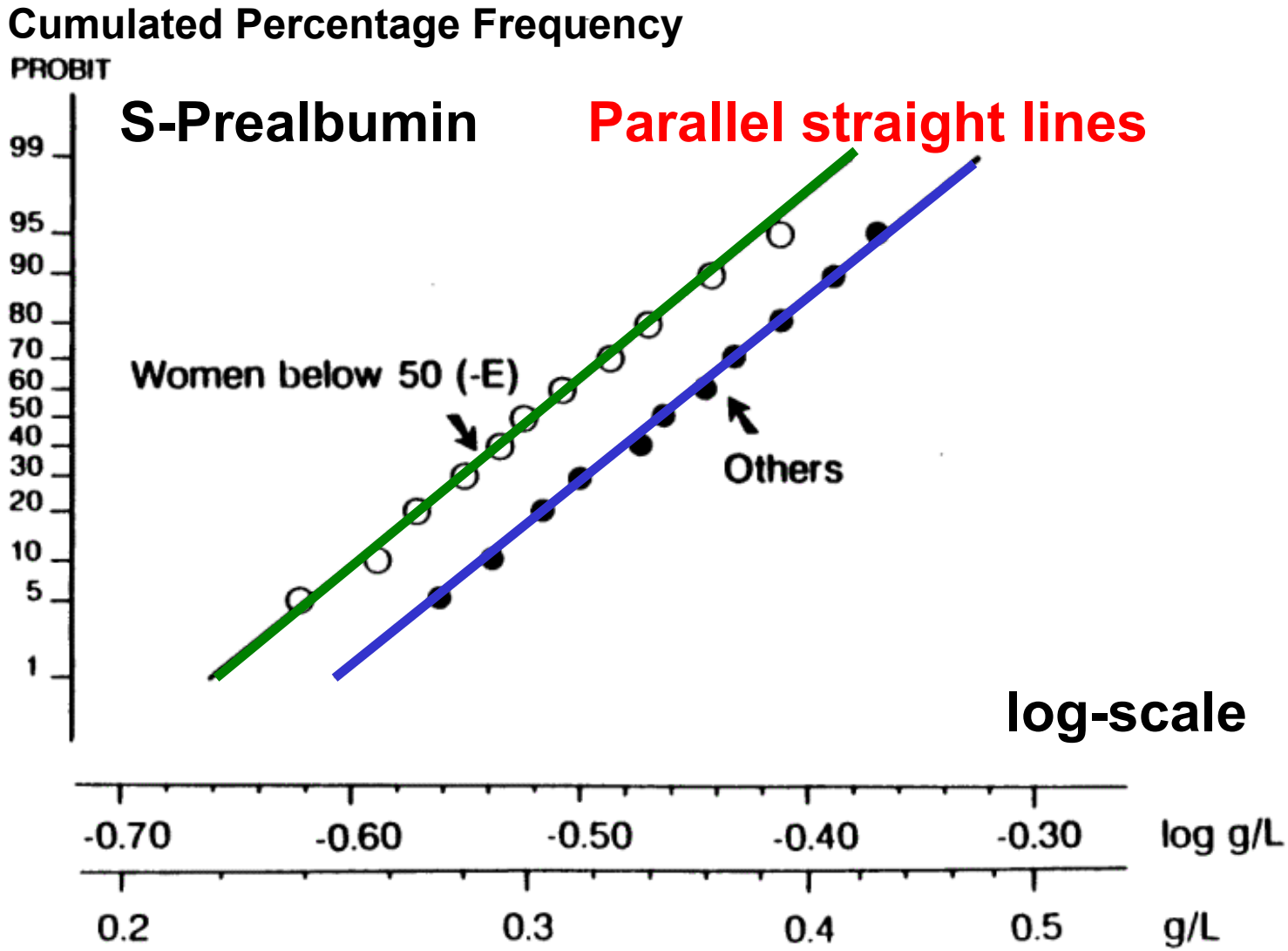
Probability paper



Probability paper



Serum-Prealbumin



Blaabjerg *et al.* Upsala J Med Sci 1994;99:315-38

Criteria for reference individuals

The rule-out criteria for reference individuals are different for different quantities

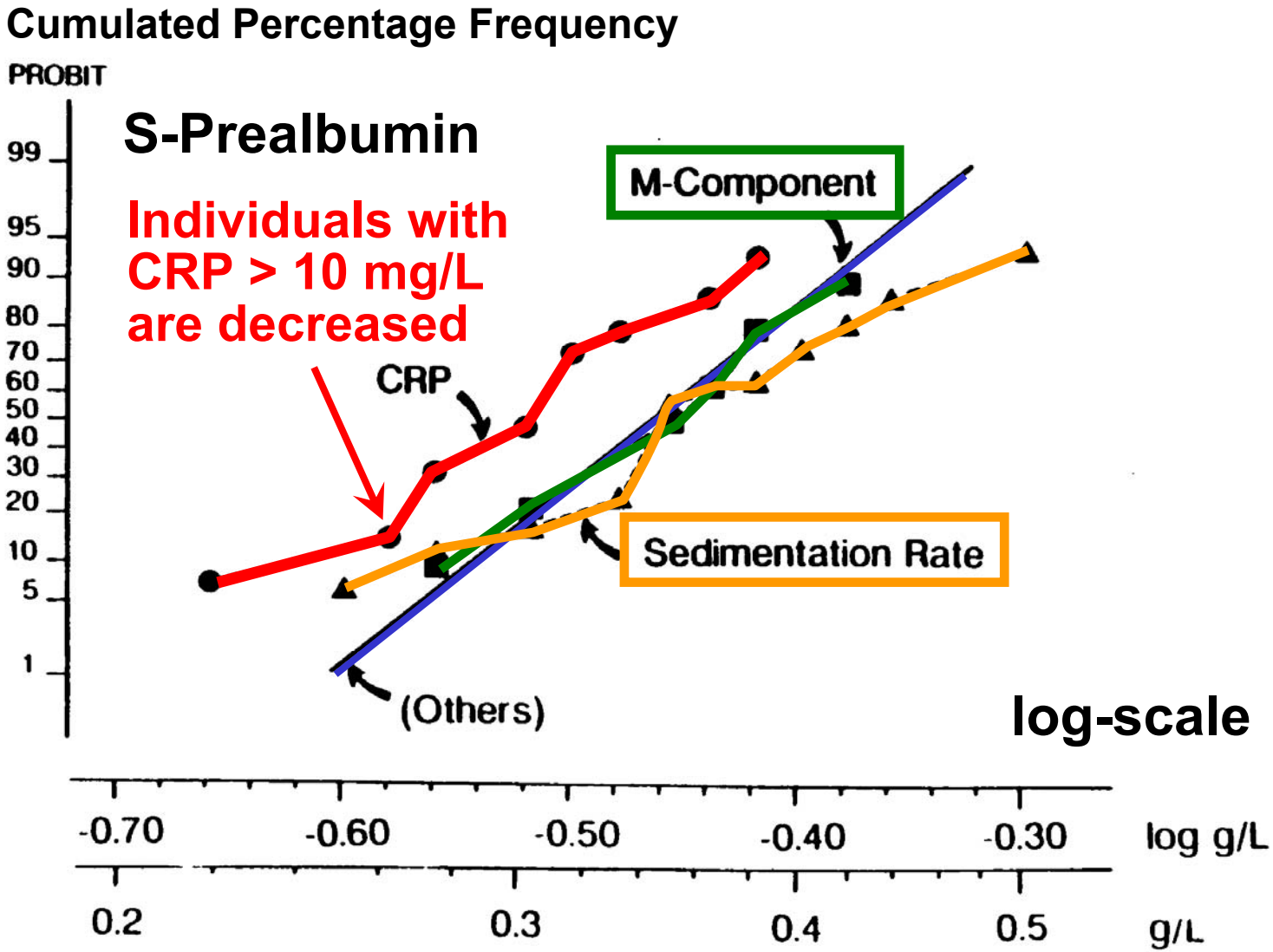
For plasma-proteins:
High CRP, high SR, M-component

For fasting plasma-glucose:
First generation DM and related diseases

For thyroid components:
Age, gender, family history etc.

Ritchie & Palomaki. Clin Chem Lab Med 2004;42:702-9

Serum-Prealbumin



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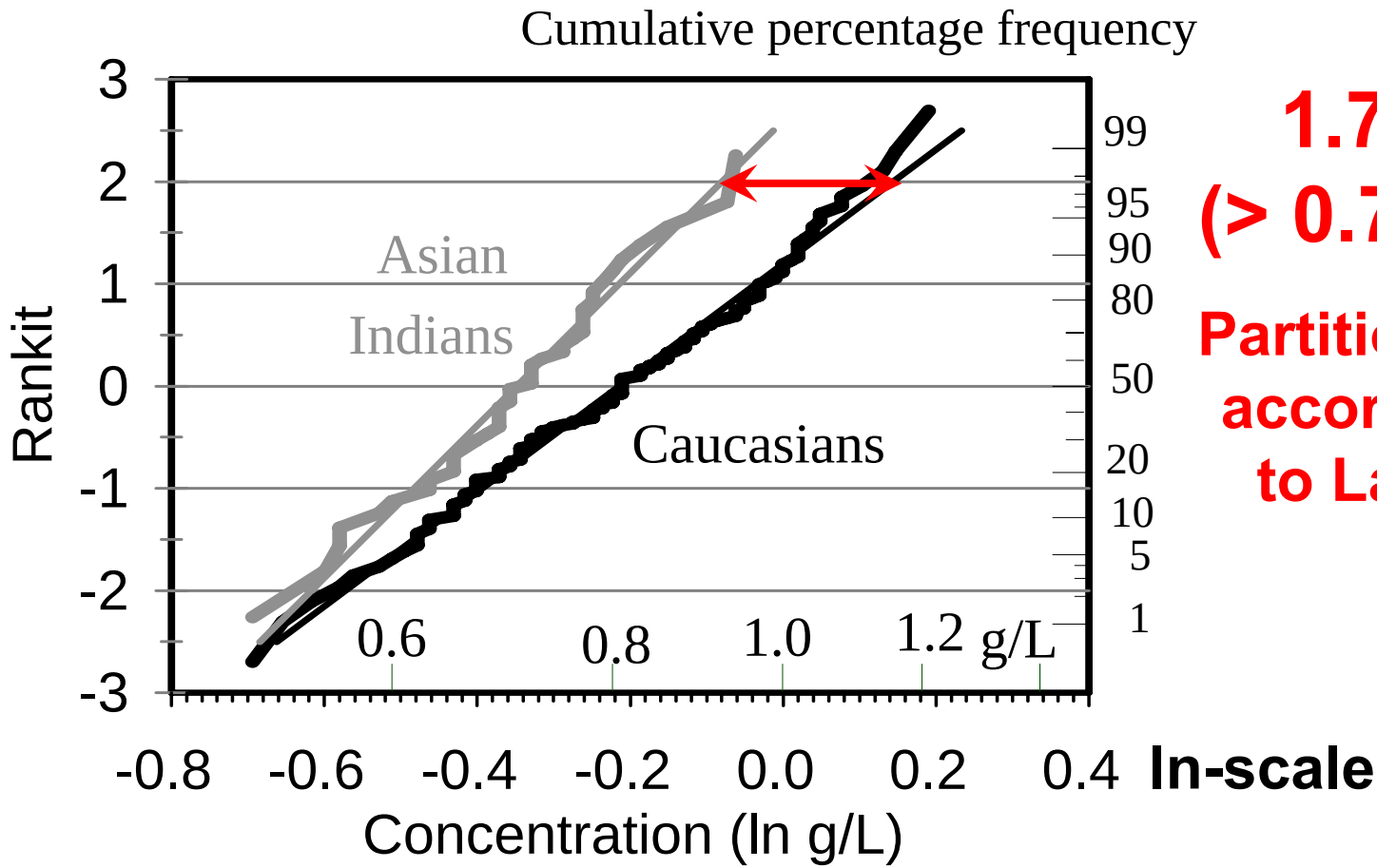
Comparison of Proteins

Comparison of Orosomucoid Values

Caucasians and Asian Indians in Leeds



1.7*s

(> 0.75*s)

