Sample materials for in-vitro diagnostics

Blood

Serum/plasma

Urine

Feces

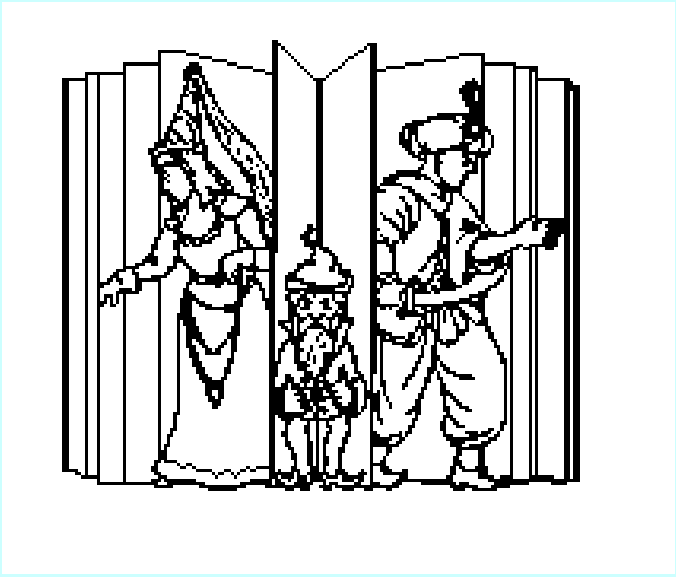
Cerebrospinal fluid

Saliva

Sweat

Punctation fluid

Methods in clinical analysis - I



Clinical analysis include a broad spectrum of

Separation techniques

Detection methods

Immunological methods

Methods in clinical analytics - II

Separation techniques:

Filtration

Chromatography

Electrophoresis

Isoelectric focussing

Ultracentrifugation

Formation of immunecomplexes

Diffusion and dialysis

Sedimentation

Methods in clinical analytics - III

Detection methods:

Mikroskopy

Osmometry

Immunochemistry

Particle count

Flow cytometry

Cytochemistry

Electrochemistry

- Potentiometry
- Amperometry
- Coulometry

Molecular analytics

Spectrometry

- Photometry
- Fluorometry
- Nephelometry
- Turbidimetry
- Mass spectrometry
- IR-spectrometry
- NMR-spectrometry
- Luminometry

Special methods

- Coagulometry
- Fluorescence polarisation

Method evaluation - I

Analytical validity

Imprecision
- Within batch
- Between batch

Incorrectness
Analytical sensitivity
Functional sensitivity
Linearity
Interferences
Matrix effect
Interferences between samples
Method comparisons

Diagnostic validity

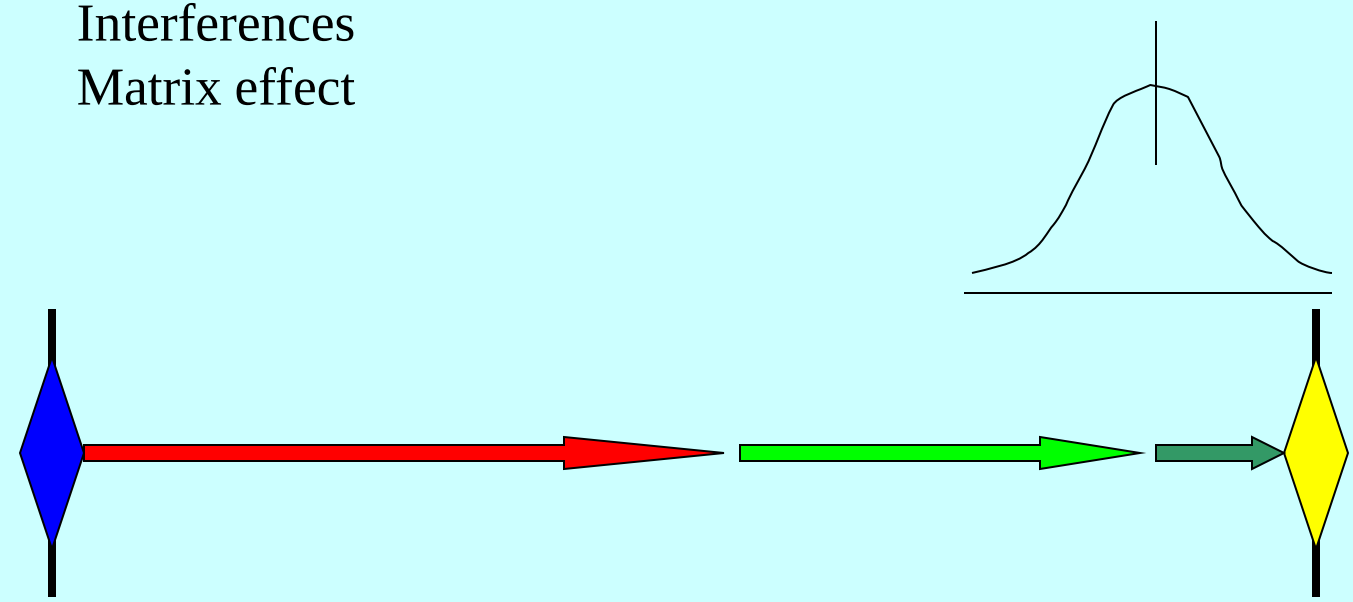
Reference values
Diagnostic sensitivity
Diagnostic specificity
Postitive predictive value
Negative predictive value
Receiver-operator-curves (ROC)

