Monitoring in healthy people and patients

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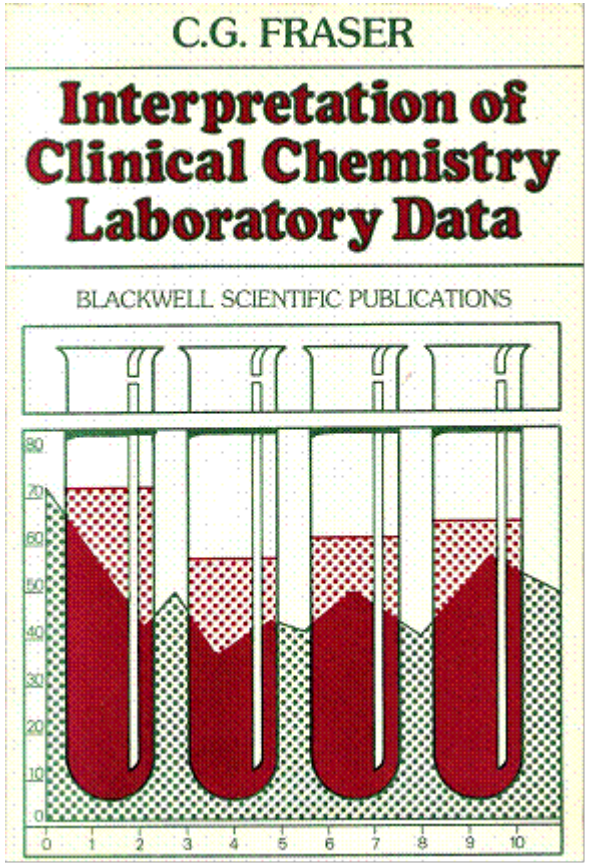
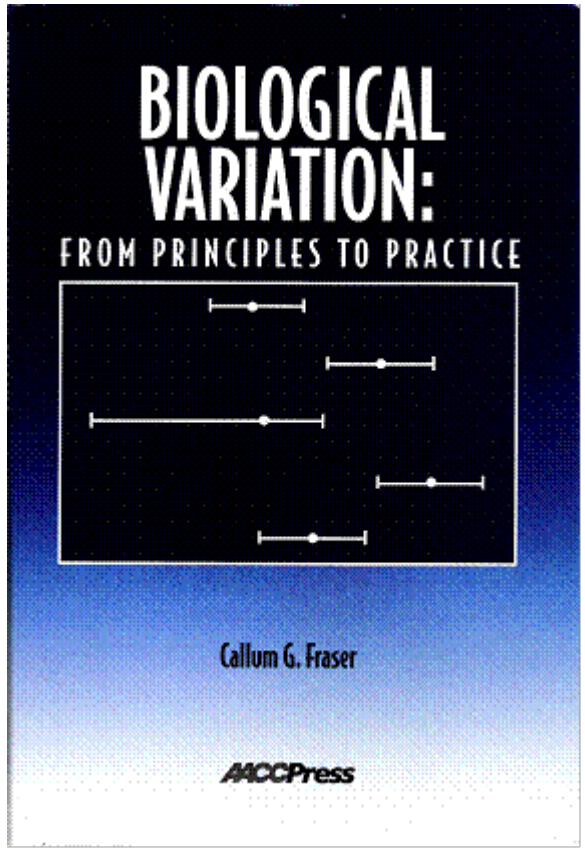
OVERVIEW

- Predictable and unpredictable variation
- Biological rhythms
- Within- and between variation
- Variance homogeneity
- Reference change value (RCV)
  - Number of decimals
  - Power
  - False positives and false negatives
- Regression to the mean

Two excellent books from Callum G. Fraser





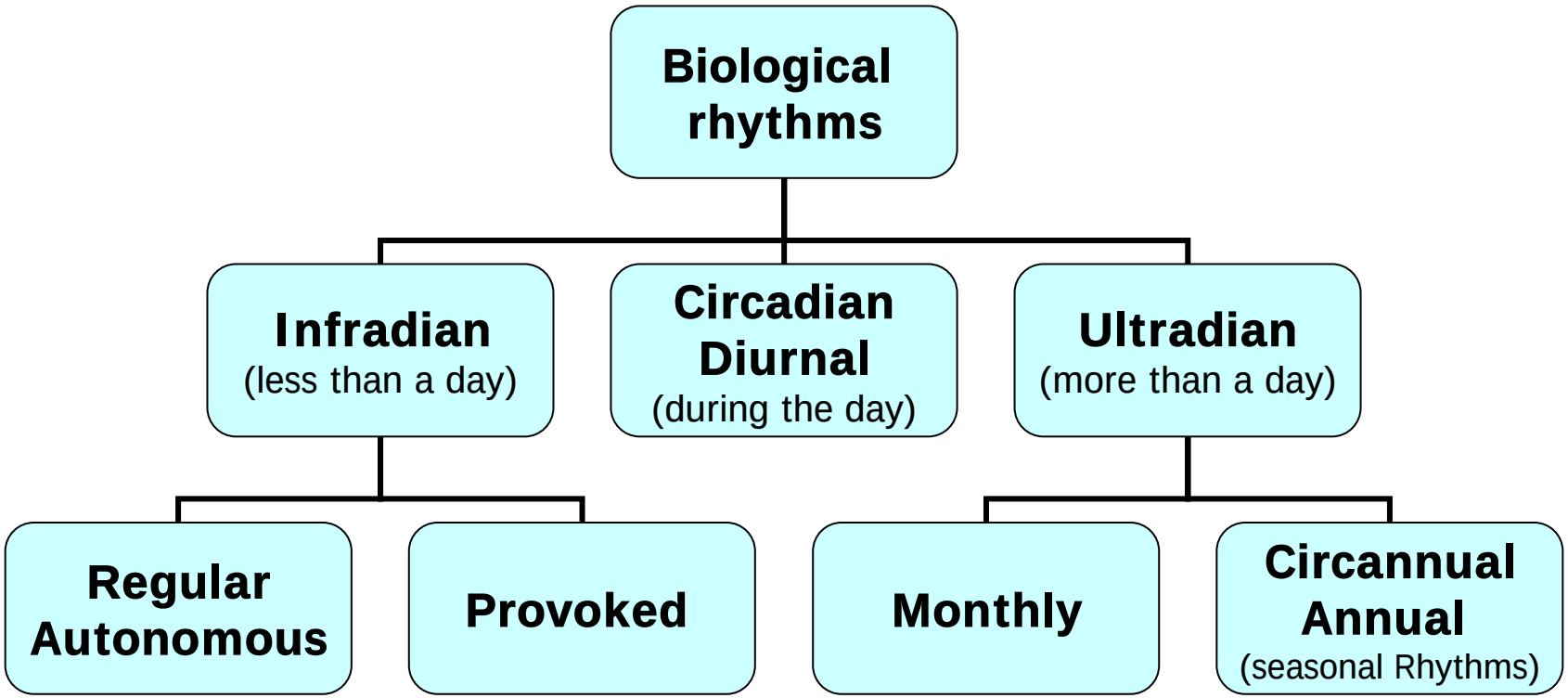
Fraser CG. Interpretation of clinical chemistry laboratory data. 1986. Blackwell Scientific Publications

Fraser CG. Biological variation. AACCPress 2001

Types of biological variation

	Unpredictable variation (inherent)	Predictable variation
Within the individual	$CV_{within\text{-}subject}$	Cyclic and other variations
Between individuals	$CV_{between\text{-}subject}$	Gender Age Race Environmental

Predictable biological variation



Biological variation during the lifespan

Biological variation during pregnancy

Biological variation during the lifespan

Age: 0 to 14 years

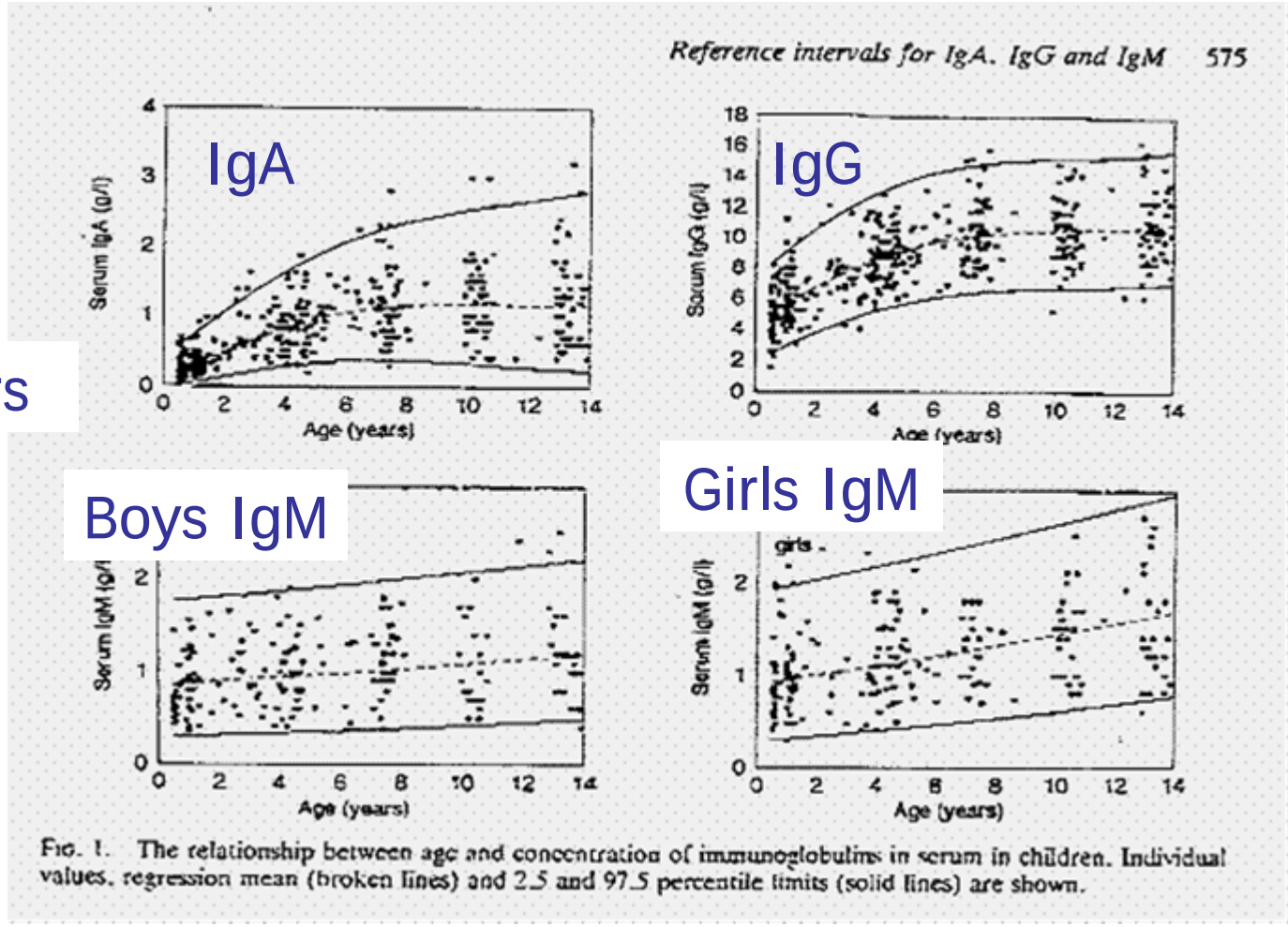
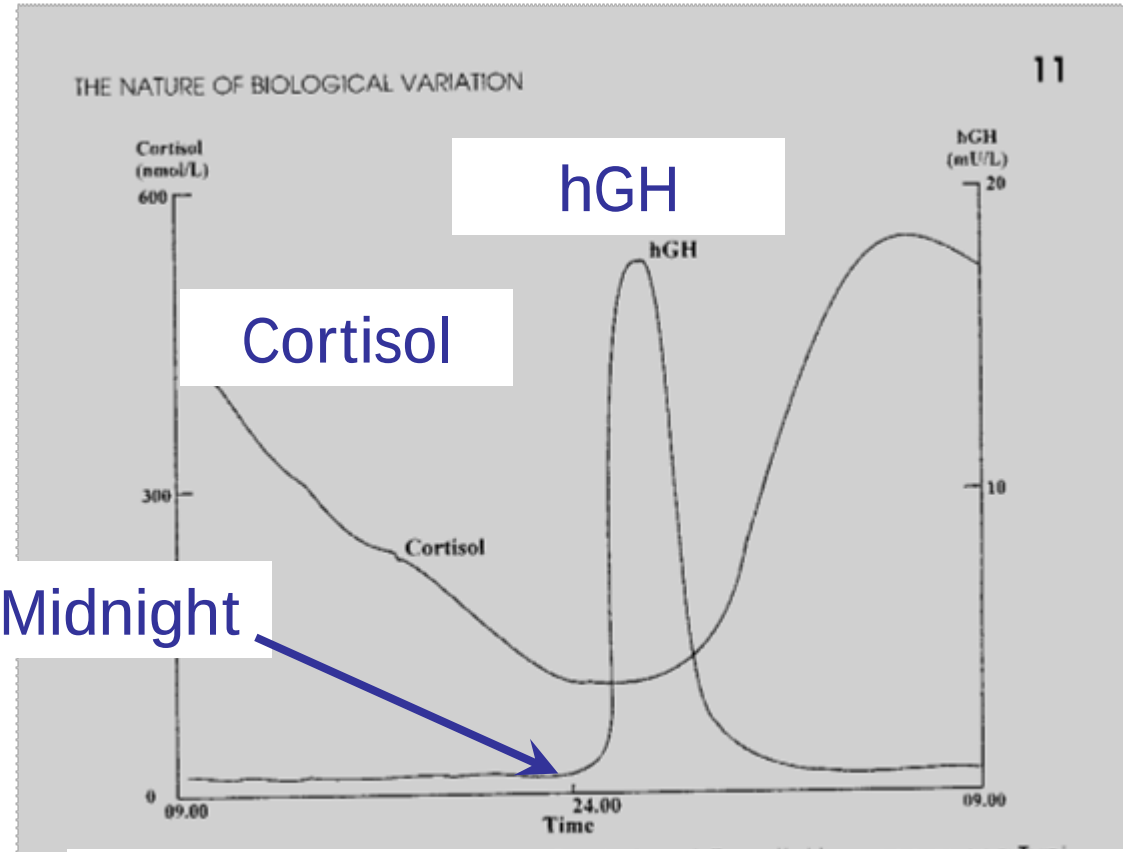


FIG. 1. The relationship between age and concentration of immunoglobulins in serum in children. Individual values, regression mean (broken lines) and 2.5 and 97.5 percentile limits (solid lines) are shown.

Irjala et al. Scand J Clin Lab Invest 1990;50:573-7

Diurnal variation



Concentrations of Cortisol and Growth hormone (hGH) 9 o'clock to next morning

Fraser CG. Biological variation. AACCPress 2001

Unpredictable biological variation

Calculations:

