EQALM symposium, Barcelona October 2016

Adam Uldall Lecture

Elements of analytical quality – a historical review

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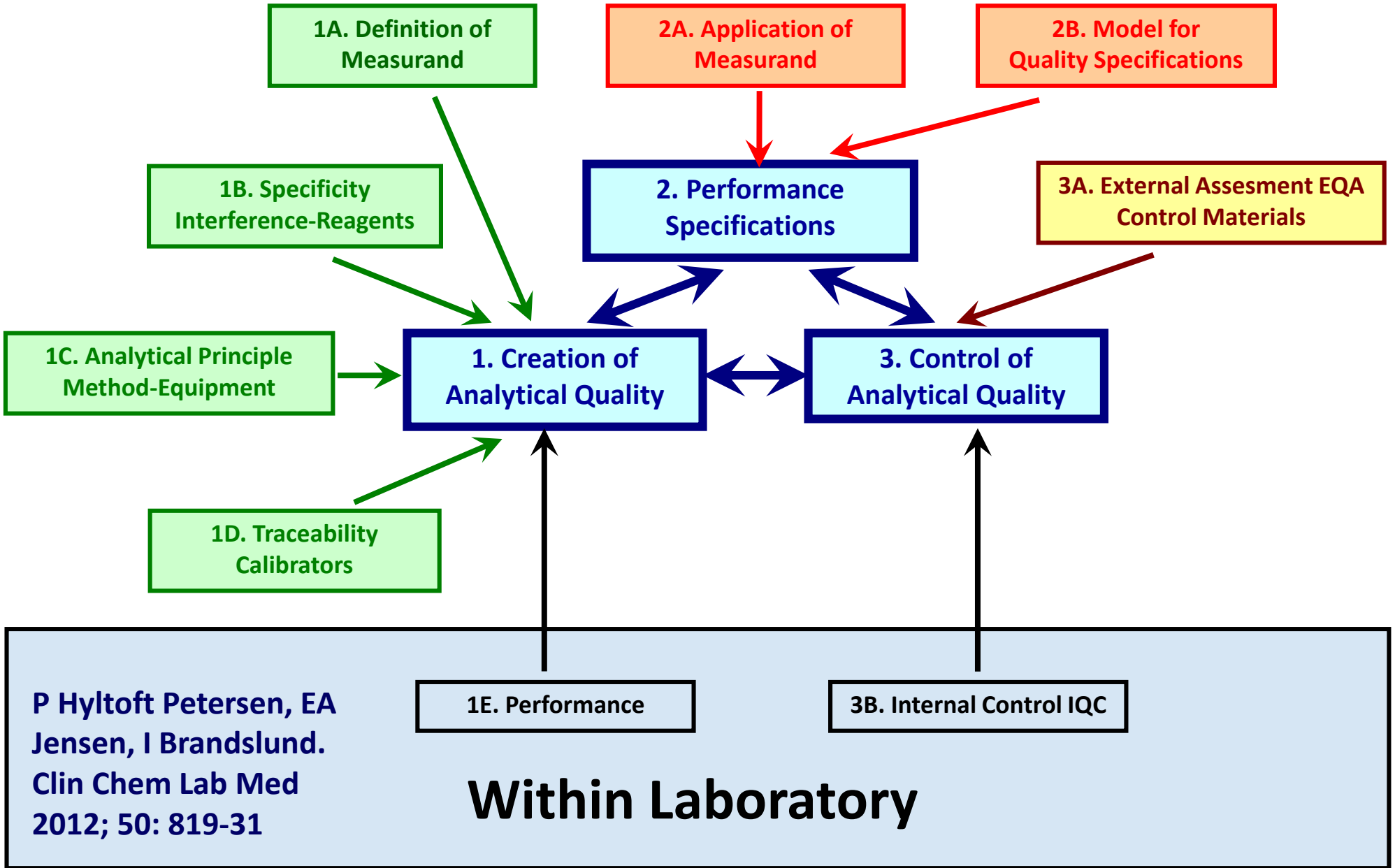
Outside Laboratory



Elements of analytical quality

The Nordic Protein Project 1986 – 94

Assessing Quality in Measurements of Plasma Proteins
Edited by Per Hyltoft Petersen, Ole Blaabjerg, Kerttu Irjala
Upsala Journal of Medical Sciences 1994;99-3:195-389

Denmark
Finland
Norway
Iceland
Sweden

A NORDKEM project
Organized in cooperation
with Labquality, Finland

Elements of analytical quality

The Nordic Protein Project

Background

Anarchy in measurements of plasma proteins in 1986

No reliable standardisation

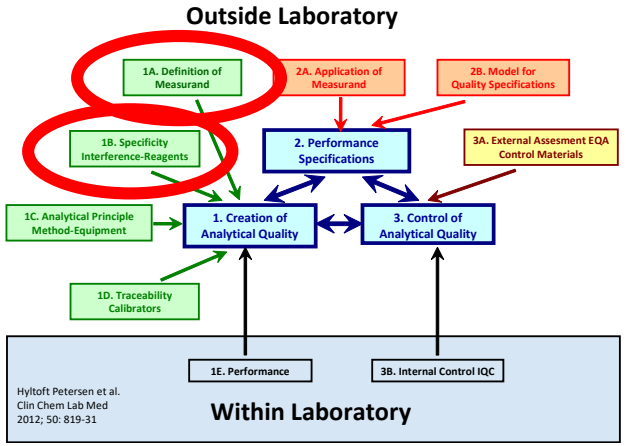
No reliable reference intervals

No reliable control

But excellent analytical specificity

Elements of analytical quality

The Nordic Protein Project



1A. Definition of the Measurand

S-Prealbumin
S-Albumin
S-Orosomucoid
S-α_1-Antitrypsin
S-Haptoglobin
S-Transferrin
S-IgA
S-IgG
S-IgM

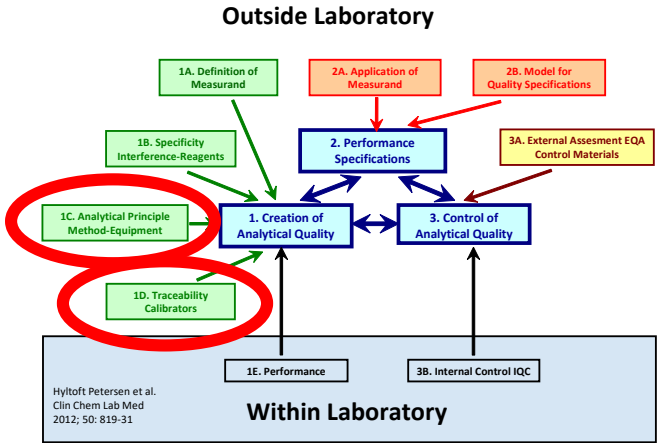
1B. Specificity – Interference - Reagents

Excellent specific antibodies from

DAKO, Denmark (Niels Harboe)
and
Behringwerke, Germany (S. Baudner)

Blaabjerg O et al. Upsala J Med SCI 1994;99:315-38

Elements of analytical quality



The Nordic Protein Project

1C. Analytical Principle – Method - Equipment

Gel methods:

Electro Immuno Assay
(Rocket electrophoresis)
Radio Immuno Assay
(RIA)

Turbidimetric methods

Turbidimetry
Nepelometry

1D. Traceability - Calibrators

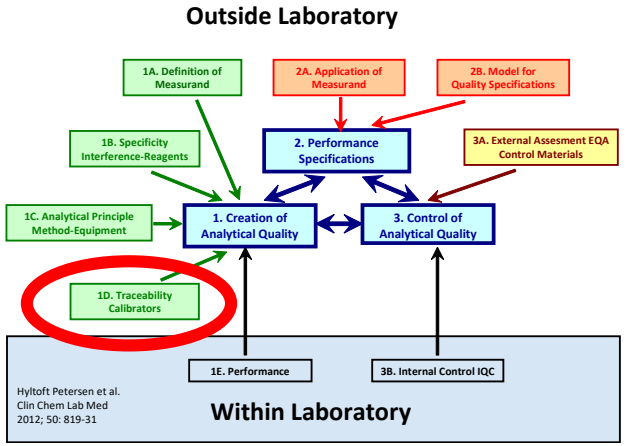
Nordic Plasma Protein Calibrator:

Serum pool from
1000 male blood donors

Reproducibility and stability
from three new samplings
Better than ± 1 %
(three pools over 3 years at –80 ^{o}C)

Hyltoft Petersen et al . Upsala J Med SCI 1994;99:277-306

Elements of analytical quality



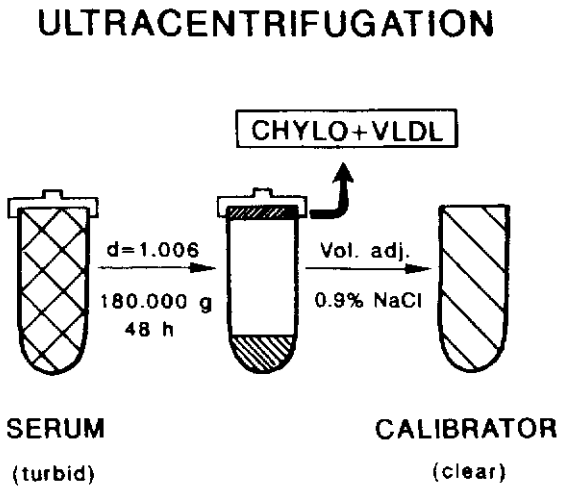
The Nordic Protein Project

1D. Traceability - Calibrators

Nordic Plasma Protein Calibrator:

The pool was ultra centrifuged
in order to clarify the serum

and to make it useful for
the four different analytical methods



The chylomicrons and very low density lipids were discharged
And the volume reconstituted with 0.9 % NaCl

Blaabjerg O et al. Upsala J Med SCI 1994;99:259-66

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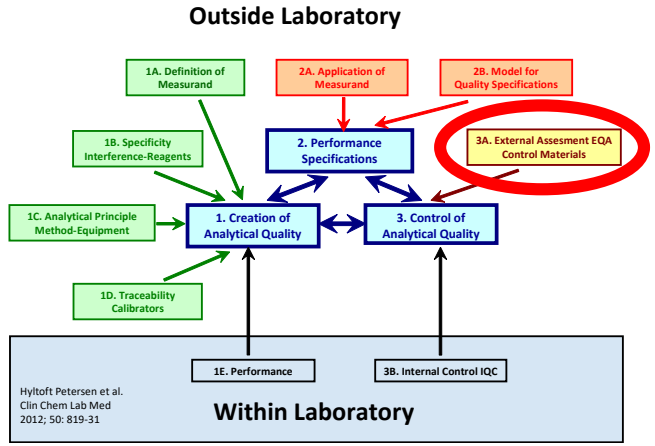
The Nordic Protein Project



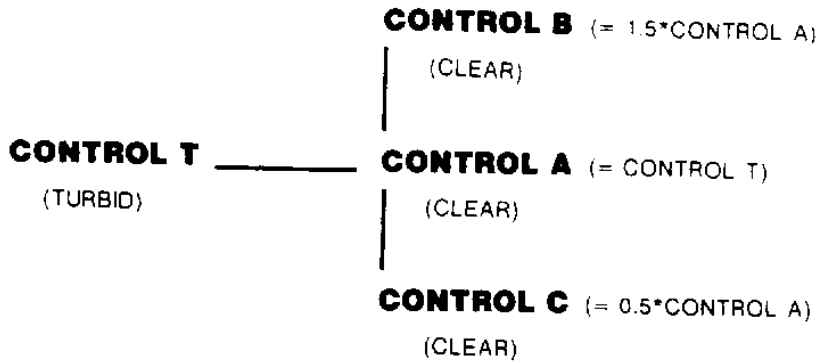
3A. External Assessment EQA Control Materials

Control Materials:

The controls were also human serum pools based on other serum pools but treated differently

Control T: The turbid control was not centrifuged
Control A: Same procedure as the Calibrator
Control B: 1½ times Control A
Control C: ½ times Control A

Later the Calibrator and Controls were Traced to the Certified Reference Material CRM 470



Concentrations of
Control T and Control A are the same, so
Control T – Control A = Lack of specificity

Controls A, B and C controls the Linearity of calibration curve.

Hyltoft Petersen P et al. Upsale J Med SCI 1994;99:277-306

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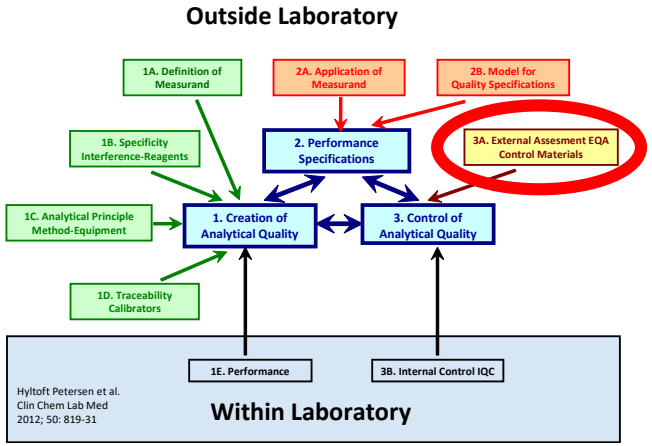
The Nordic Protein Project



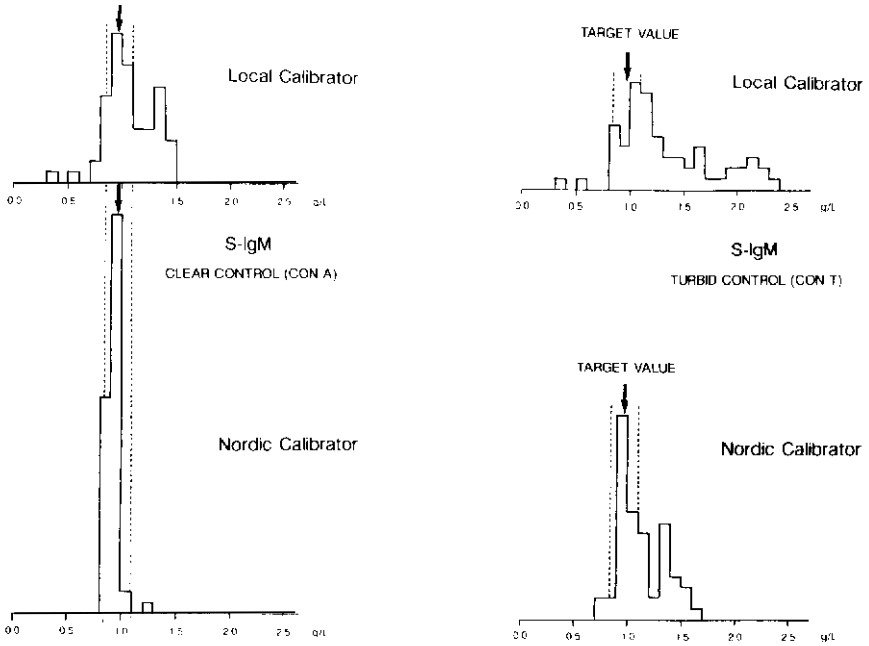
3A. External Assessment EQA Control Materials

S-IgM:

Control A (clear) **Control T (turbid)** **Control T (turbid) with Nordic Calibrator**