Method evaluation - II

Analytical imprecision

Sample dependent bias
- Unspecificity
- Influence factors
- Interferences
- Matrix effect

System dependent bias
- Random error
- Calibration



„True value“

„Method dependent value“

Method evaluation - III

Reference values:

Population (e. g. kaukasians, blacks)

Gender (male, female)

Age (e. g. newborn, infant, adolescent, adult)

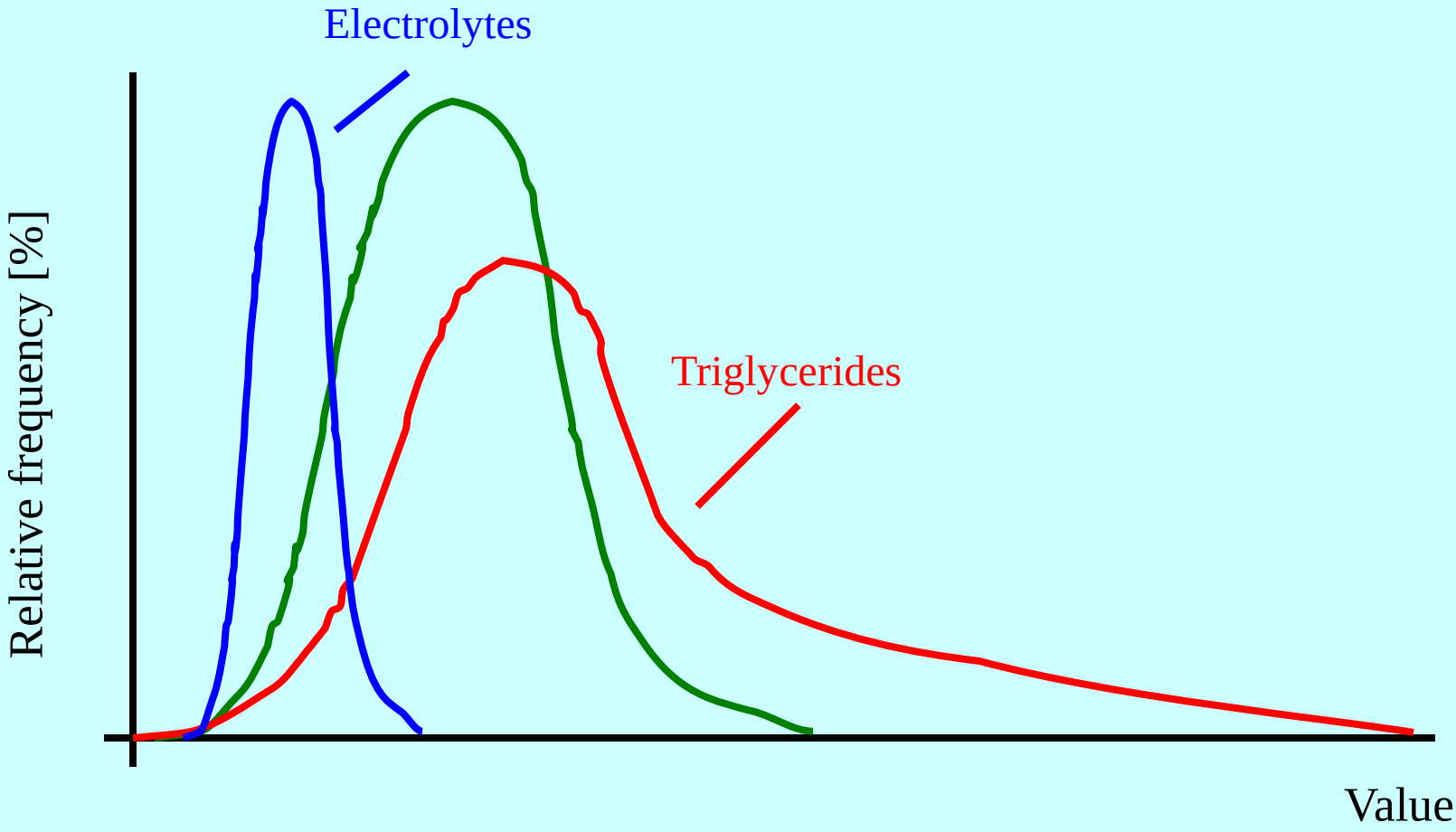
Daytime (circadian rhythms)