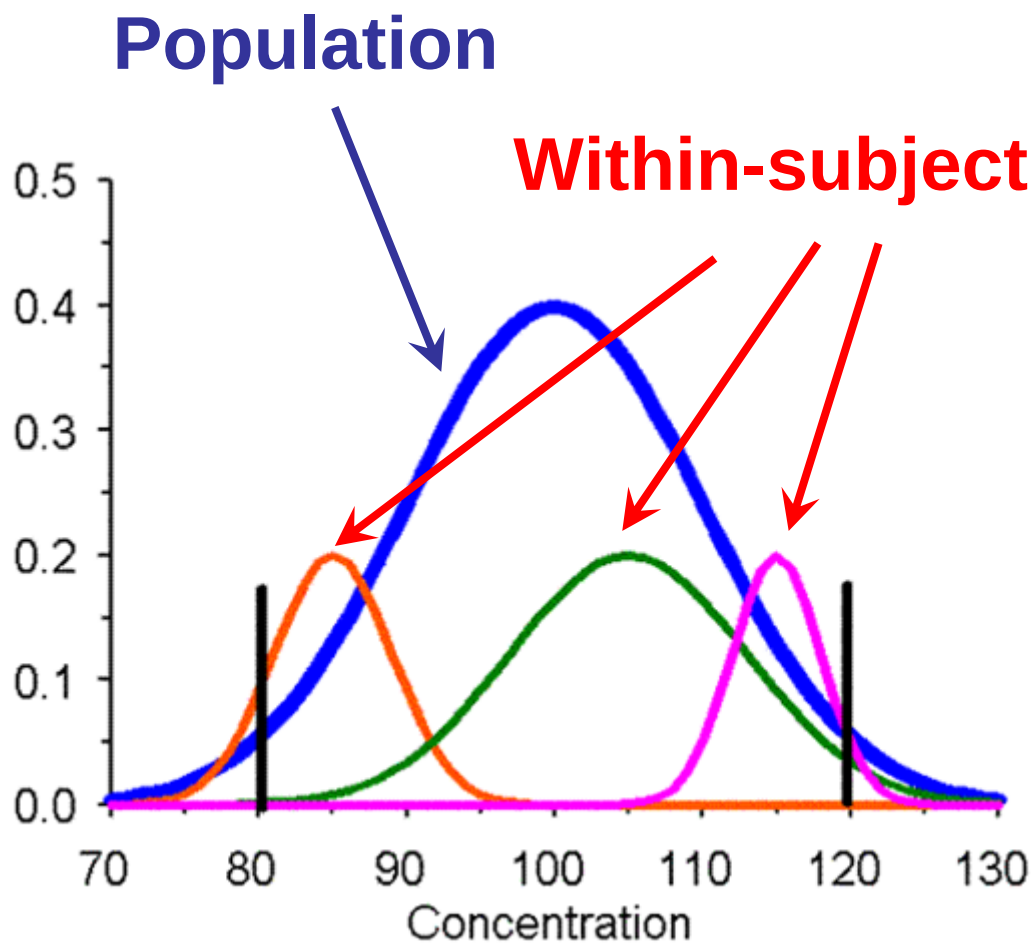
Coefficient of variation

$CV = \sigma/\mu$

and CV % = 100*CV

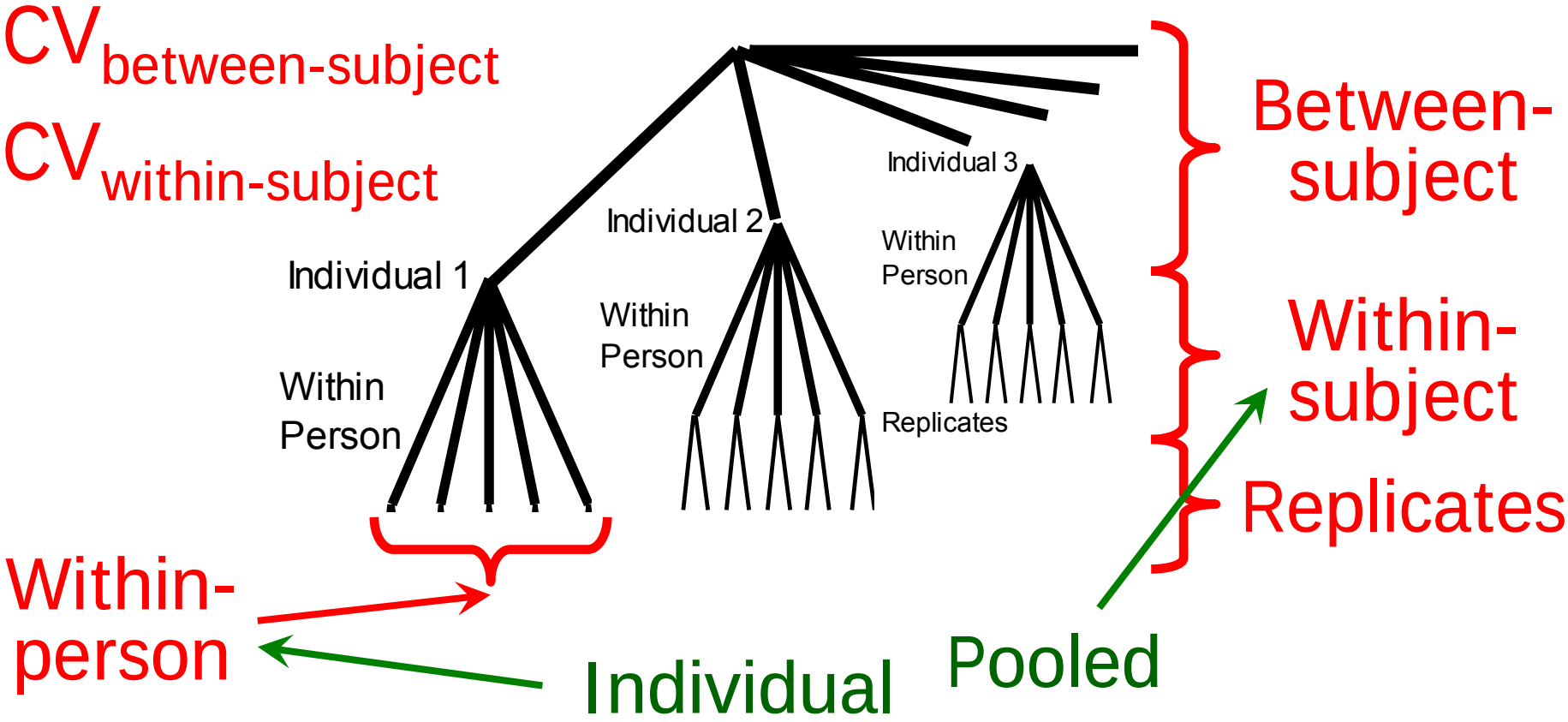
$CV_{within\text{-}subject}$

$CV_{between\text{-}subject}$

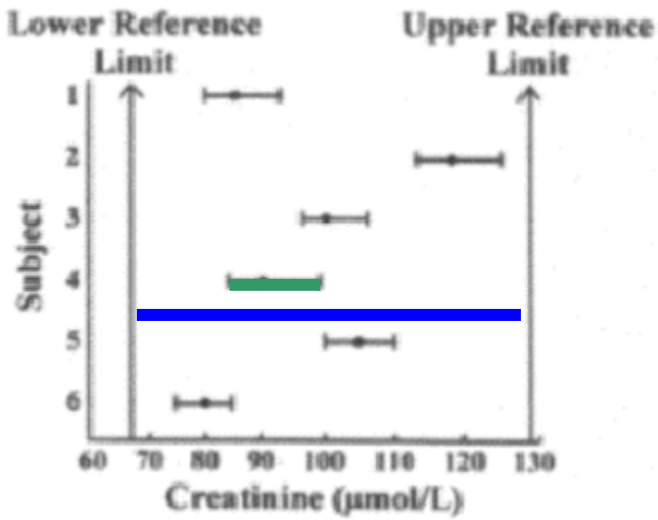


Unpredictable biological variation

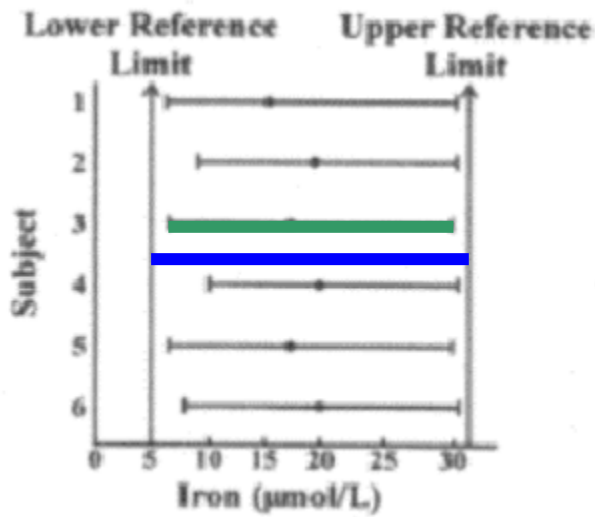
Monitoring: Experimental design



Unpredictable biological variation



S-Creatinine



S-Iron

Callum G. Fraser:

Biological variation

AACC Press 2001

Unpredictable biological variation

$CV_{between\text{-}subject}$

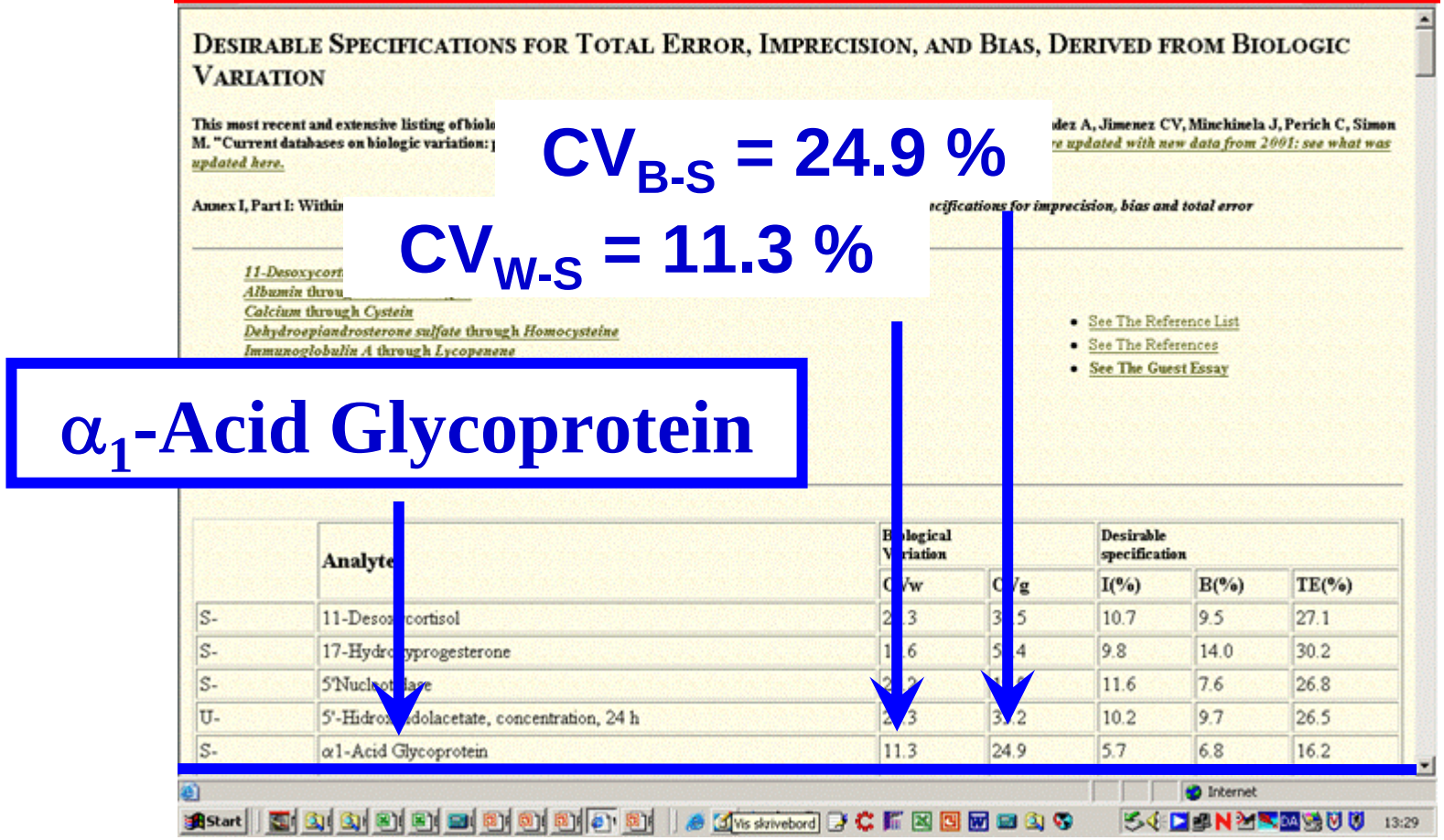
$CV_{within\text{-}subject}$

Data on Biological Variation

Component	$CV_{W\text{-}S}$	$CV_{B\text{-}S}$
S-Sodium	0.7 %	0.6 %
S-Potassium	5.4 %	4.8 %
S-Carbamide	13.9 %	13.8 %
S-Thyroxine	5.1 %	10.2 %
U-Albumin	70.0 %	55.0 %

Data base for biological variation and goals

http://www.westgard.com/guest36.htm



DESIRABLE SPECIFICATIONS FOR TOTAL ERROR, IMPRECISION, AND BIAS, DERIVED FROM BIOLOGIC VARIATION

CV_{B-S} = 24.9 %

CV_{W-S} = 11.3 %

α_1-Acid Glycoprotein

	Analyte	Biological Variation		Desirable specification		
		CVw	CVg	I(%)	B(%)	TE(%)
S-	11-Deso[illegible]cortisol	2[illegible].3	3[illegible].5	10.7	9.5	27.1
S-	17-Hydro[illegible]yprogesterone	1[illegible].6	5[illegible].4	9.8	14.0	30.2
S-	5'Nucle[illegible]ase	2[illegible].2	[illegible]	11.6	7.6	26.8
U-	5'-Hidro[illegible]dolacetate, concentration, 24 h	2[illegible].3	3[illegible].2	10.2	9.7	26.5
S-	α1-Acid Glycoprotein	11.3	24.9	5.7	6.8	16.2

Unpredictable biological variation

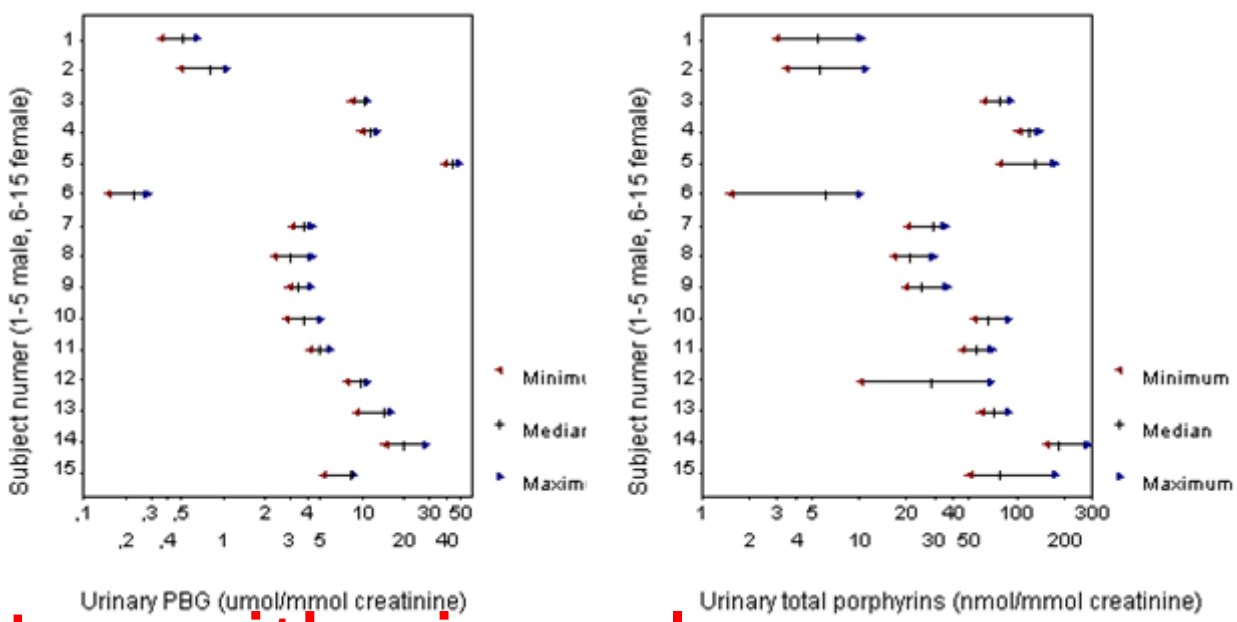
Example: Patients with Acute Intermittent Porphyria

Urine-PBG: Urine-Porphyrins

Porphobilinogen
µmol/mmol creatinine

Patients

10 weeks



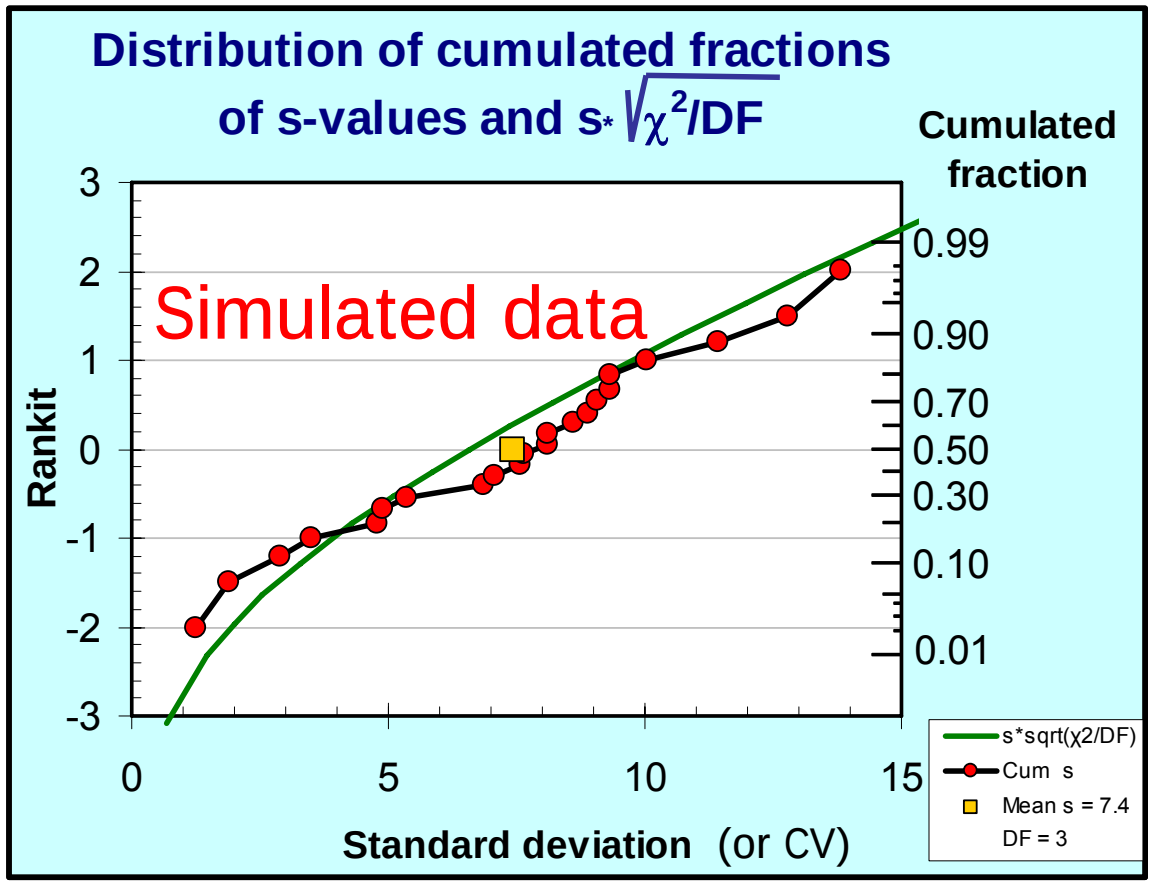
Logarithmic scale

Aarsand *et al.*
Clin Chem
2006;52:650-6

Distribution of homogeneous standard deviations

Cumulated frequency of standard deviations

Theoretical distribution of standard deviations according to χ^2 for DF = 3 when pooled s = 7.4



Distribution of homogeneous standard deviations

In order to use the pooled $CV_{within\text{-}subject}$ for calculation of RCV

The CV-values must be distributed homogeneously

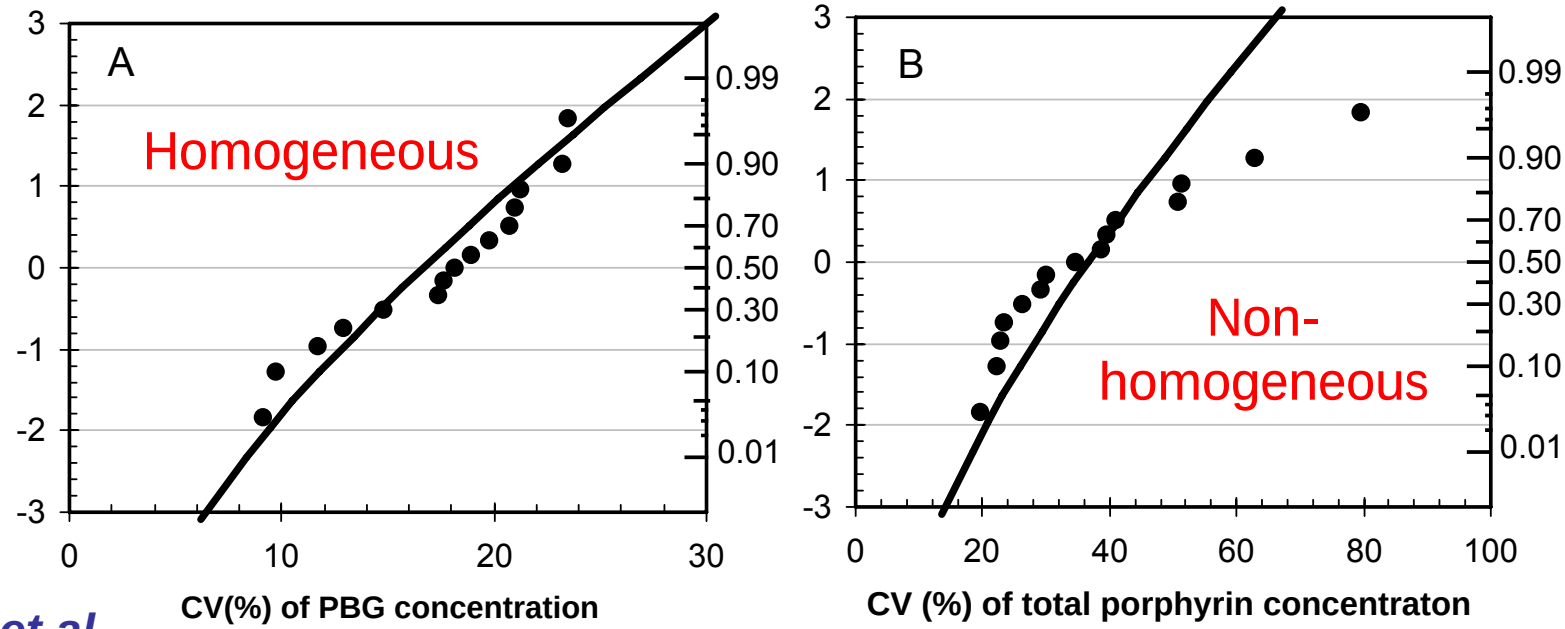
Otherwise the $CV_{within\text{-}person}$ must be estimated for each individual