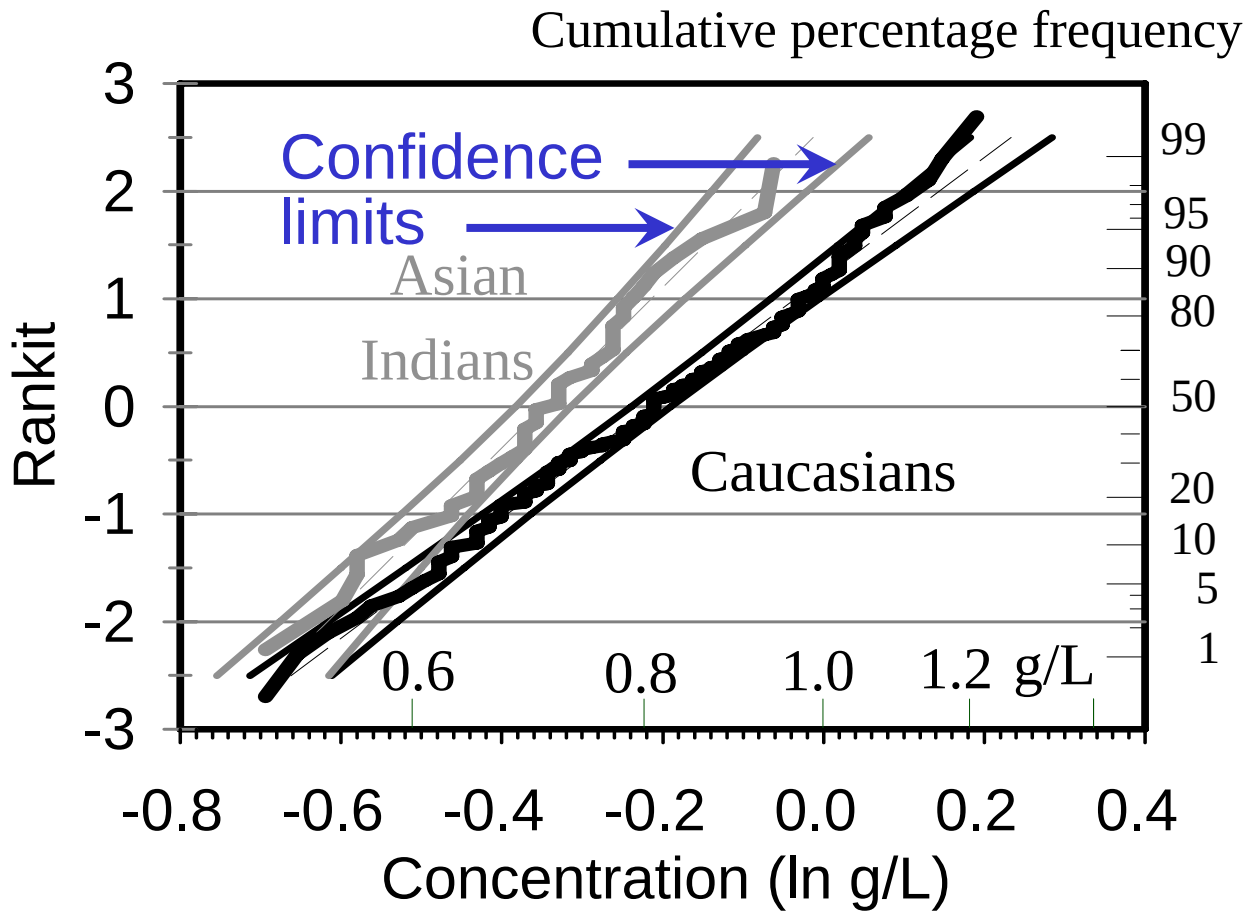
Partitioning according to Lahti

Johnson *et al.* CCLM 2004;42:792-9

Comparison of Proteins

Comparison of Orosomucoid Values

Caucasians and Asian Indians in Leeds

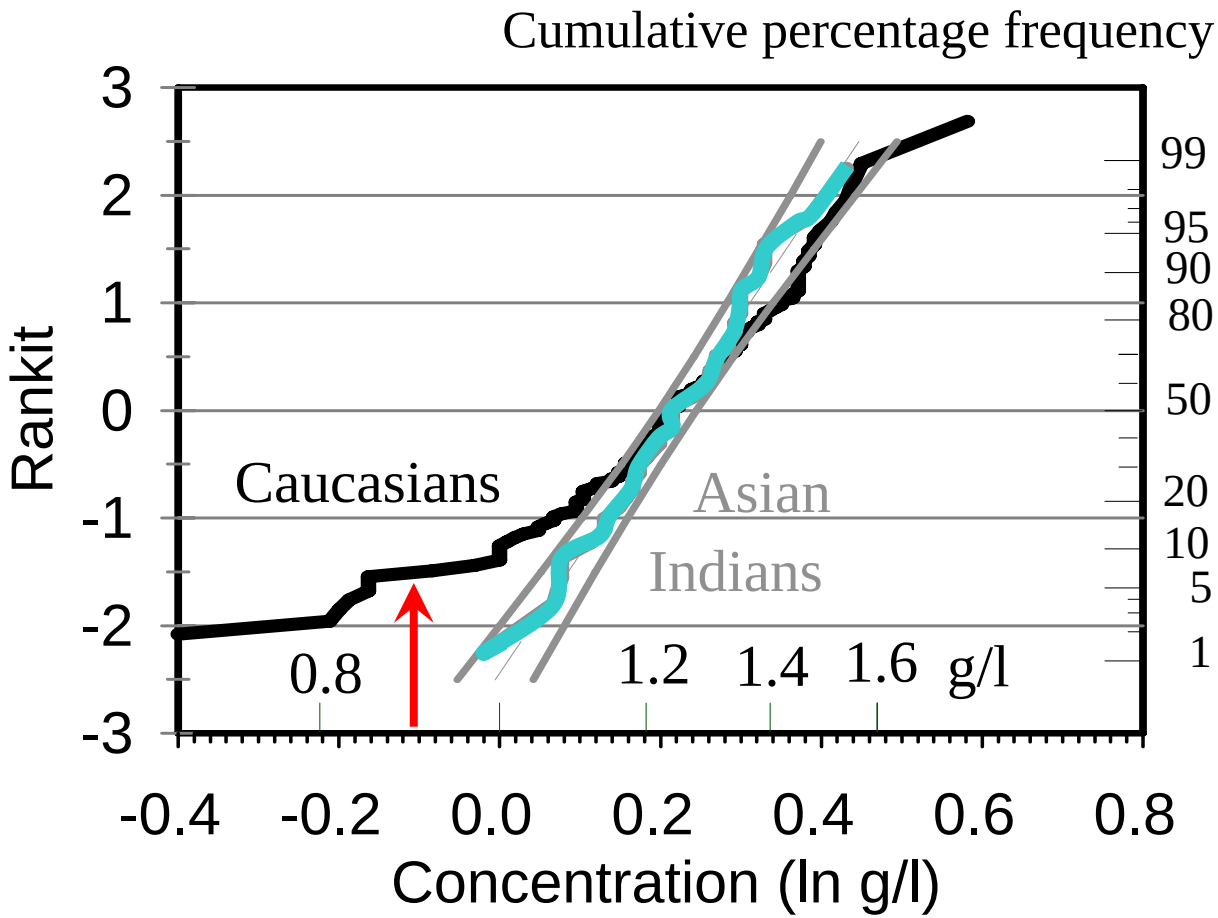


Johnson *et al.* CCLM 2004;42:792-9

Comparison of Proteins

Comparison of Antitrypsin Values

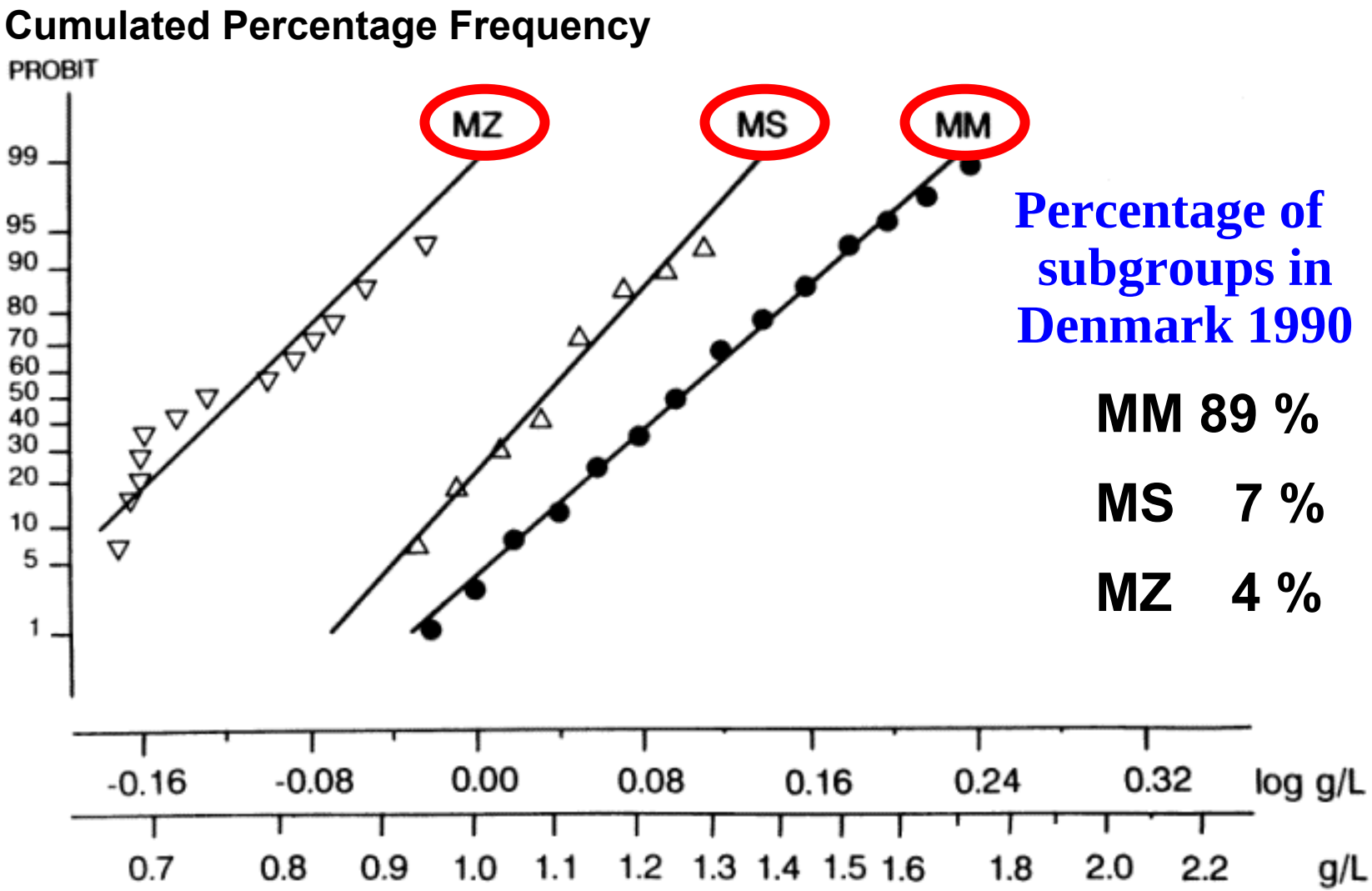
Caucasians and Asian Indians in Leeds



Johnson *et al.* CCLM 2004;42:792-9

Comparison of Proteins

Distribution of α_1-Antitrypsin genotypes



Percentage of subgroups in Denmark 1990

MM 89 %

MS 7 %

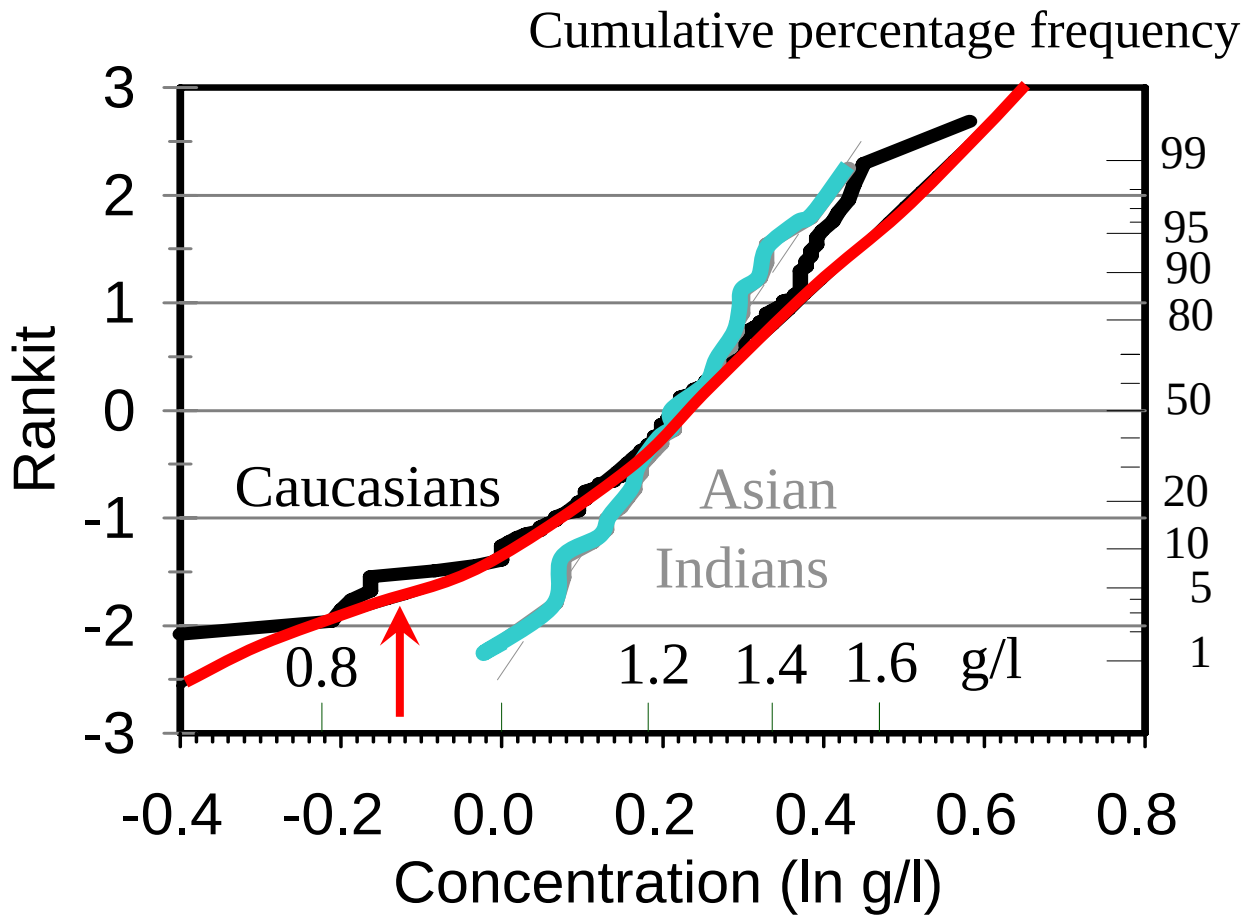
MZ 4 %

Lund *et al.* Upsala J Med Sci 1994;99:339-46

Comparison of Proteins

Comparison of Antitrypsin Values

Caucasians and Asian Indians in Leeds



Johnson *et al.* CCLM 2004;42:792-9

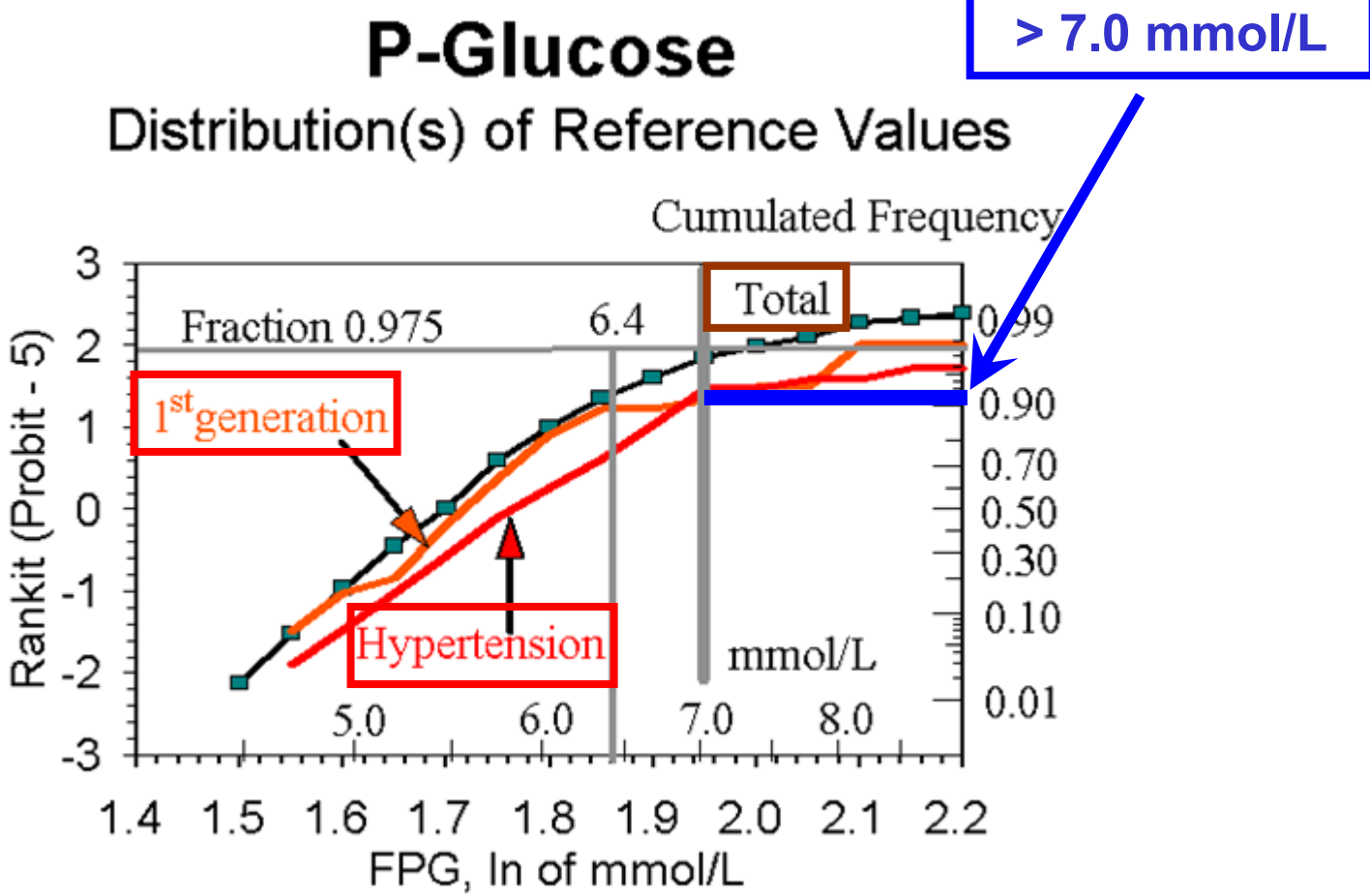
Plasma-Glucose
Low risk population

Invited population (social security no)	2100
Responders	755
Diseased	29
At risk	290
(First-generation diabetes relatives, Hypertension, Cardiac diseases, Indicrine diseases, etc.)	
Increased HbA_{1C}	12
Low risk population	424

Jørgensen *et al.Scand J Clin Lab Invest* 2001; 61:181-190.