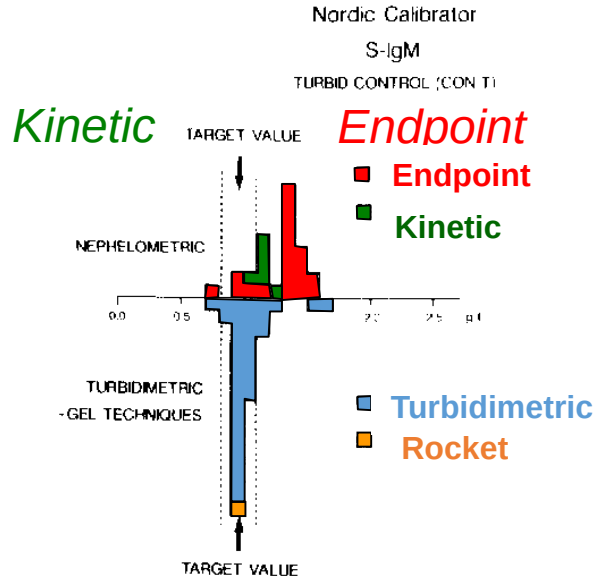
Local laboratory calibrator

Nordic calibrator



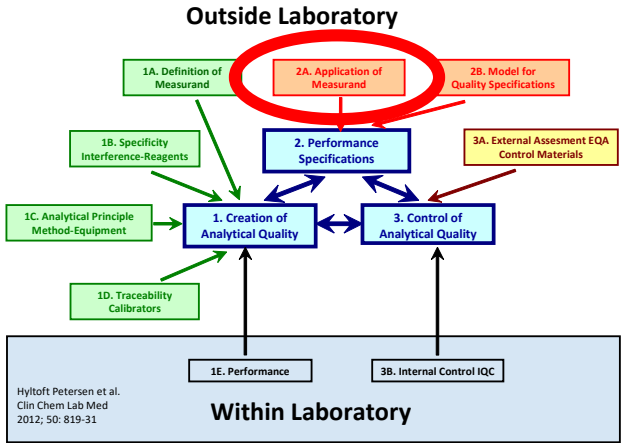
Nephelometric methods

Turbidimetric methods and Rocket



Hyltoft Petersen P et al. Upsala J Med SCI 1994;99:277-306

Elements of analytical quality



The Nordic Protein Project

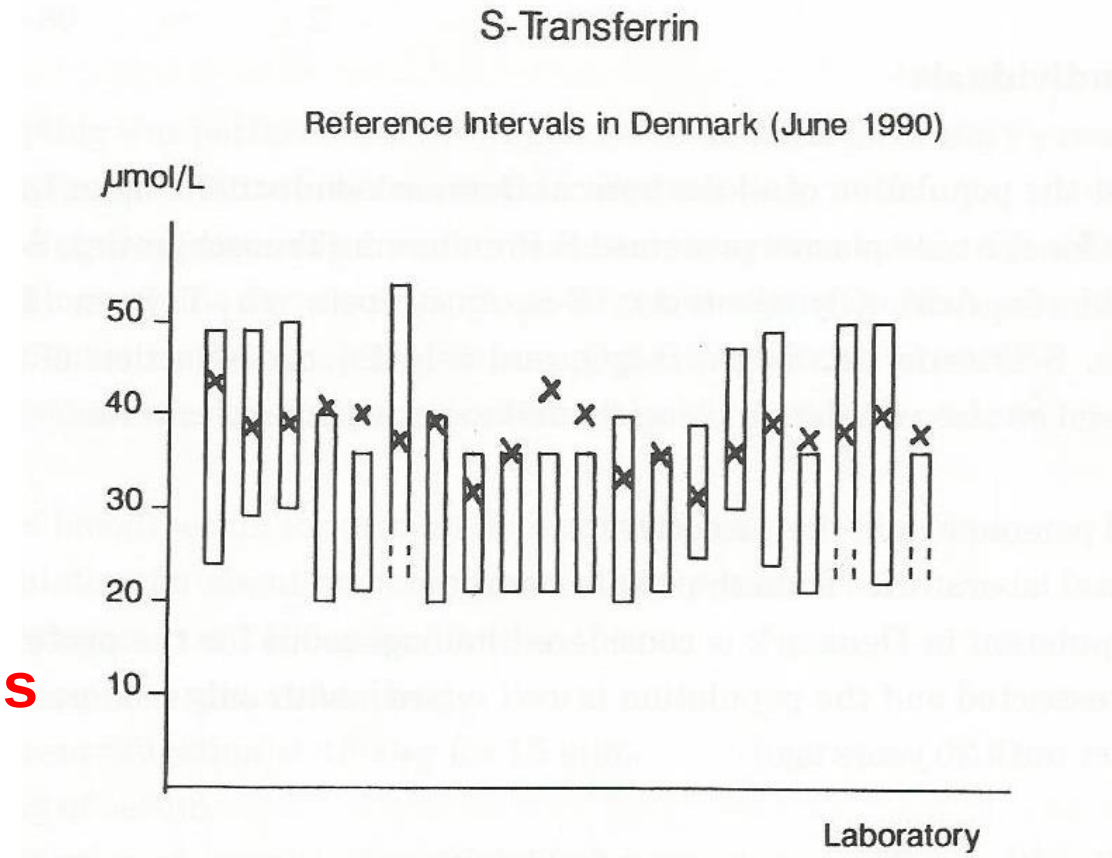
2A. Application of the Measurand

Poor Reference Intervals

S-Transferrin:

Original Local
Reference Intervals and
Control A (clear)

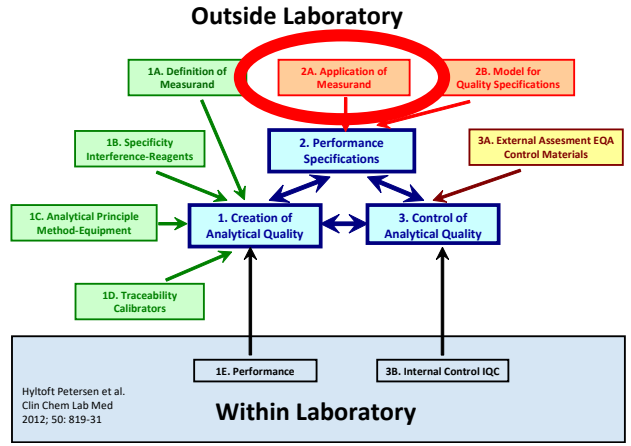
Therefore:
New Common reference intervals

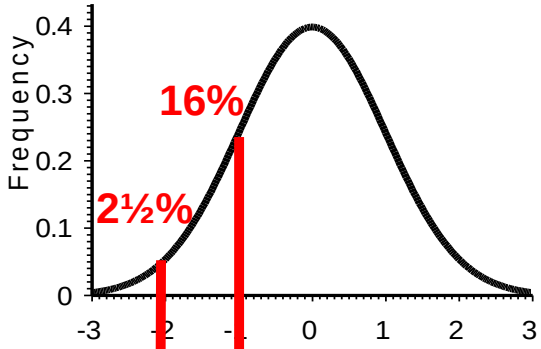


Blaabjerg O et al . Upsale J Med SCI 1994;99:315-38

Elements of analytical quality

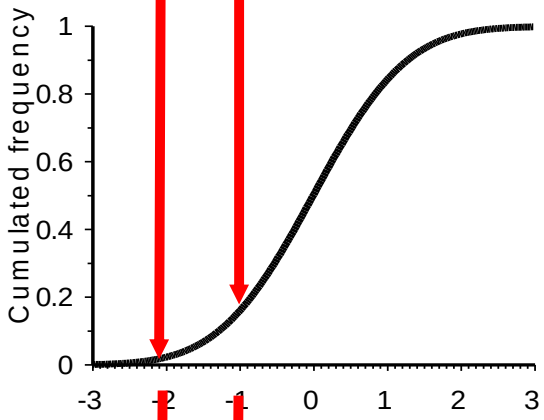
Rankit-transformation





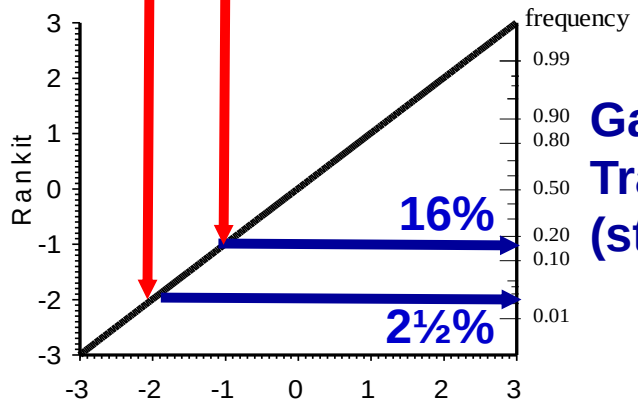
Gaussian distribution
Frequency distribution
(Bellshaped)

Cumulated frequency



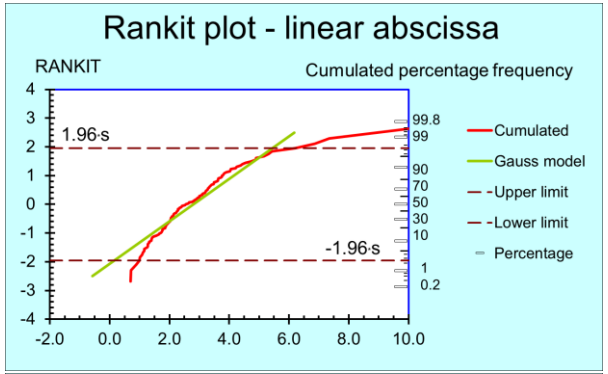
Gaussian distribution
Cumulated frequency
(S-shaped)

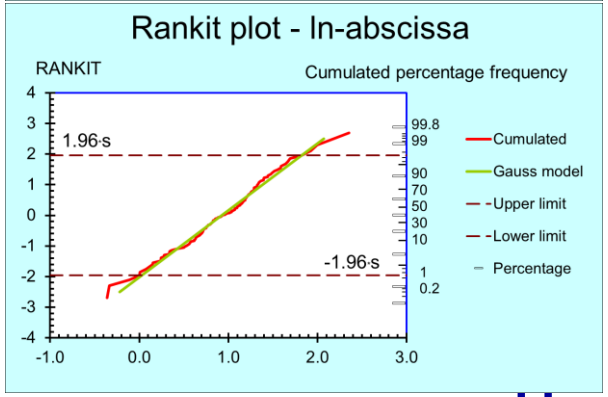
RANKIT transformation



Gaussian distribution
Transformed to RANKIT
(straight line)

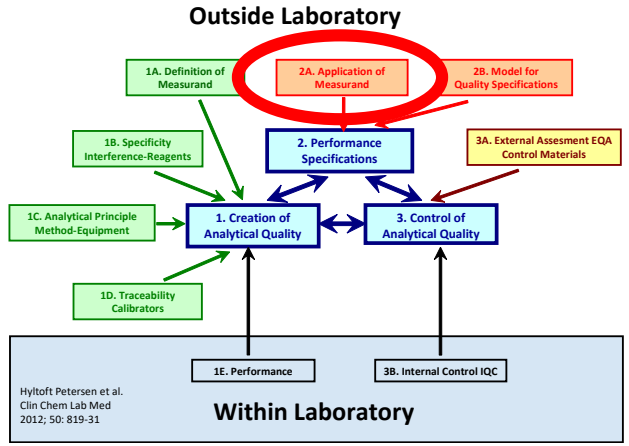
RANKIT
from Gauss to lnGauss





Hyltoft Petersen et al.
Clin Chem Lab Med
2004;42:715-24

Elements of analytical quality

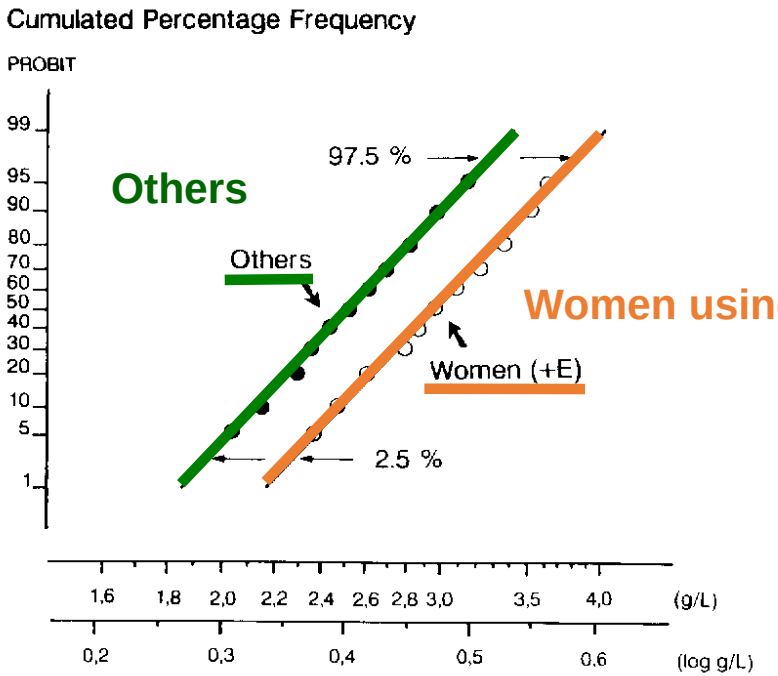


The Nordic Protein Project

2A. Application of the Measurand

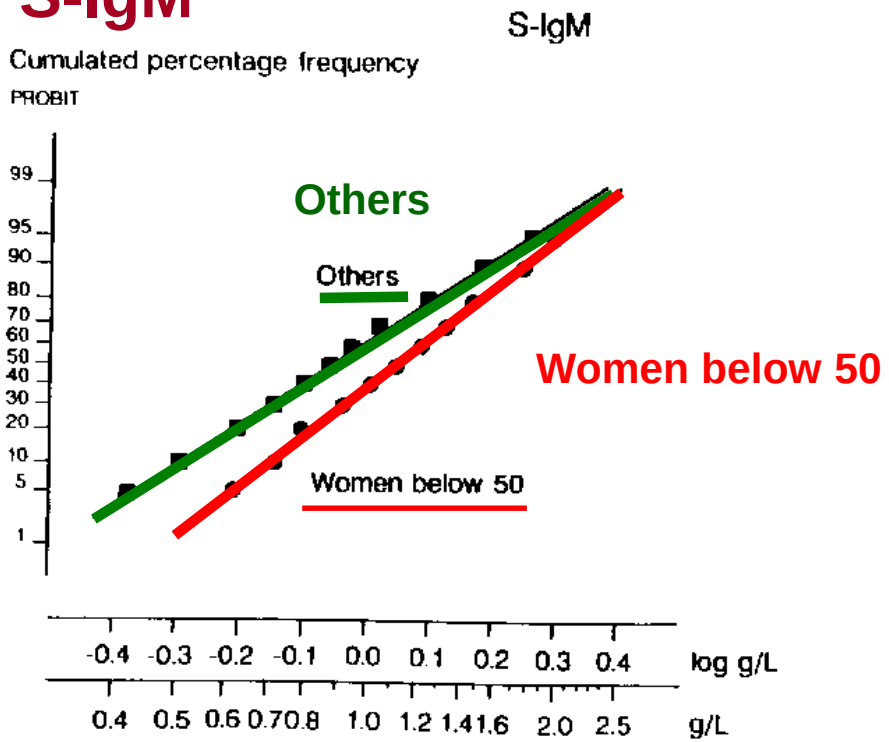
Estimation of Common Reference Intervals with Partitioning (N = 720)