Distribution of coefficients of variation

Patients with Acute Intermittent Porphyria

PBG: Porphobilinogen

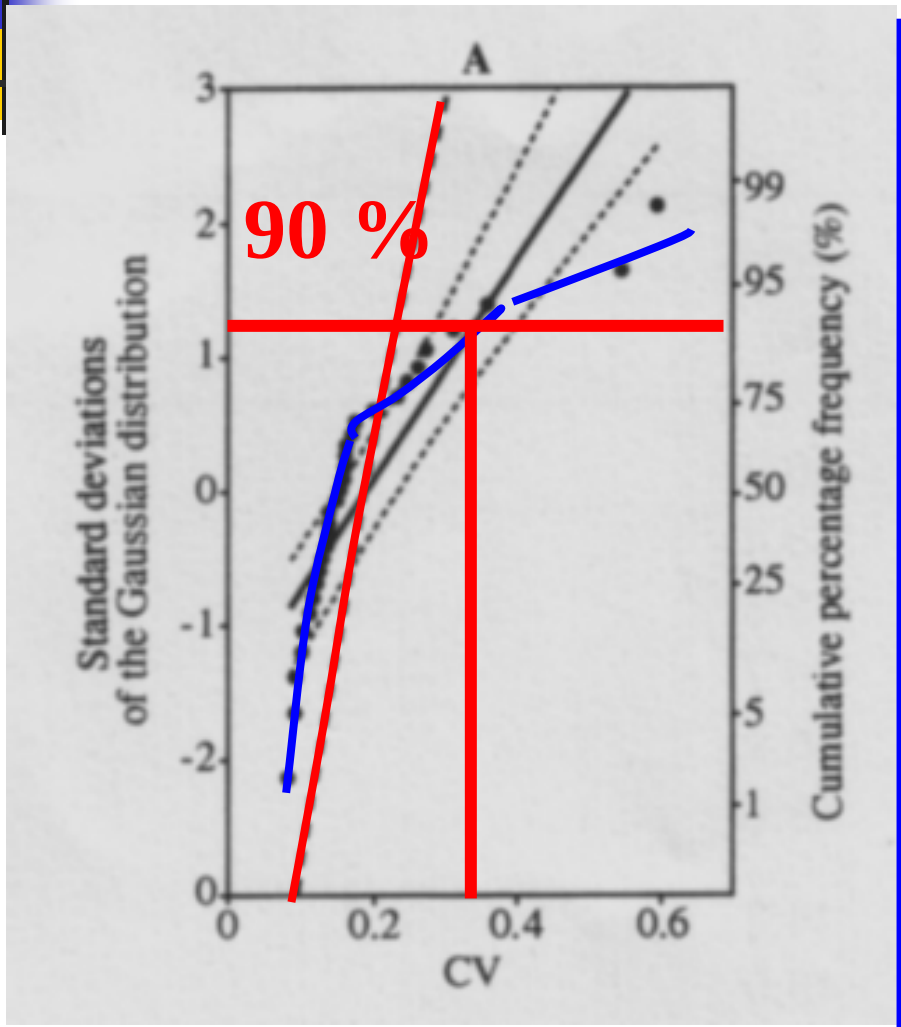
Porphyrins



Aarsand *et al.*
Clin Chem
2006;52:650-6

CV-values

Non-homogeneous variances



The tumour-marker CA 125 in all healthy women:

Distribution of CV-values demonstrates non-homogeneity of variances (at least two distributions)

Tuxen *et al.*

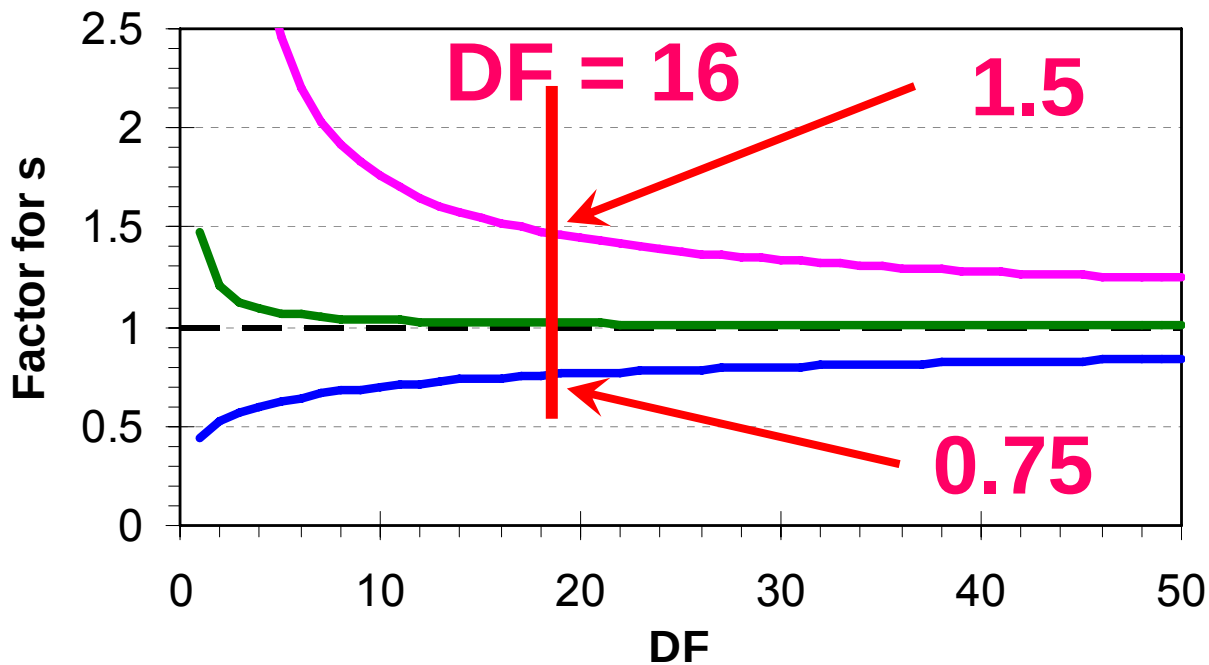
Clin Chem Lab Med 2001; 39:531-8

Variation of CV (Degrees of Freedom (DF))

Confidence interval for sigma, σ (or CV)

$$DF*s^2/\chi_2^2 < \sigma^2 < DF*s^2/\chi_1^2$$

95% CI for calculated standard deviation, s = 1.0



What is a coefficient of variation?

$$CV = \sigma/\mu \quad \text{or } CV\,\% = 100*\sigma/\mu$$

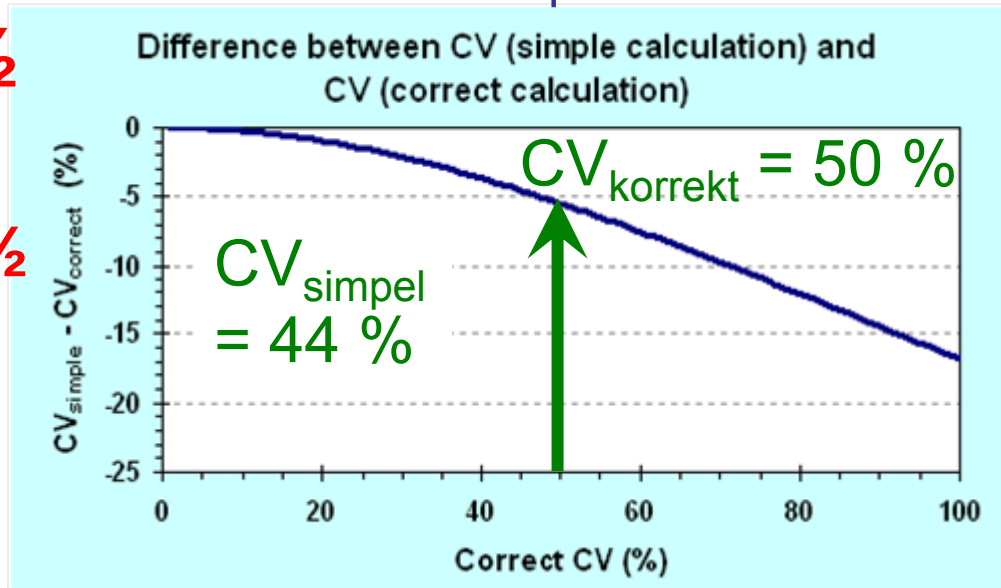
Simple CV-calculation $\sigma_{lnx} = CV_x$

Correct calculation

$$CV_x = (e^{\sigma_{lnx}^2}-1)^{1/2}$$

$$\sigma = (\ln(CV_x^2+1))^{1/2}$$

CV_{simple} - $CV_{correct}$



BIOLOGICAL VARIATION OF THE THYROID AUTOANTIBODIES

24 fertile women

One menstrual cycle, mean 9 samples (7 to 11)

Harmonised:

1. ***According to first day in menstrual cycle***
2. ***Length of cycle adjusted to 28 days***
3. ***Samples grouped in periods of four days***

Normalised:

By division of individual values from each women by the mean of all values from the same woman

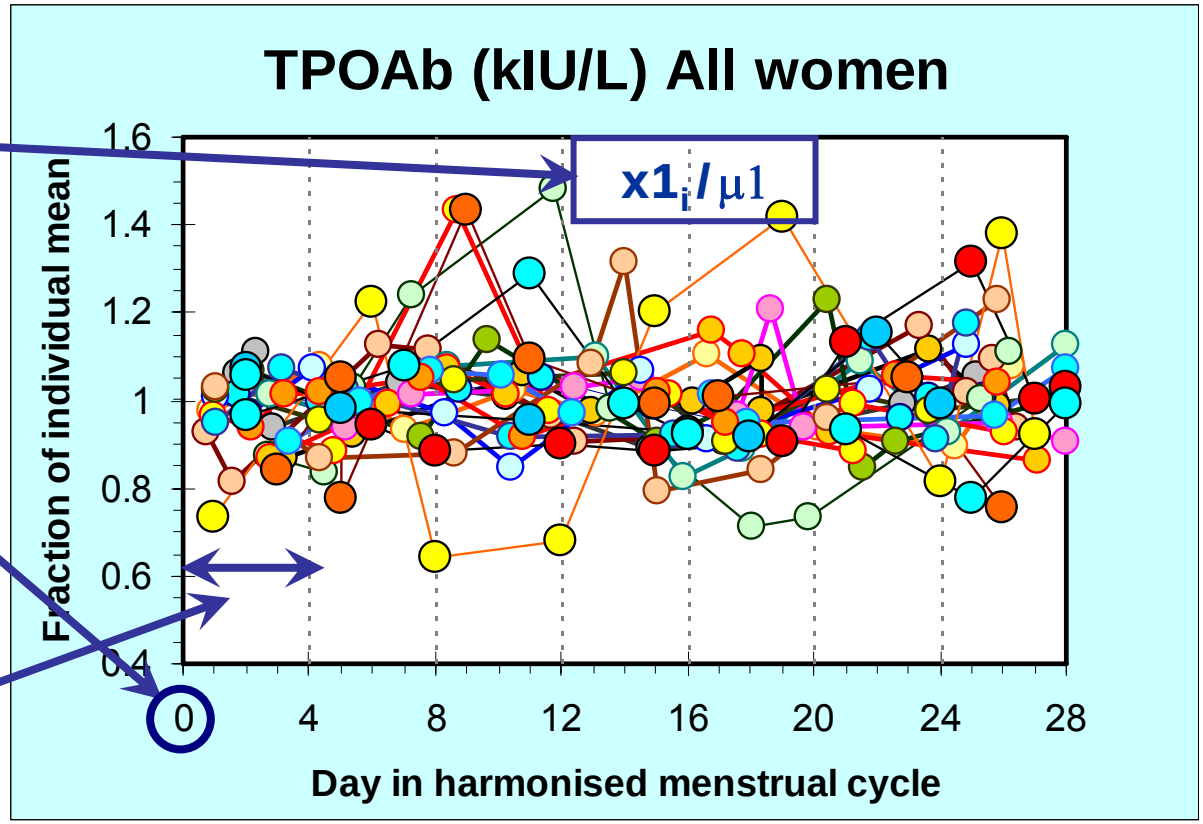
BIOLOGICAL VARIATION OF THE THYROID AUTOANTIBODIES

Normalisation:

Each value is divided by the mean of same woman

Harmonisation of days in menstrual cycle

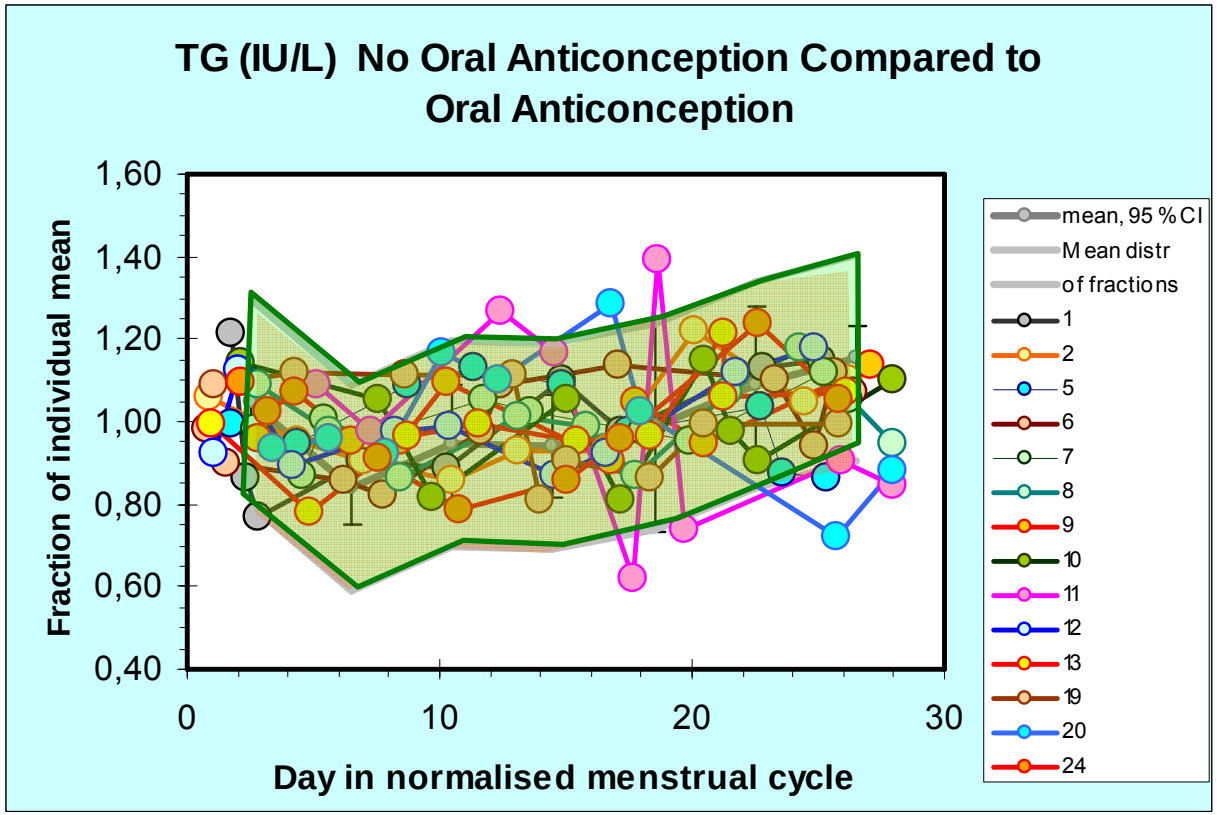
Groups of four days



Jensen et al. ***Clin Chem Lab Med 2007;45:1058-64***

Biological variation of Thyroglobulin

Six women using estrogens as anticonception



Jensen et al. ***Clin Chem Lab Med 2007;45:1058-64***

BIOLOGICAL VARIATION OF THE THYROID AUTOANTIBODIES

Biological variation of TPOAb and TGAb in fertile women harmonised according to menstrual cycle

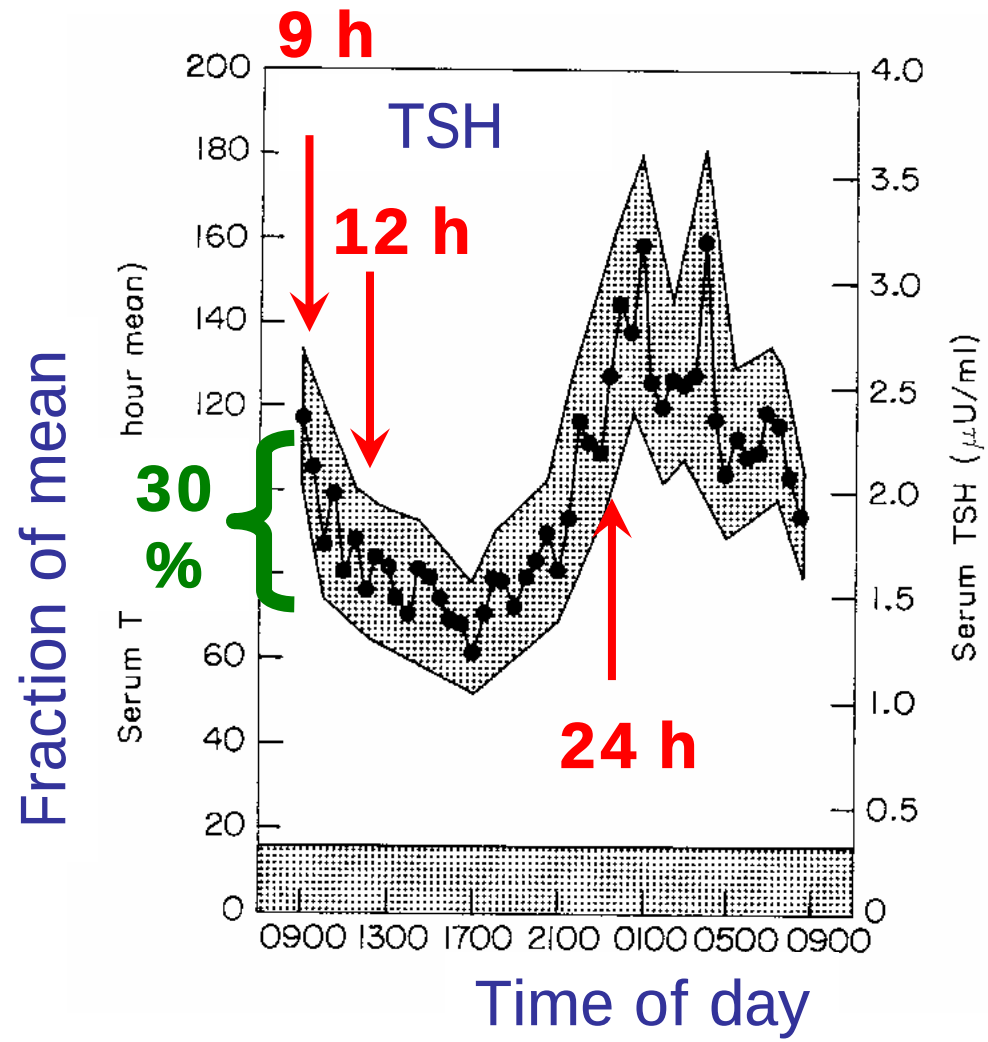
Component	$CV_{between-group}$	$CV_{within-group}$
TPOAb (n = 24)	1.2 %	13.0 %
TGAb (n = 24)	1.8 %	10.3 %
TG (n = 21 ('all'))	2.7 %	17.9 %
TG (n = 6 (+E))	9.6 %	12.5 %

Jensen et al. *Clin Chem Lab Med 2007;45:1058-64*

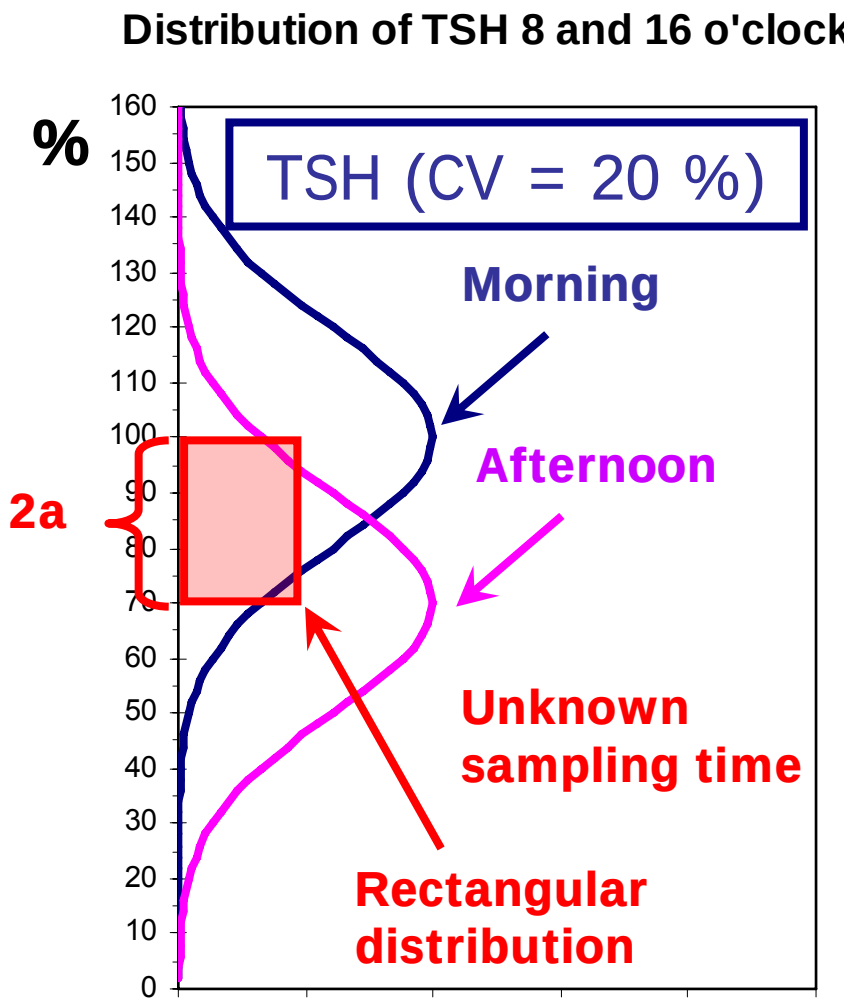
Diurnal variation

Daily rhythm of serum TSH in healthy euthyroid subjects

Fraser CG. Interpretation of clinical chemistry laboratory data, Blackwell Scientific Publications.1986