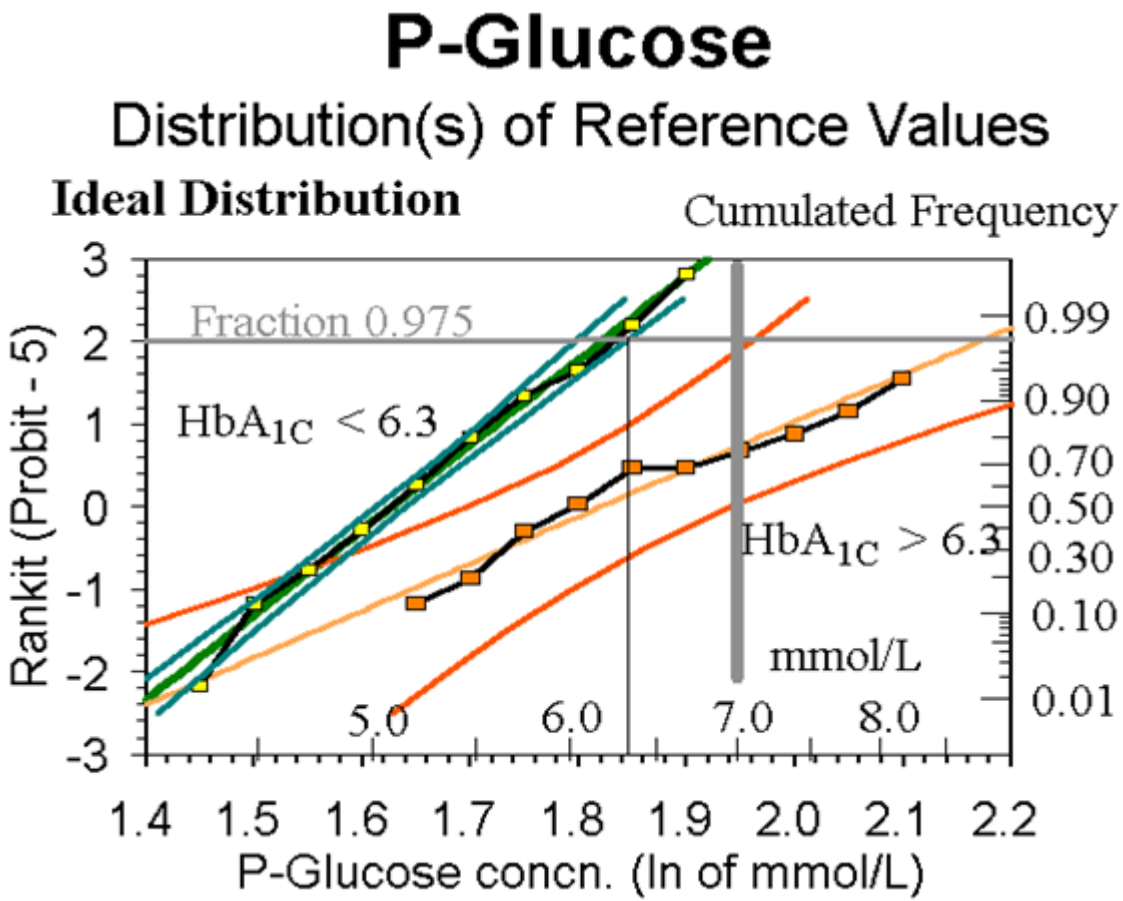
Plasma-Glucose

Low risk population



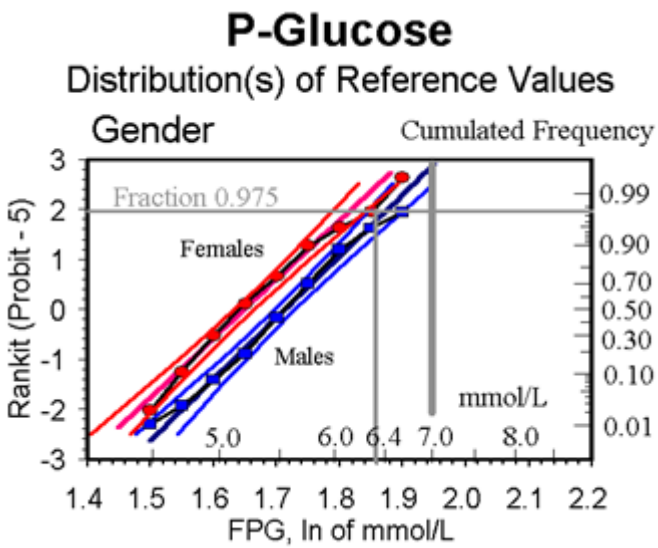
Jørgensen *et al.Scand J Clin Lab Invest* 2001; 61:181-190.

Plasma-Glucose
Low risk population

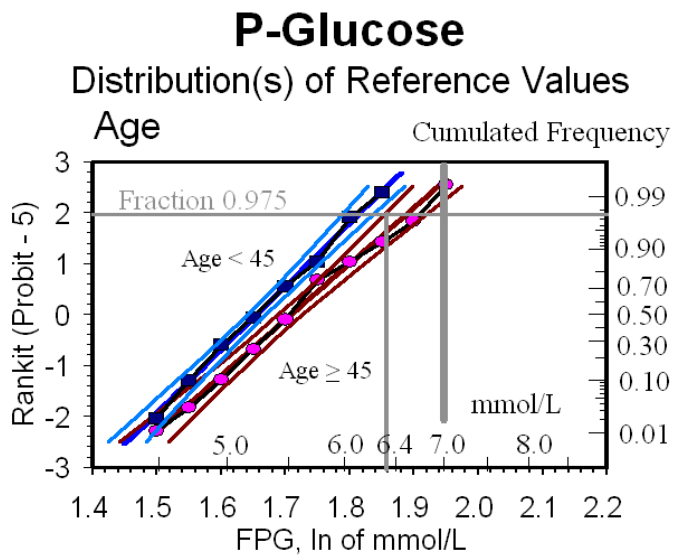


Jørgensen *et al.Scand J Clin Lab Invest* 2001; 61:181-190.

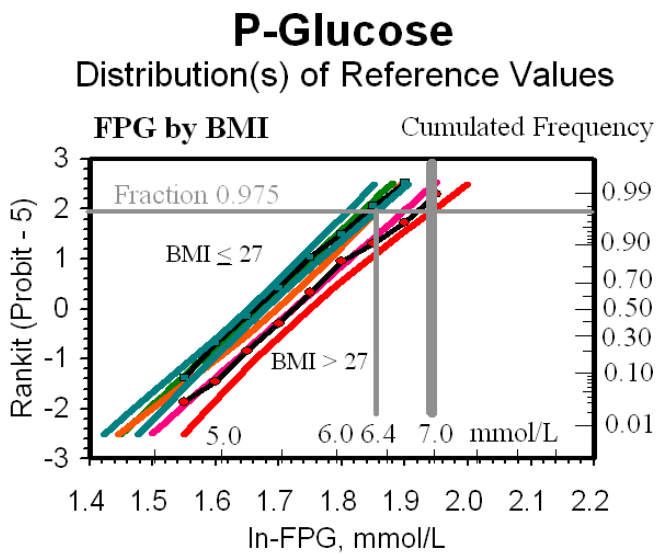
Plasma-Glucose - *Low risk population*



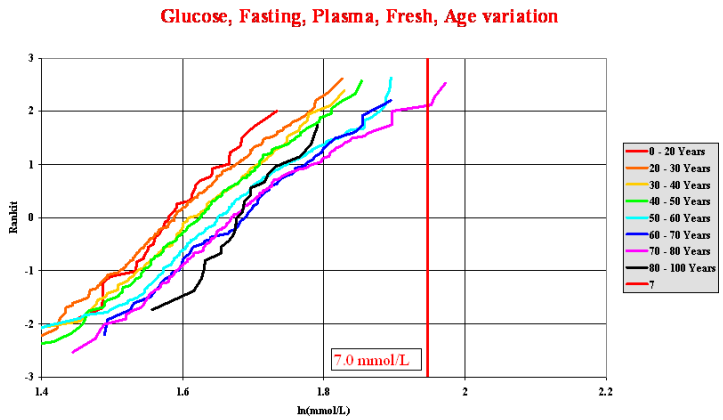
Gender



Age



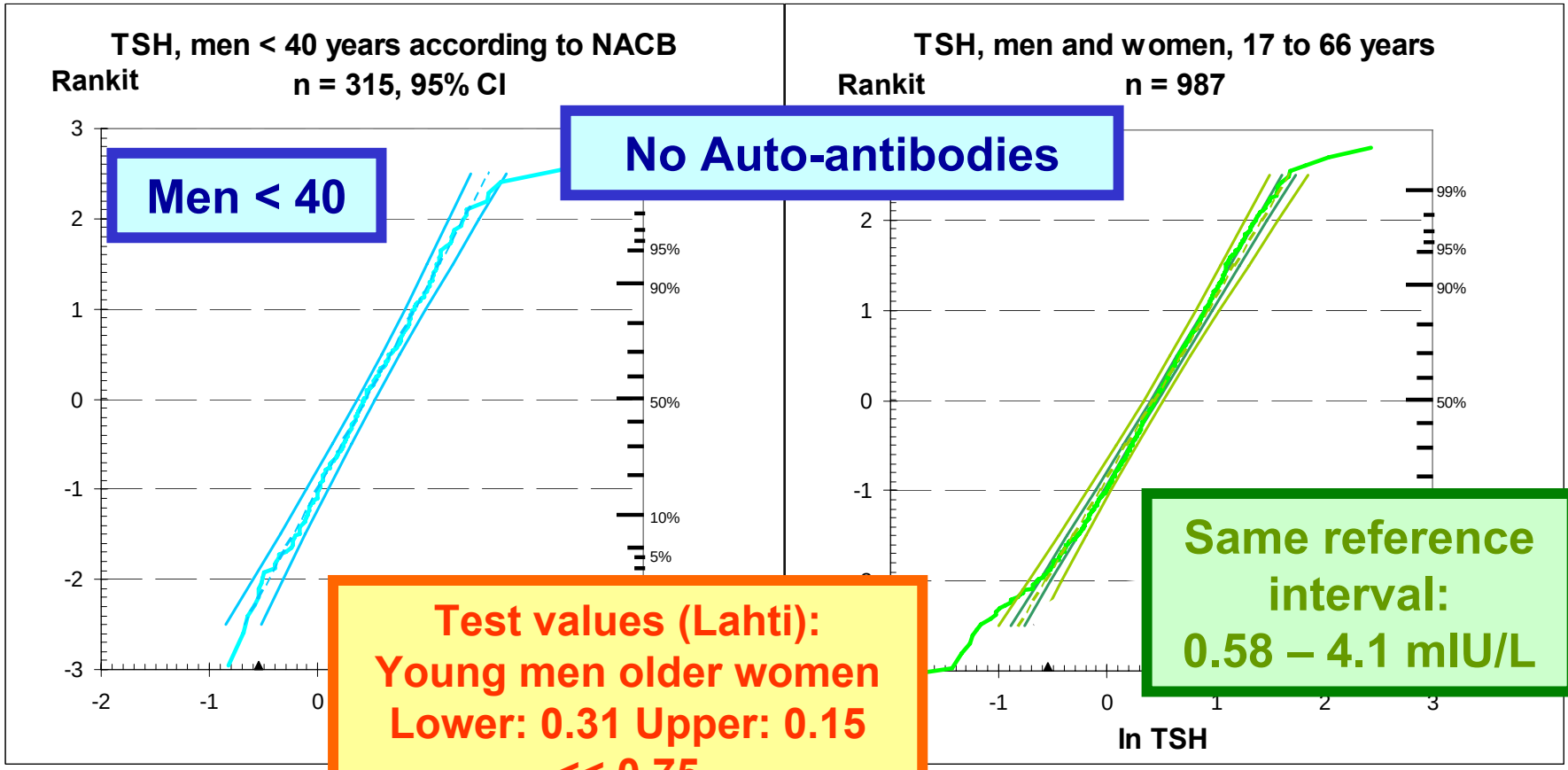
BMI



Jørgensen *et al.Scand J Clin Lab Invest* 2001; 61:181-190.

Serum-TSH

Low risk population



Jensen *et al. Clin Chem Lab Med* 2004; 42:824-32.