S-Transferrin



Blaabjerg O. et al. Upsale J Med SCI 1994;99:315-38

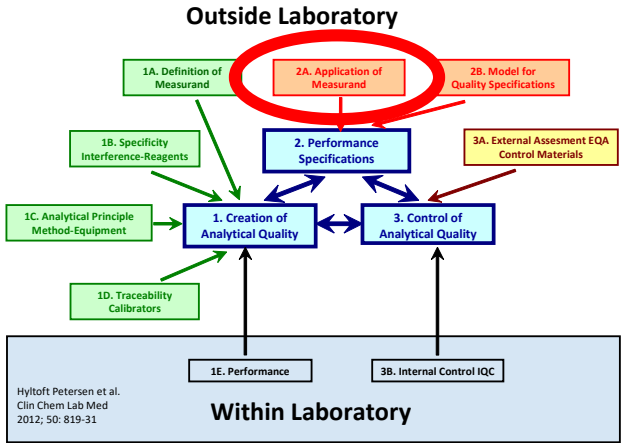
S-IgM



Elements of analytical quality

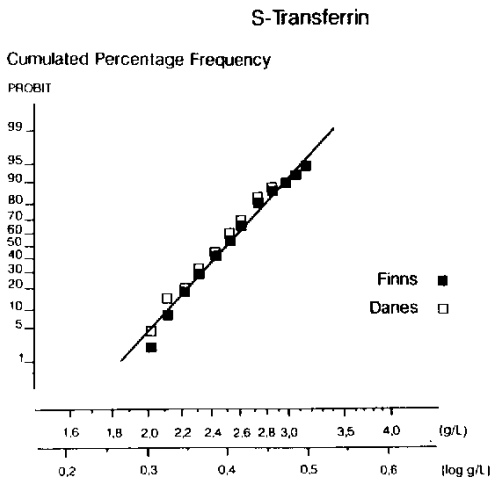
The Nordic Protein Project

2A. Application of the Measurand



Reference Intervals from other Races

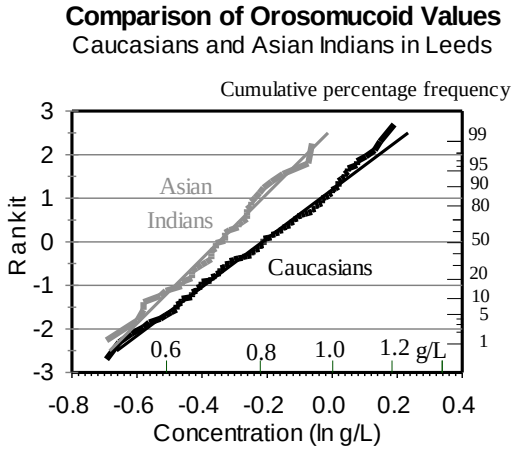
S-Transferrin



Finns and Danes

Irjala K et al. Upsale J Med SCI 1994;99:347-56

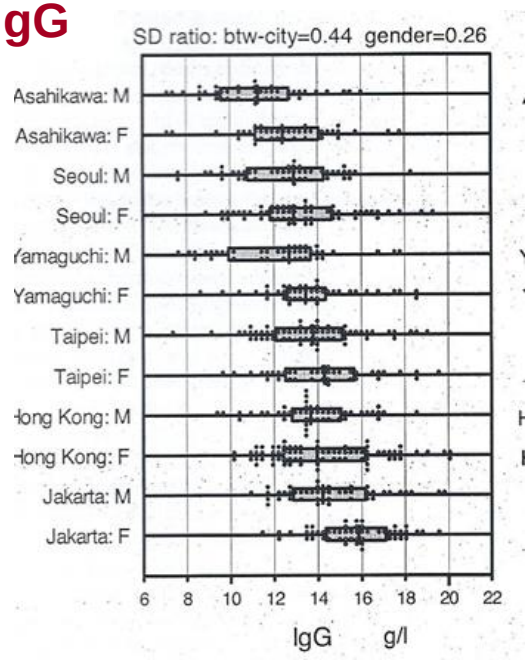
S-Orosomucoid



Asian Indians and Caucasians in Leeds, England

Johnson AM, Whicher JT et al.
Clin Chem Lab Med 2004;42:792-9

S-IgG



Individuals in Different Towns in East Asia

Kiyoshi Ichihara et al.
Clin Chem 2008;54:356-65

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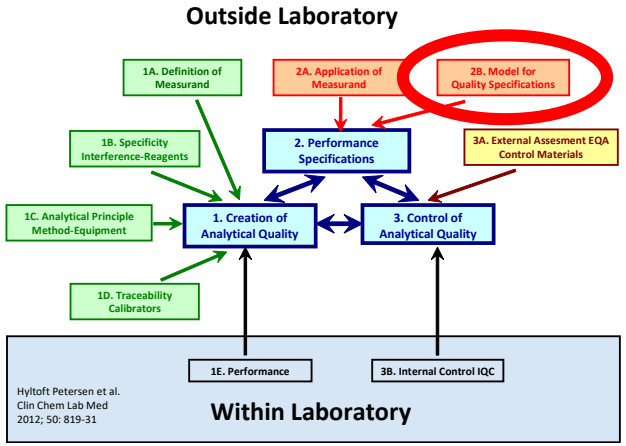
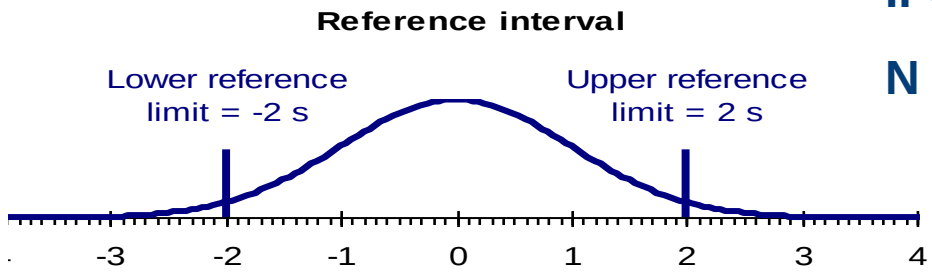
2B. Model for Quality Specificatios

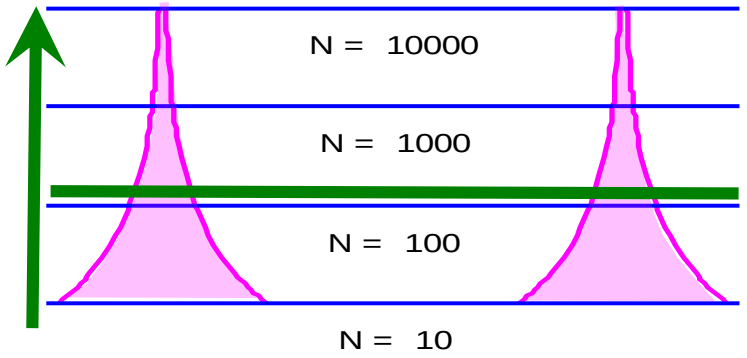


Creation of Quality Specifications for Reference Intervals

Confidence Intervals for Reference Interval Limits

Solberg HE. Approved Recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J Clin Chem Clin Biochem 1987;25:645-56





Decreasing CI for increasing N

IFCC Recommendations

N ≥ 120 Individuals

N = 120

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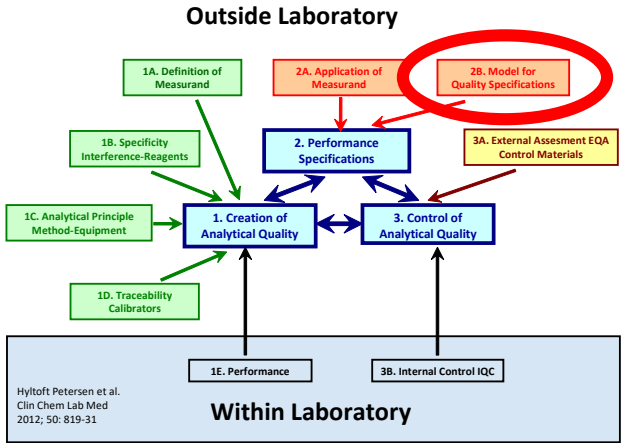
The Nordic Protein Project

2B. Model for Quality Specificatios



The Gowans Quality Specifications Bias in Common Reference Intervals

The goal is to establish a Common Reference Interval based on a sample size of e.g. N > 800

If each laboratory has a bias below the CI according to the IFCC criteria when using the Common Reference Interval

Then all laboratories perform as good as if they had used the IFCC criteria

And the benefit is that all has the same reference interval

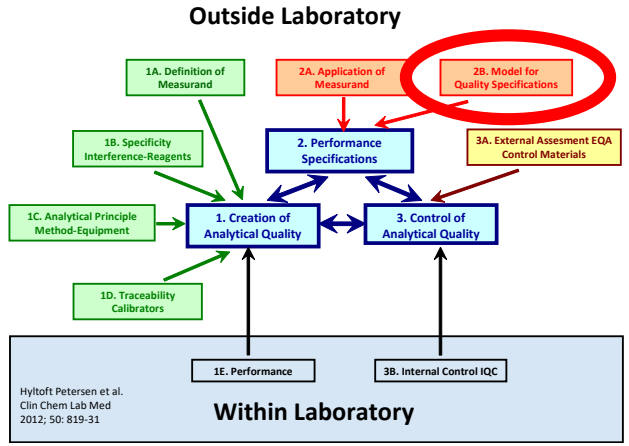
This defines the maximum allowable bias to

$|Bias| < 0.25 * s_{Population}$ (Standard Deviation)!

Gowans E *et al. Scand J Clin Lab Invest* 1988;48:757-64

Elements of analytical quality

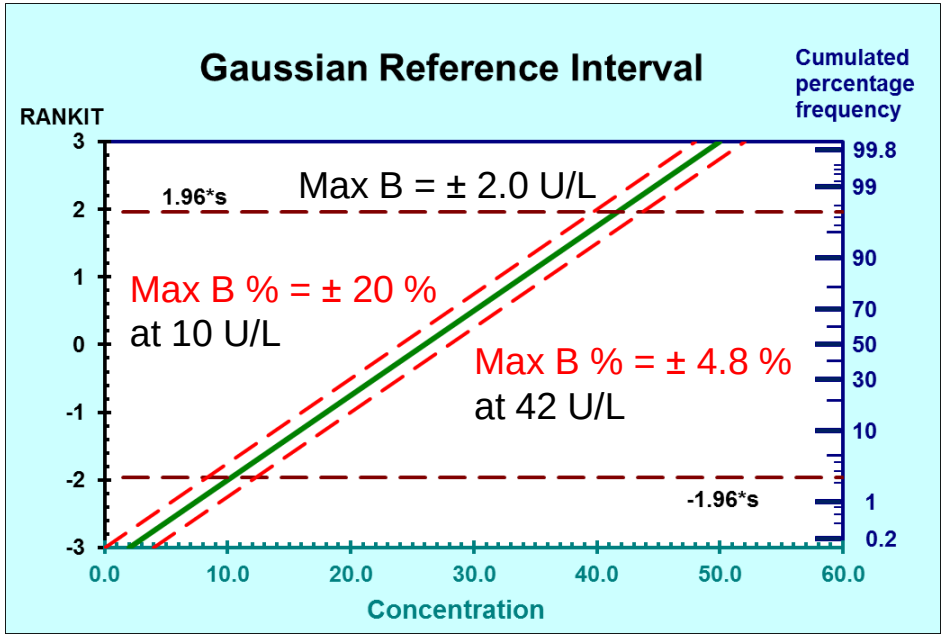
The Nordic Protein Project



2B. Model for Quality Specificatios

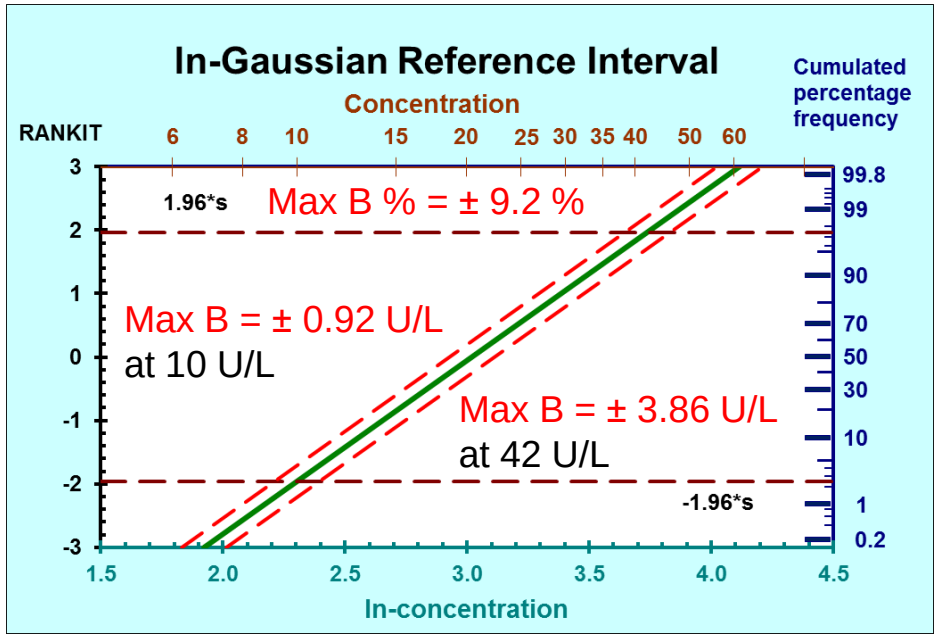
Maximum Specifications for Gaussian and In-Gaussian Distributions for Reference Interval from 10 to 42 U/L

Gaussian reference interval: 10 – 42 U/L



Gowans EMS *et al. Scand J Clin Lab Invest* 1988;48:757-64

In-Gaussian reference interval: 10 – 42 U/L



Hyltoft Petersen et al. *Scand J Clin Lab Invest* 1989;49:727- 37.

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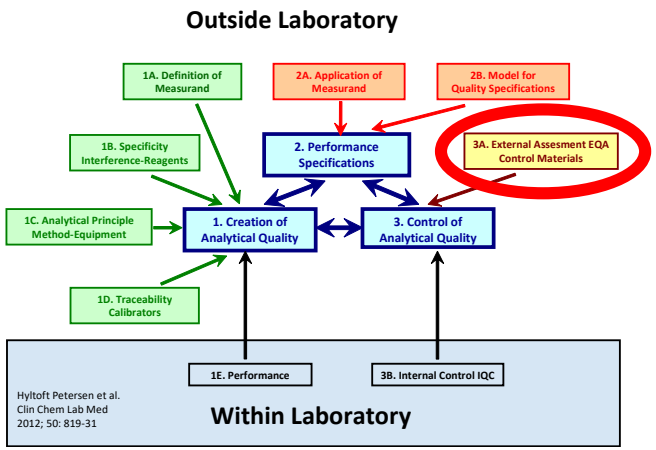