Diurnal variation



Rectangular distribution:

$s \sim a/3^{½}$ or

$CV_R = 15\ \% / 3^{½}$

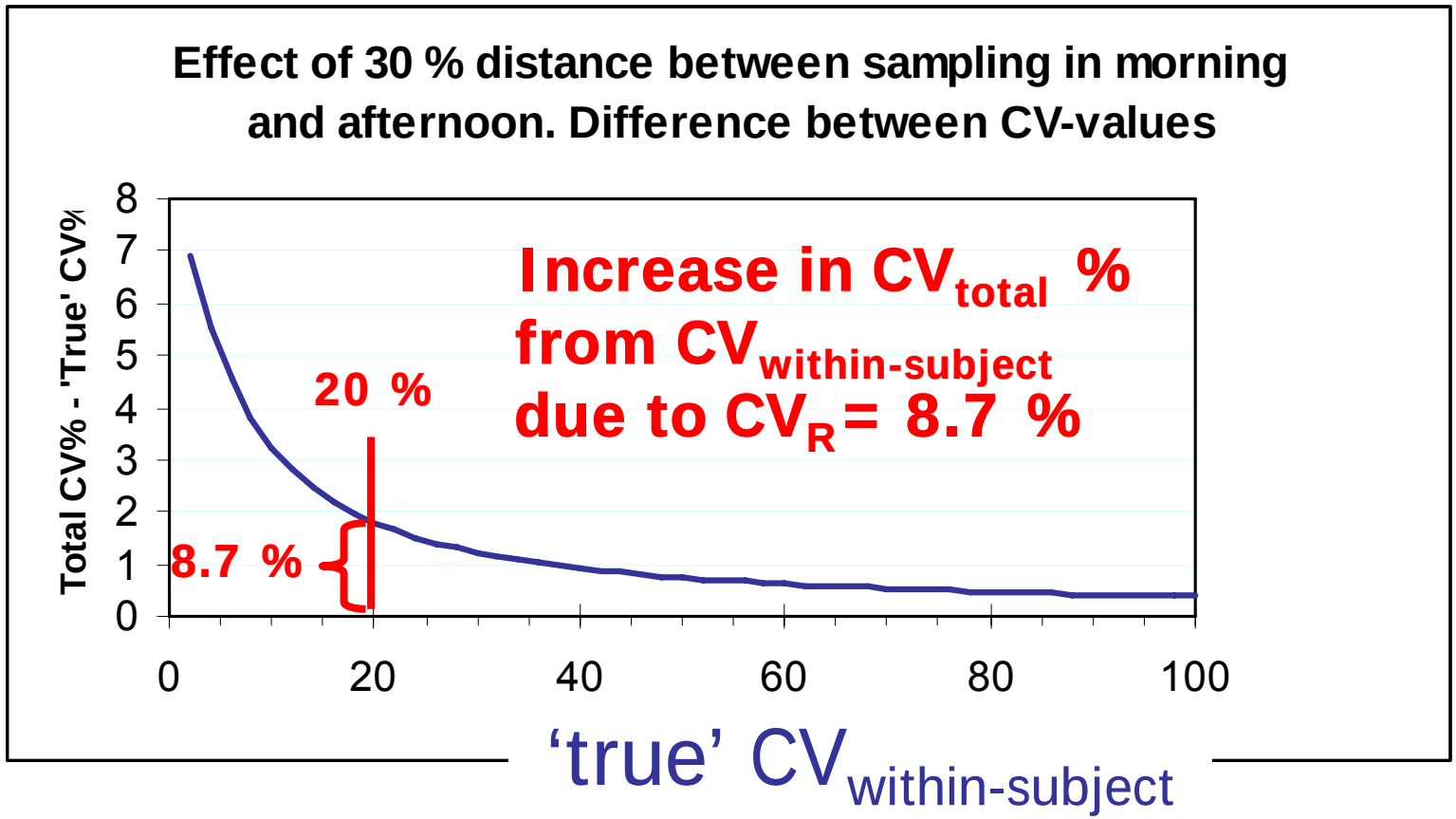
$= 8.7\ \%$

$CV_{total}^2 = CV_{within\text{-}subject}^2 + CV_R^2 = 20\ \%^2 + 8.7\ \%^2 = 22\ \%^2$

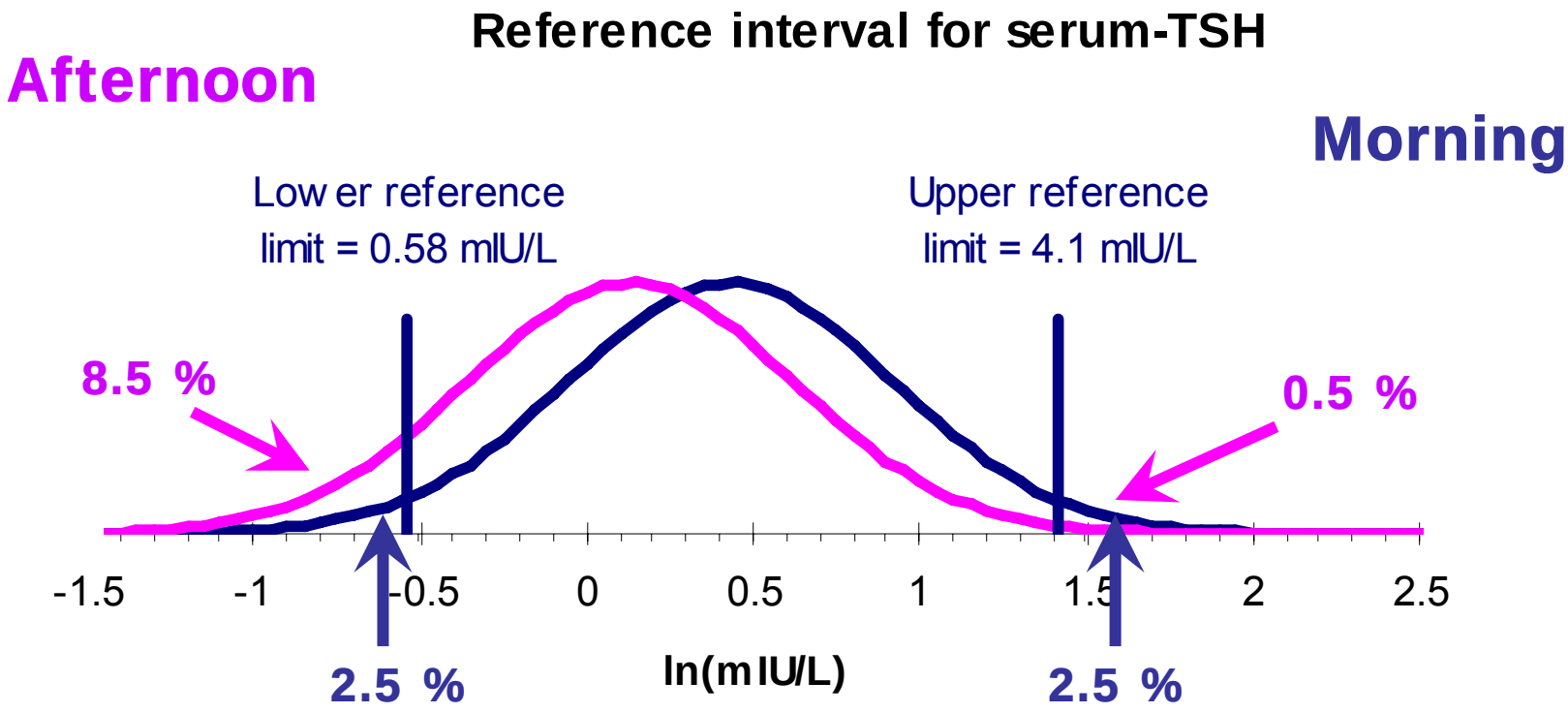
$CV_{total} = 22\ \%$

Diurnal variation

TSH

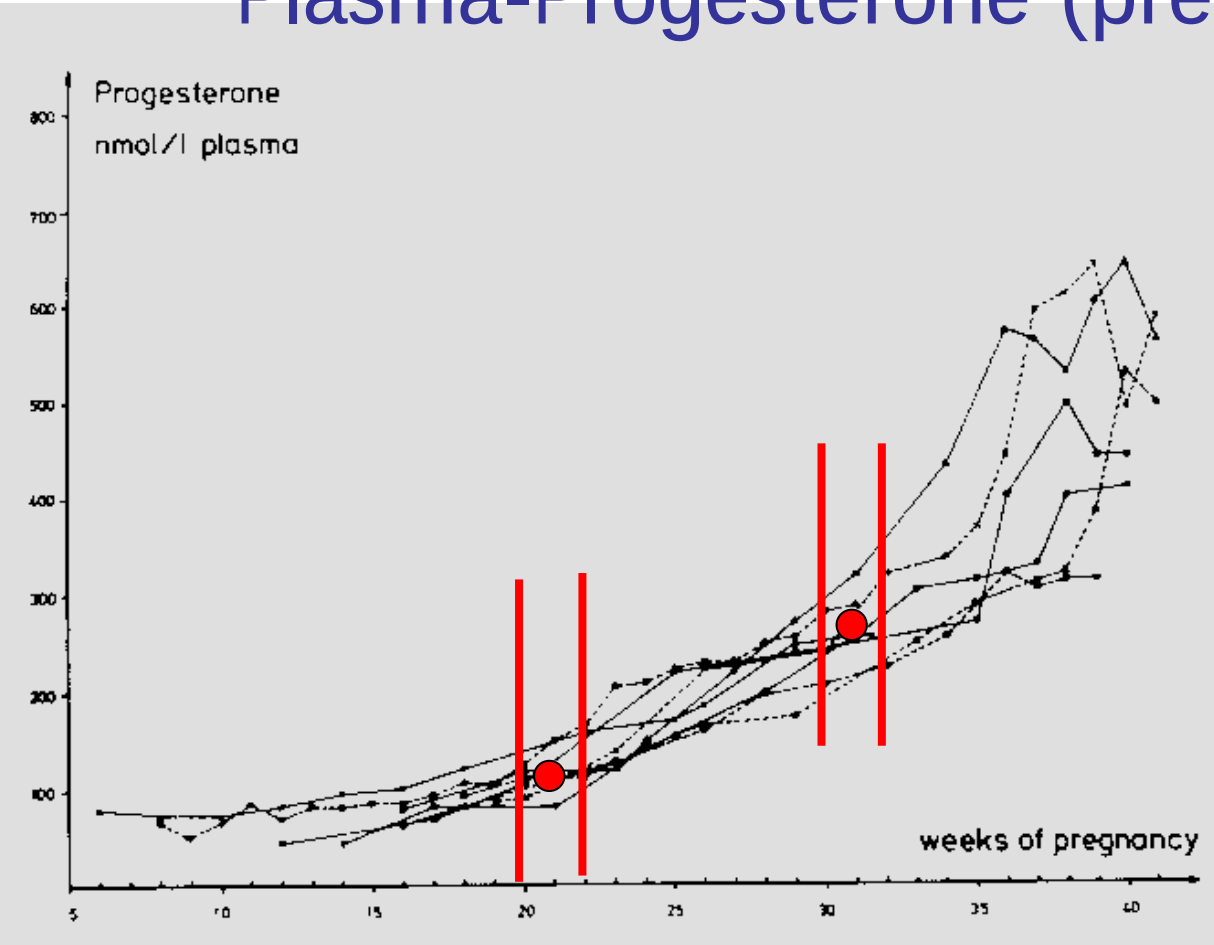


Diurnal variation and reference interval for TSH



MOM: Multiple Of Median

Plasma-Progesterone (pregnancy)



Interval

Median of interval

All points within the interval are divided by the median: MOM

Same procedure

Results are now normalised

Winkel et al. Clin Chem 1976;22:422-8

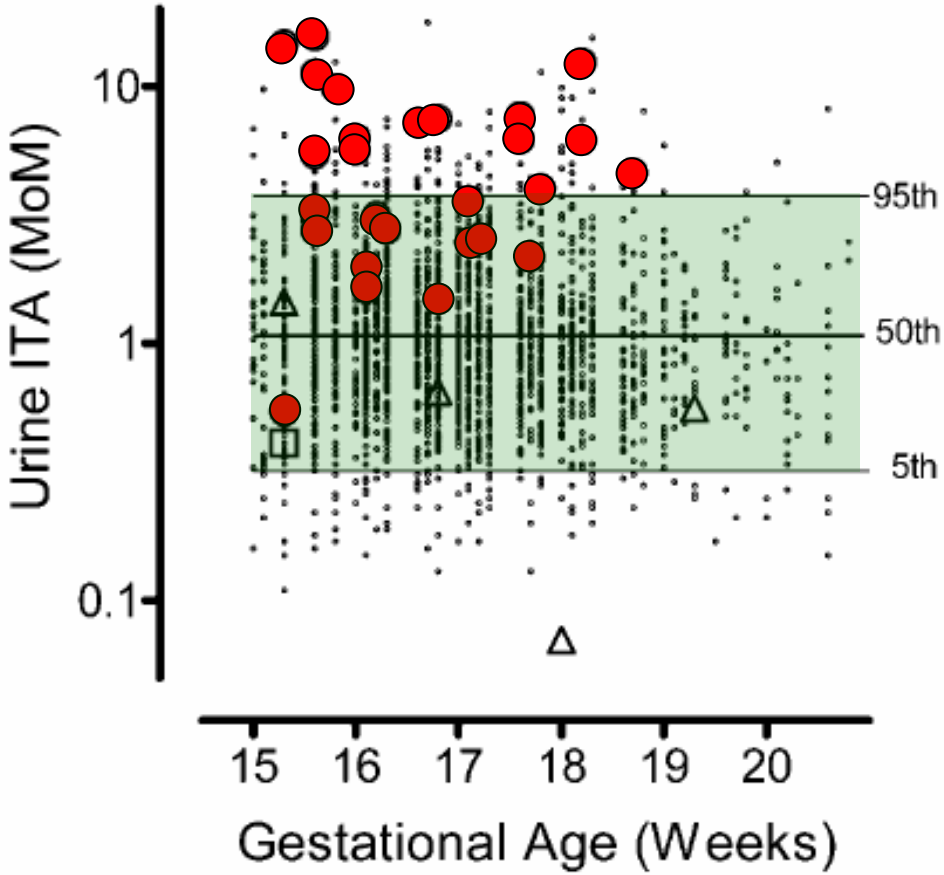
MOM: Multiple Of Median

Urine-ITA: Invasive trophoblast antigen

[Hyperglycosylated human chorionic gonadotropin (hCG)]

A marker for Down Syndrome

MOM (logarithmic scale)



Palomaki et al. Clin Chem 2004;50:182-9

Diurnal variation

Monitoring by use of a1-Antitrypsin and Albumin

Healthy Person: *steady-state* (48 hours)

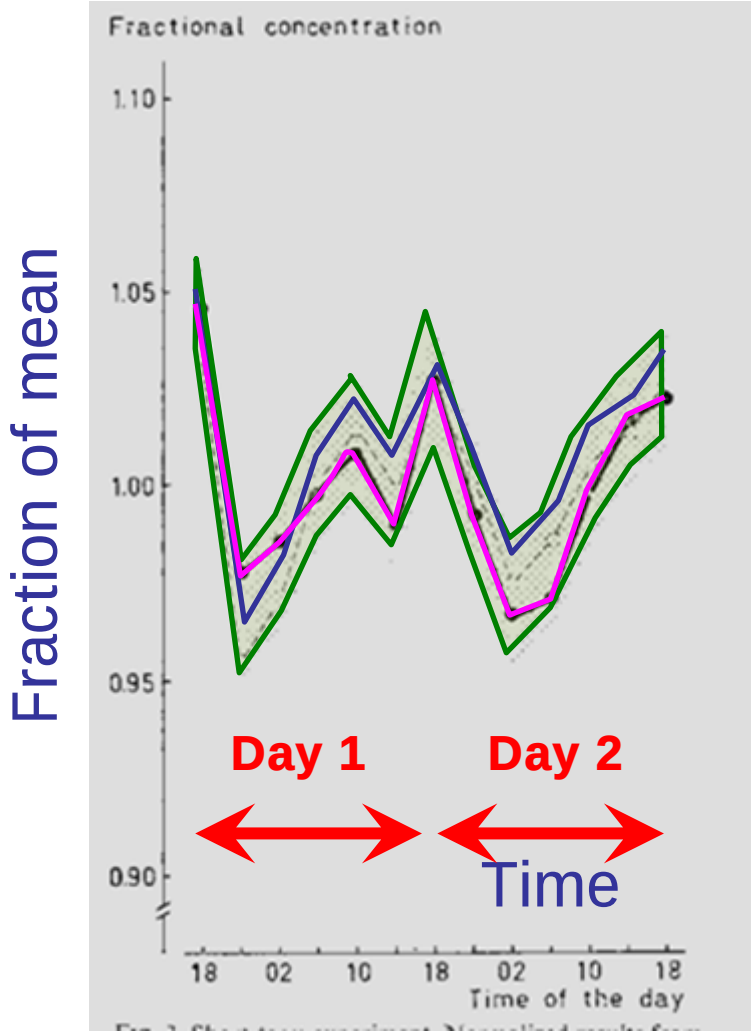
Ratio plot

Normalised data of the two proteins during 48 hours

Each protein divided by its mean of 12 values

Hatched area:

mean ± 2s(analytical)