Reference interval for serum-Testosterone

Healthy young men 20 – 29 years

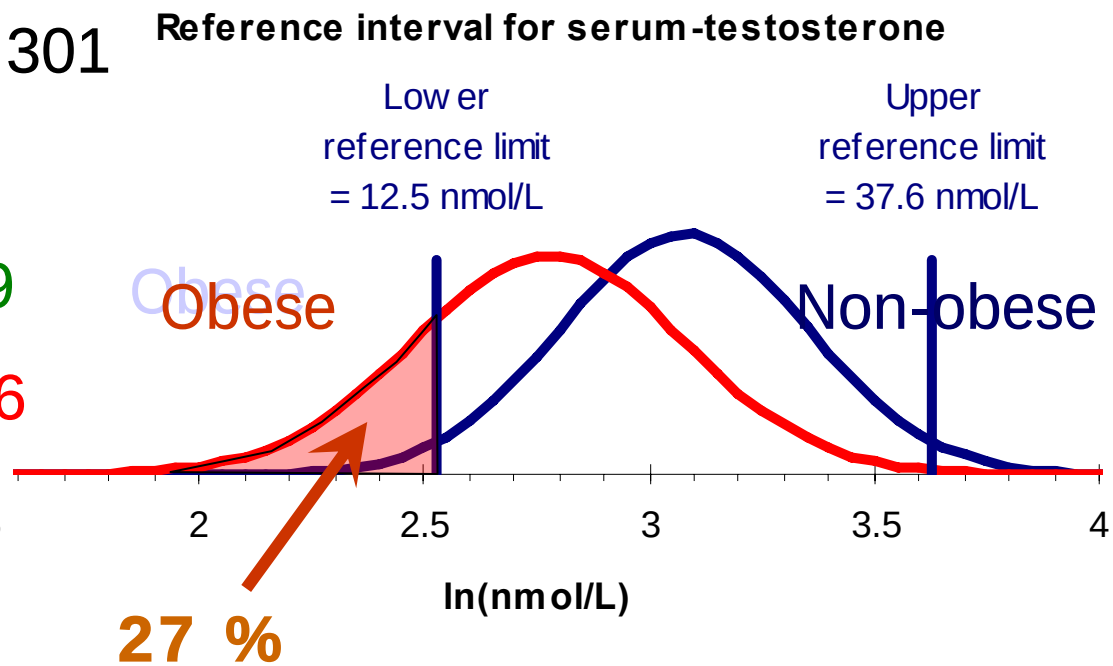
Non-obese: Obese:
s(ln) = 0.275 s(ln) = 0.301

Difference: Low High

0.351 0.249

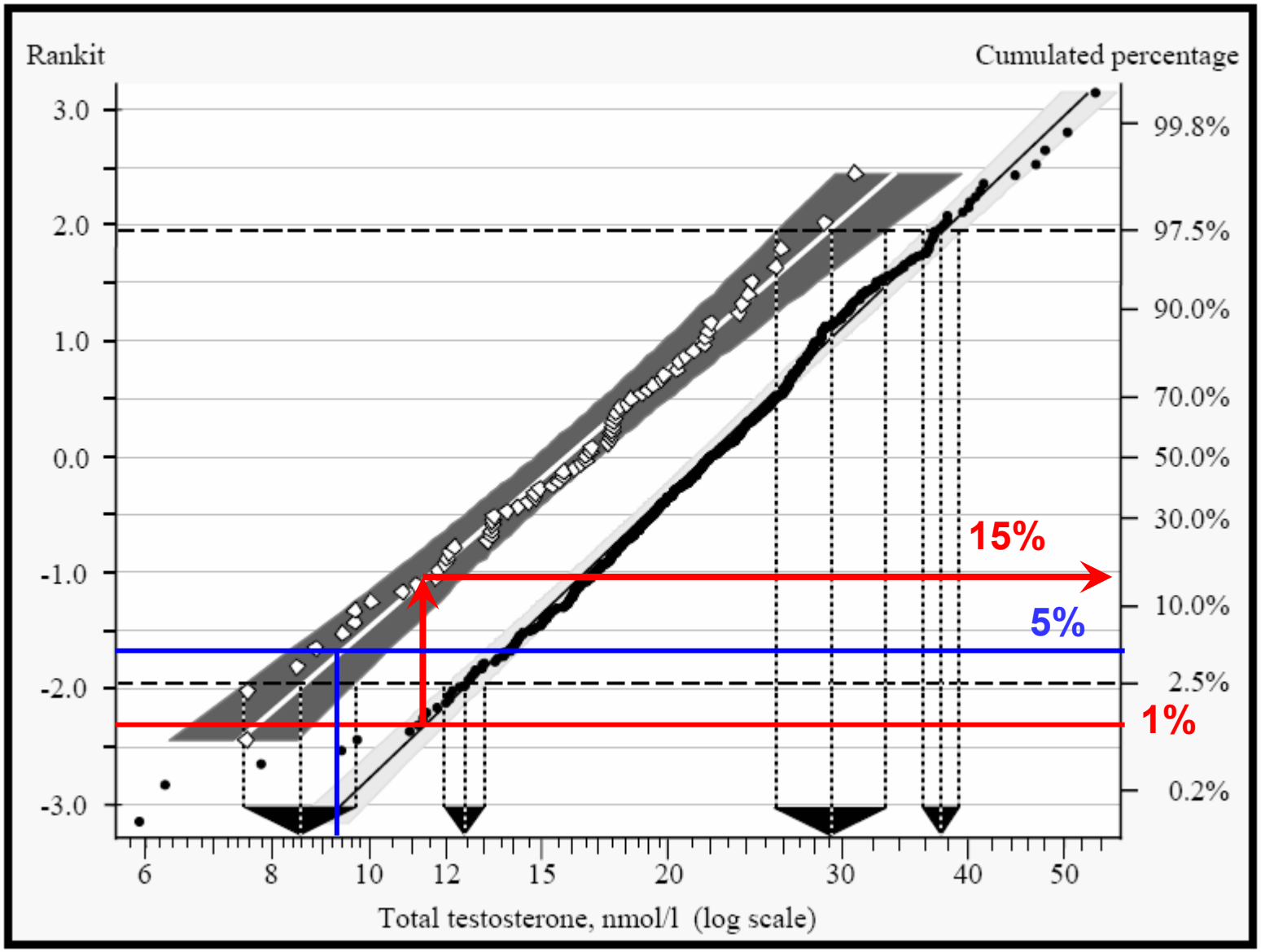
Ratio: 1.275 0.906

Partitioning



Nielsen et al. J Clin Endocrin Metabolism 2007;92:2696-705

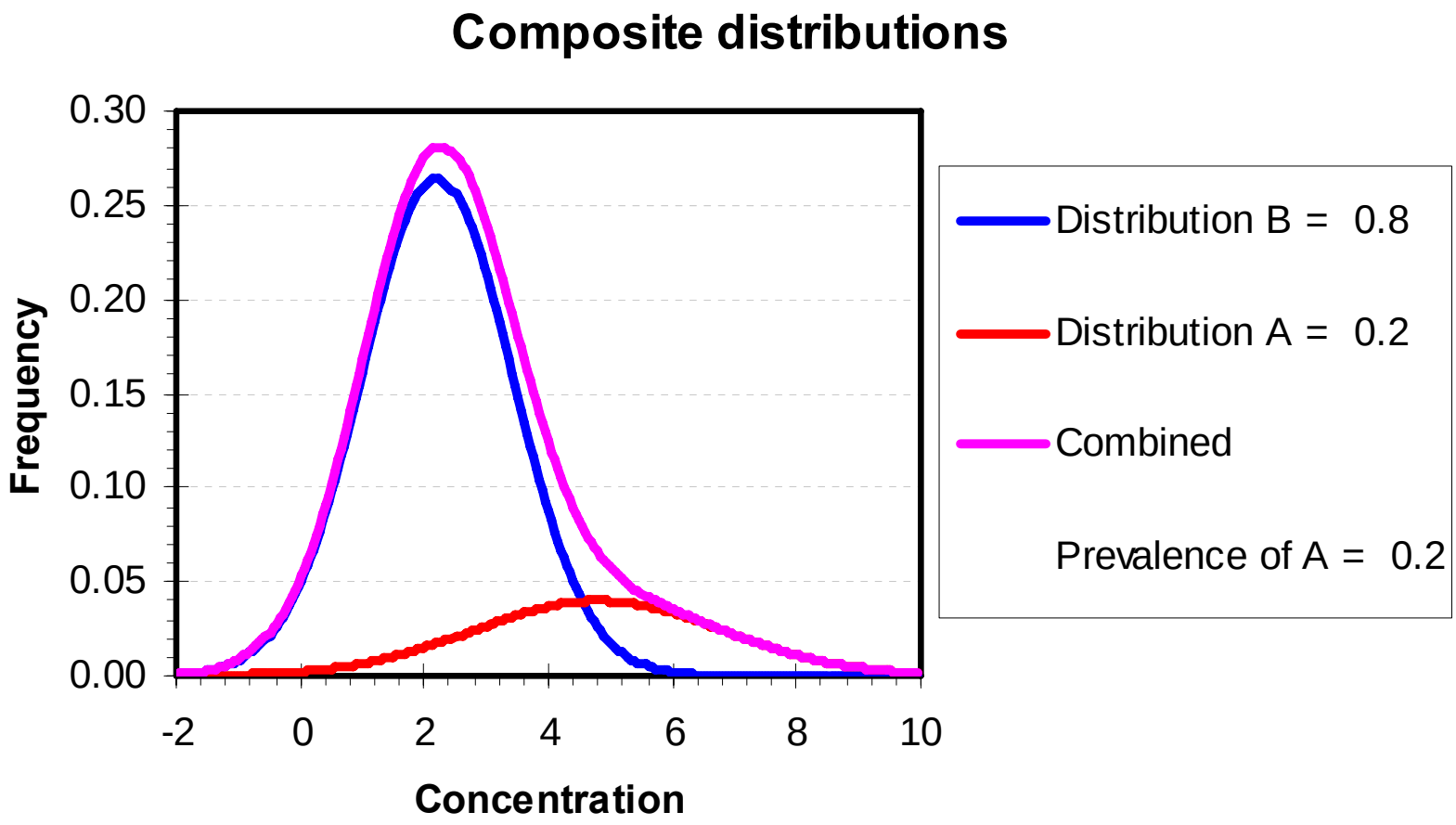
Reference interval for serum-Testosterone



Nielsen et al. J Clin Endocrin Metabolism 2007;92:2696-705

Composite distributions

Theory - Model

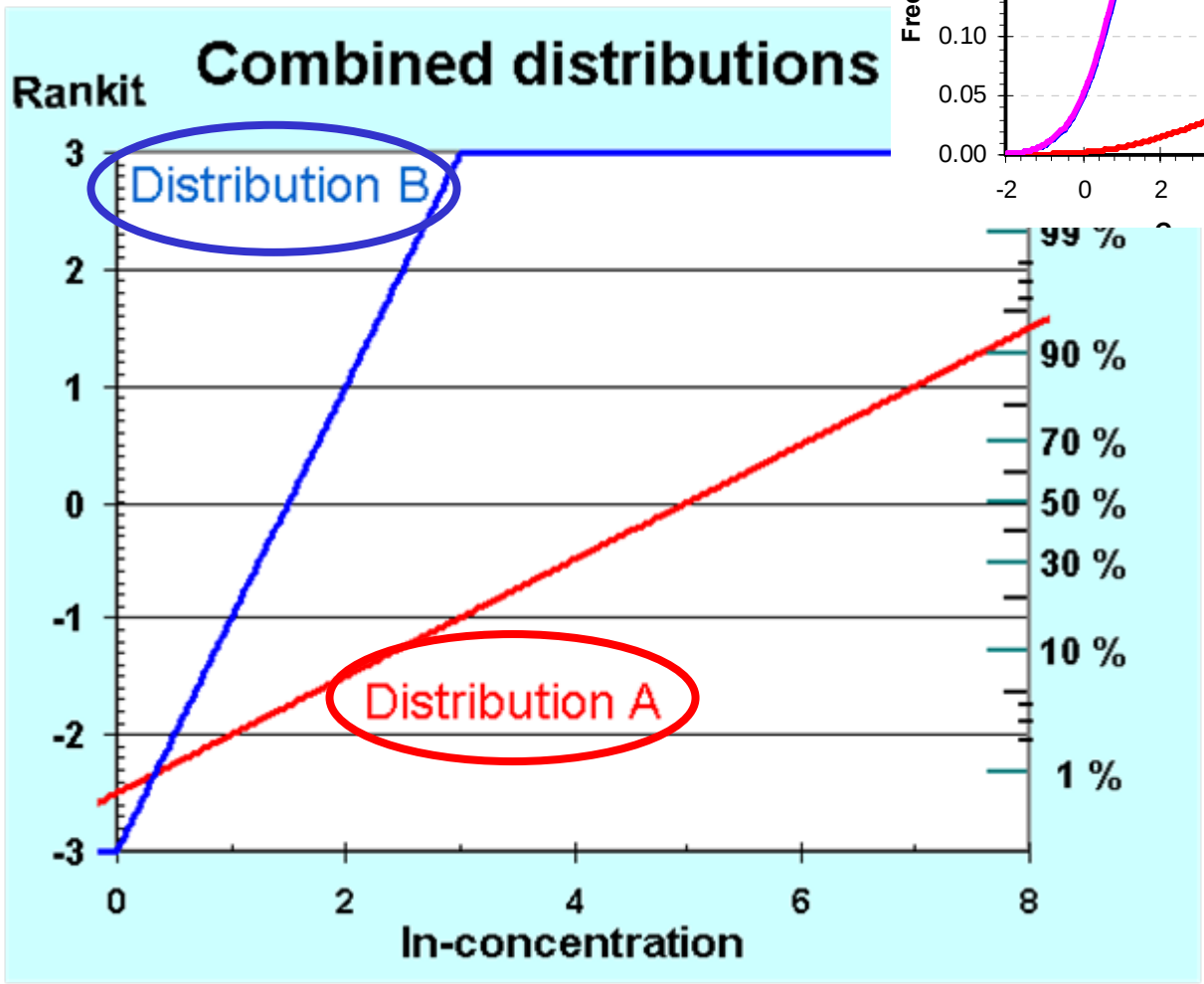


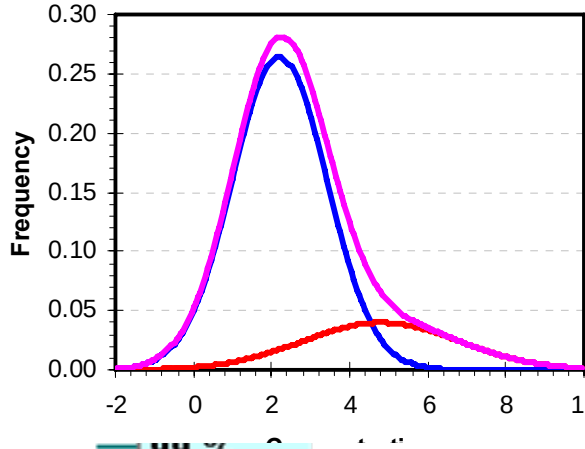
Jensen *et al.* . Clin Chem Lab Med 2006;44:991-8