3A. External Assessment EQA Control Material

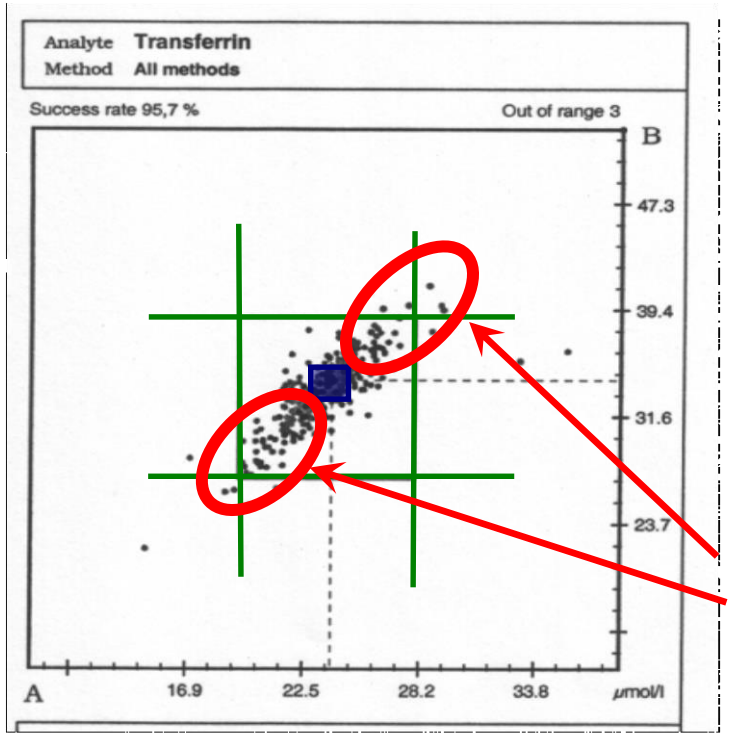
EQA Results for S-Transferrin

After CRM 470

Target ± 17 %

Gowans specifications

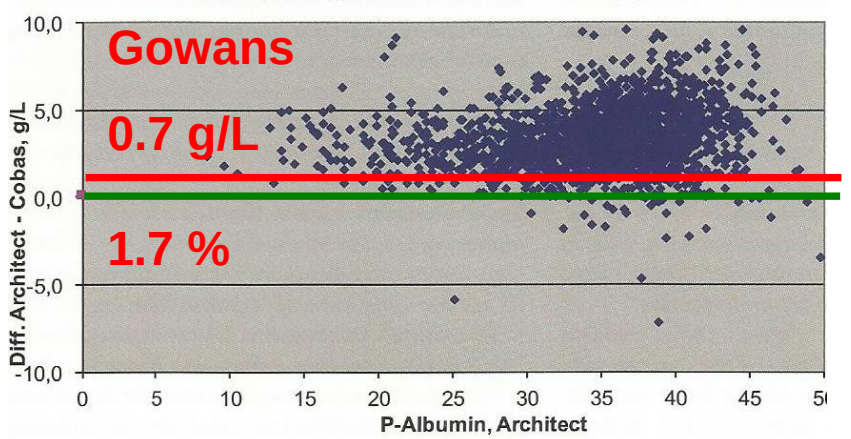
Target ± 3.2 %



Poor traceability

0.0 g/L

Comparison of Albumin from Cobas and Architect



Helmersson-Karlquist et al.
Klinisk Biokemi i Norden 2016;3:38-5

Deutche Gesellschaft für Klinische Chemie

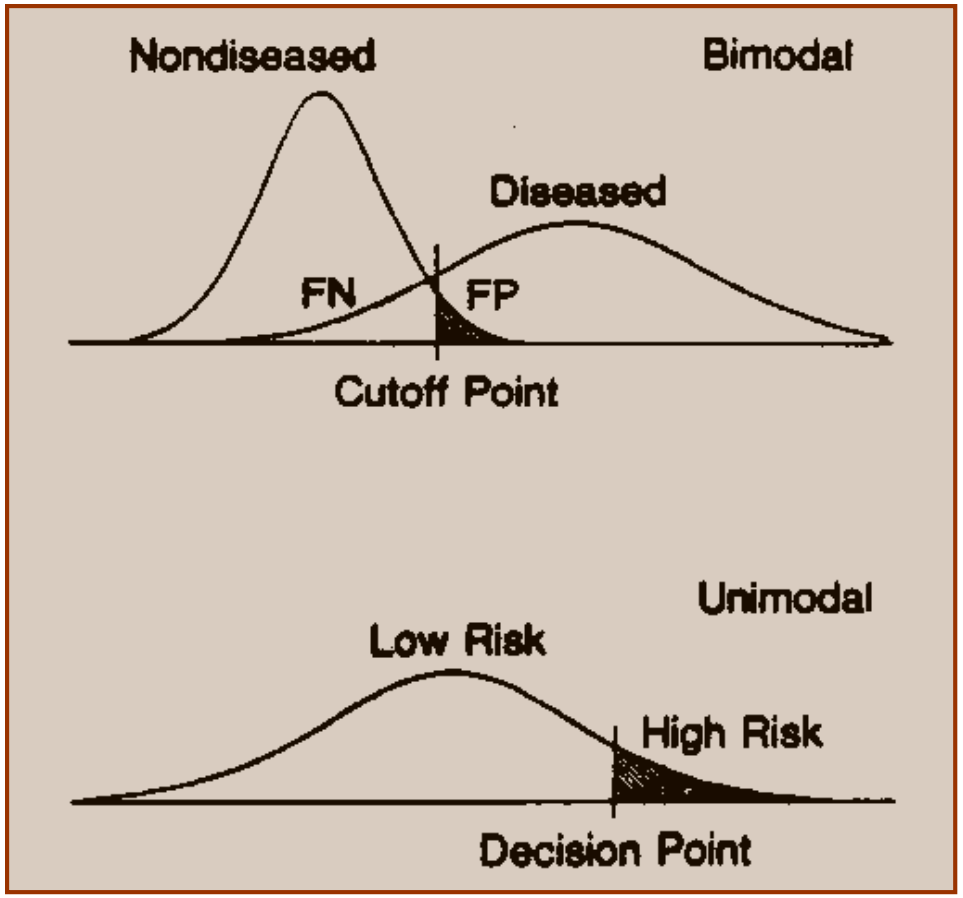
Elements of analytical quality

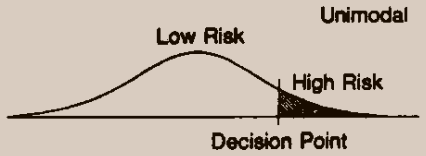
Today reference intervals are often outdated and substituted by decision limits

Diagnostic test:

Bimodal accepts FP and FN
(Galen & Gambino)

Unimodal has a sharp point
(Decision Limit)





Uni-modal decision model

Influence of Analytical Bias and Imprecision on Guideline-Driven Medical Decision Limits

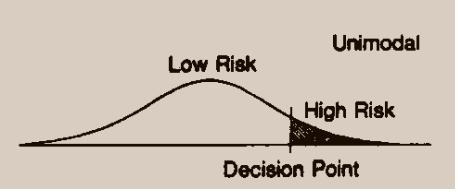
Example:

HbA1c in diagnosis of diabetes mellitus

Decision: HbA1c above or below 48 mmol/mol (6.5 % HbA1c)

Sacks et al. *Diabetes Care* 2011;34:c61-c99

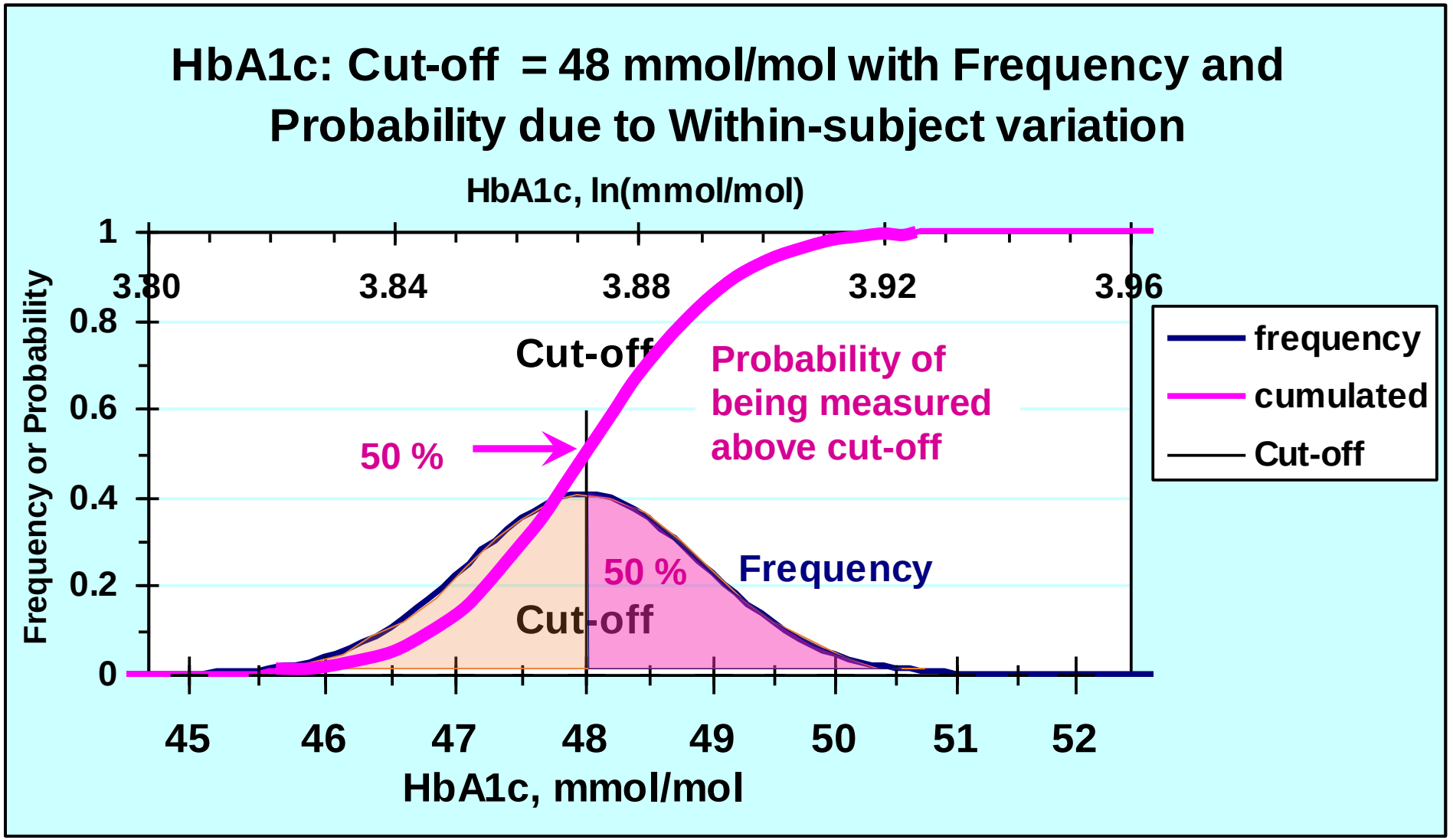
Hyltoft Petersen P, Klee GG.
Influence of Analytical Bias and Imprecision on the Number of False Positive Results Using Guideline-Driven Medical Decision Limits.
Clin Chem Acta 2014;430:1-8



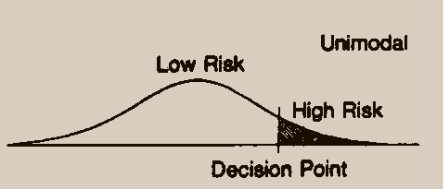
"Guideline-Driven Medical Decision Limits".

Distribution for a person with set-point = cut-off

HbA1c: Frequency Cumulated frequency (probability)

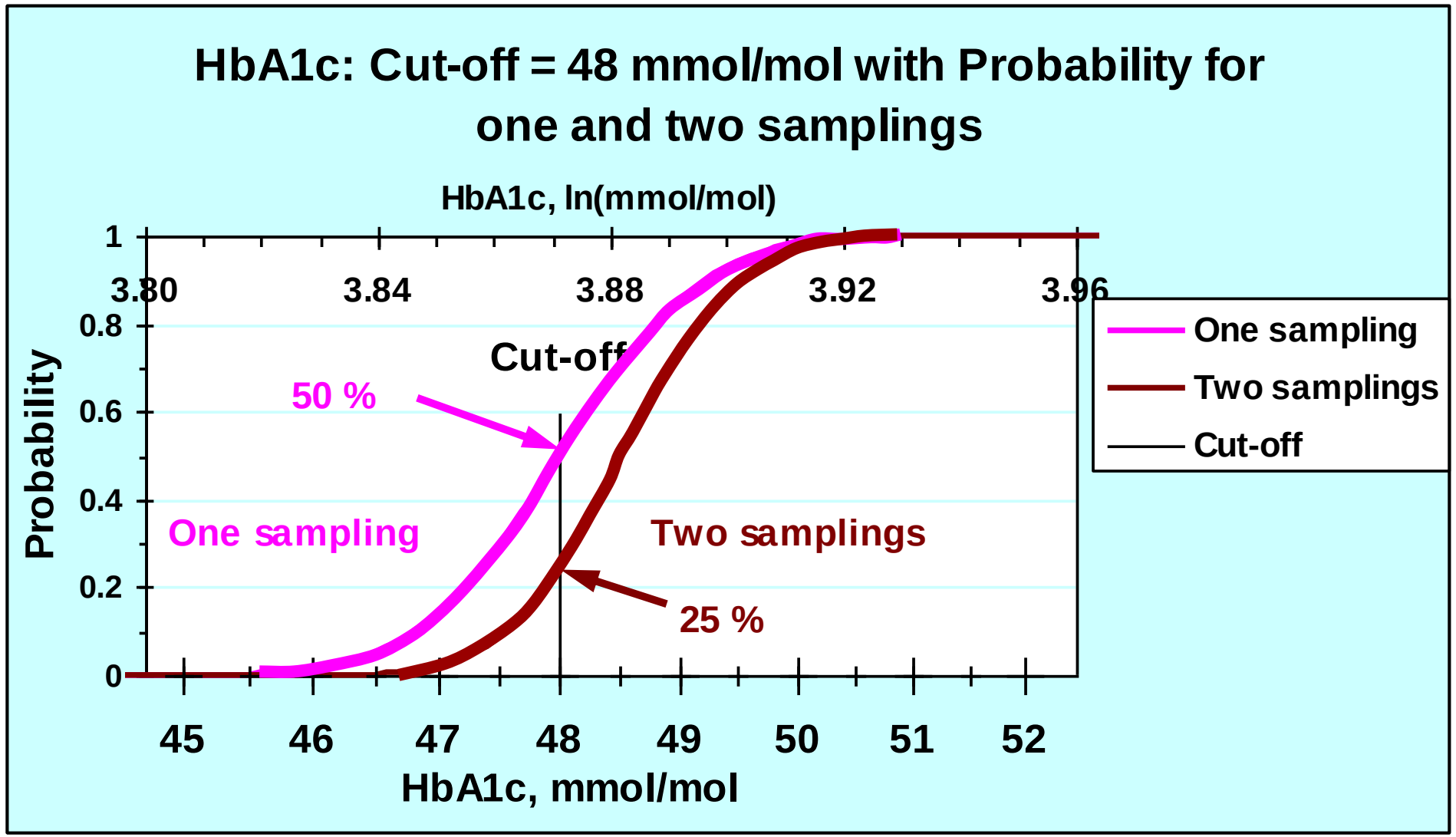


Hyltoft Petersen P, Klee GG. Clin Chem Acta 2014;430:1-8

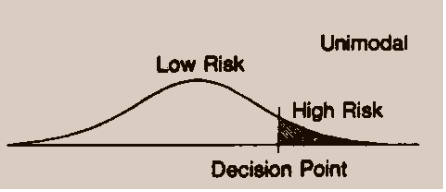


Distribution for a person with set-point = cut-off

HbA1c: Effect of one and two samplings

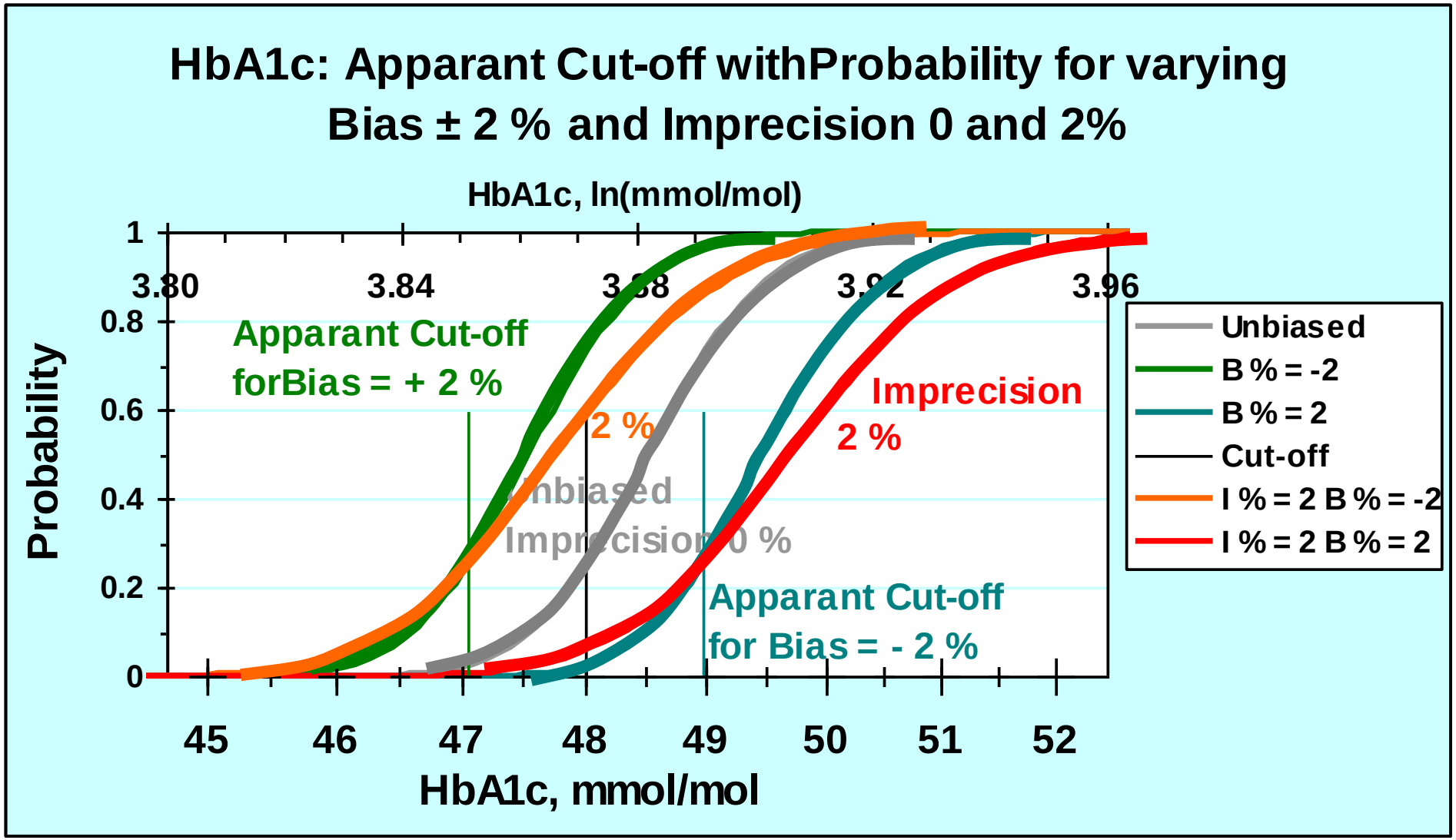


Hyltoft Petersen P, Klee GG. Clin Chem Acta 2014;430:1-8

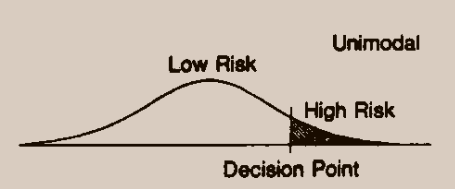


Two samples from same patient with set-point = cut-off

HbA1c: Effect of combined Bias and Imprecision



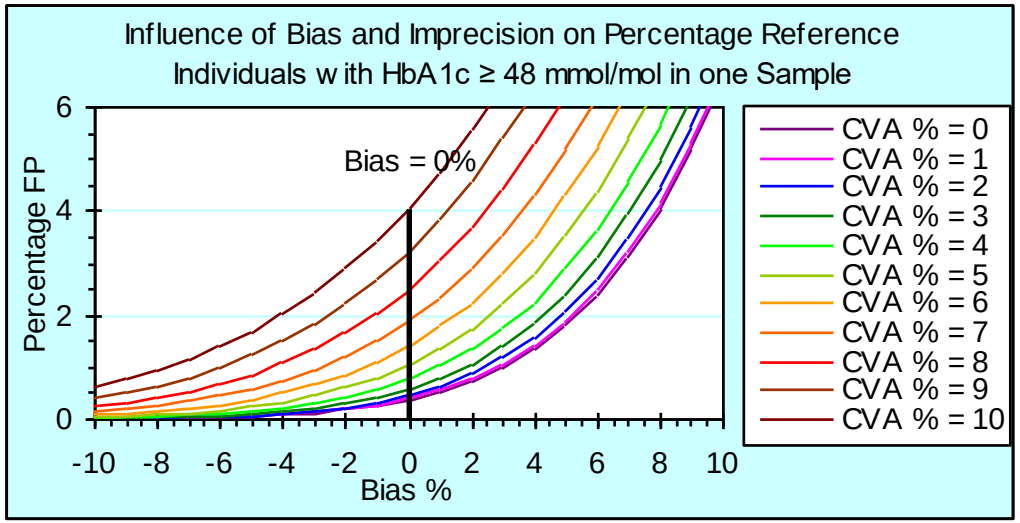
Hyltoft Petersen P, Klee GG. Clin Chem Acta 2014;430:1-8