Method (e. g. Fe by photometry or atomic absorption)

Problem: Study groups may be different - who is healthy?

Which study group is the best and re-presents the population?

Method evaluation - IV



The distribution pattern of reference values differs strongly for various parameters.

Comparison of analytical methods - I

Comparison of random samples:

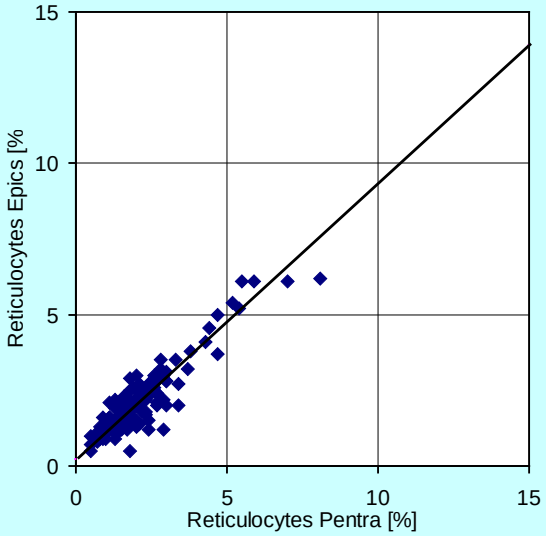
Student´s t-test
Wilcoxon´s Rank-test
Mann-Whitney-test

Regression analysis:

Linear regression
Passing.Bablock
Feldmann

Comparison of analytical methods - II

Comparison of three methods for reticulocyte determination



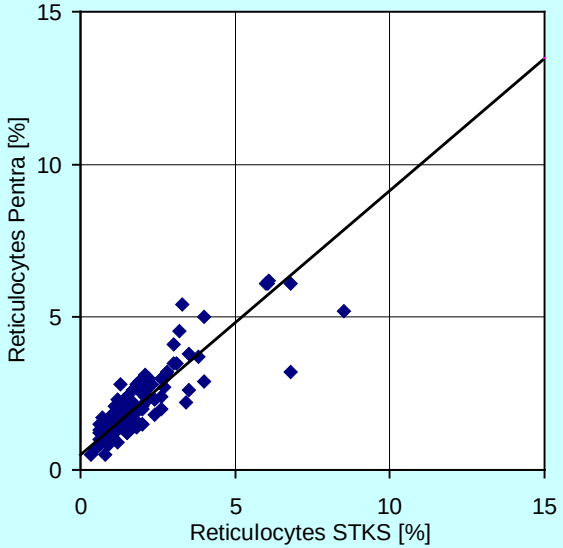
Epics (thiazole orange) vs. Pentra (thiazole orange)

n = 130 samples

Linear regression according to Passing and Bablock:

y = 0.911 • x + 0.215

r_S = 0.913; p < 0.001



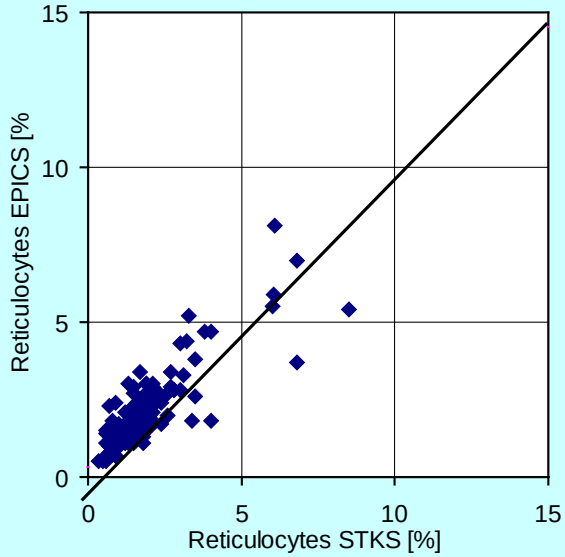
Pentra (thiazole orange) vs. STKS (methylene blue)

n = 130 samples

Linear regression according to Passing and Bablock:

y = 0.865 • x + 0.501

r_S = 0.859 ; p < 0.001



Epics (thiazole orange) vs. STKS (methylene blue)