Composite distributions

Theory

Model



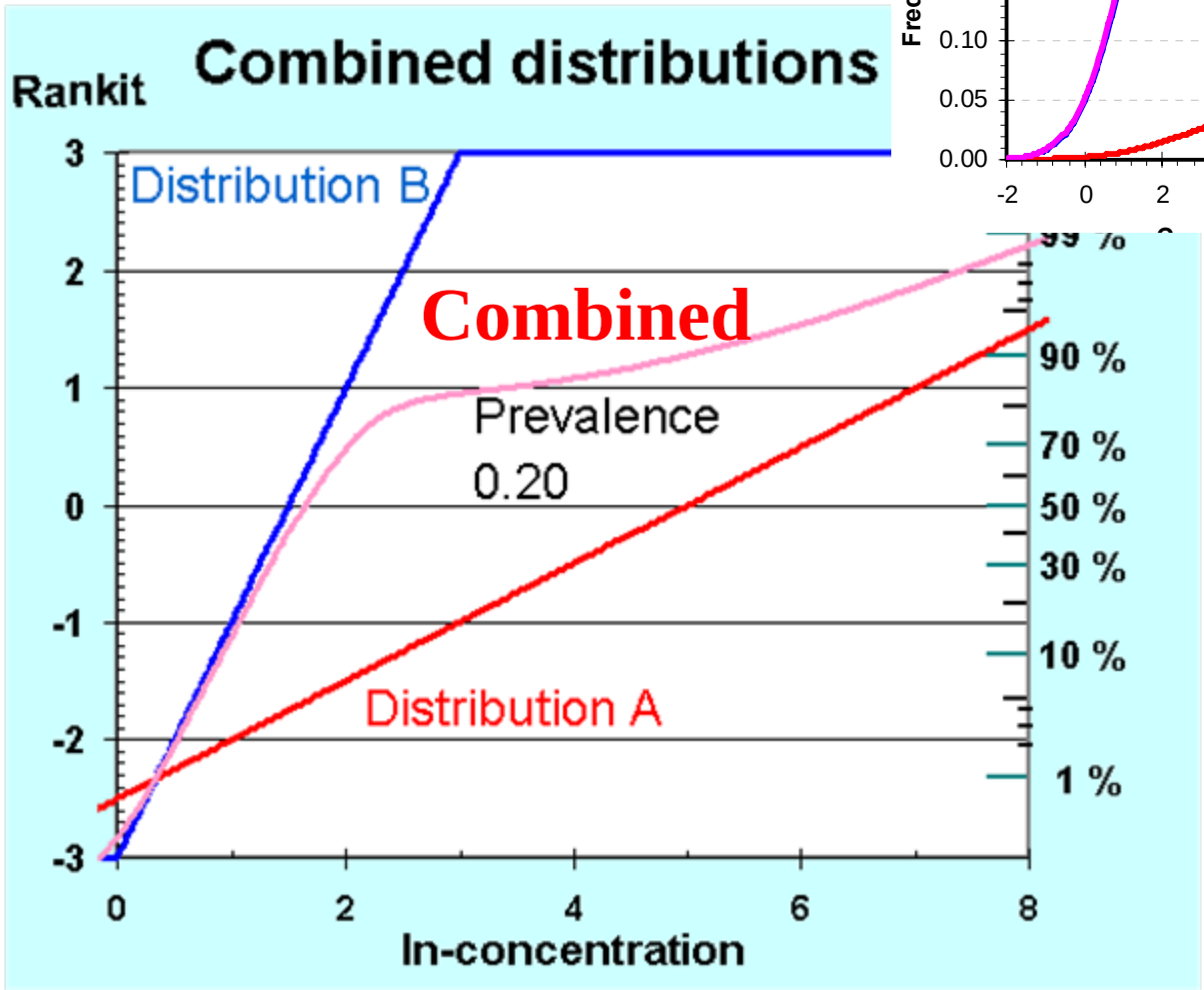


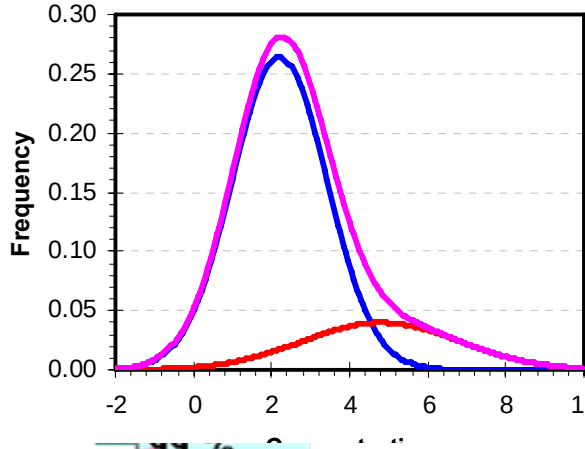
Jensen *et al.* . Clin Chem Lab Med 2006;44:991-8

Composite distributions

Theory

Model



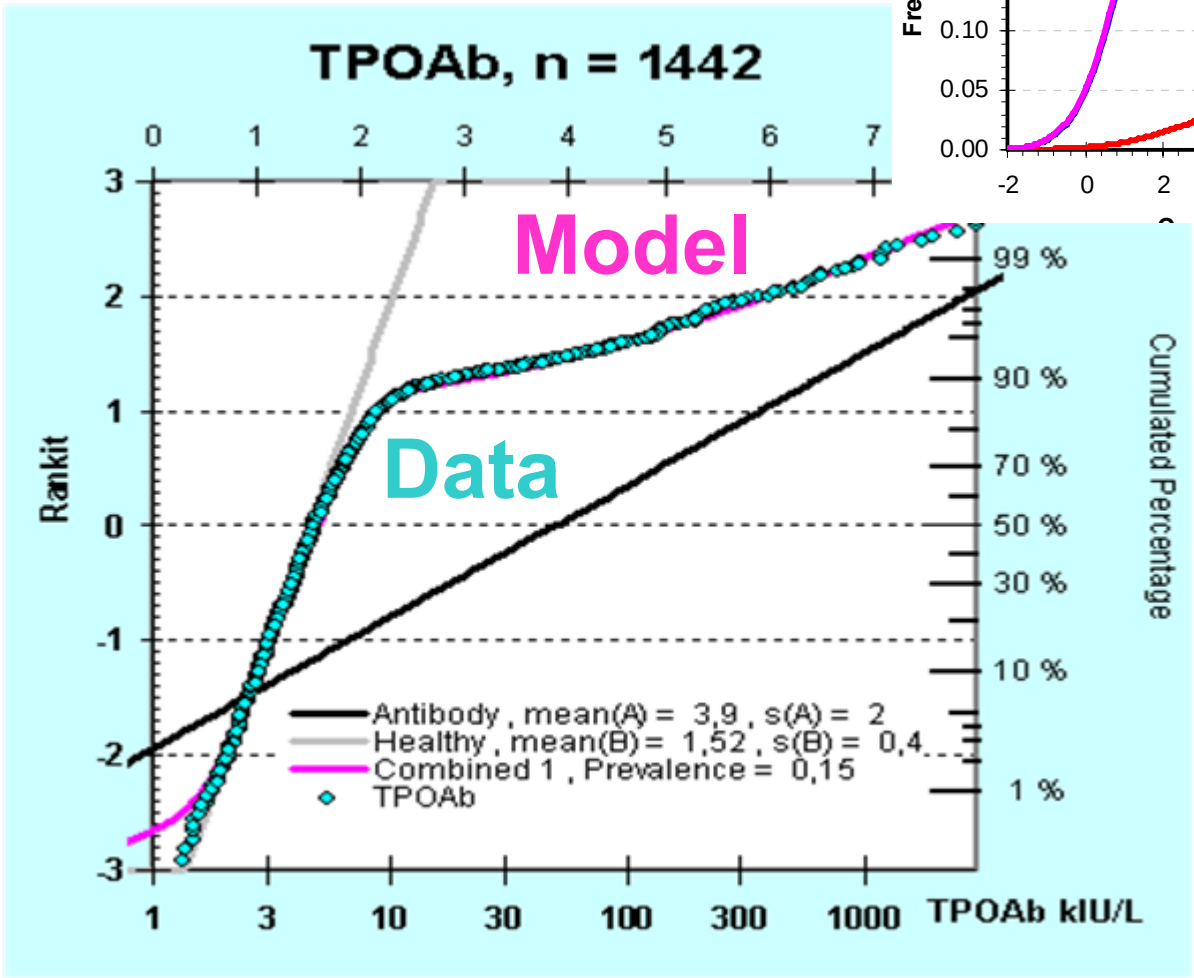


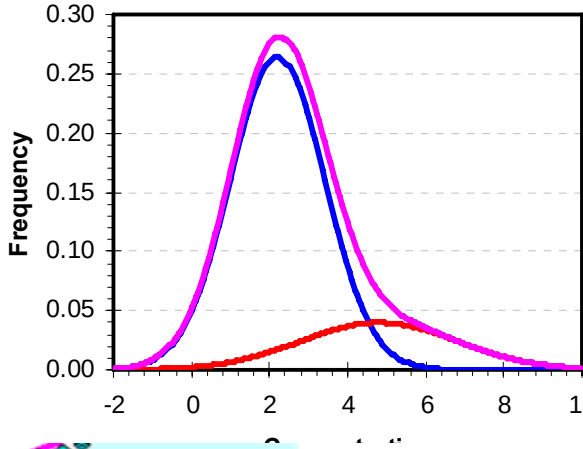
Jensen *et al.* . Clin Chem Lab Med 2006;44:991-8

Composite distributions

TPOAb

Data and Model

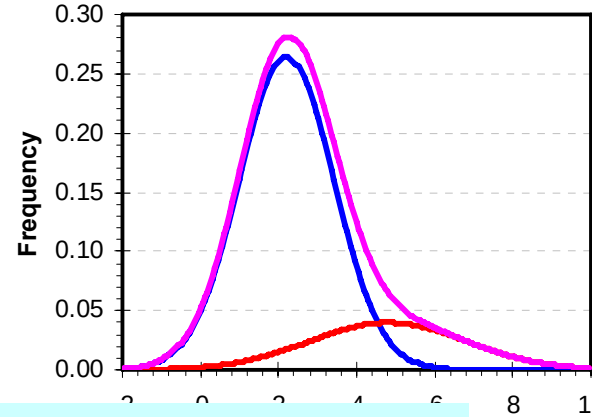


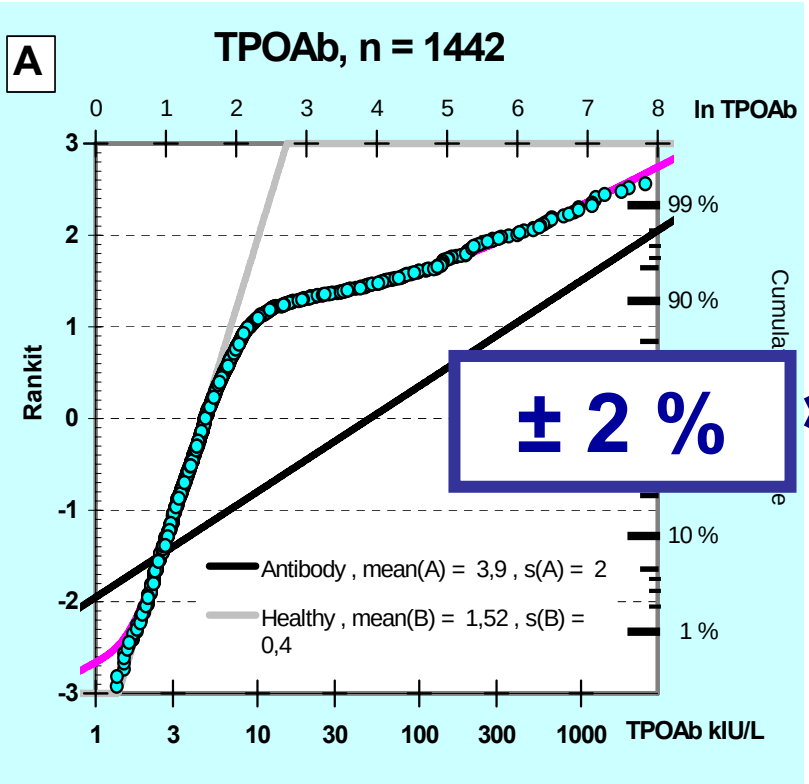


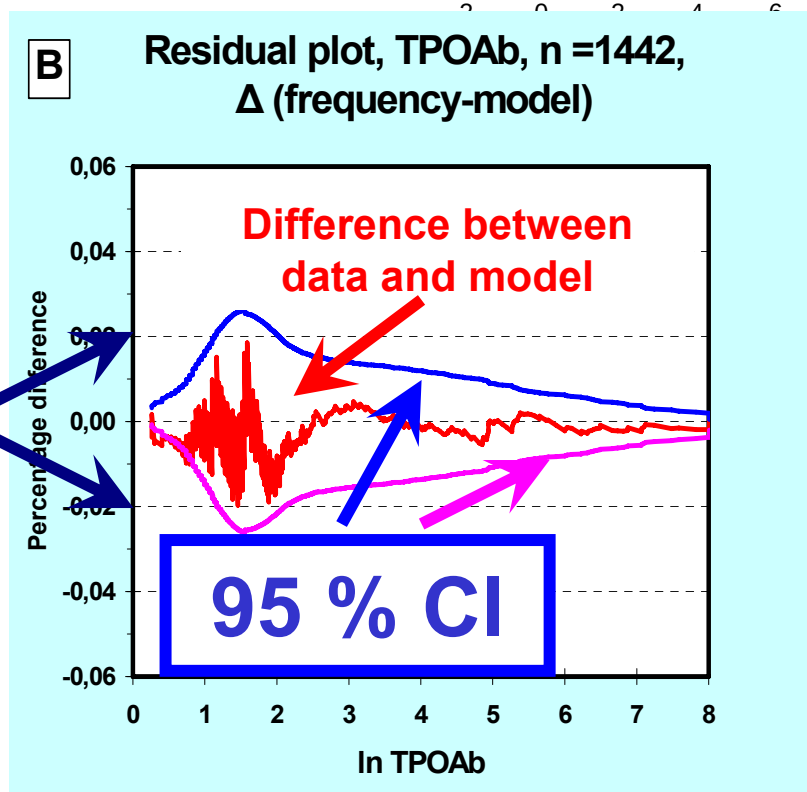
Jensen *et al.* . Clin Chem Lab Med 2006;44:991-8

Composite distributions

Testing of fit for TPOAb







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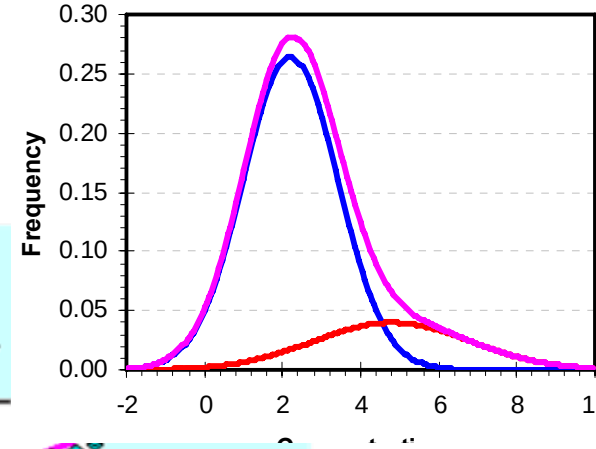
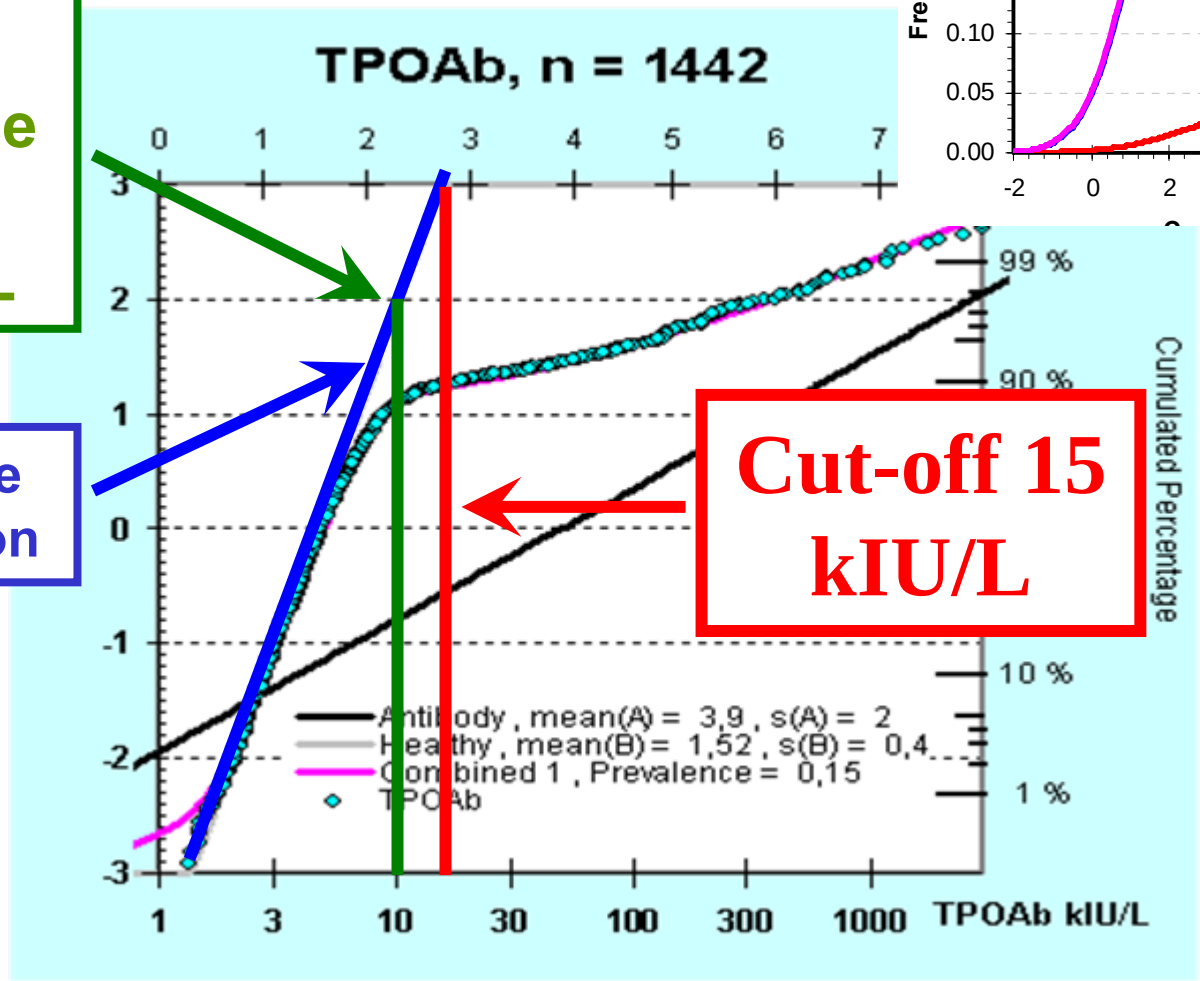
Composite distributions