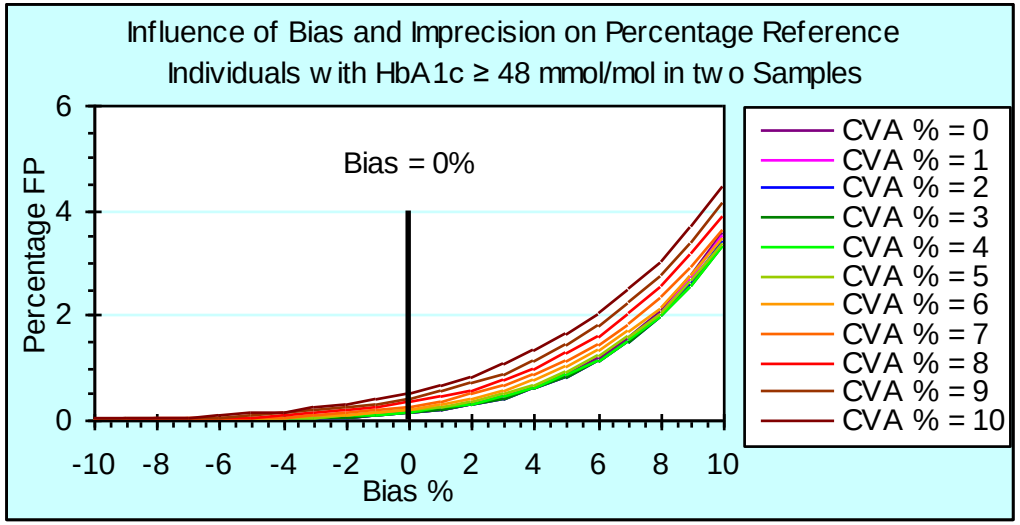
Percentage of false positive (healthy population)

HbA1c:

As function of bias % for varying percentages of imprecision



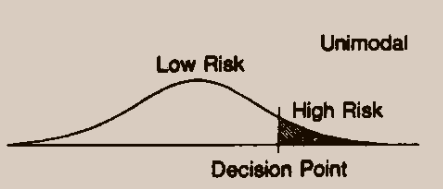
For one sampling



For two samplings

Two samplings reduces the effect of imprecision and modify the effect of bias

Hyltoft Petersen P, Klee GG. Clin Chem Acta 2014;430:1-8



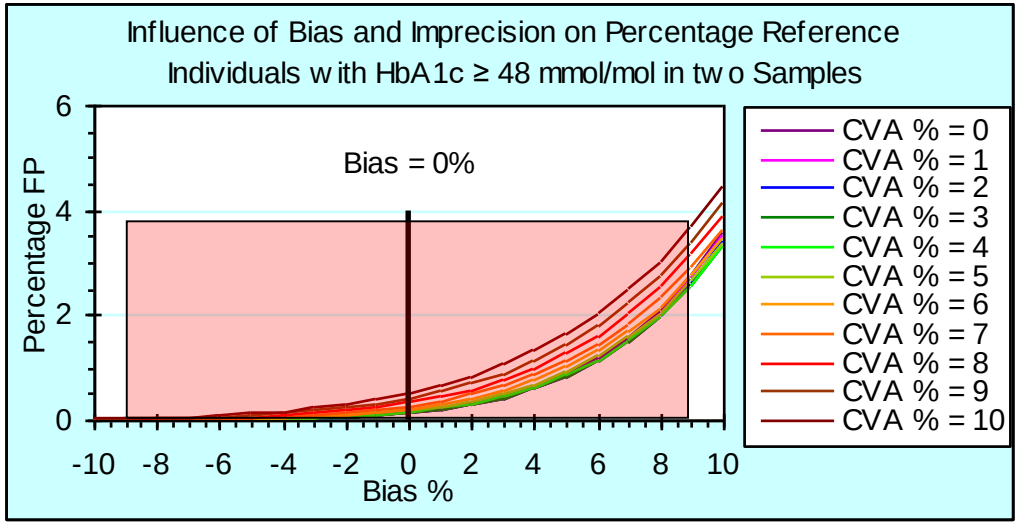
HbA1c:

What are the recommended quality specifications from Sacks et al. *Clin Chem* 2011;57:793-8

Desirable specifications for HbA1c measurement are an intralaboratory CV < 2% and an interlaboratory CV < 3.5 %

The CV 3.5 % DCCT units corresponds to 5.2 % at 48 mmol/mol in IFCC units, and reduced by the 2 %, the final allowable bias is ca. ± 9 % at a 95 % interval

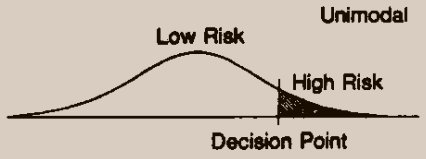
and false positives could be from 0 to 2.8 %



Hyltoft Petersen P, Klee GG. Clin Chem Acta 2014;430:1-8

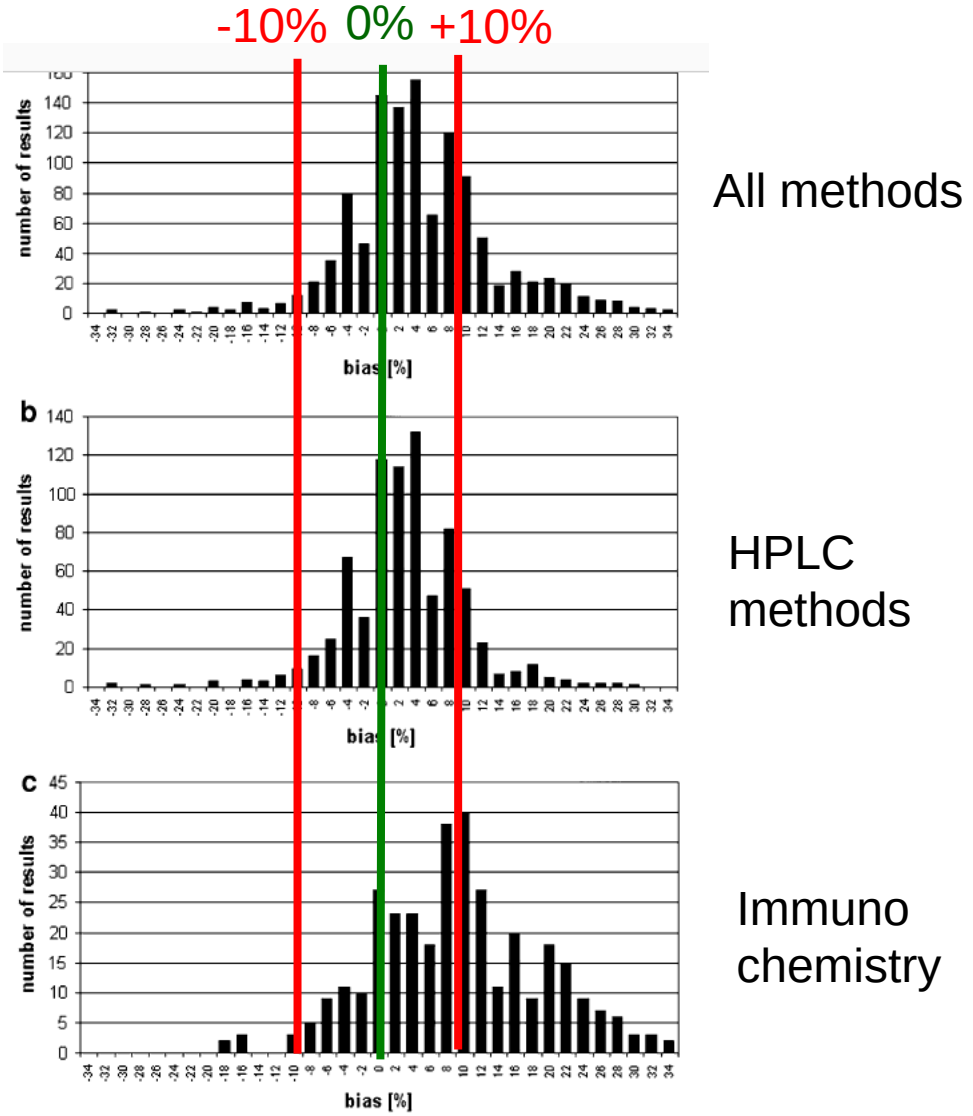
$$CV(IFCC) = \frac{CV(DCCT) \times \bar{X}(DCCT) \times 10.93}{\bar{X}(DCCT) \times 10.93 - 23.52}$$

Personal information from
Thomas Røraas and Sverre Sandberg
NOKLUS, Bergen, Norway



HbA1c:

Extern control of HbA1c In the Czech Republic



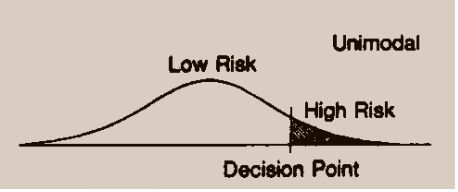
All methods

HPLC methods

Immuno chemistry

Fig. 3 Comparison of bias (deviation from certified value based on IFCC reference method) in HbA_{1c} measurement in 2007 EQA survey. Comparison is based on the method of measurement used by EQA participants: **a** all methods used in the survey, **b** only HPLC methods, and **c** only immunochemistry methods

Friedercky et al. Accred Qual Assur 2010;15:239-43



HbA1c bias in Denmark over 5 years

Bias av HbA1c upptäckt med EQA i Skandinavien

Inger Plum[1], Marie Lundberg[2], Poul Jørgen Jørgensen[3], Ivan Brandslund[4], Gunnar Nordin[2]

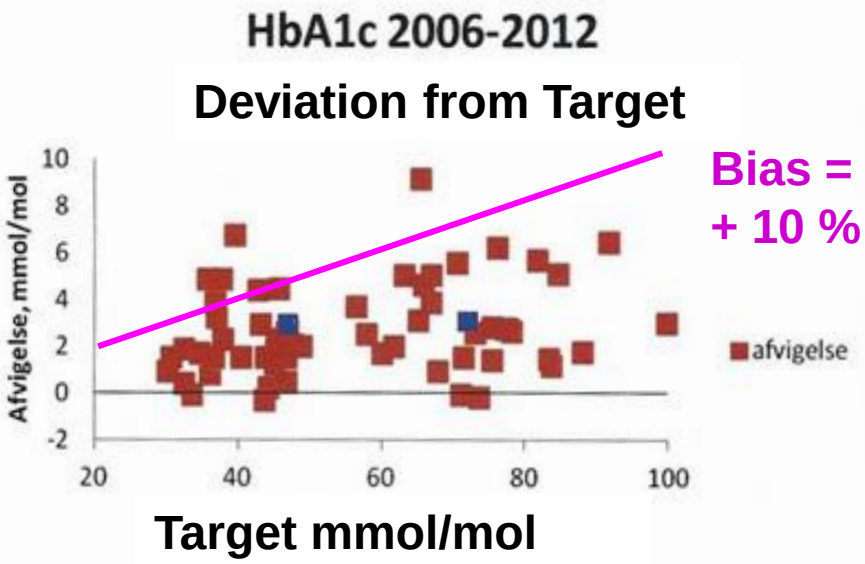
[1]DEKS, Herlev Hospital, [2]Equalis, Uppsala, [3]Klinisk Biokemisk Afdeling, Sygehus Lillebælt, Kolding, [4]Laboratoriecentret, Sygehus Lillebælt, Vejle

inger.plum@deks.dk

I artiklen bliver det vist
- *at problemet har eksisteret i mere end fem år,*
- *at det ikke skyldes gammelt EQA-prøvemateriale ell forsendelse*
- *at det ikke skyldes forkert opbevaring eller håndteri af kalibratorerne*
- *at kontrolmateriale fremstillet med samme meto som kalibratorerne ikke er egnet til at kontrolle kalibratorerne, men*
- *at det skyldes fremstillingsprocessen for kalibratorer i form af frysetørringsmetoden og værditillæggelsen*

På baggrund af det nordiske samarbejde er artikl flettet af danske og svenske indlæg.

Figur 1. *Resultater fra 48 EQA-udsendelser i Danmark. Hvert punkt repræsenterer differensen mellem gennemsnittet af 10-27 laboratorieresultater og targetværdien/facitværdet. Denne sidste er bestemt med HPLC direkte kalibreret op mod IFCCs referencemetode af MCA/ERL. De to med blå markerede punkter markerer resultatet fra apriludsendelsen 2012.*



Klinisk Biokemi i Norden 2013;2:11-5

In Danish and Swedish language

Monitoring of Patients

When is a Measured Difference the same as a Clinical Change in a Patient?