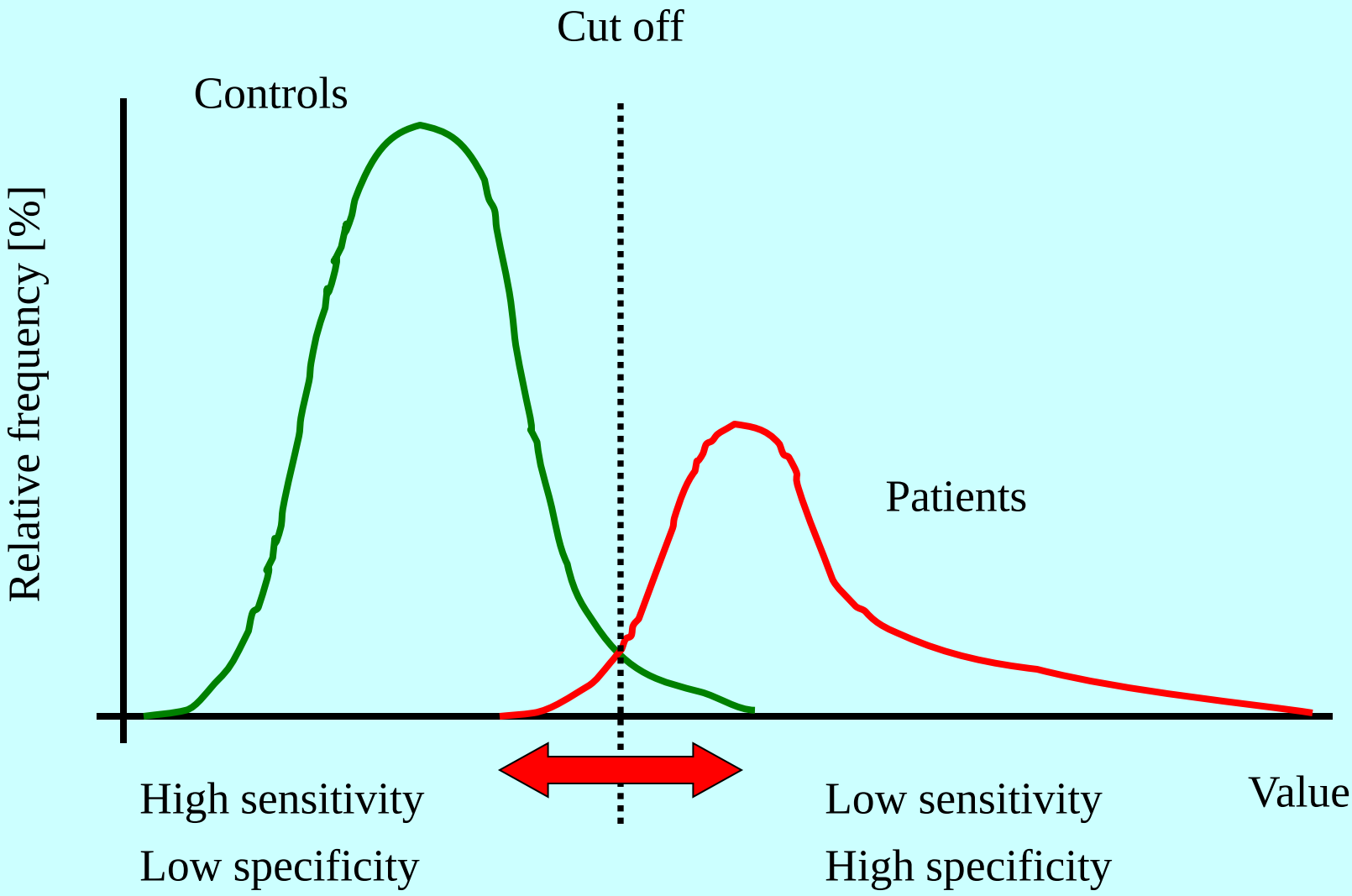
n = 130 samples

Linear regression according to Passing and Bablock:

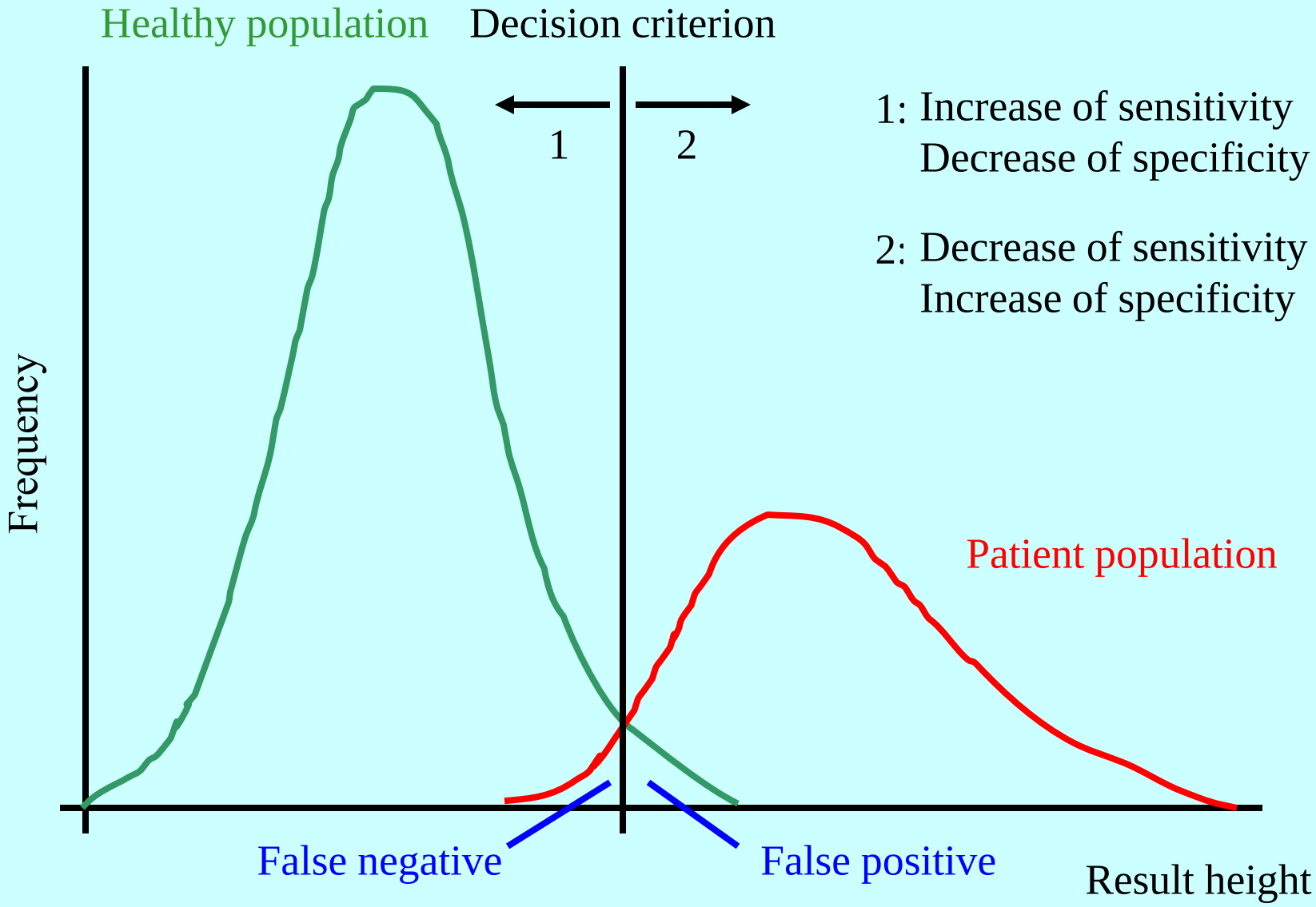
y = 0.947 • x + 0.319

r_S = 0.843; p < 0.001

Sensitivity and specificity of laboratory tests - I



Sensitivity and specificity of laboratory tests - II



Sensitivity and specificity of laboratory tests - III

Diagnostic sensitivity:

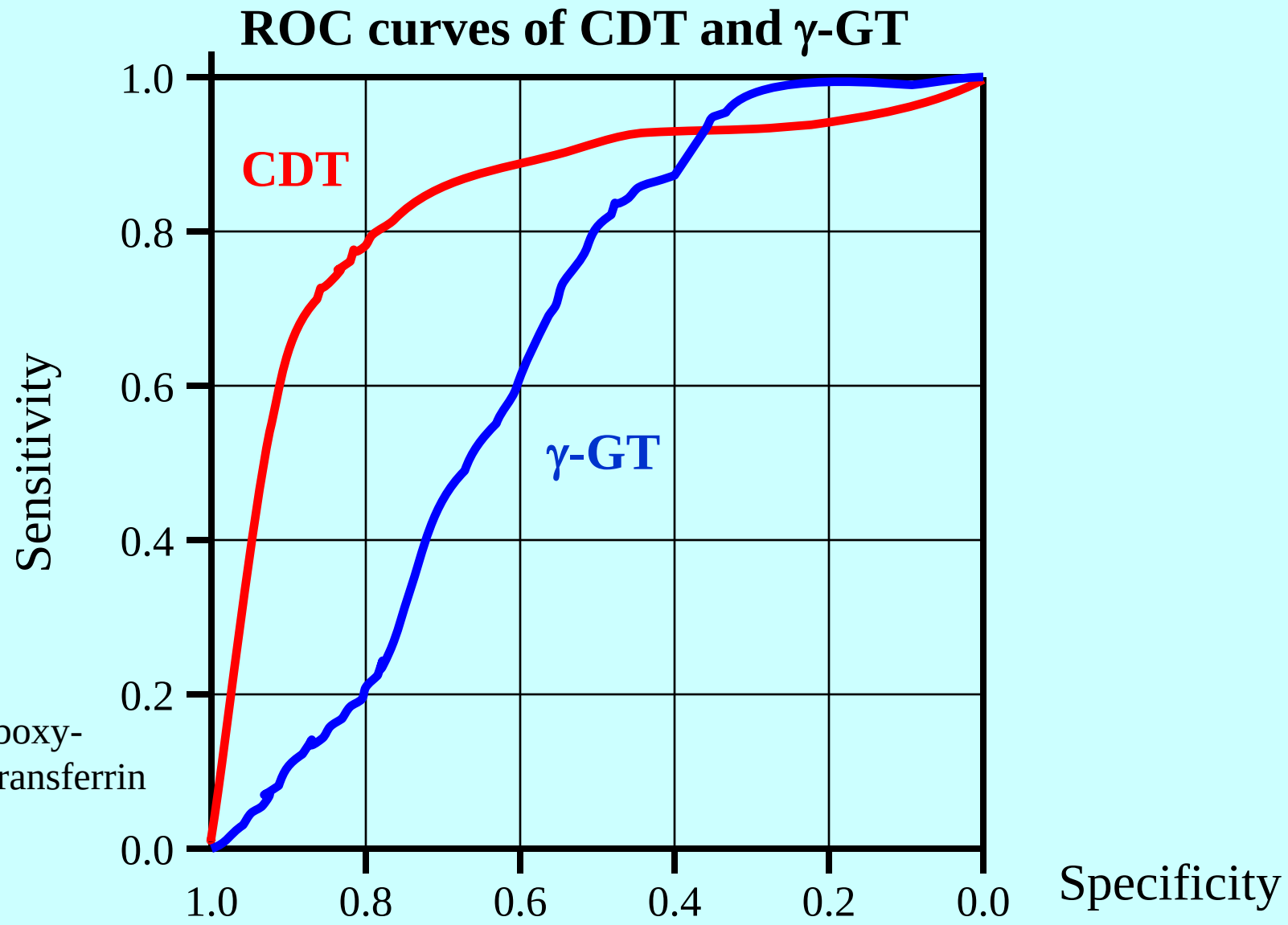
$$\frac{\text{Correct positive}}{\text{Correct positive + false negative}} \bullet 100\ \%$$