Upper reference limit = 10 kIU/L

Reference distribution



Cut-off 15 kIU/L

Jensen *et al.* . Clin Chem Lab Med 2006;44:991-8

Composite distributions

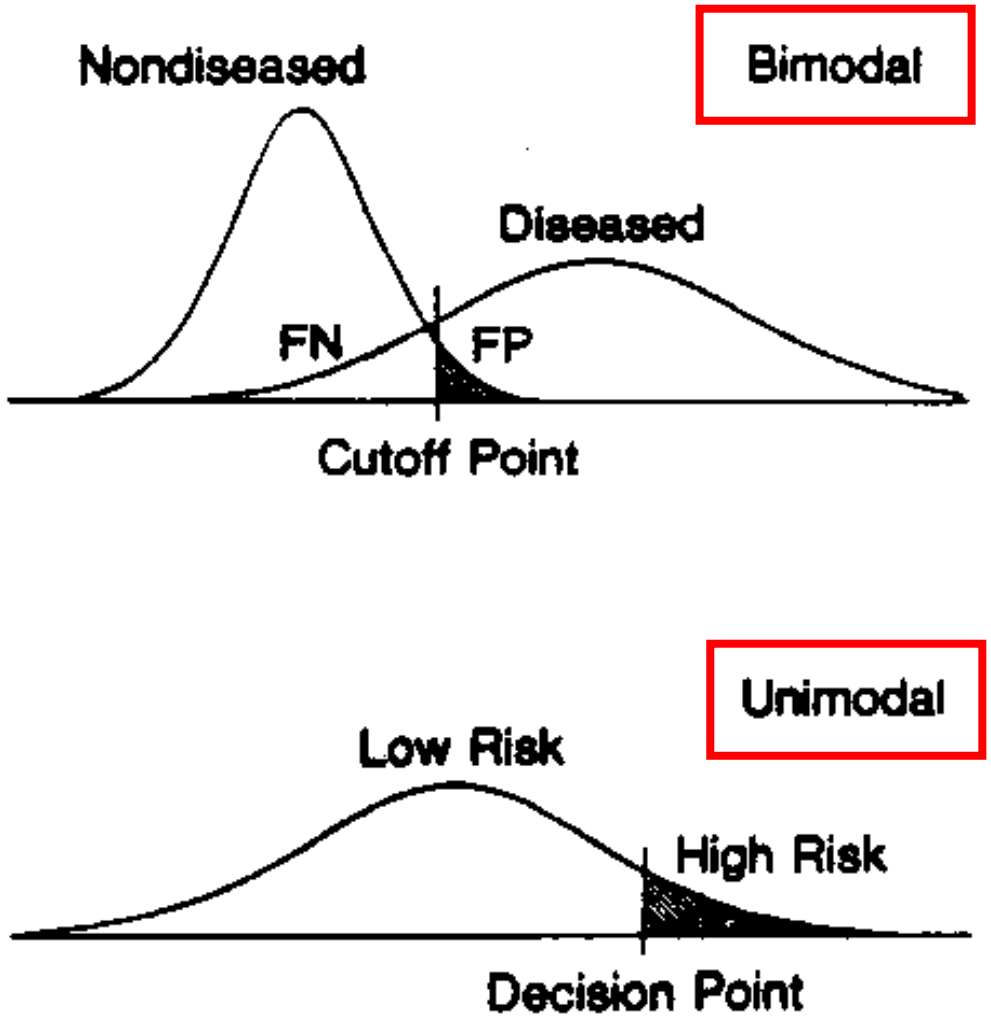
In the TPOAb example

Upper reference limit is part of the description of a low risk population based on mean + 2*s of ln-values (10 kIU/L cutting 2½ %)

Decision limit is chosen as mean + 3*s of ln-values in order to keep false positives below 0.2 % (15 kIU/L)

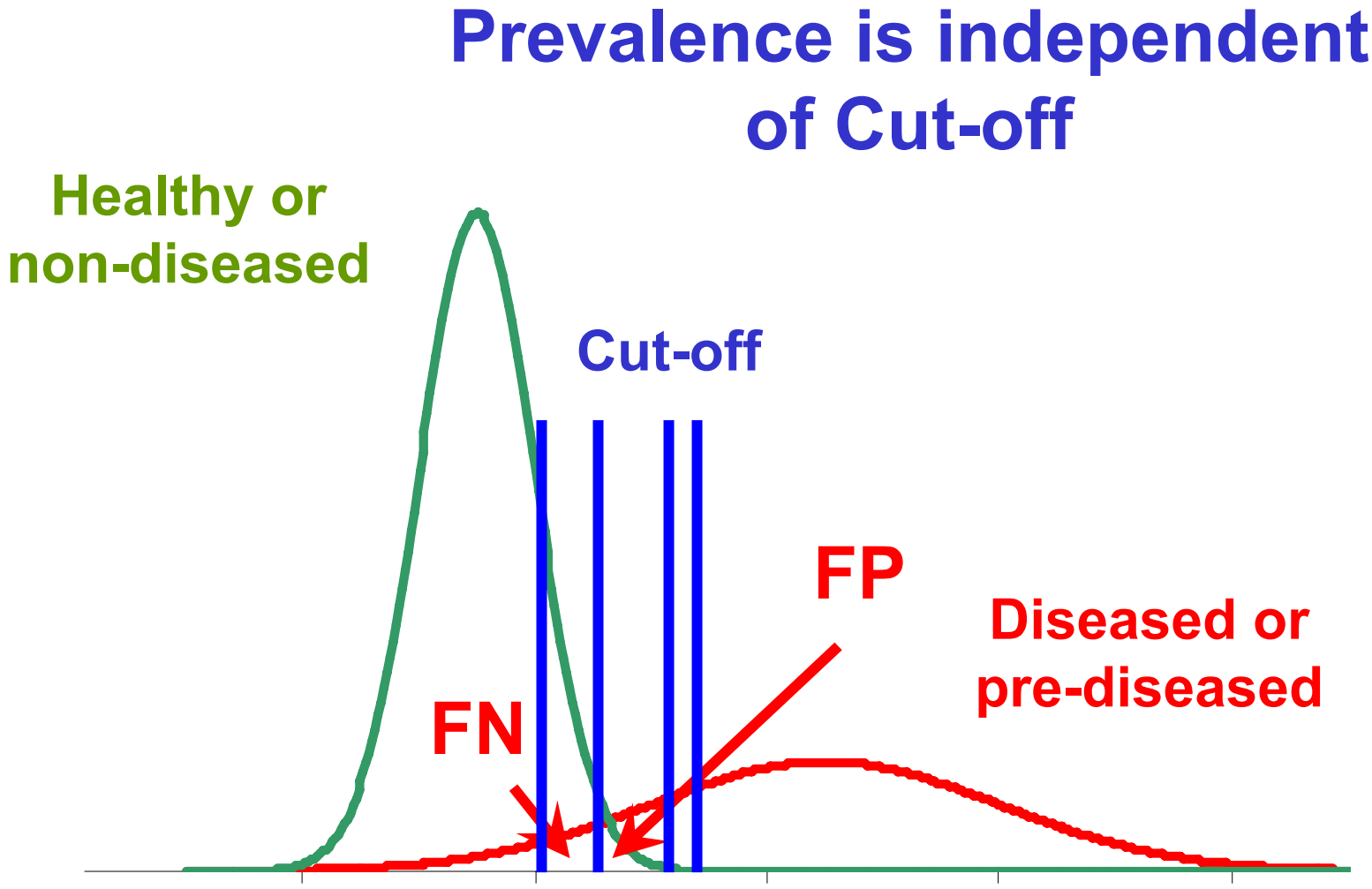
Jensen *et al.* . Clin Chem Lab Med 2006;44:991-8

Bi-modal and uni-modal decision models



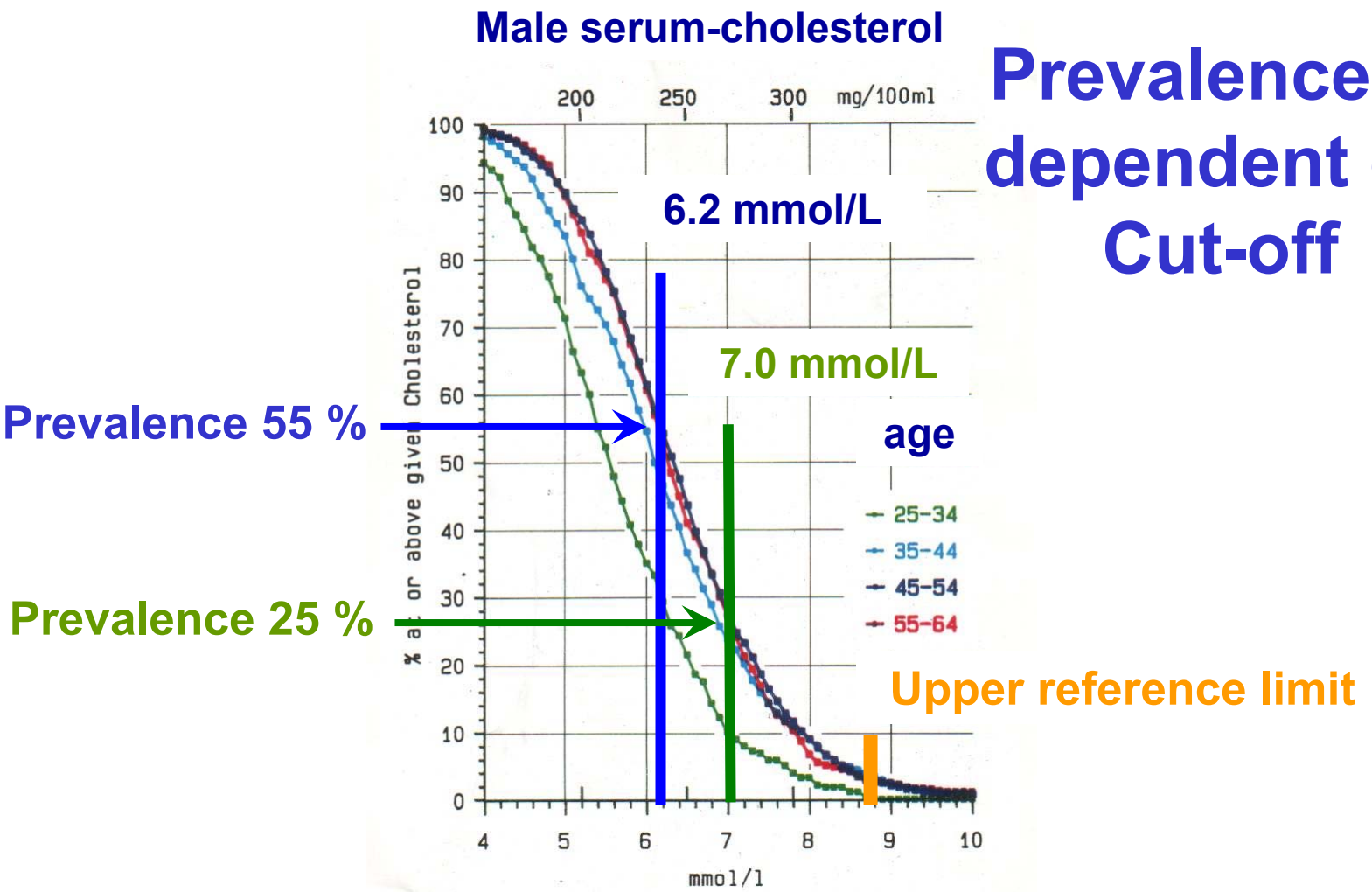
Hyltoft Petersen P, Hørder M. *Arch Pathol Lab Med* 1988; 112: 435-43