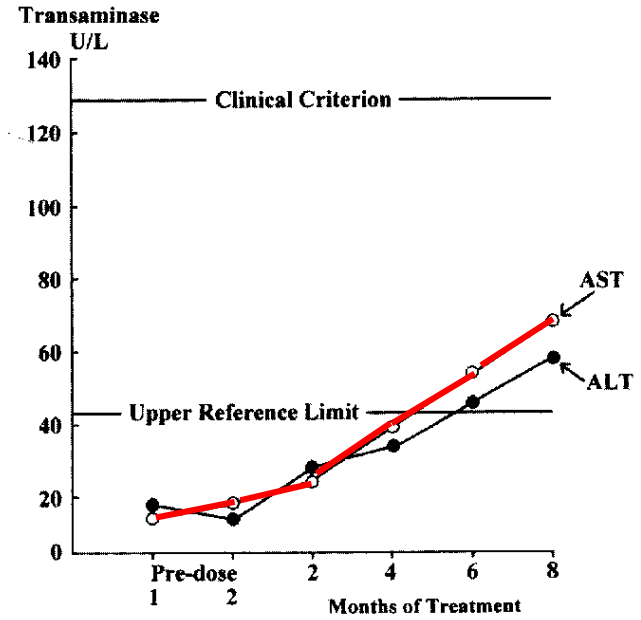


Figure 3.1 Changes in Serum ALT and AST Activities with Time

Reference Change Value (RCV)
= Critical Difference (CD)
= Significant difference

$RCV = z*2^{½}*s_T$ where $s_T = (s_I^2+s_A^2)^{½}$ and $z = 1.96$

$RCV = z*2^{½}*CV_T$ where $CV_T = (CV_I^2+CV_A^2)^{½}$ and $z = 1.96$

Applicable to steady-state situations

Callum Fraser. Biological Variation. AACCPress 2001

Harris & Yasaka. Clin Chem 1983;29:25-30

Monitoring of Patients

Influence of analytical quality

Cotlove's rule (for imprecision):

$s_A \leq ½ * s_I$ or $CV_A \leq ½ * CV_I$

$RCV = 2.77 * (CV_I^2 + CV_A^2)^{1/2}$

$|\Delta B| \leq 3.10 * CV_I - 2.77 * (CV_I^2 + CV_A^2)^{½}$

$|\Delta B|/s_I \leq 3.10 - 2.77 * [1^2 + (s_A/s_I)^2]^{1/2}$

Cotlove et al. Clin Chem 1970,16:1028-32

Maximum combination of $|\Delta B|/s_I$ and s_A/s_I

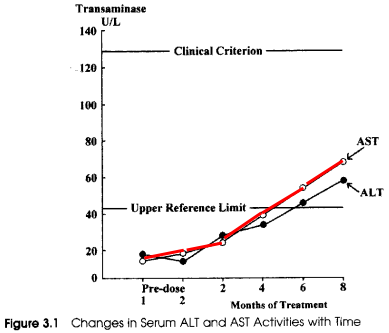
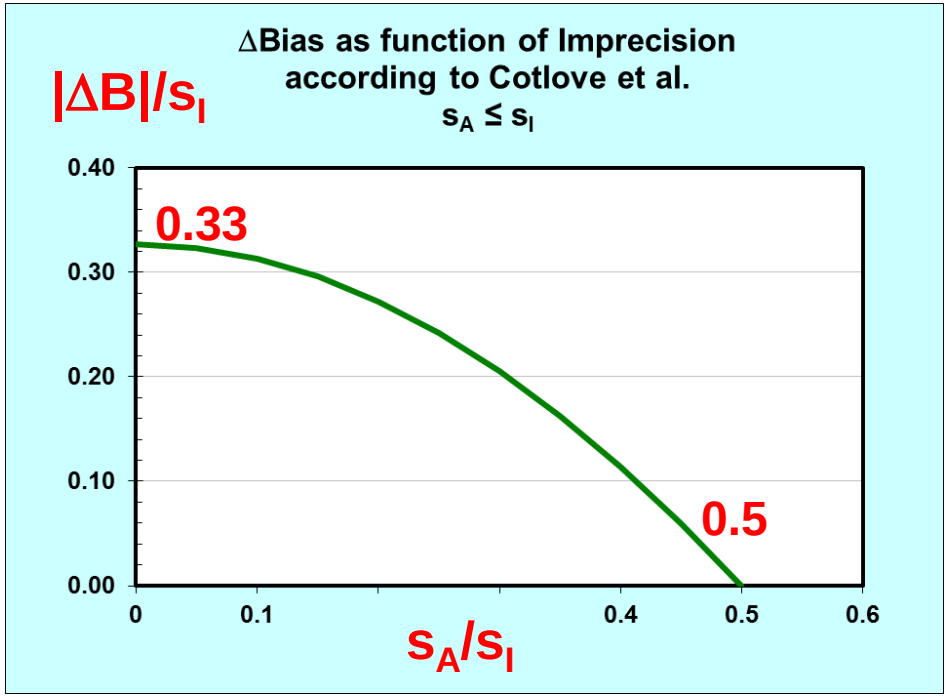


Figure 3.1 Changes in Serum ALT and AST Activities with Time



Lytken Larsen et al. Ann Clin Biochem 1991;28:272-8

ΔBias in Routine

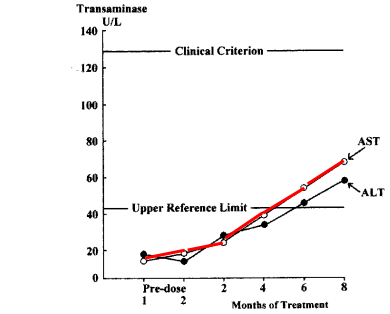
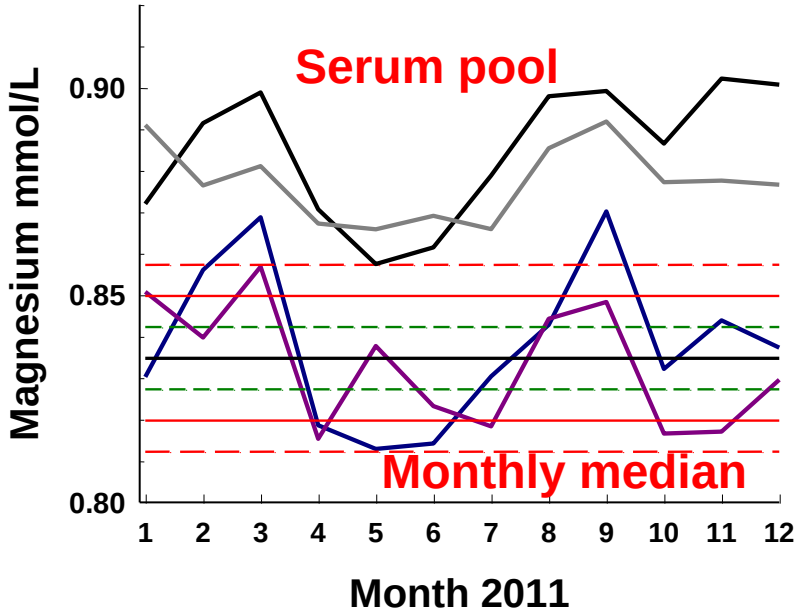


Figure 3.1 Changes in Serum ALT and AST Activities with Time

Serum pool and Patient median on 2 Instruments

How to protect against variations in batches?

Magnesium



Sodium

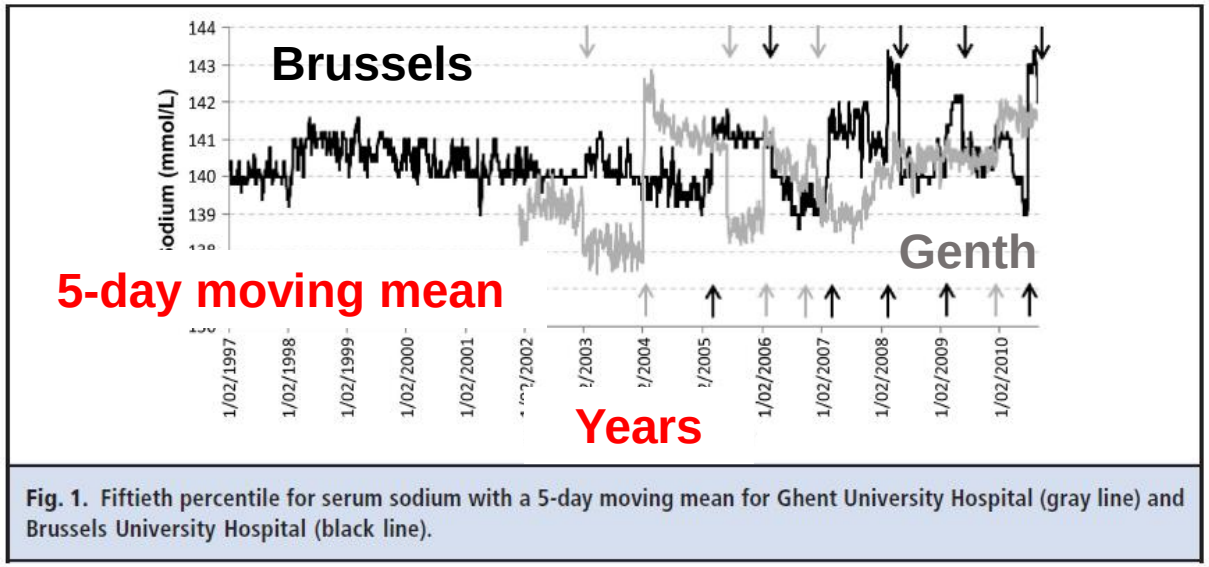


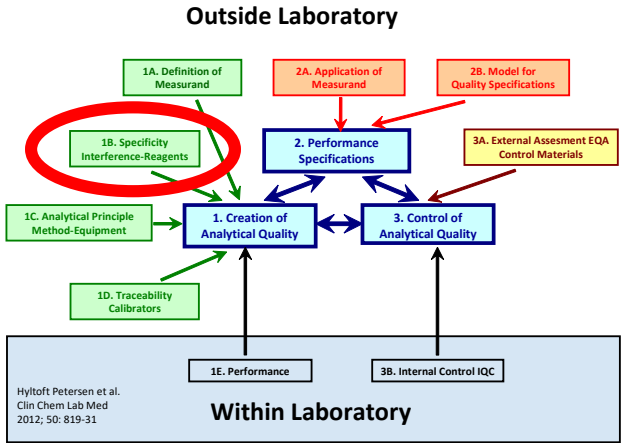
Fig. 1. Fiftieth percentile for serum sodium with a 5-day moving mean for Ghent University Hospital (gray line) and Brussels University Hospital (black line).

Mønsted Jørgensen L et al. Clin Chim Acta 2015;446:186-91

Stepman et al. Clin Chim 2011;57:1616-7

Elements of analytical quality

1B. Specificity – Interference - Reagents



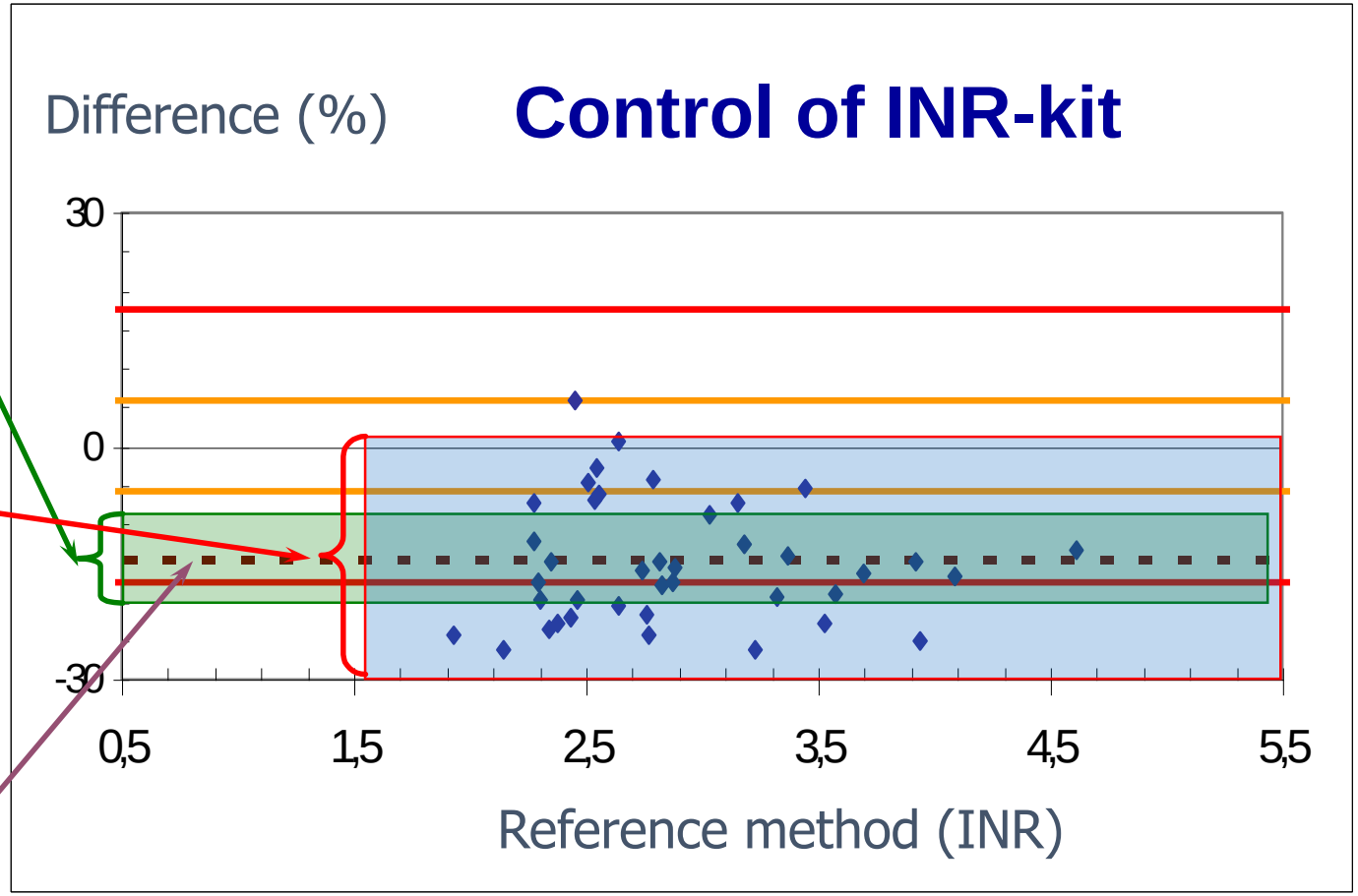
Estimated CV_A
CV_A = 3.1 %

$CV_{Total}^2 = CV_{Matrix}^2 + CV_A^2$

CV_{Total} = 8.5 %

CV_{Matrix} = 7.9 %

Bias = – 15 %



Borrowed from Esther A. Jensen, Denmark

POC instruments

It is difficult to get sufficient amounts of commutable materials to control POC instruments spread over numerous participants.

Stavelin solved it with a model where selected participants estimated the bias of the kit and at the same time analyzed a non-commutable control material and gave a target for the rest of participants.