Hyltoft Petersen et al.
***Scand J Clin Lab Invest* 1981;41:143-50**

Slovakia 2009

Acute phase reaction

Monitoring by use of α_1-Antitrypsin and Albumin

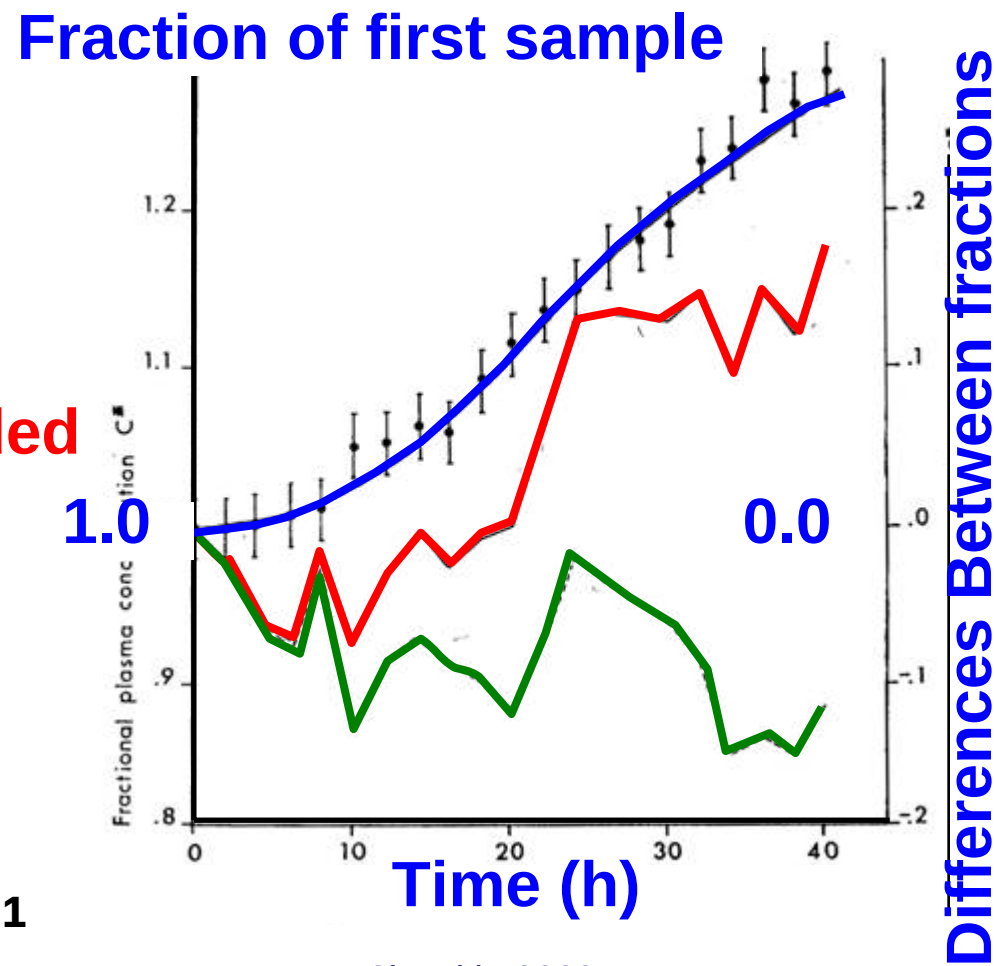
Differences
Between fractions

α_1-Antitrypsin

Each concentration divided by first conc

Albumin

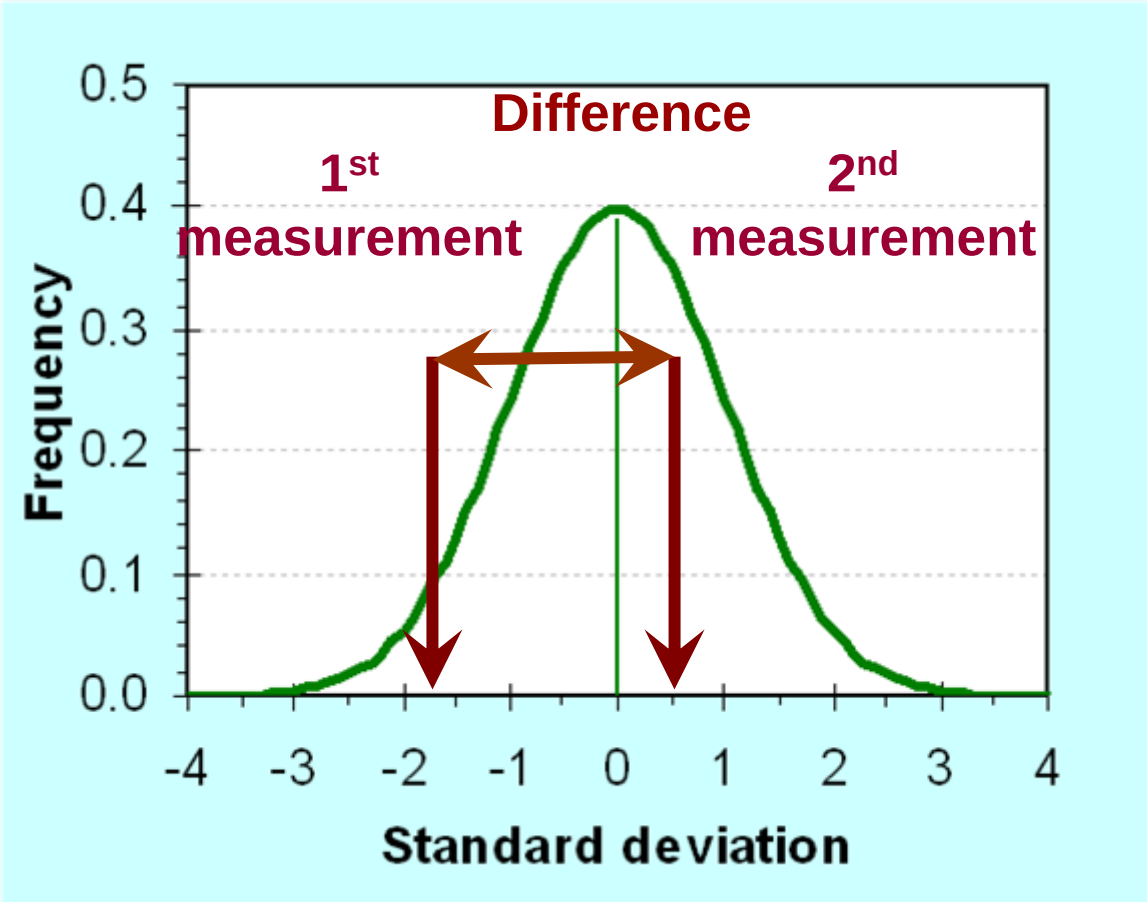
Each concentration divided by first conc



Hyltoft Petersen et al.
***J Clin Computing* 1979;8:180-201**

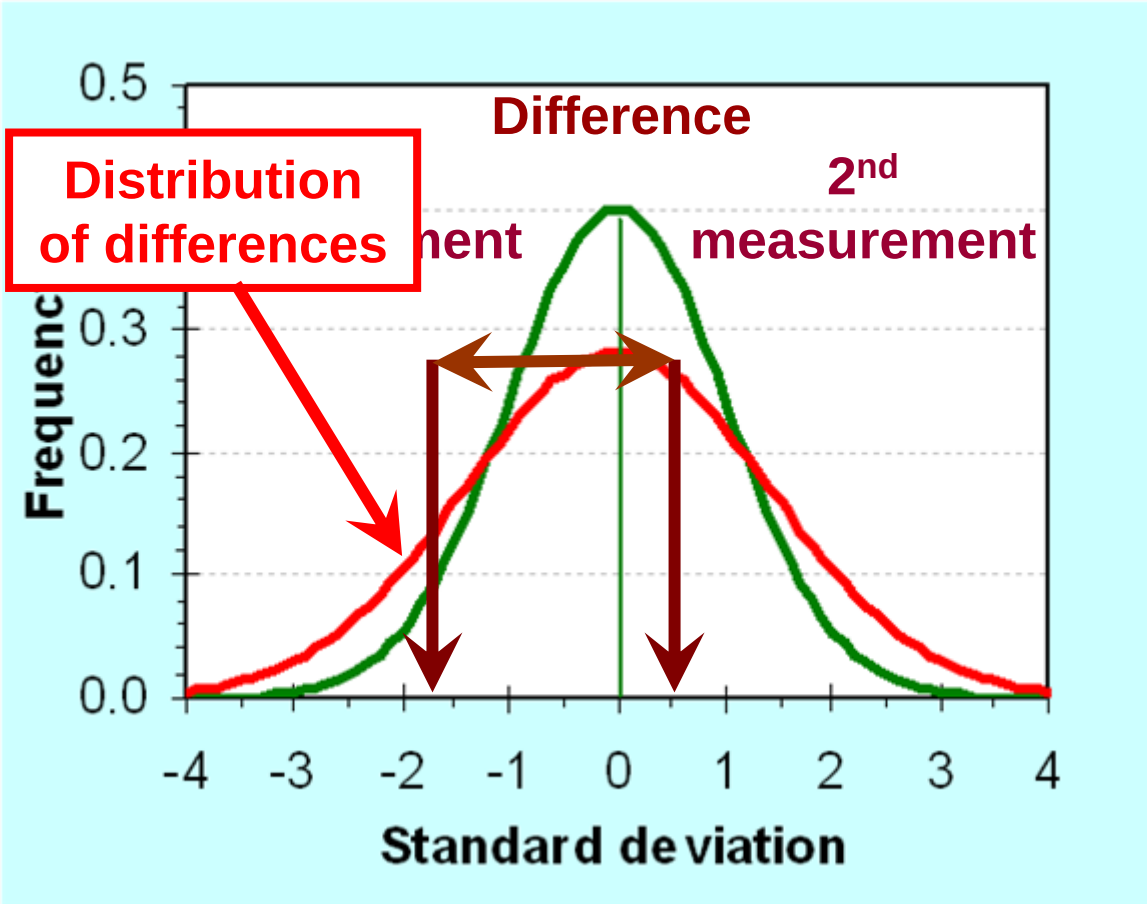
Interpretation of serial data

Distribution of measured values from a healthy individual



Interpretation of serial data

Distribution of measured differences from a healthy individual



$$s_D^2 = s_{W-S}^2 + s_{W-S}^2$$

$$s_D = 2^{1/2} * s_{W-S}$$

Interpretation of serial data

Reference Change Value (RCV)

$$RCV = \boxed{z} * \boxed{2^{1/2}} * CV_{W\text{-}S}$$

$$= \boxed{1.96} * 2^{1/2} * CV_{W\text{-}S}$$

$$= \boxed{2.77} * CV_{W\text{-}S} \quad = 1.96 * CV_D$$

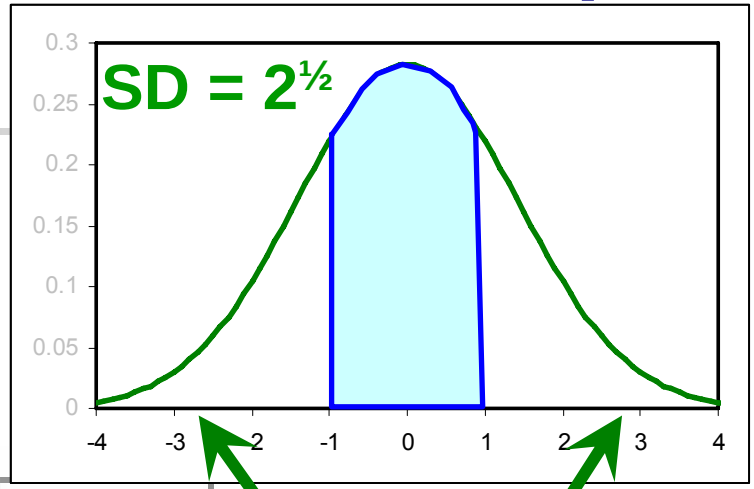
Probability 5 % (two tailed)

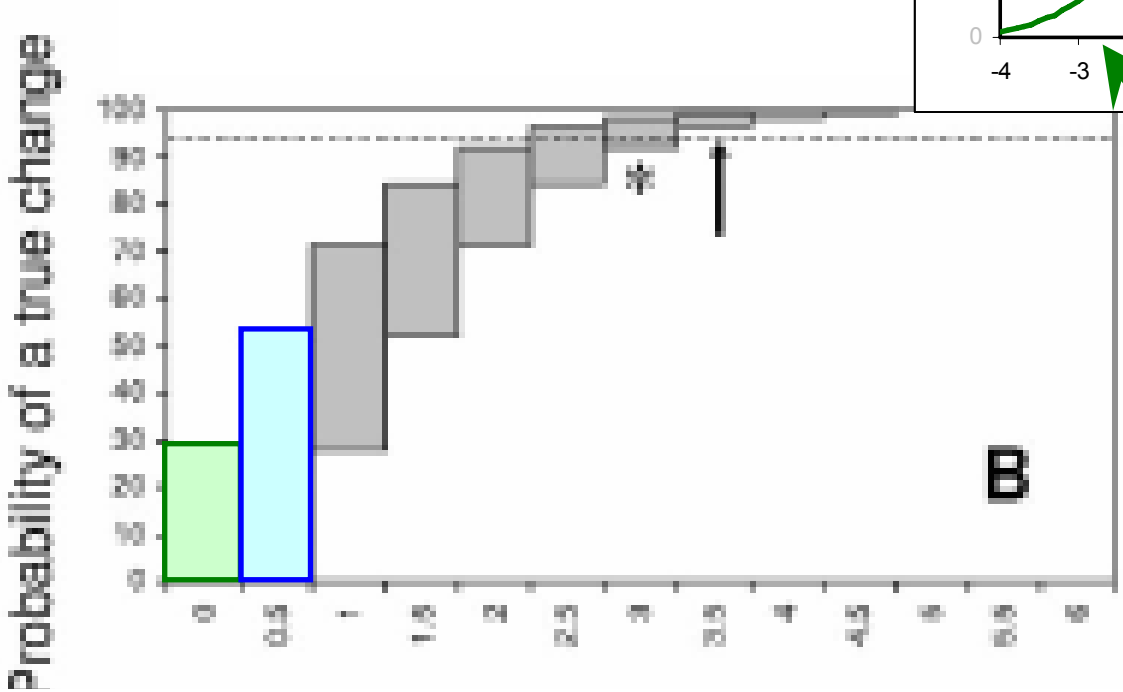
$$CV_D = \sqrt{CV_{W\text{-}S}^2 + CV_{W\text{-}S}^2} = \sqrt{2} * CV_{W\text{-}S}$$

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

RCV (number of reported decimals)

Reporting Interval Size
= 0.5*SD



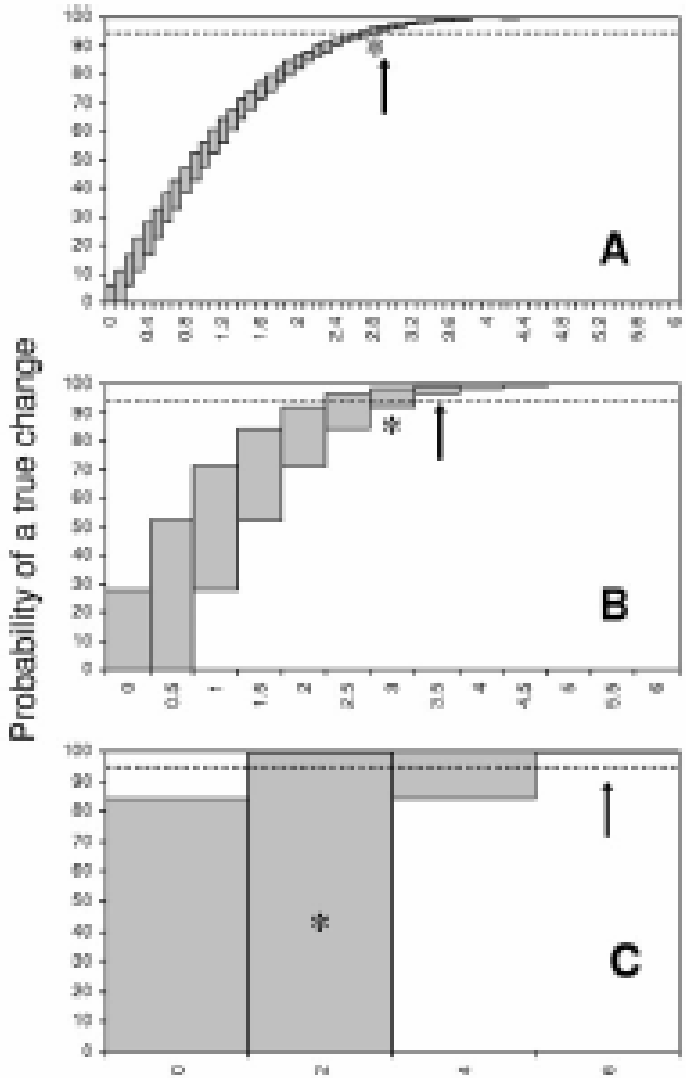


$\pm 1.96 * 2^{1/2} * SD_{w-s}$
$= 2.77 * SD_{w-s}$

Jones R.D.G.

Clin Chem 2006;52:880-5

RCV (number of reported decimals)



Reporting Interval Size = 0.1*SD

Reporting Interval Size = 0.5*SD

Reporting Interval Size = 2.0*SD

Jones R.D.G.

Clin Chem 2006;52:880-5

Interpretation of serial data

When is a measured difference reflecting a real change?

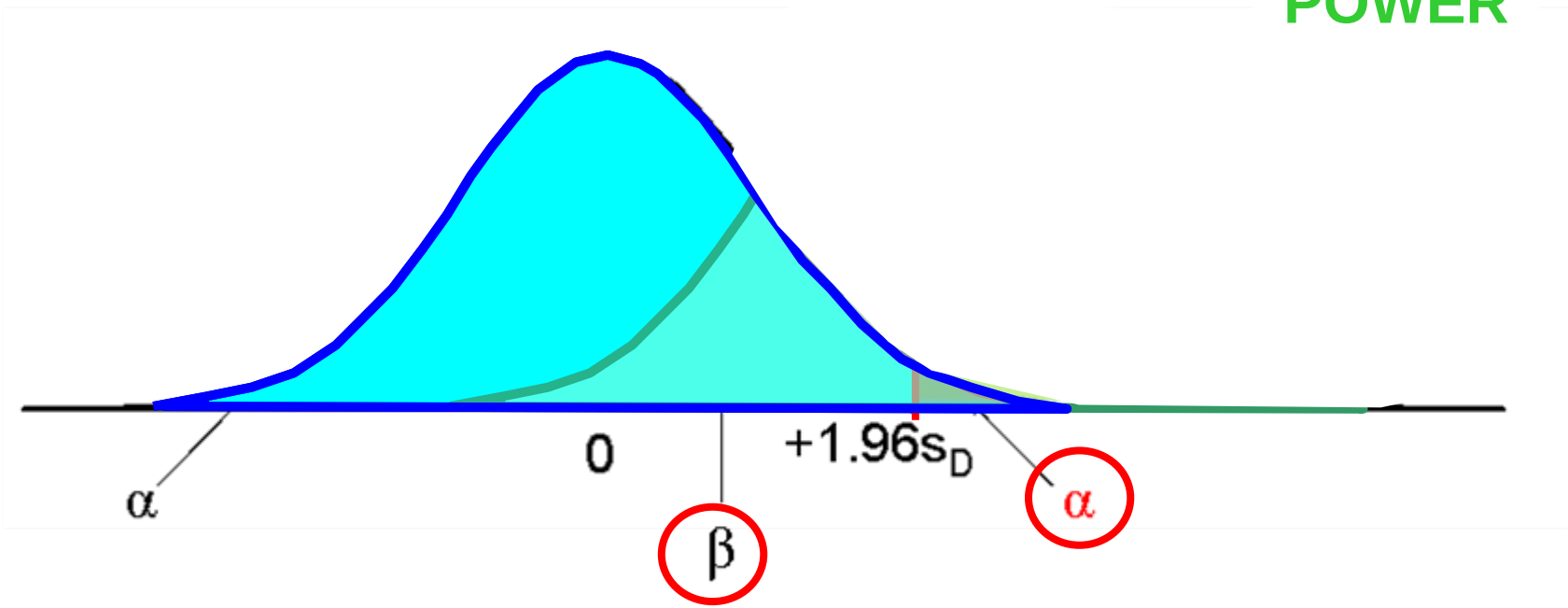
and when is it just random variation?