Bi-modal model



Galen & Gambino. Beyond Normality. Johv Wiley & Sons. New York 1975

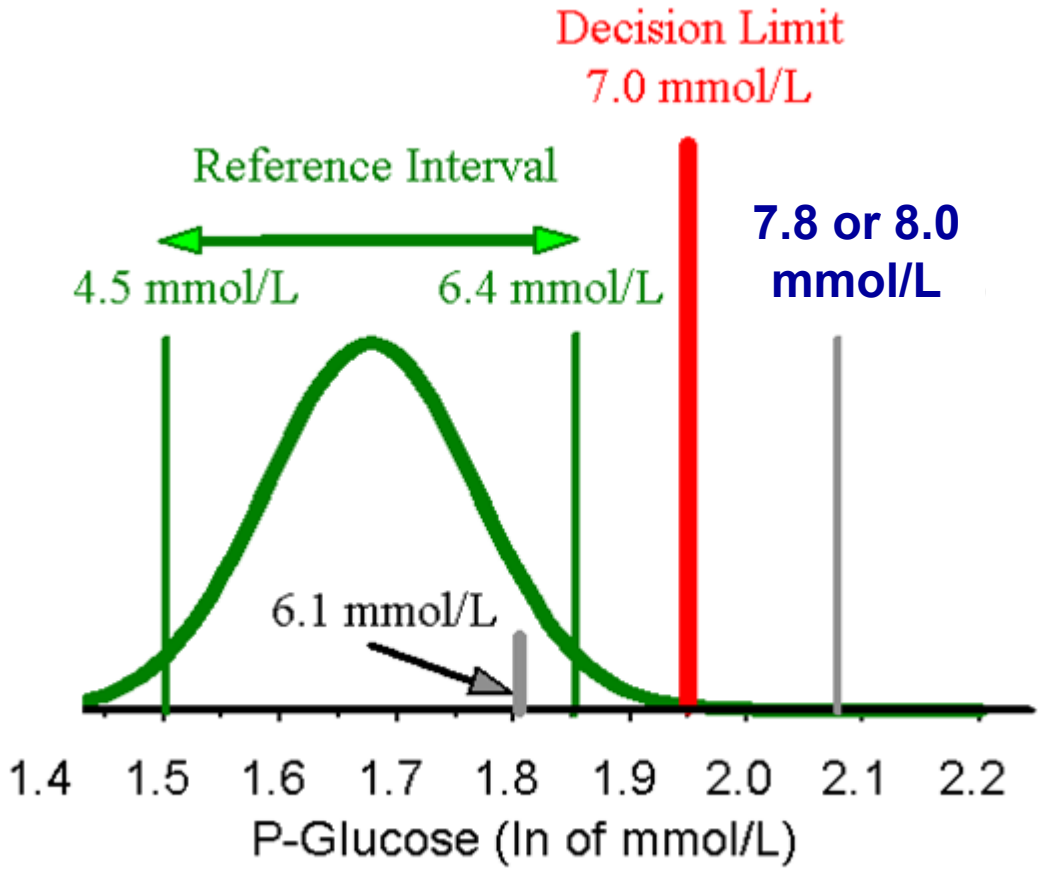
Uni-modal model



Prevalence is dependent on Cut-off

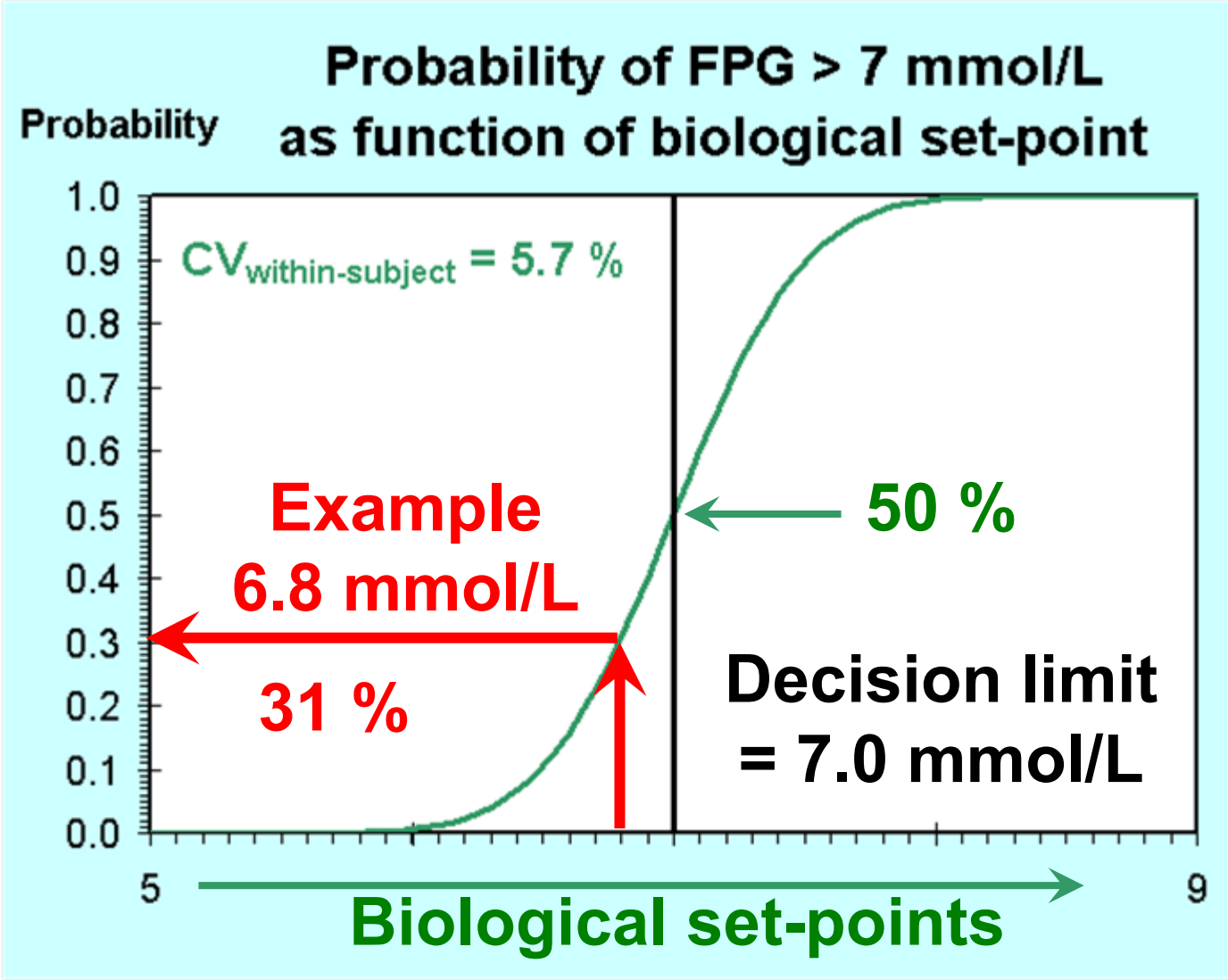
Tunstall-Pedoe *et al. Lancet* 1989; 1:540-2

Uni-modal model
Diagnosis of Diabetes



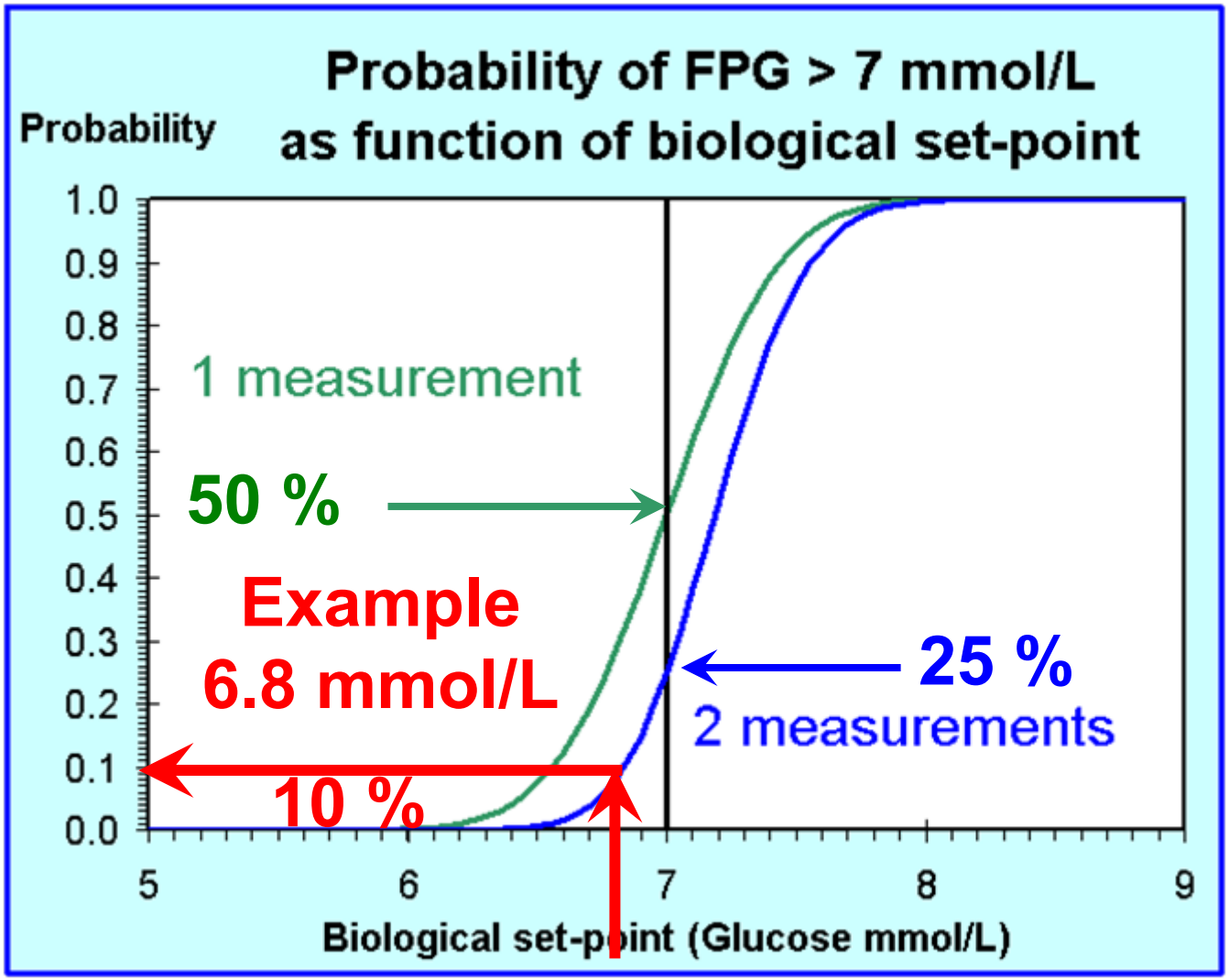
Hyltoft Petersen *et al. Scand J Clin Lab Invest* 2001; 61:191-204

Diagnosis of Diabetes



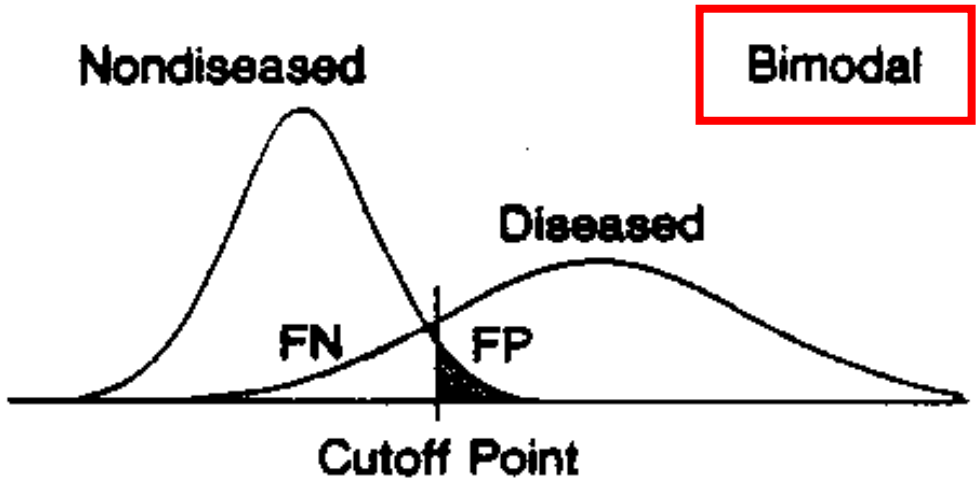
Jørgensen *et al.* *Int J Risk Assessment and Management* 2005; 5:358-73.

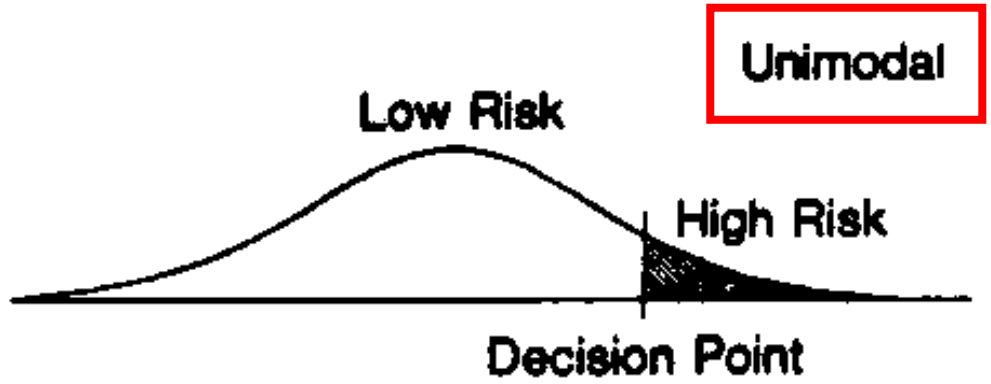
Diagnosis of Diabetes



Jørgensen *et al.* *Int J Risk Assessment and Management* 2005; 5:358-73.