Difference between method bias and individual deviation

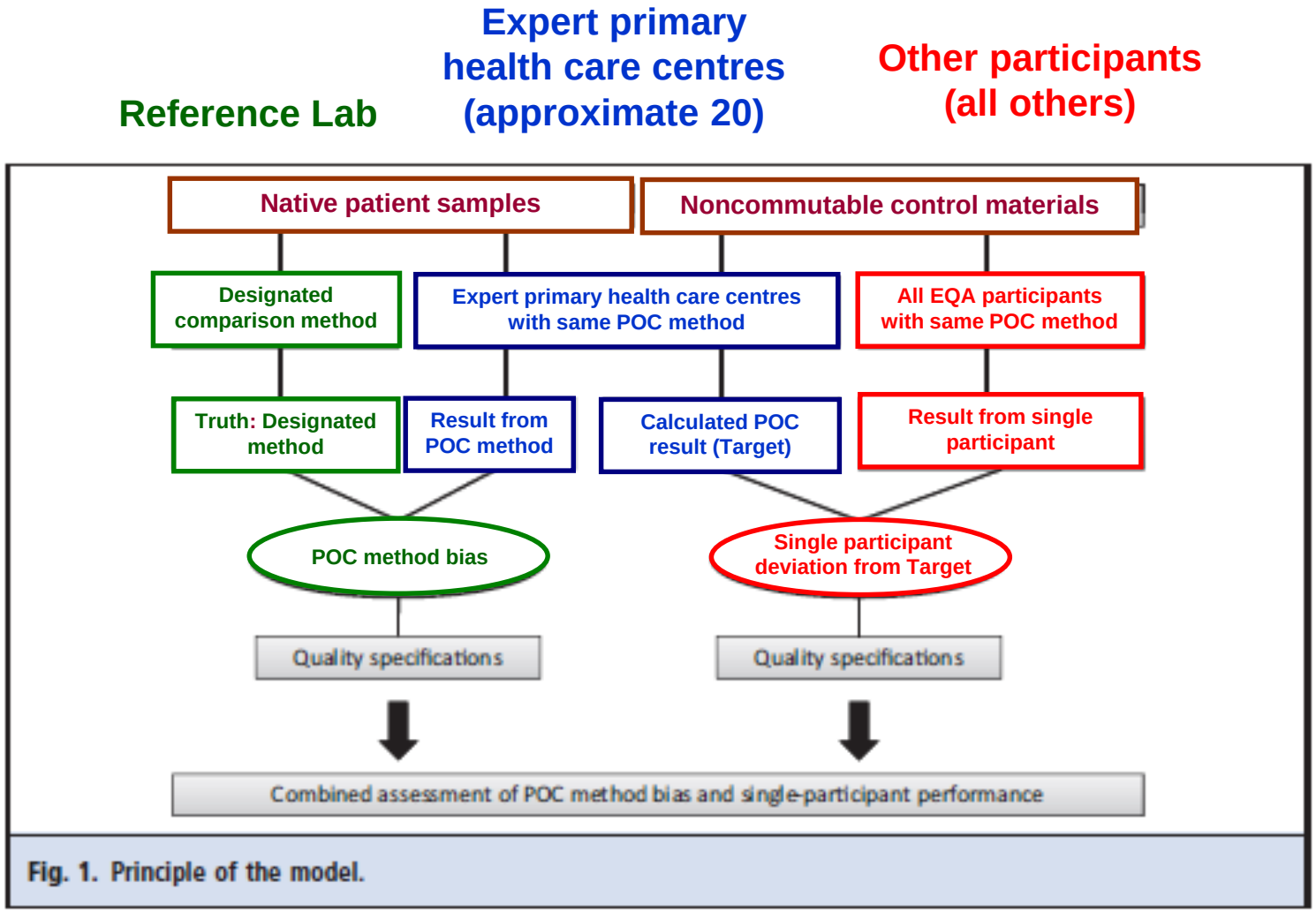
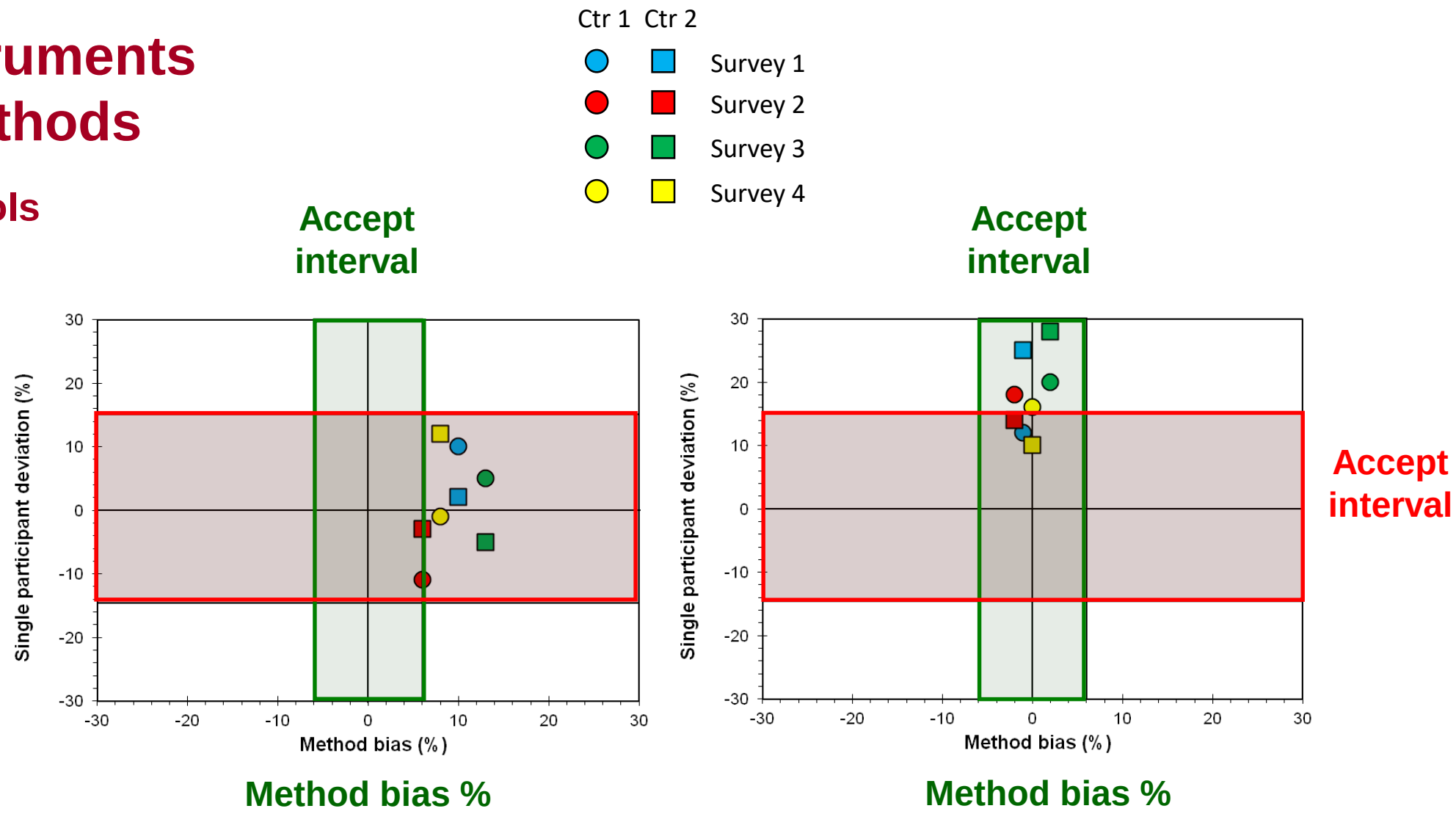


Fig. 1. Principle of the model.

Stavelin A et al. Clin Chem 2013;59:363-71

POC Instruments
INR methods

Two controls



Stavelin A et al. Clin Chem 2013;59:363-71

Measurement Scales

Properties of Measurement Scales

Ratio Scale of Measurements

identity, magnitude, equal intervals, and minimum value of zero

Plasma concentration of Sodium

Interval Scale of Measurements (Differens Scale)

identity, magnitude, and equal intervals, (3-2 = 20-19)

Celsius degrees (^{o}C)

Ordinal Scale of Measurements

identity and magnitude (ordered relationship)

First Class – Business Class – Ordinary Class

Nominal Scale of Measurements

identity (descriptive)

Colour (red and green)

Measurement Scales

Properties of Measurement Scales

Ratio Scale of Measurements
identity, magnitude, equal intervals, and minimum value of zero
Plasma concentration of Sodium

Interval Scale of Measurements (Differens Scale)
identity, magnitude, and equal intervals, (3-2 = 20-19)
Celsius degrees (°C)

Ordinal Scale of Measurements
identity and magnitude (ordered relationship)
First Class, Business Class, Ordinary Class

Nominal Scale of Measurements
identity (descriptive)
Colour (red and green)

Ordinal scale

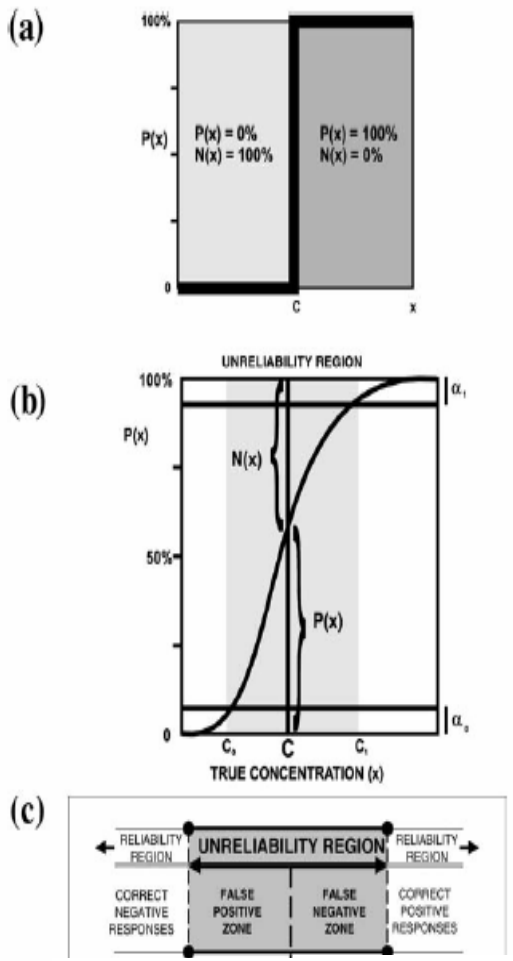
MEQUALAN

Dichotome tests for each concentration

Percentage or Fraction of Positive Tests

Accred Qual Assur (2003) 8:68–77
DOI 10.1007/s00769-002-0556-x

From 0 % to 100 % positive

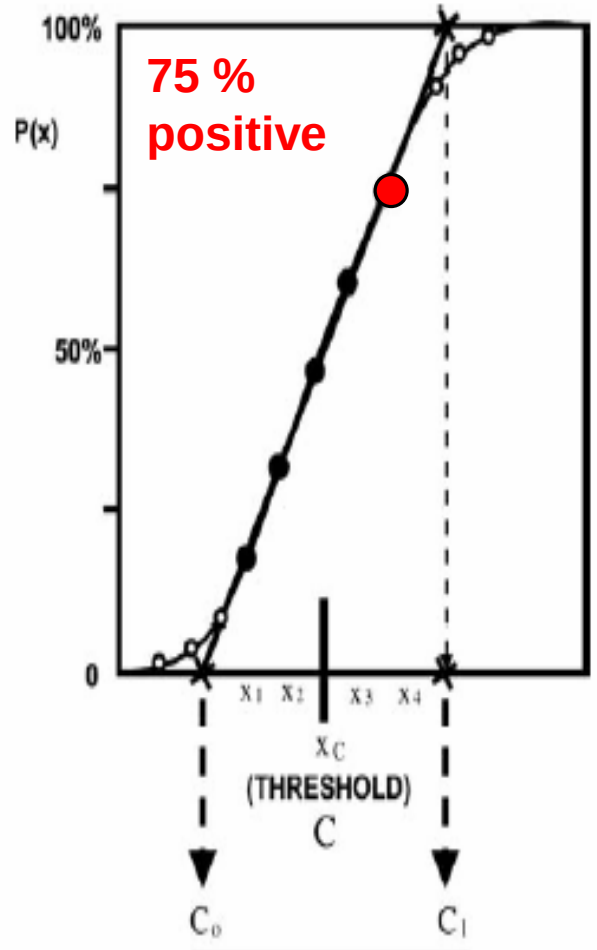


Ideal

Reality

False positive

False negative



Unreliable region

Measurement Scales

Properties of Measurement Scales

Ratio Scale of Measurements
identity, magnitude, equal intervals, and minimum value of zero
Plasma concentration of Sodium

Interval Scale of Measurements (Differens Scale)
identity, magnitude, and equal intervals, (3-2 = 20-19)
Celsius degrees (°C)

Ordinal Scale of Measurements
identity and magnitude (ordered relationship)
First Class, Business Class, Ordinary Class

Nominal Scale of Measurements
identity (descriptive)
Colour (red and green)

Transformation to RANKIT

U-Haemoglobin

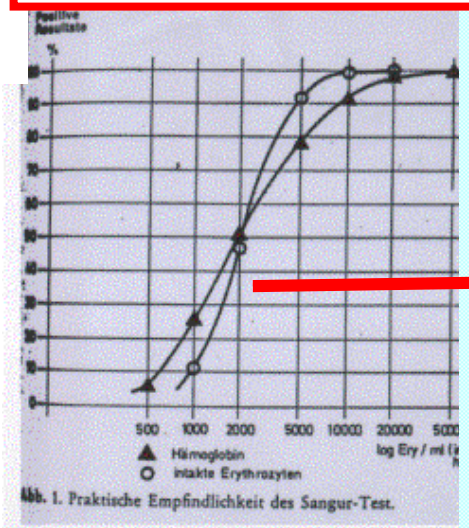
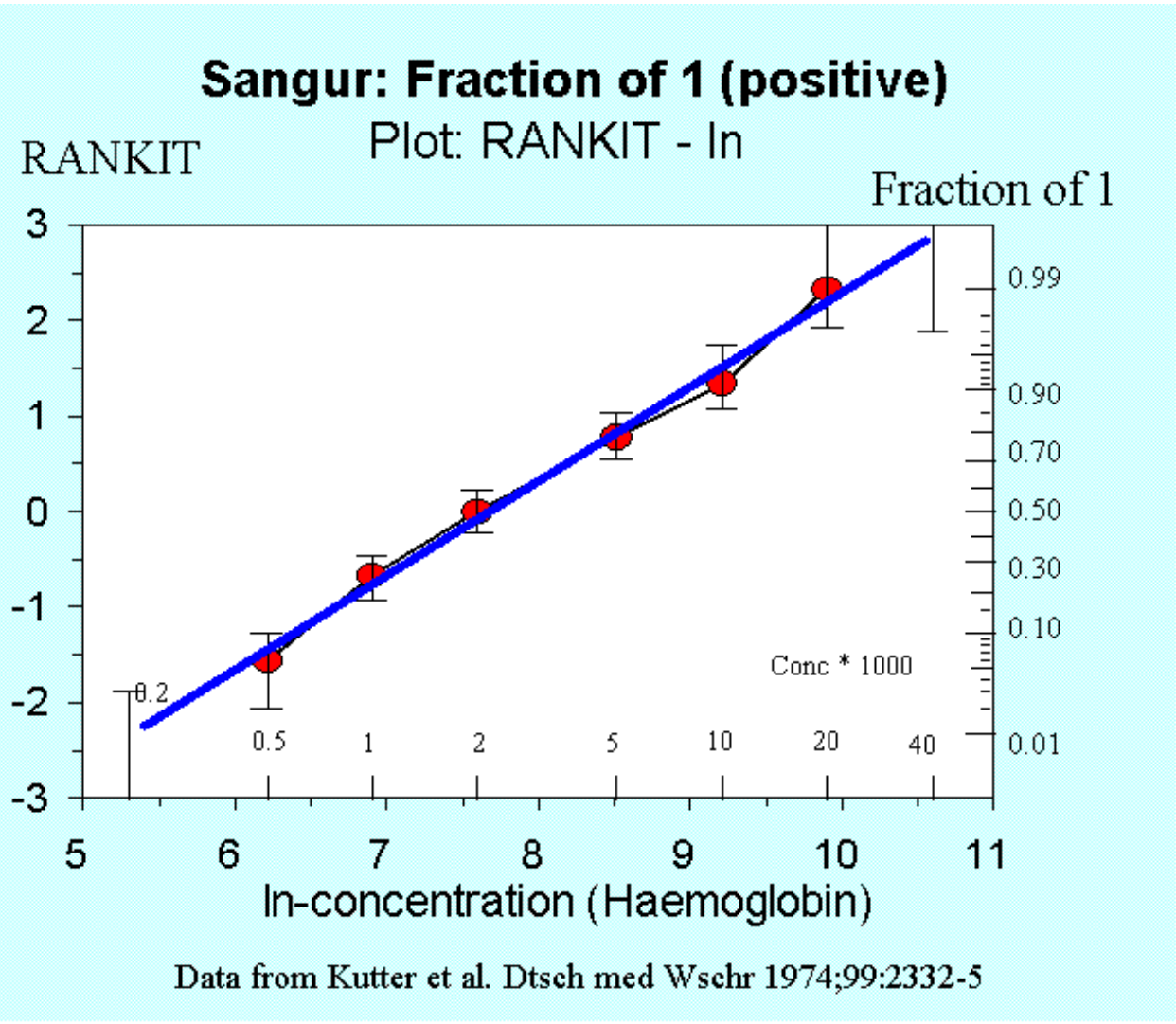


Abb. 1. Praktische Empfindlichkeit des Sangur-Test.