Interpretation of serial data

No change

Change = RCV

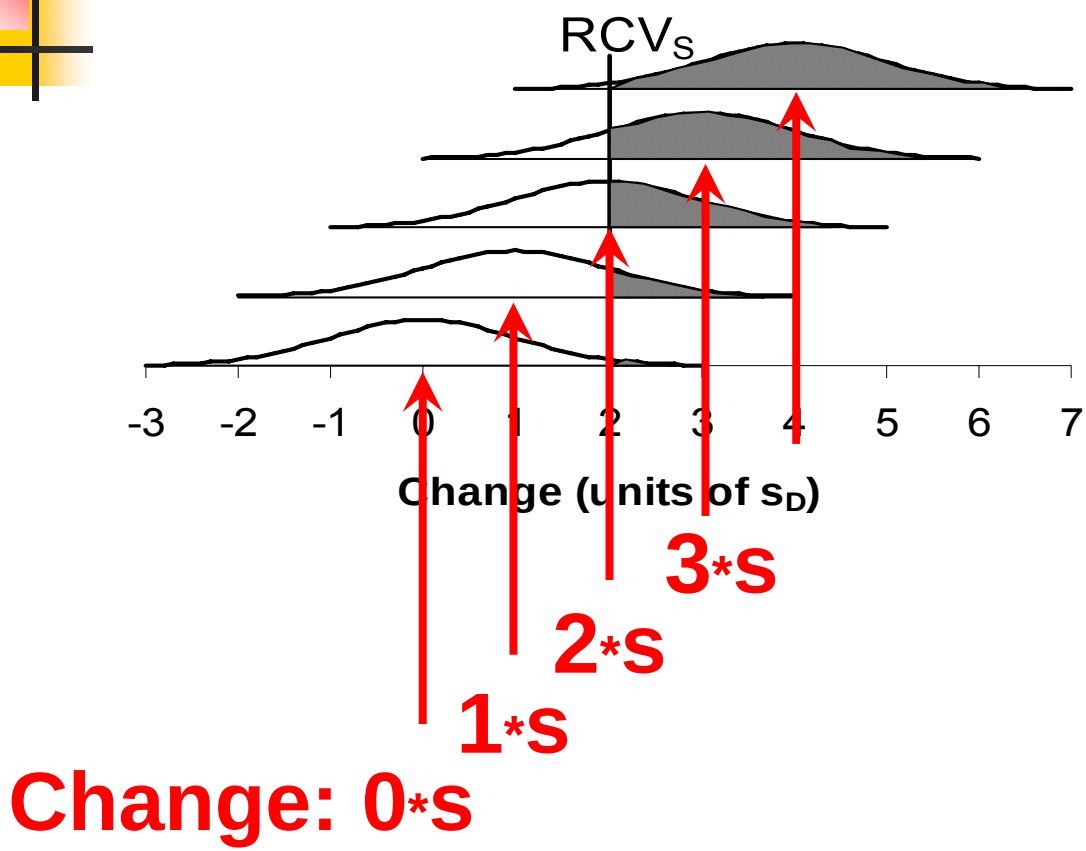
POWER



$$s_D = 2^{1/2} * s_{W-S} \qquad RCV = 1.96 * s_D = 1.96 * 2^{1/2} * s_{W-S}$$

Iglesias Canadell *et al*. *Clin Chem Lab Med* 2004; 42:415-22.

Power function

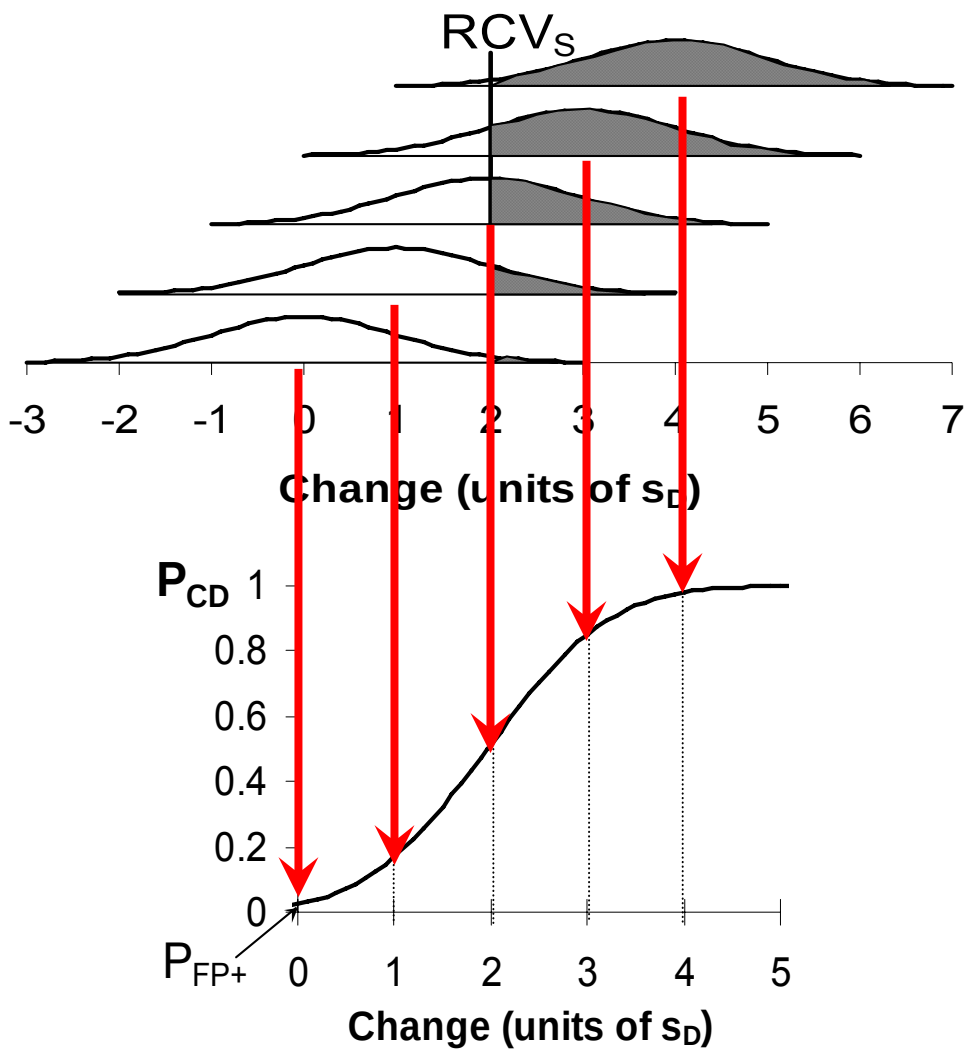


Changes	P_{CD} (1–β)
+4	97.9%
+3	85%
+2	52%
+1	17%
0	2.5% (P_{FP+})

Iglesias Canadell *et al*.

***Clin Chem Lab Med* 2004; 42:415-22.**

Power function

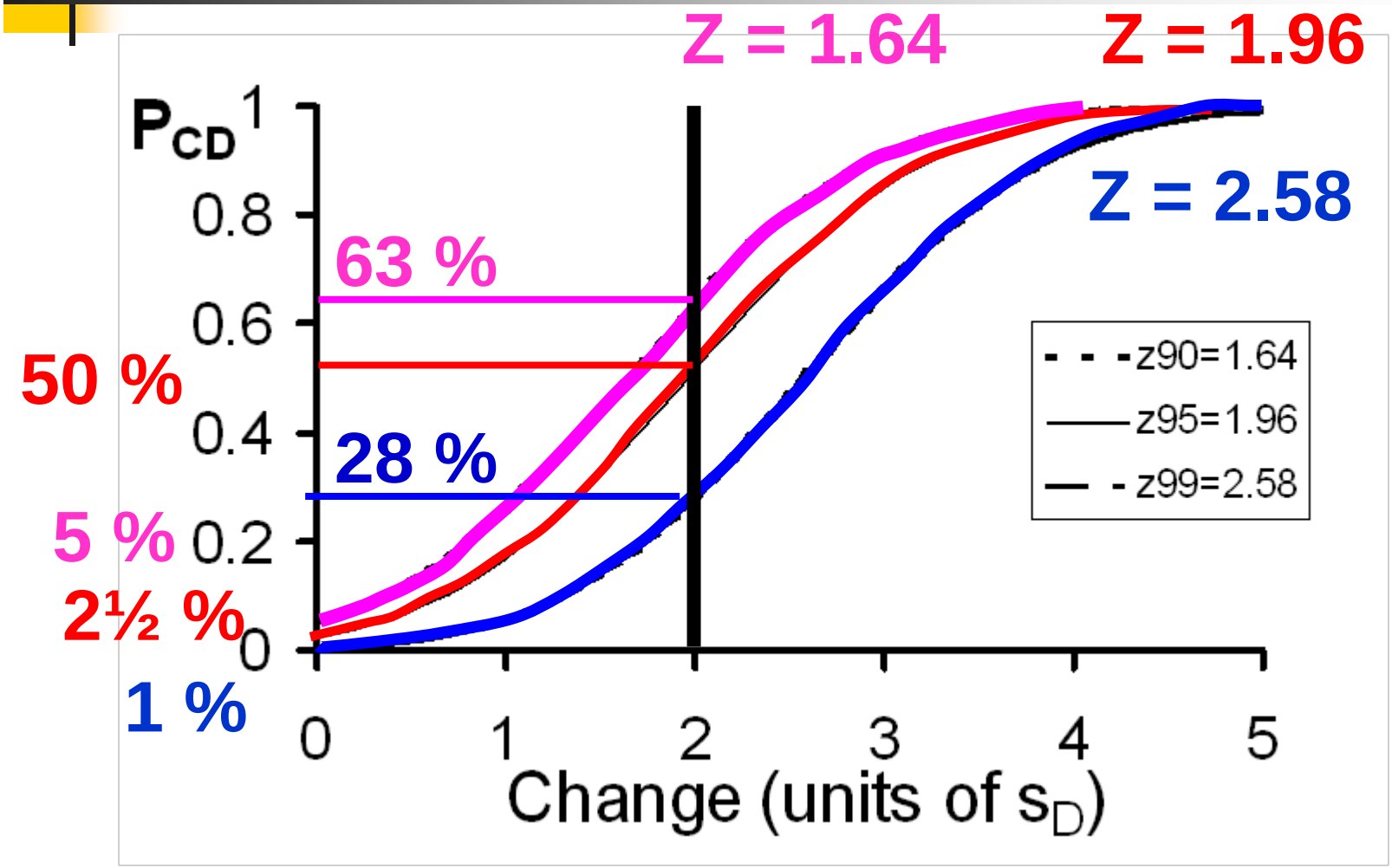


Changes	P_{CD} $(1-\beta)$
+4	97.9%
+3	85%
+2	52%
+1	17%
0	2.5% (P_{FP+})

Iglesias Canadell *et al*.

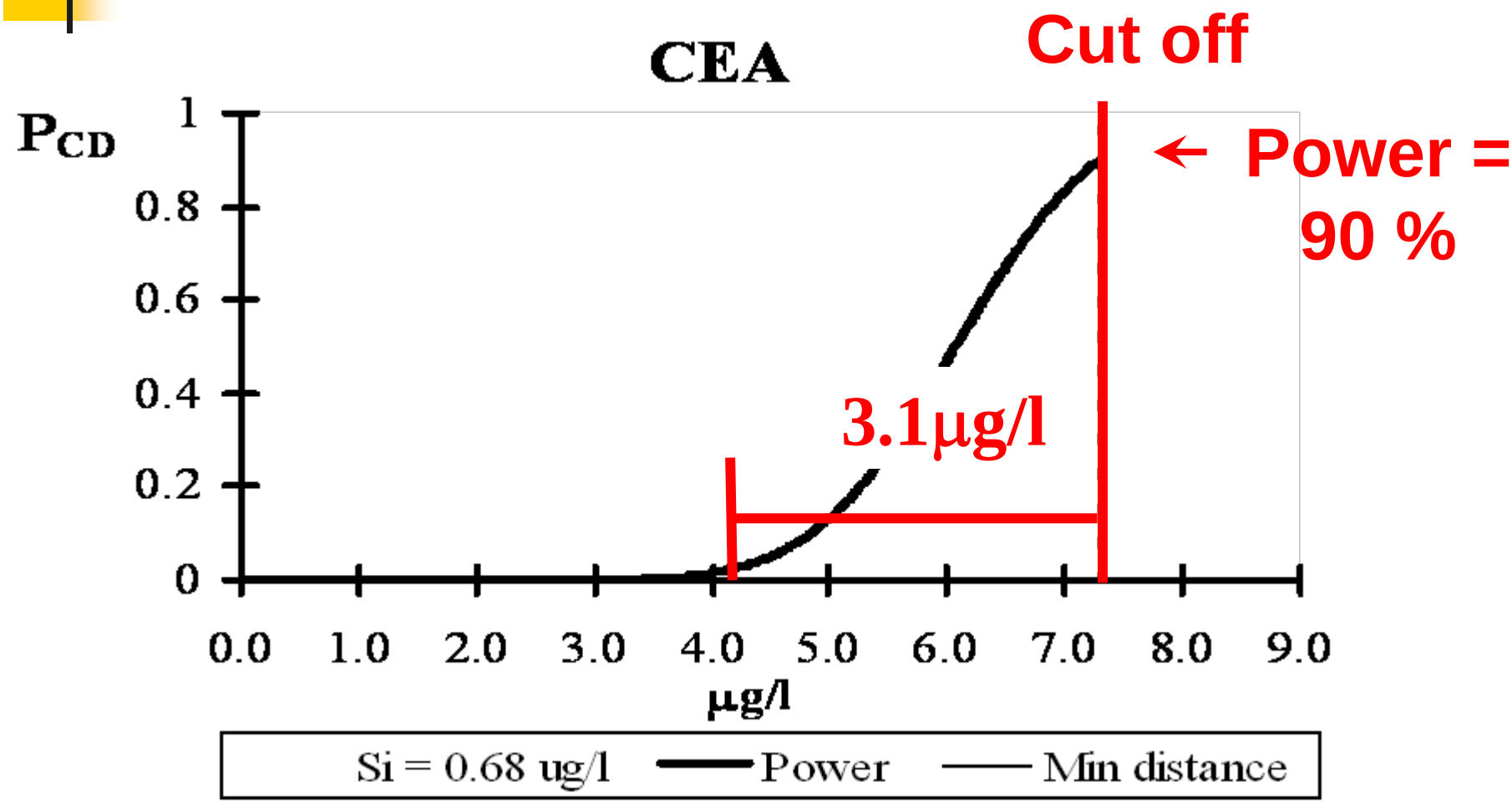
***Clin Chem Lab Med* 2004; 42:415-22.**

Diferent reference change values



Iglesias Canadell *et al*. *Clin Chem Lab Med* 2004; 42:415-22.

Power function for CEA



Iglesias Canadell *et al*. *Clin Chem Lab Med* 2005; 43:441-8

Index-of-individuality

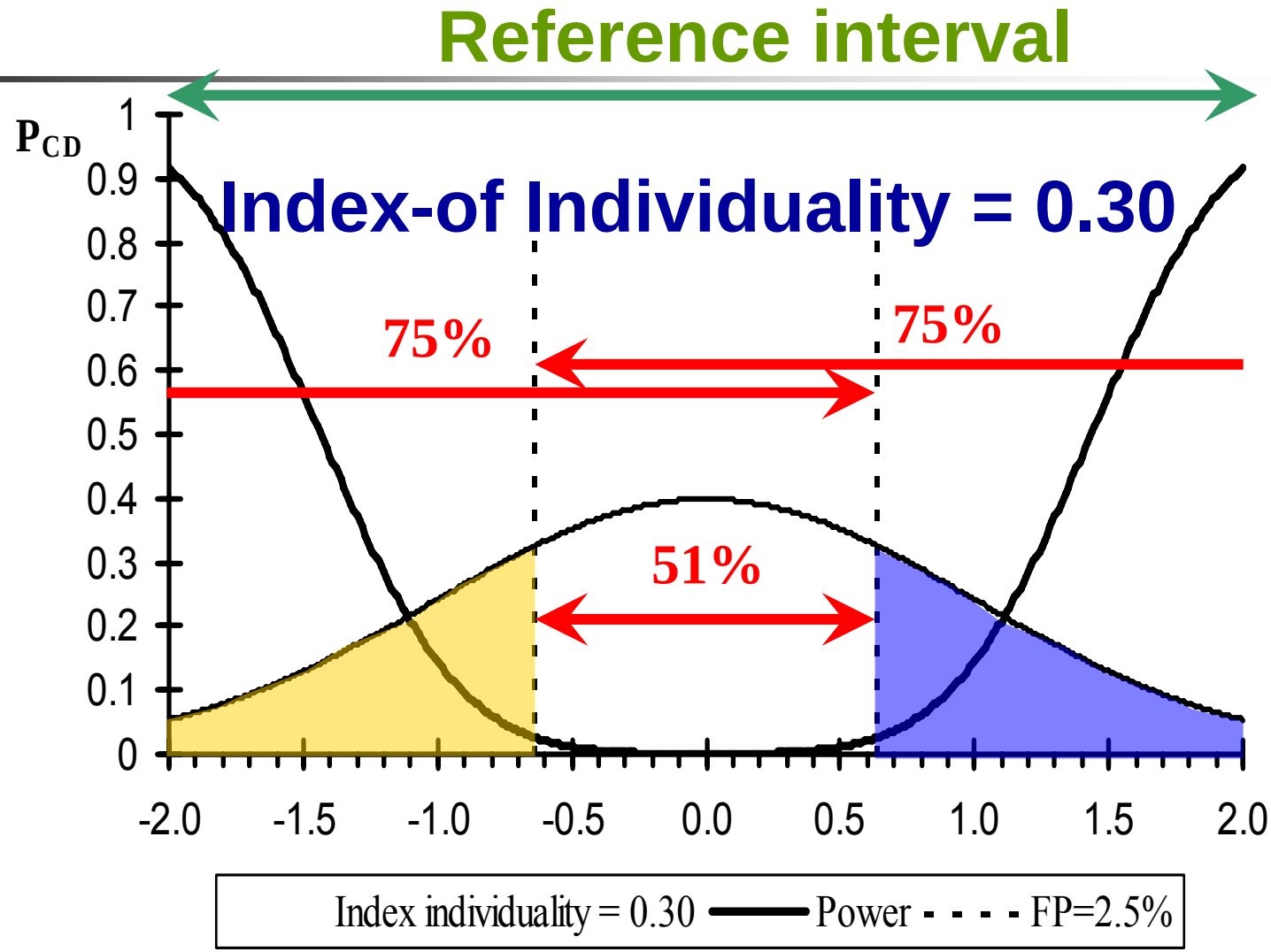
Ratio between within- and between-subject variation

Index-of-individuality:

$$(CV_{W\text{-}S}^2 + CV_A^2)^{1/2} / CV_{B\text{-}S}$$

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

RCV and reference interval



Iglesias Canadell *et al*. *Clin Chem Lab Med* 2005; 43:441-8

RCV and reference interval

Index Individuality	Power 80%		Power 90%		Power 95%	
	one way	both ways	one way	both ways	one way	both ways
0.1	95	89	94	88	93	86
0.2	89	78	86	73	84	68
0.3	81	61	75	51	70	41
0.4	70	40	62	23	54	8
0.48	61	23	50	0	42	0
0.5	59	18	48	0	39	0
0.6	49	0	36	0	27	0
0.7	39	0	27	0	18	0
0.8	32	0	19	0	12	0
0.9	26	0	14	0	8	0
1.0	21	0	11	0	5	0

Iglesias Canadell *et al*. *Clin Chem Lab Med* 2005; 43:441-8

Repeated Testing

Probability of false positive (FP)

$$P_{total} = 1 - (1 - P_{single})^n$$

$$P_{total} = 1 - (1 - 0.05)^2$$
$$= 1 - 0.95^2 = 1 - 0.9025$$
$$= 0.0975 = 9.75\ \%$$