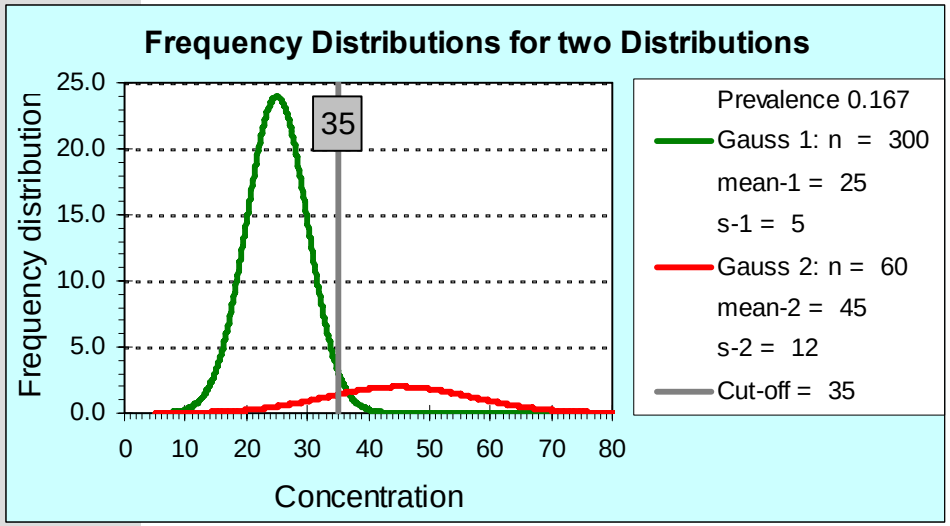
Bi-modal and uni-modal decision models





Hyltoft Petersen P, Hørder M. *Arch Pathol Lab Med* 1988; 112: 435-43

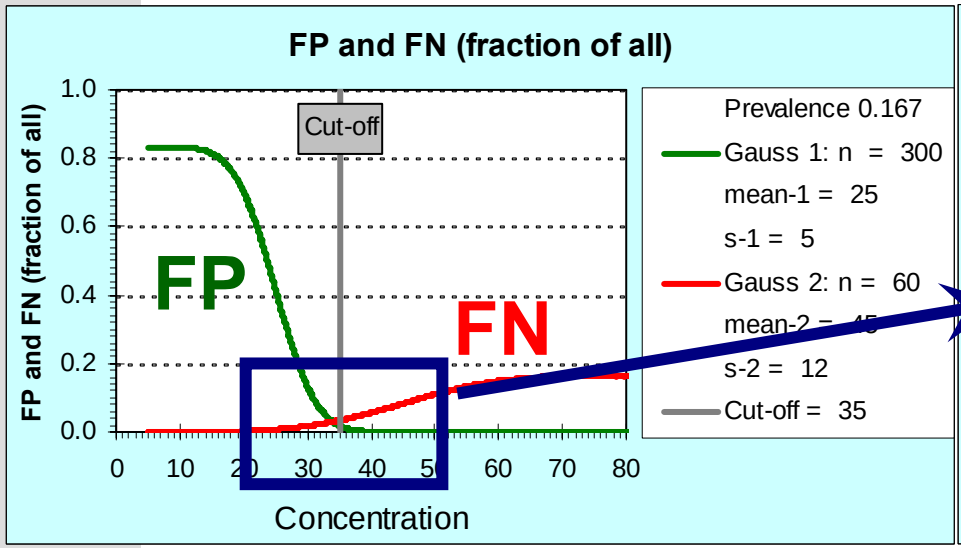
Bi-modal Distributions

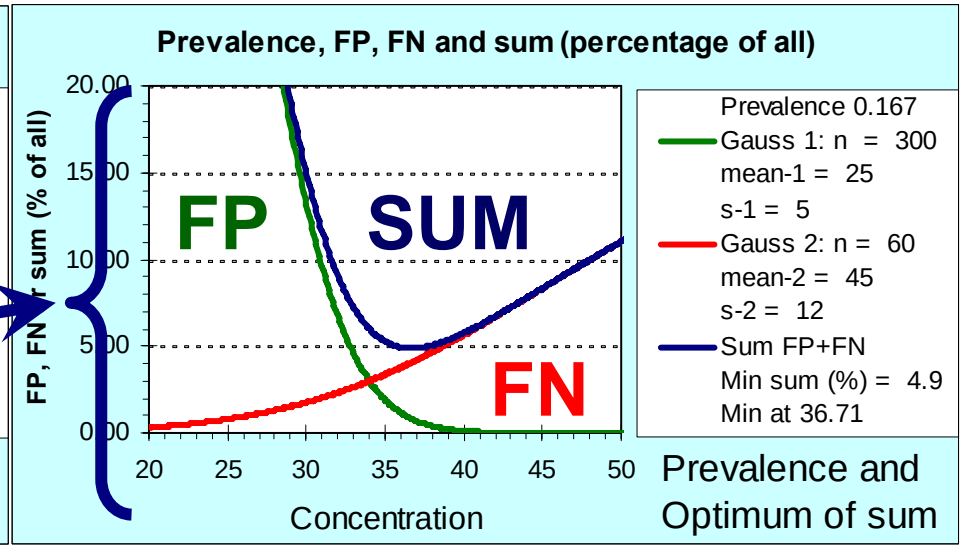


Pre-valence: 0.167

Minimum misclassifications: 4.9 %

Minimum at 36.7 U/L





Prevalence and Optimum of sum

Bi-modal Distributions

Weighting FP and FN

FP: weighting-factor 0.4

FN: weighting-factor 2.0

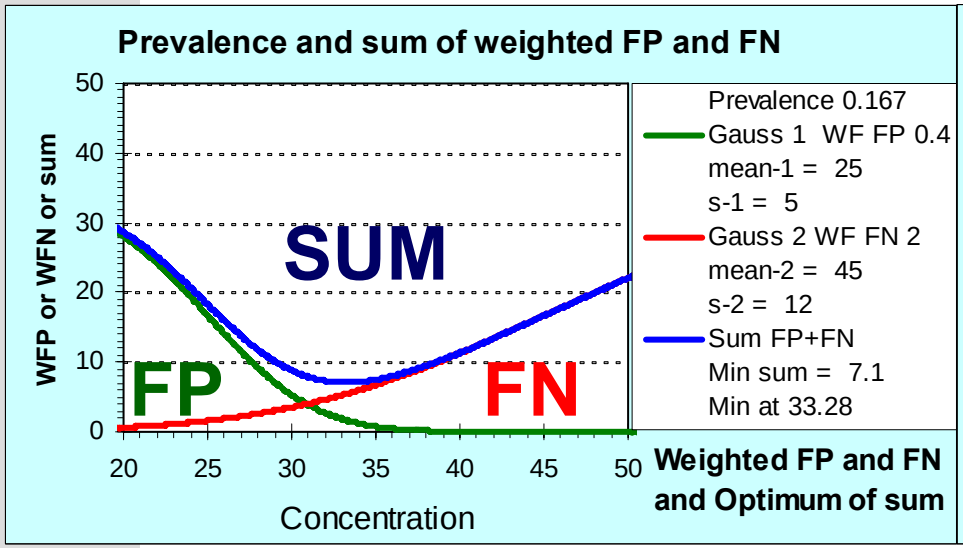
Pre-valence: 0.167

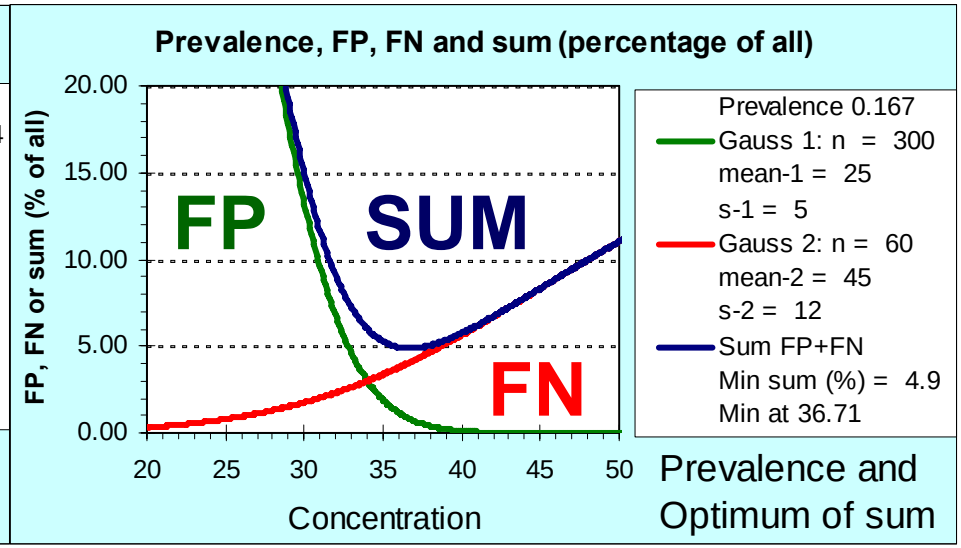
Minimum sum: 7.1 Units

Minimum at 33.3 U/L

Minimum misclassifications: 4.9 %

Minimum at 36.7 U/L





Bi-modal Distributions

Weighting FP and FN

FP: weighting-factor 0.4

FN: weighting-factor 2.0

Pre-valence: 0.167

FP: weighting-factor 0.4

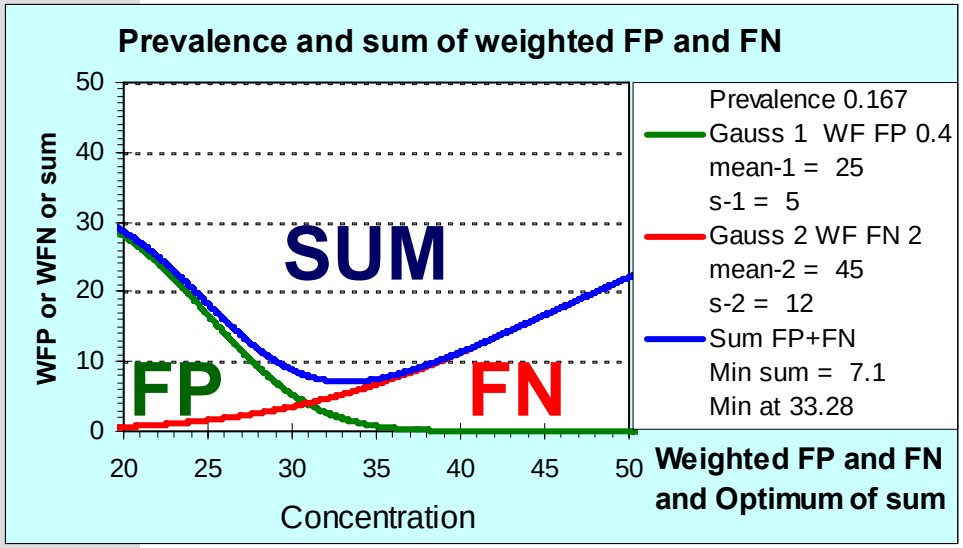
FN: weighting-factor 8.0

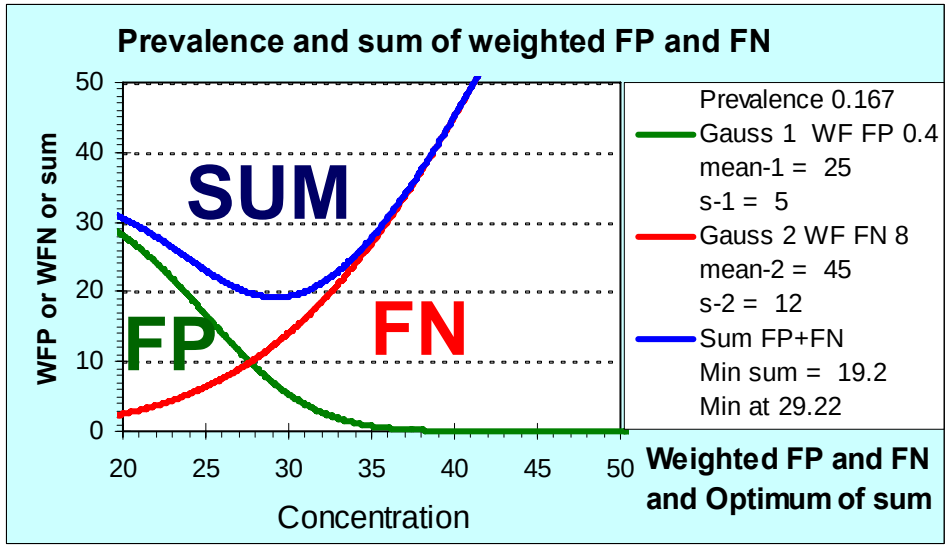
Minimum sum: 7.1 Units

Minimum at 33.3 U/L

Minimum sum: 19.2 Units

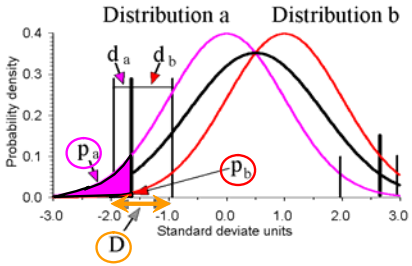
Minimum at 29.2 U/L





Summery

Criteria for Partitioning



Lahti *et al.* Clin Chem 2002;48:338-52

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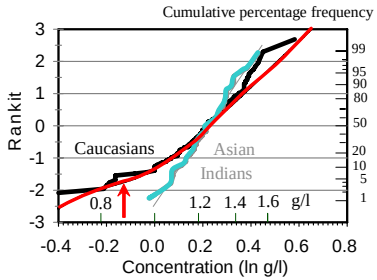
Comparison of Proteins

Comparison of Antitrypsin Values
Caucasians and Asian Indians in Leeds



Johnson *et al.* CCLM 2004;42:792-9

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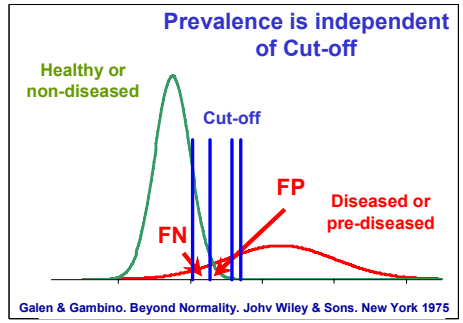
Bi-modal model



Galen & Gambino. Beyond Normality. Johv Wiley & Sons. New York 1975

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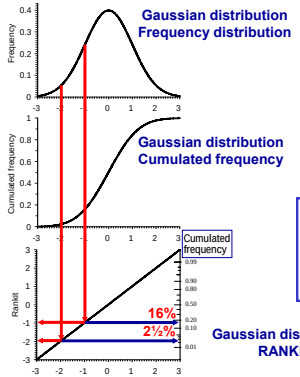
Rankit-transformation 3



Transformation of the ordinate so that the S-shaped curve becomes a straight line

When the cumulated frequency shows up as a straight line, the distribution can be considered Gaussian

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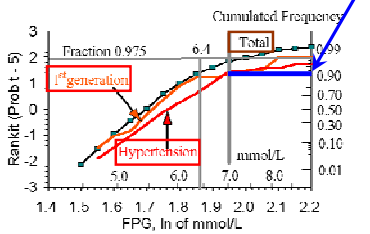
Plasma-Glucose

Low risk population

P-Glucose
Distribution(s) of Reference Values



Jørgensen *et al.Scand J Clin Lab Invest* 2001; 61:181-190.

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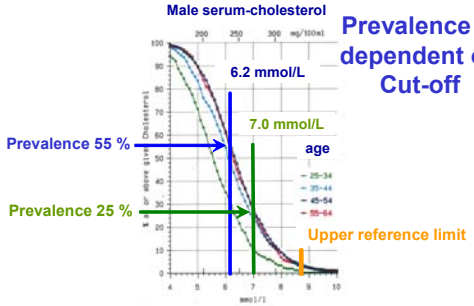
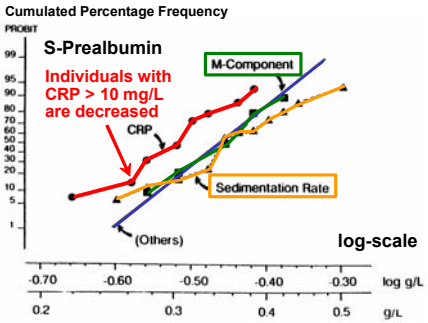
Uni-modal model



Tunstall-Pedoe *et al. Lancet* 1989; 1:540-2

Serum-Prealbumin



Blaabjerg *et al.* Upsala J Med Sci 1994;99:315-38

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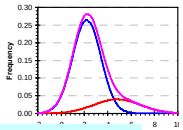
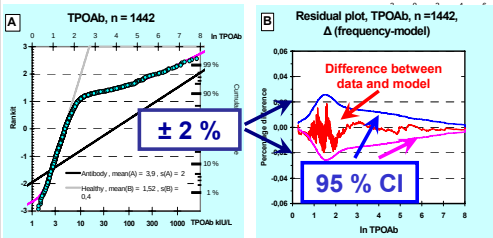
Composite distributions

Testing of fit for TPOAb





Jensen *et al.* . Clin Chem Lab Med 2006;44:991-8

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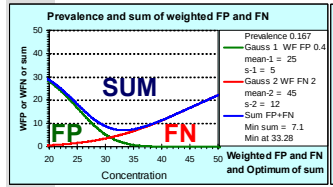
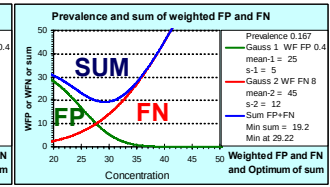
Bi-modal Distributions

Weighting FP and FN
FP: weighting-factor 0.4
FN: weighting-factor 2.0

Pre-valence: 0.167
FP: weighting-factor 0.4
FN: weighting-factor 8.0

Minimum sum: 7.1 Units
Minimum at 33.3 U/L

Minimum sum: 19.2 Units
Minimum at 29.2 U/L