Kutter *et al. Dtsch med Wschr* 1974; 99:2332-5

Petersen et al. Clin Chem Lab Med 2000; 38:545-51

Data from Kutter et al. Dtsch med Wschr 1974; 99:2332-5



Ordinal scale transformed to Rankit Scale

Ordinal scale where there is an underlying ratio scale

Measurement Scales

Properties of Measurement Scales

Ratio Scale of Measurements
identity, magnitude, equal intervals, and minimum value of zero
Plasma concentration of Sodium

Interval Scale of Measurements (Differens Scale)
identity, magnitude, and equal intervals, (3-2 = 20-19)
Celsius degrees (°C)

Ordinal Scale of Measurements
identity and magnitude (ordered relationship)
First Class, Business Class, Ordinary Class

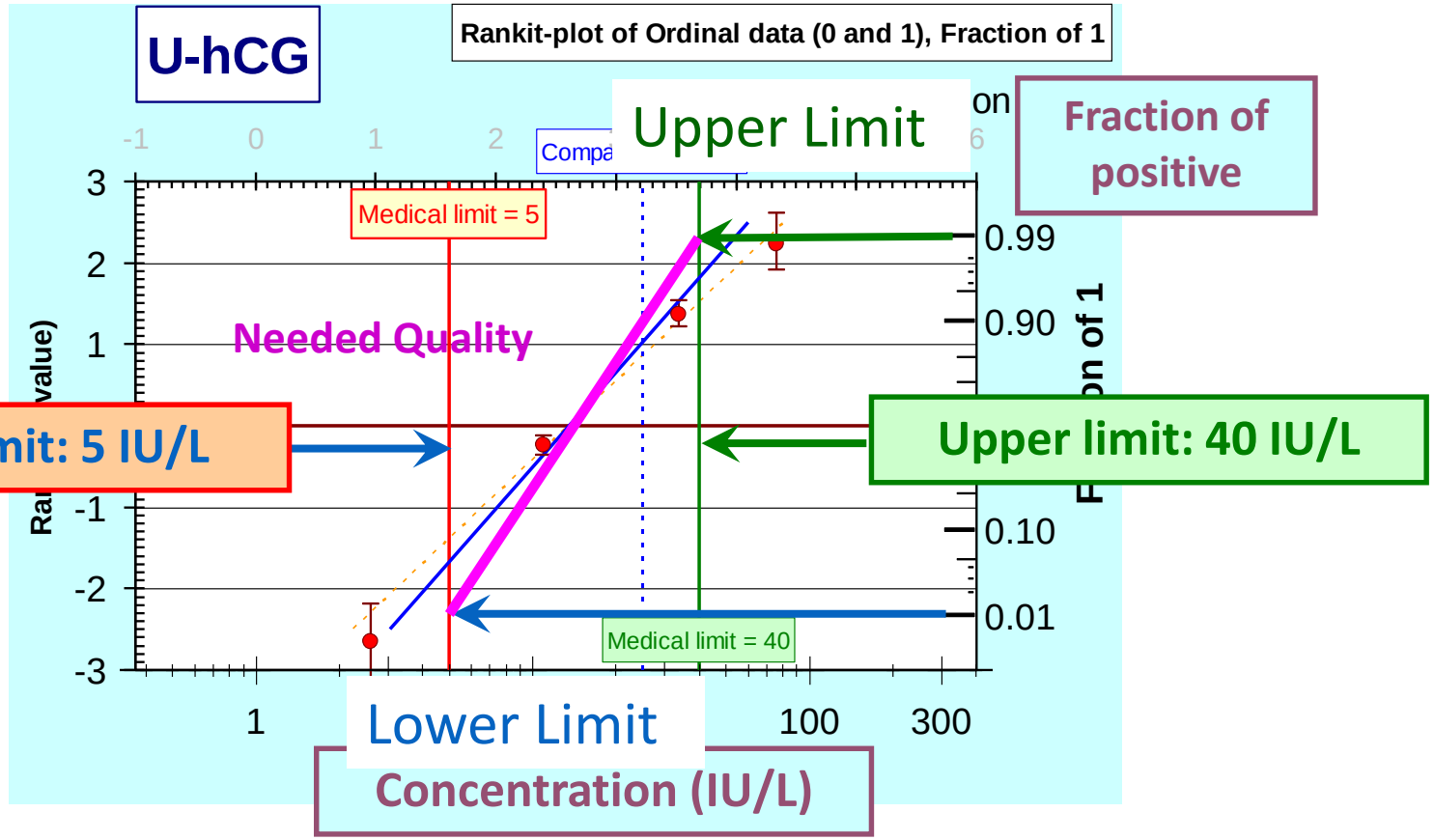
Nominal Scale of Measurements
identity (descriptive)
Colour (red and green)

Ordinal scale – dichotomous test

Clinical Specifications: 5 IU/L ≤ 1 % Positive Results
40 IU/L ≥ 99 % Positive Results

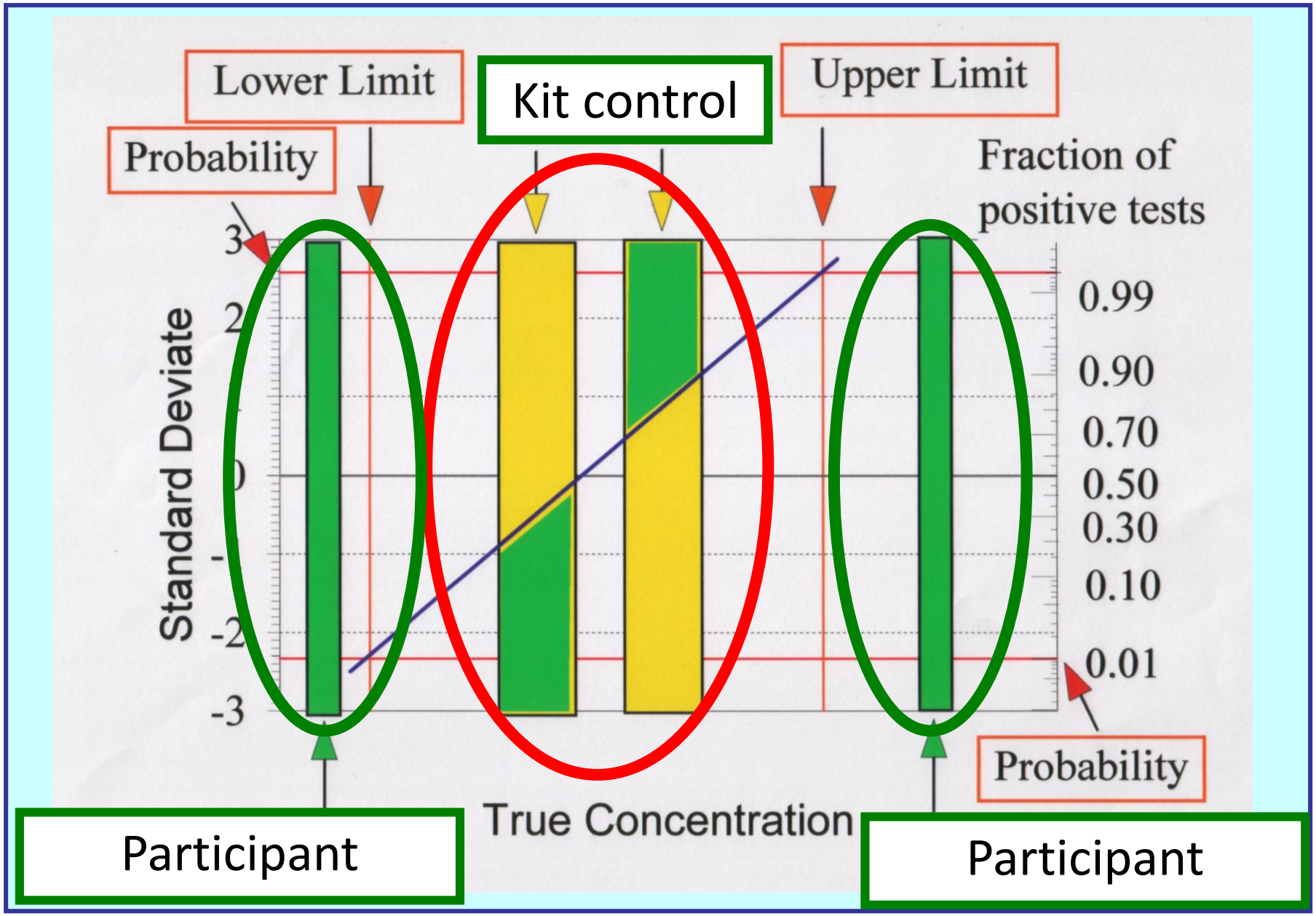
Urine-hCG

Ordinal scale where there is an underlying ratio scale



Hyltoft Petersen P, Gade Christensen N, et al. Scand J Clin Lab Invest 2008;68:298-311

Ordinal-skala og Probit-transformation



Measurement Scales

Properties of Measurement Scales

Ratio Scale of Measurements
identity, magnitude, equal intervals, and minimum value of zero
Plasma concentration of Sodium

Interval Scale of Measurements (Differens Scale)
identity, magnitude, and equal intervals, (3-2 = 20-19)
Celsius degrees (°C)

Ordinal Scale of Measurements
identity and magnitude (ordered relationship)
First Class, Business Class, Ordinary Class

Nominal Scale of Measurements
identity (descriptive)
Colour (red and green)

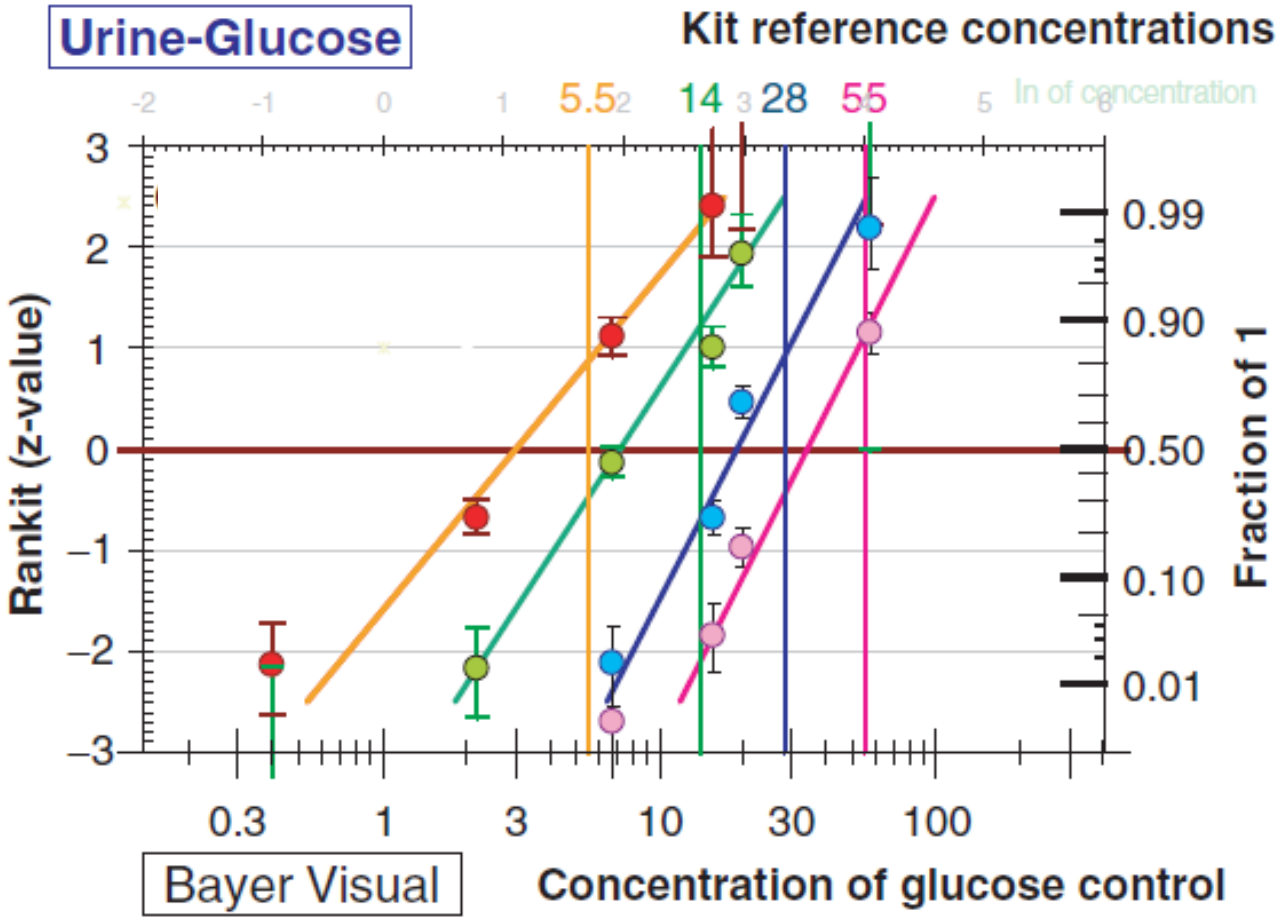
Ordinal scale – Semi-quantitative test

for U-Glucose with four concentration steps

Urine-Glucose

Concentration Steps

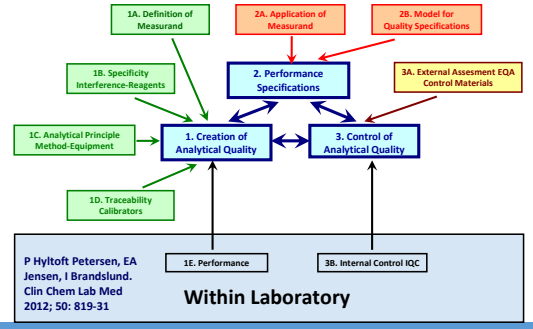
5.5 mmol/L
14 mmol/L
28 mmol/L
55 mmol/L



Hyltoft Petersen P, Gade Christensen N, et al. Scand J Clin Lab Invest 2009; 69:662-72

Concentration (mmol/L)

Outside Laboratory



P Hyltoft Petersen, EA Jensen, I Brandslund. Clin Chem Lab Med 2012; 50: 819-31

Elements of analytical quality

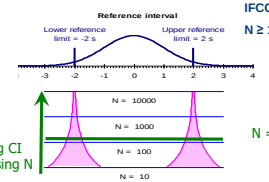
The Nordic Protein Project

2B. Model for Quality Specificatios

Creation of Quality Specifications for Reference Intervals

Confidence Intervals for Reference Interval Limits

Solberg HE. Approved Recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J Clin Chem Clin Biochem 1987;25:645-56



Decreasing CI for increasing N

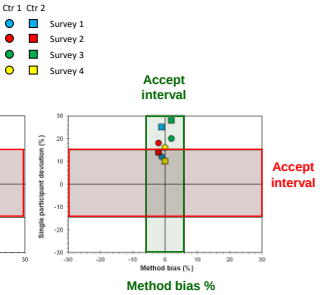
IFCC Recommendations

N ≥ 120 Individuals

N = 120

POC Instruments INR methods

Two controls



Stavelin A et al. Clin Chem 2013;59:363-71

Elements of analytical quality

The Nordic Protein Project

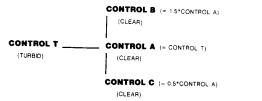
3A. External Assessment EQA Control Materials

Control Materials:

The controls were also human serum pools based on other serum pools but treated differently

Control T: The turbid control was not centrifuged
Control A: Same procedure as the Calibrator
Control B: 1½ times Control A
Control C: ½ times Control A

Later the Calibrator and Controls were Traced to the Certified Reference Material CRM 470



Concentrations of Control T and Control A are the same, so Control T – Control A = Lack of specificity

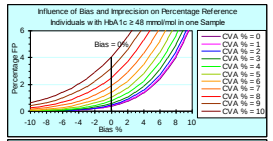
Controls A, B and C controls the Linearity of calibration curve.

Hyltoft Petersen P et al. Upsale J Med SCI 1994;99:277-306

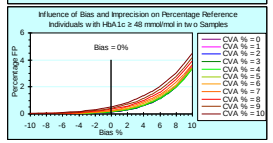
"Guideline-Driven Medical Decision Limits".

Percentage of false positive (healthy population)

HbA1c: As function of bias % for varying percentages of imprecision



For one sampling



For two samplings

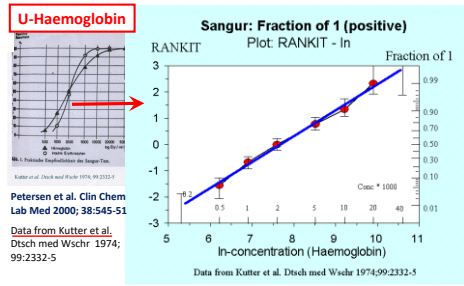
Two samplings reduces the effect of imprecision and modify the effect of bias

Hyltoft Petersen P, Klee GG. Clin Chem Acta 2014;430:1-8

Measurement Scales

Transformation to RANKIT

U-Haemoglobin



Ordinal scale transformed to Rankit Scale

Ordinal scale where there is an underlying ratio scale

Petersen et al. Clin Chem Lab Med 2000; 38:545-51

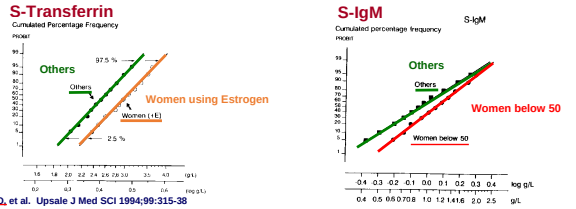
Data from Kutter et al. Dtsch med Wschr 1974; 99:2332-5

Elements of analytical quality

The Nordic Protein Project

2A. Application of the Measurand

Estimation of Common Reference Intervals with Partitioning (N = 720)



Blaabjerg O. et al. Upsale J Med SCI 1994;99:315-38

Monitoring of Patients

Influence of analytical quality

Cotlove's rule (for imprecision):

$s_A \leq ½*s_I$ or $CV_A \leq ½*CV_I$

This means that the total $CV_T = (CV_I^2 + CV_A^2)^{1/2} \leq 1.12*CV_I$

$RCV = 2.77*(CV_I^2 + CV_A^2)^{1/2}$

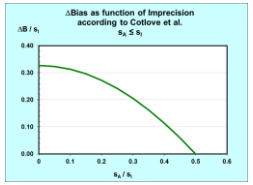
$|\Delta B| \leq 3.10*CV_I - 2.77*(CV_I^2 + CV_A^2)^{½}$

$|\Delta B|/CV_I \leq 3.10 - 2.77*[1^2 + (CV_A/CV_I)^2]^{1/2}$

$|\Delta B|/s_I \leq 3.10 - 2.77*[1^2 + (s_A/s_I)^2]^{1/2}$

Cotlove et al. Clin Chem 1970,16:1028-32

Maximum combination of $|\Delta B|/s_I$ and s_A/s_I



Lytken Larsen et al. Ann Clin Biochem 1991;28:272-8

Ordinal-skala og Probit-transformation