RCV and repeated testing

$$RCV = 1.96 * 2^{1/2} * CV_{W\text{-}S}$$

P(single sample) = 5 %

P(patient) = 1- (1-P(single sample))^n

P(patient) = 9.8 % for n = 2

P(patient) = 18.5 % for n = 4

Computer simulation of monitoring

Steady-state and linear increase

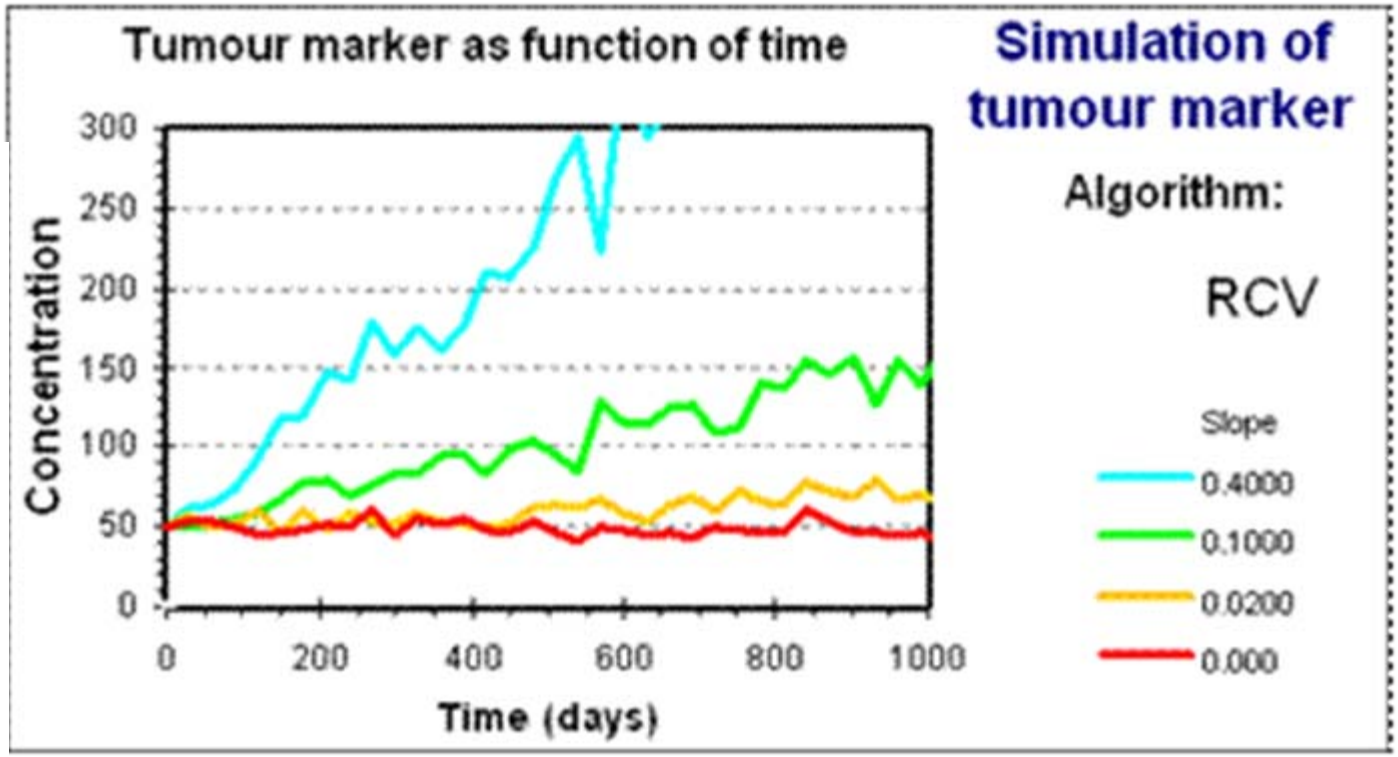
Increase

0.00 U/day

0.02 U/day

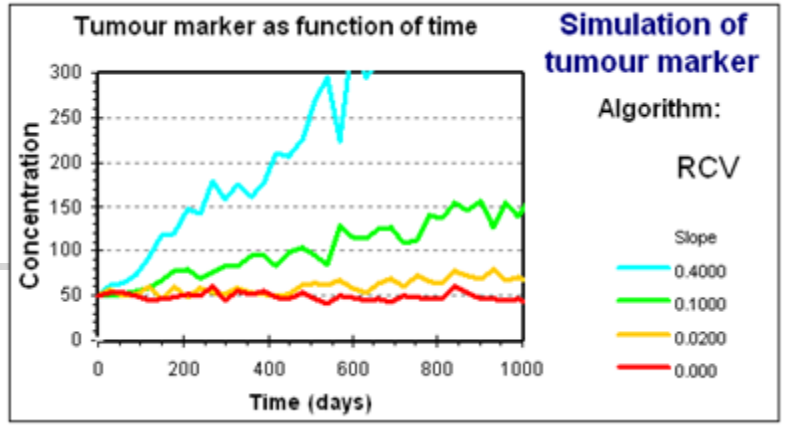
0.10 U/day

0.40 U/day



Sampling frequency

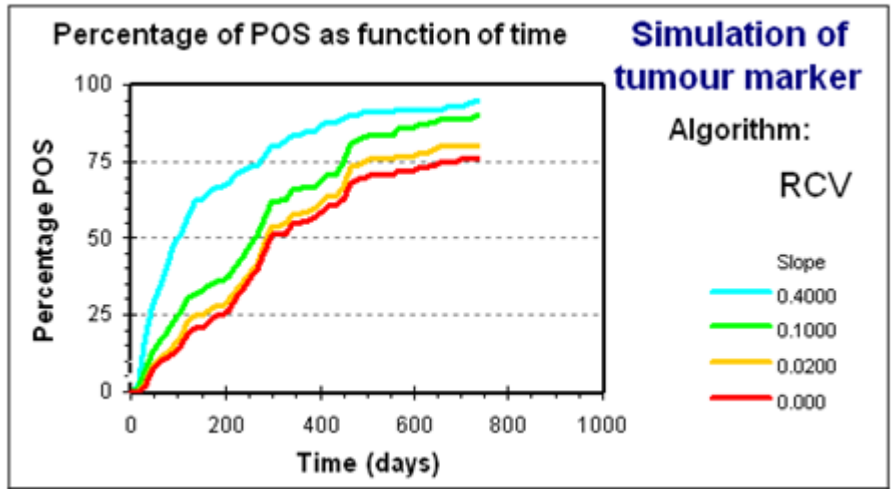
RCV (p = 5 %)



Frequent sampling: sampling / 15 days

Increased probability of FP

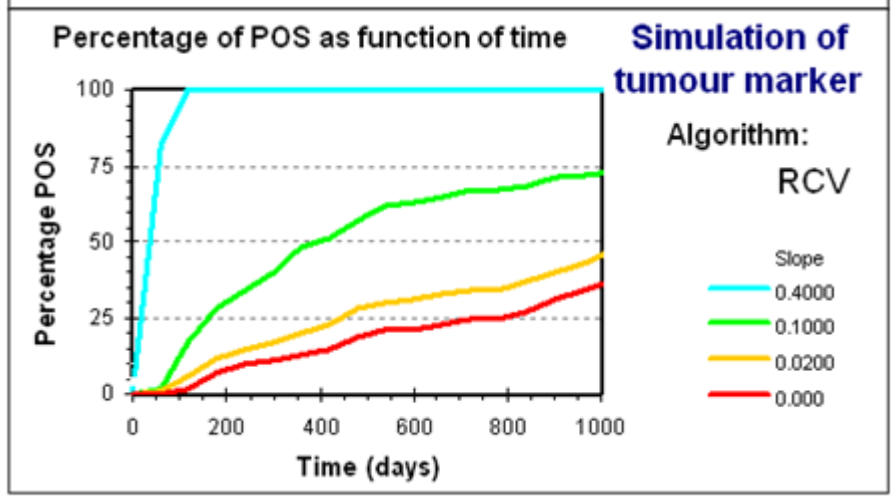
Decreased probability of TP



Infrequent sampling: sampling / 60 days

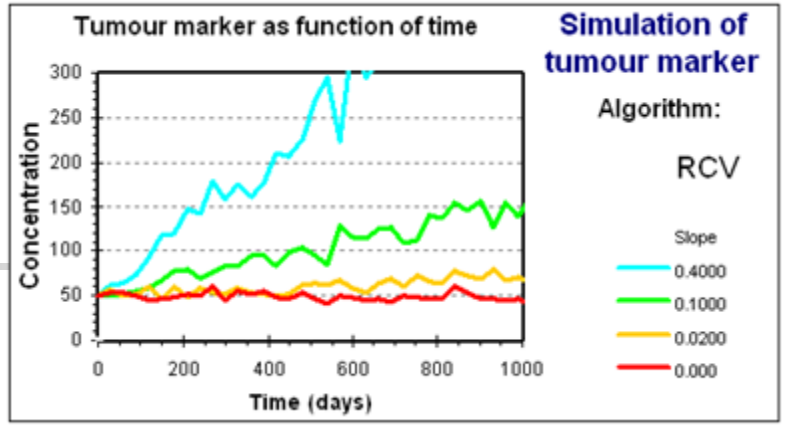
Decreased probability of FP

Increased probability of TP



Sampling frequency

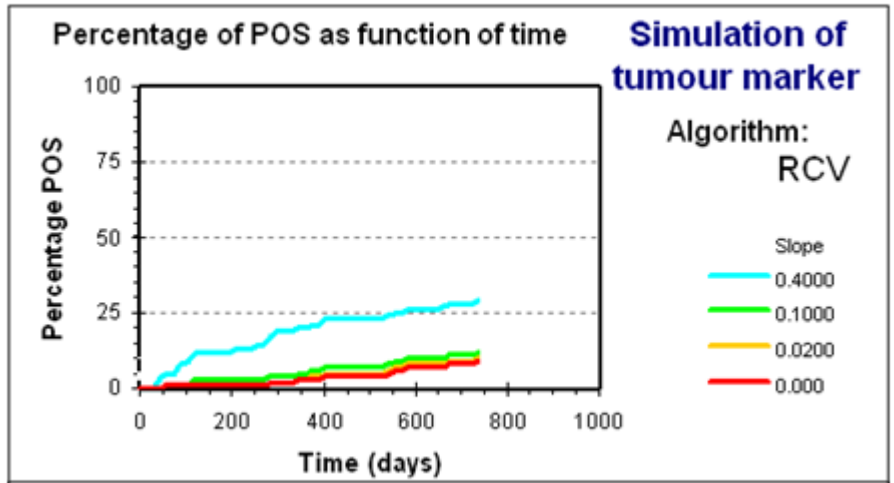
RCV (p = 0.4 %)



Frequent sampling: sampling / 15 days

Decreased probability of FP

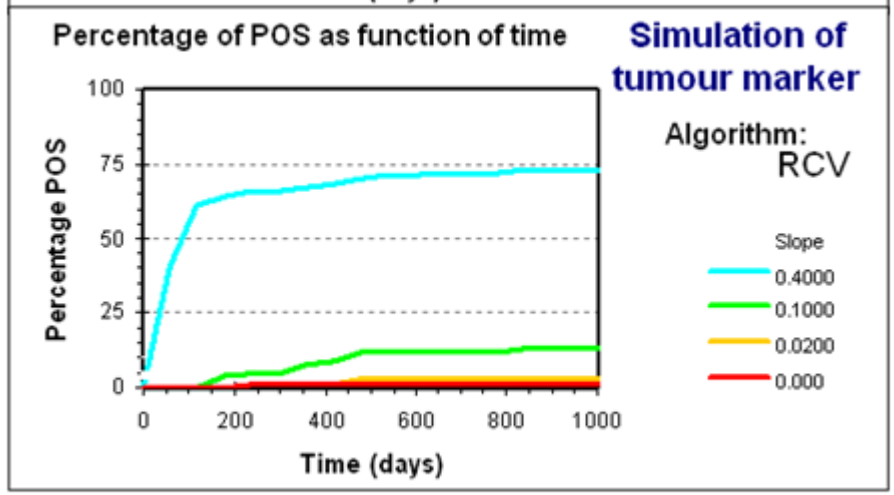
Decreased probability of TP



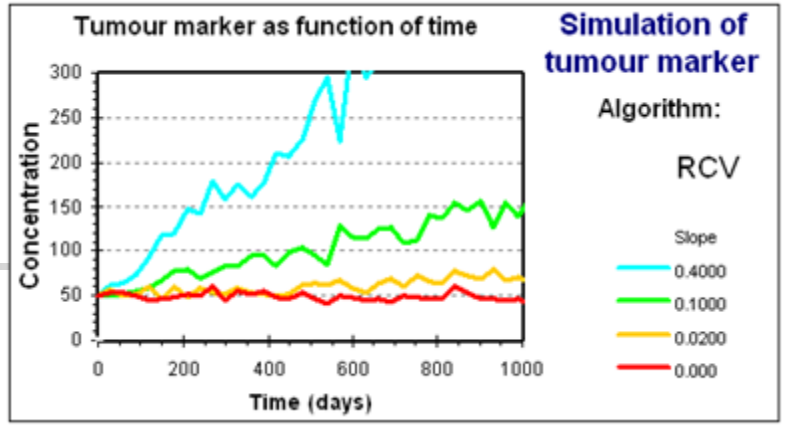
Infrequent sampling: sampling / 60 days

Decreased probability of FP

Decreased probability of TP



Sampling frequency



2*RCV (p = 0.1 % each)

A. Latest against the former

B. Latest against minimum

Frequent sampling: sampling/15 days

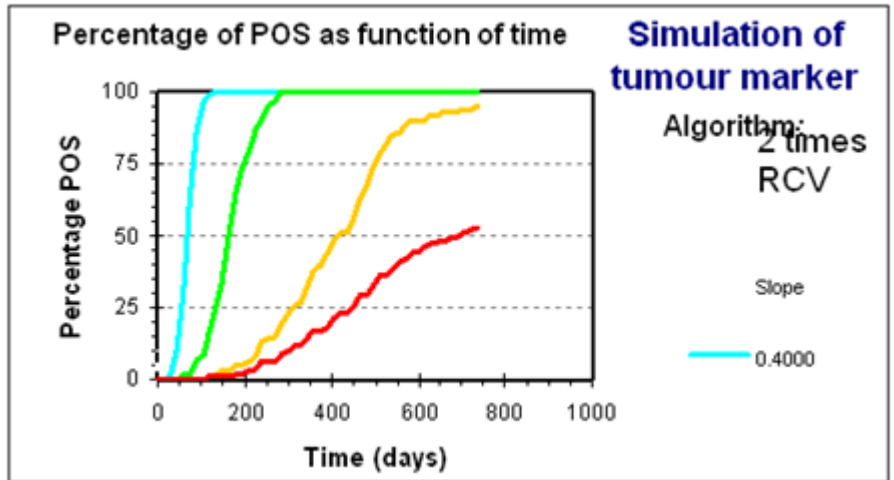
Increased probability of FP

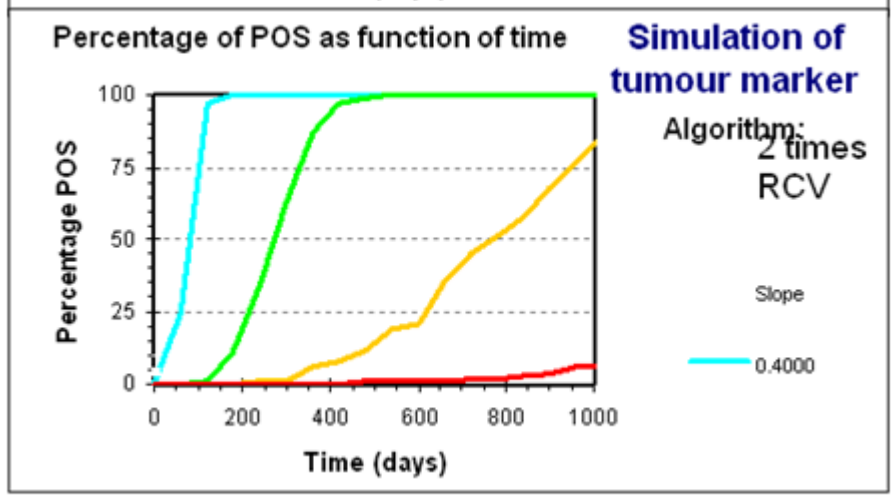
Increased probability of TP

Infrequent sampling: sampling/60 days

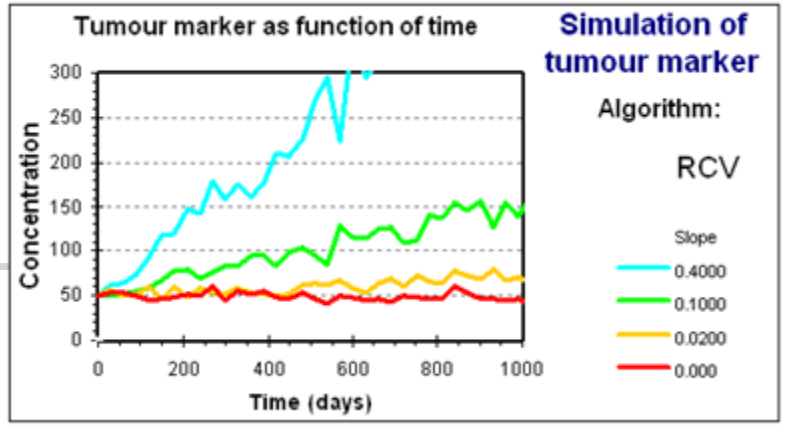
Decreased probability of FP

Increased probability of TP



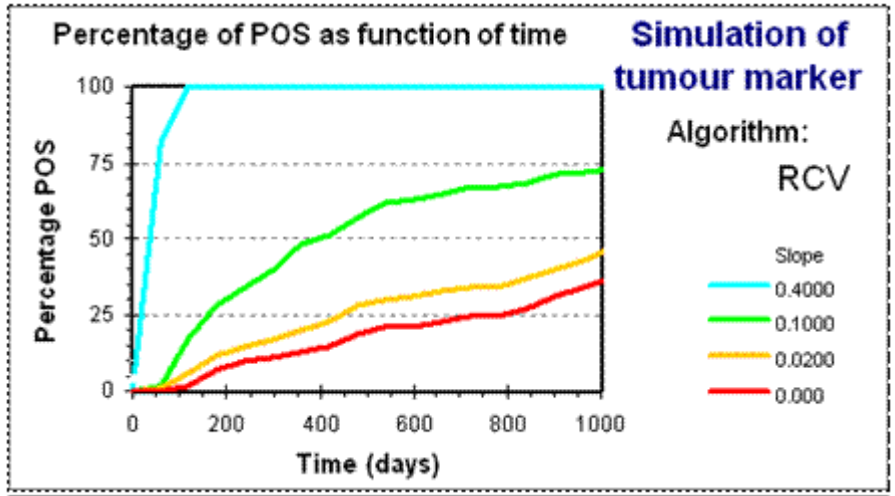


Sampling frequency



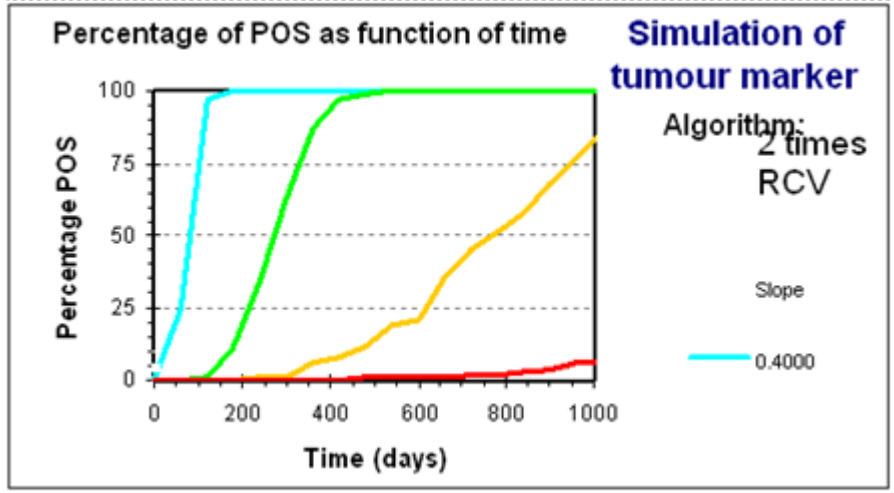
RCV (p = 5 %)
Latest against the former

Infrequent sampling: sampling/60 days



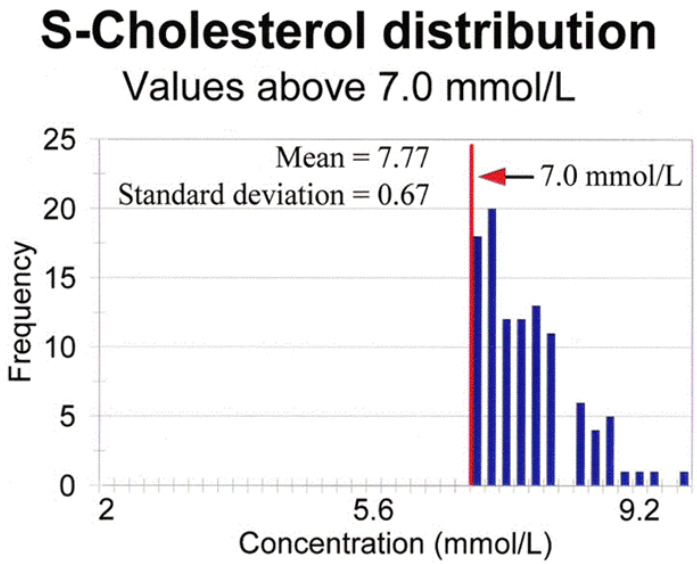
2*RCV (p = 0.1 % each)
A. Latest against the former
B. Latest against minimum

Infrequent sampling: sampling/60 days

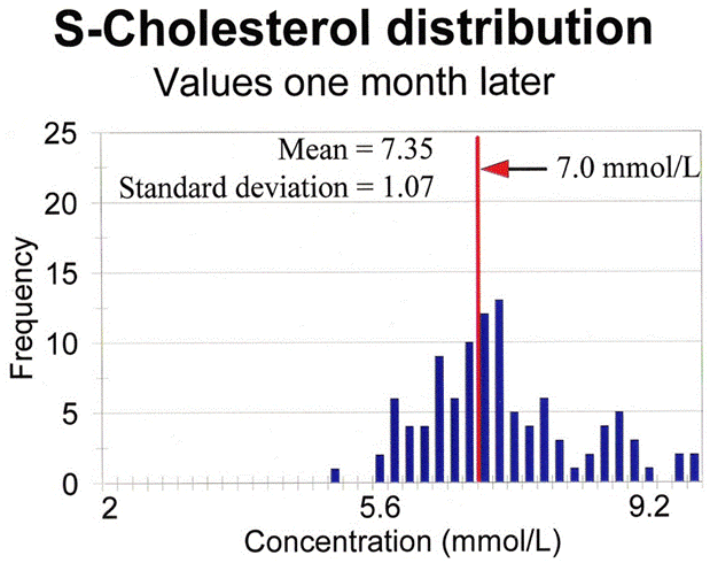


Regression to mean

S-Cholesterol



A month later

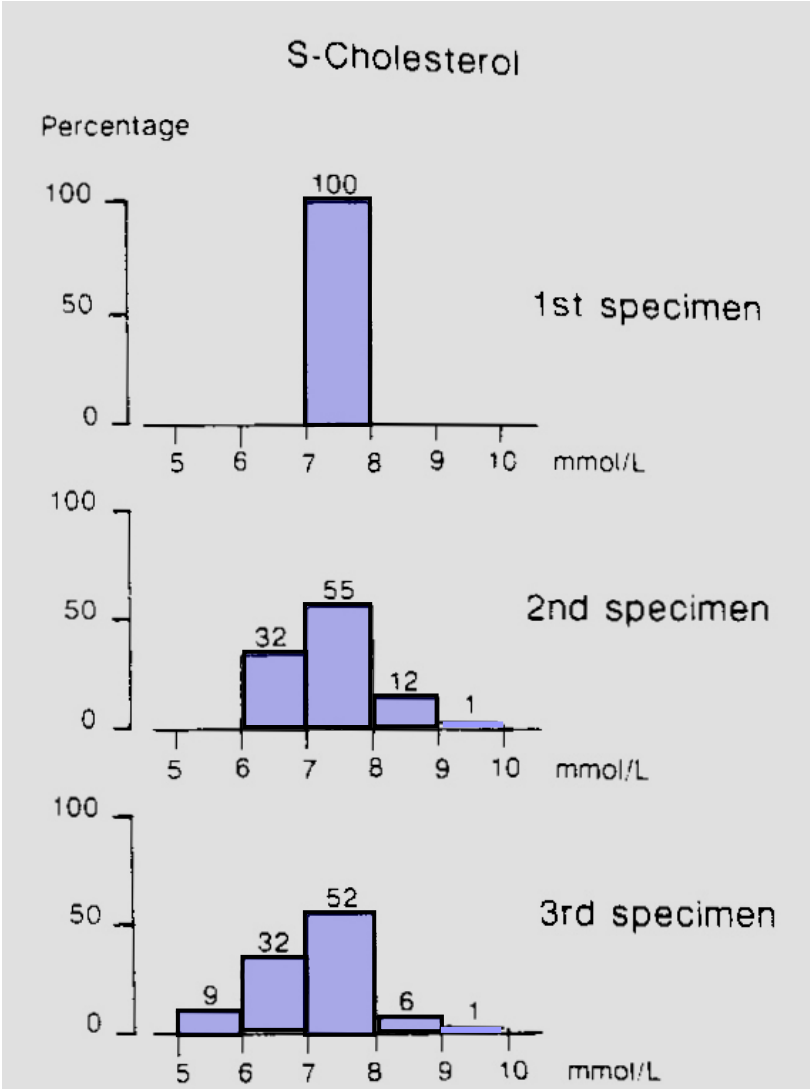


Regression to mean

S-Cholesterol

Two weeks later

One month later

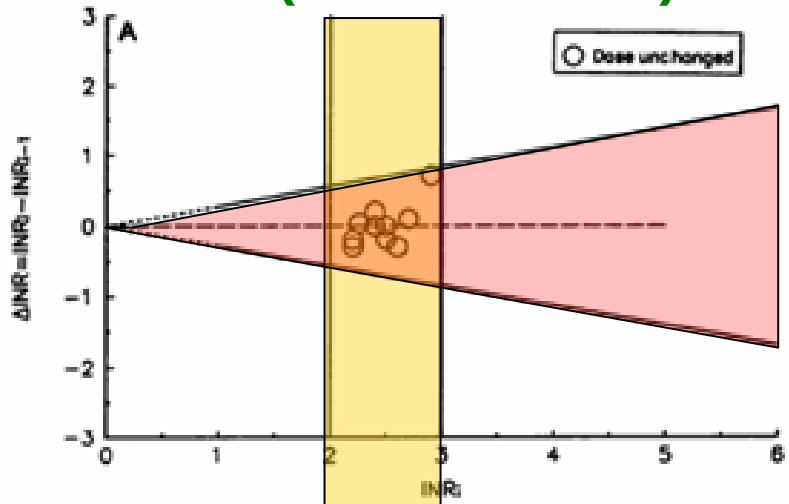


Hyltoft Petersen et al. .
Scand J Clin Lab Invest
1990;50/suppl 198:66-72.

INR measurements

Difference (last minus first)

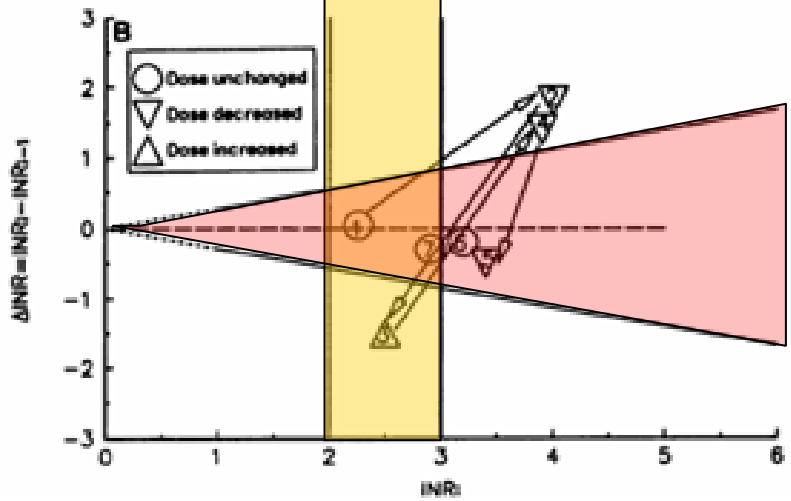
Stable patient



± RCV

INR concentration

Ping – pong treatment



± RCV

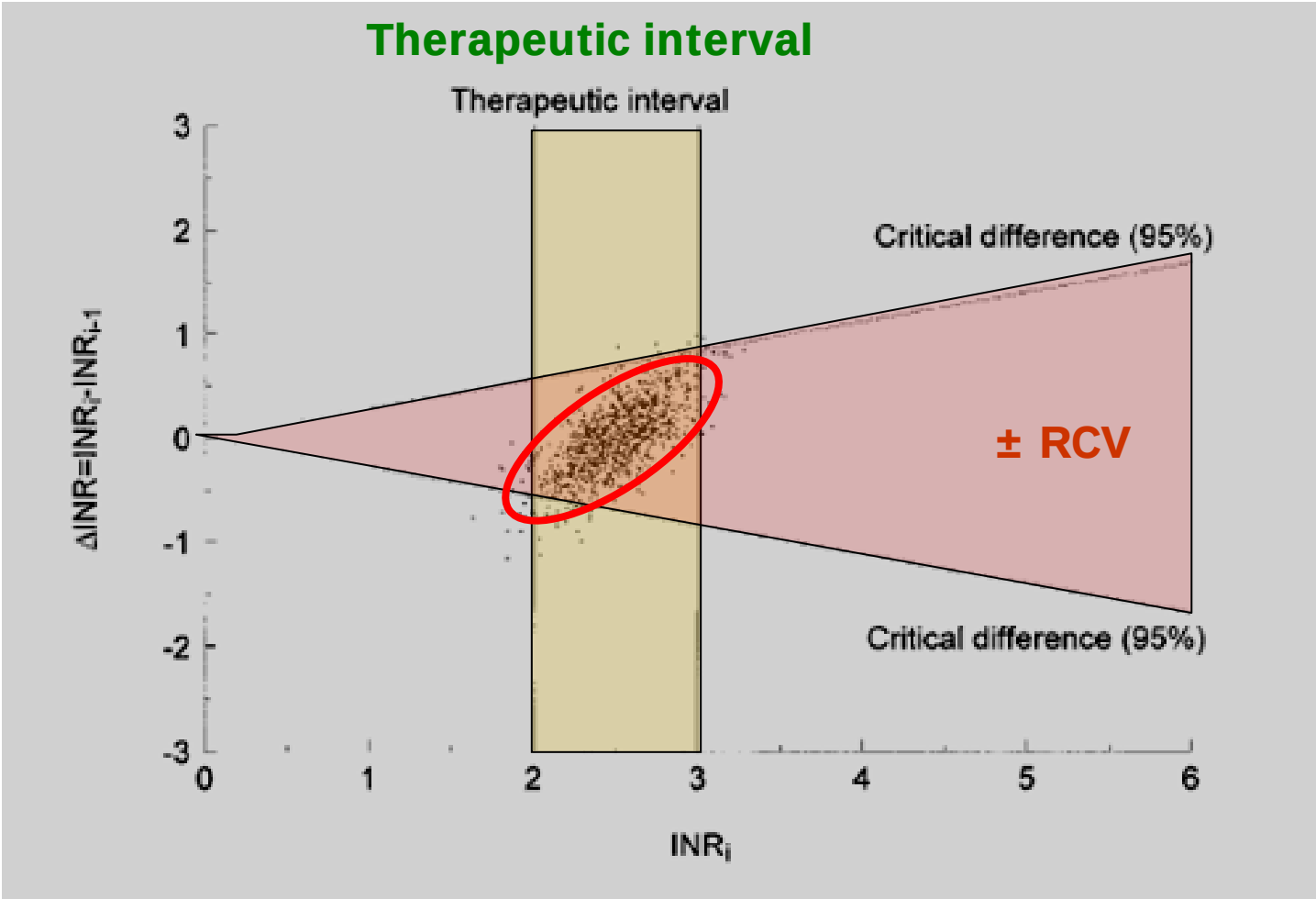
INR concentration

Therapeutic interval

Flensted Lassen et al.
Clin Chem
1995;41:1171- 6.

INR measurements *regression to mean*

Simulations



Kjeldsen et al. *Clin Chem* 1997;43::2175-82

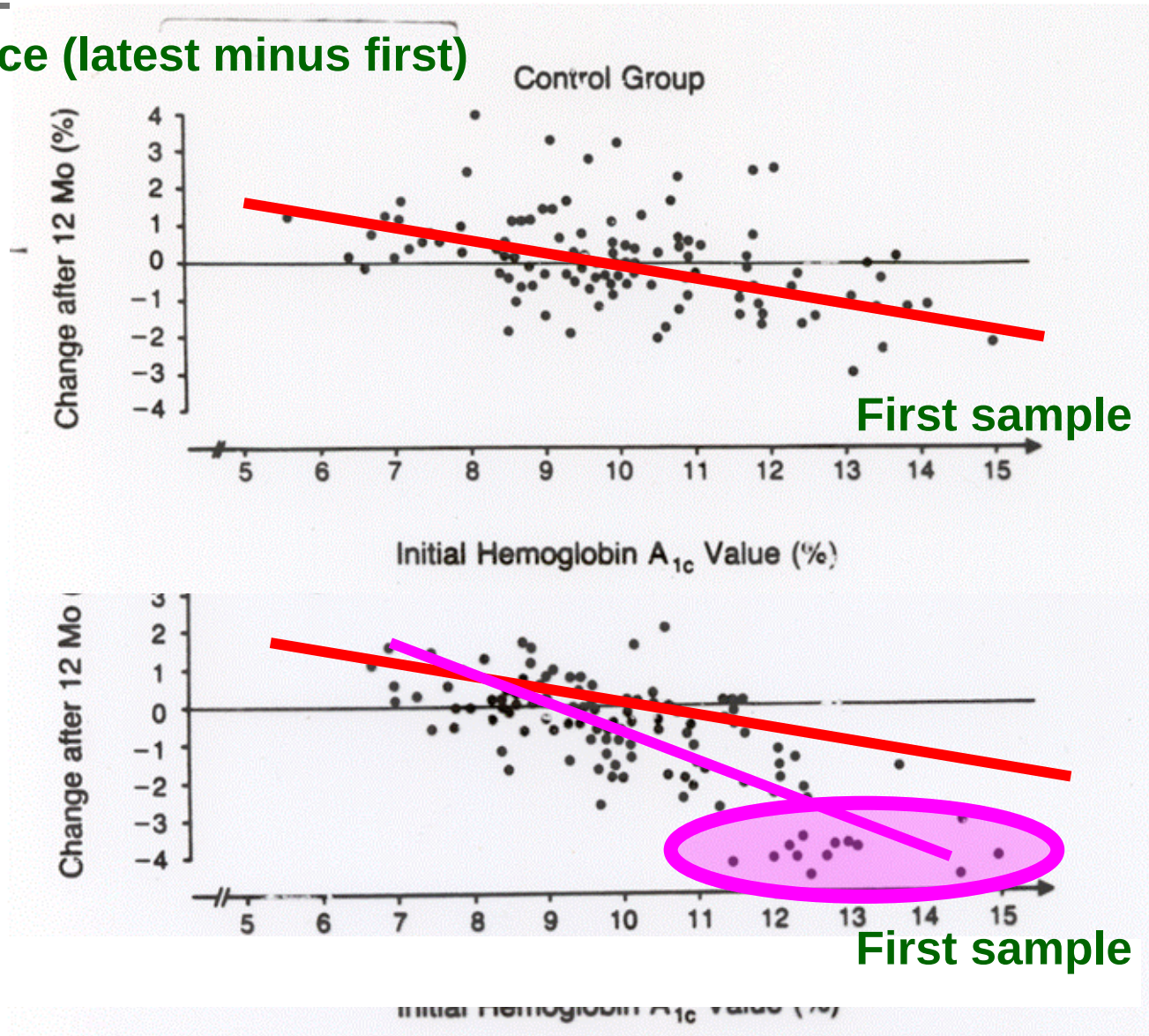
HbA_{1C} in diabetics *regression to mean*

Difference (latest minus first)

Diabetics One year between

Closed for clinicians

Open for clinicians

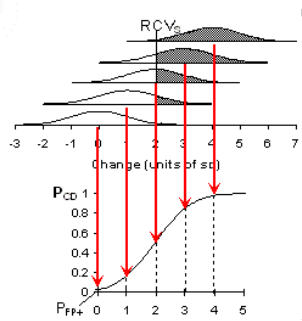


Lytken Larsen et al.

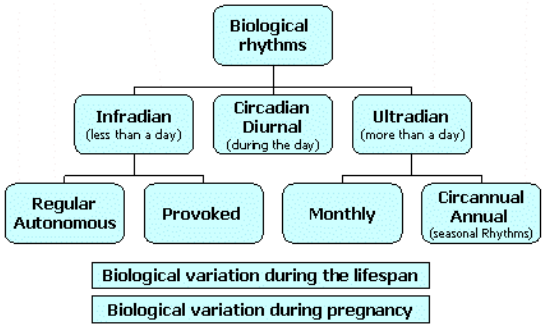
New England J Med

SUMMERY

Power function



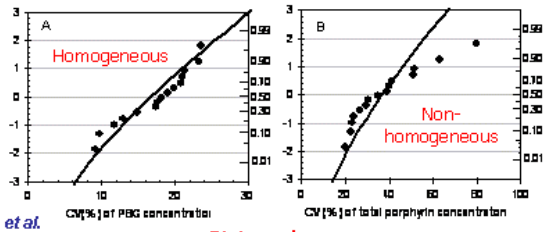
Predictable biological variation



Distribution of coefficients of variation

Patients with Acute Intermittent Porphyria

PBG: Porphobilinogen Porphyrins



Aarsand *et al.* Clin Chem 2006;52:650-6

CV-values

RCV and repeated testing

$$RCV = 1.96 * 2^{1/2} * CV_{W\text{-}S}$$

P(single sample) = 5 %

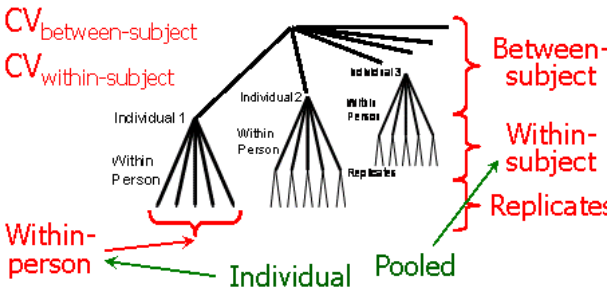
P(patient) = 1- (1-P(single sample))^n

P(patient) = 9.8 % for n = 2

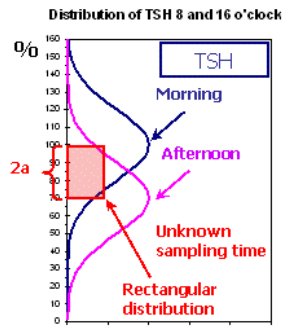
P(patient) = 18.5 % for n = 4

Unpredictable biological variation

Monitoring: Experimental design



Diurnal variation



Rectangular distribution:

$s \sim a/3^{1/2}$ or

$CV_R = 15\ \%/3^{1/2}$

$= 8.7\ \%$

$CV_{total}^2 = CV_{within\text{-}subject}^2 + CV_R^2 = 20\ \%^2 + 8.7\ \%^2 = 22\ \%^2$

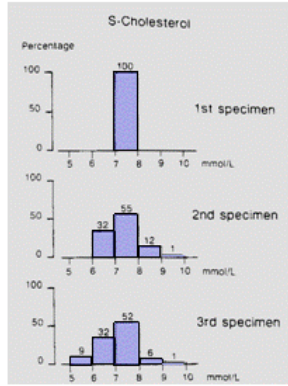
$CV_{total} = 22\ \%$

Regression to mean

S-Cholesterol

Two weeks later

One month later



Hyltoft Petersen et al. . *Scand J Clin Lab Invest* 1990;50/suppl 198:66-72.