Diagnostic specificity:

$$\frac{\text{Correct negative}}{\text{Correct negative + false positive}} \bullet 100\ \%$$

Values of diagnostic sensitivity and specificity are given in [%]

Sensitivity and specificity of laboratory tests - IV



CDT: Carboxy-deficient transferrin

Incidence and prevalence - I

Incidence:

Number of individuals in a population newly developing a disease within an observation period of 1 year. The incidence is based on 100.000 individuals.

Prevalence:

Number of individuals in a population suffering from a disease within an observation period of 1 year; the prevalence is based on 100.000 individuals.

Incidence and prevalence - II

Examples of incidence and prevalence:

Incidence and prevalence of Creutzfeld-Jacob disease (CJD) are about 1/1000000. Both values are very similar because the time course of the lethal disease is only few months.

About 17 million Americans are believed to have diabetes mellitus. This is a prevalence of 6.2 %. The incidence is about 1 million new cases occuring each year. Because of the relatively low mortality of the disease the values of incidence and prevalence are different.

Predictive value - I

In clinical diagnostics the physician is more interested for the likelihood of manifest disease in a patient after receiving a positive test result and a non-manifest disease after receiving a negative test result than for the likelihood of a pathological test result in disease and a normal test result in healthy subjects.

Predictive values of laboratory tests depend on the prevalence of the disease in the population (i. e. the relation between diseased and non-diseased patients in a population at a defined time-point.

Predictive value - II

Calculation of the positive ($PV_{pos.}$) and the negative ($PV_{neg.}$) predicted value of a diagnostic test from the data of positive and negative test results ($PV_{pos.}$ and $Pv_{neg.}$ are given in [%]).

Positive predictive value:

$$PV_{pos.}\ [\%] = \frac{\text{Number of correct positive results} \bullet 100}{\text{Total number of positive results}^{\#)}}$$

#) correct and false positive results

Negative predictive value:

$$PV_{neg.}\ [\%] = \frac{\text{Number of correct negative results} \bullet 100}{\text{Total number of negative results}^{\#)}}$$

#) correct and false negative results

Predictive value - III

Calculation of the positive ($PV_{pos.}$) and the negative ($PV_{neg.}$) predicted value of a diagnostic test from the data of diagnostic sensitivity, specificity and prevalence ($PV_{pos.}$ and $Pv_{neg.}$ are given in [%]).

Positive predictive value:

$$PV_{pos.}\,[\%] = \frac{\text{Prevalence} \bullet \text{Sensitivity} \bullet 100}{\text{Prevalence} \bullet \text{Sensitivity} + (100 - \text{Prevalence}) \bullet (100 - \text{Specificity})}$$

Negative predictive value:

$$PV_{neg.}\,[\%] = \frac{(100 - \text{Prevalence}) \bullet \text{Specificity} \bullet 100}{(100 - \text{Prevalence}) \bullet \text{Specificity} + \text{Prevalence} \bullet (100 - \text{Sensitivity})}$$

Diagnostic efficiency - I

The diagnostic efficiency describes the relation of the correct test results and all results of the investigated group.

It depends on the diagnostic sensitivity and specificity as well as the prevalence of the disease.

Diagnostic efficiency - II

Calculation of the diagnostic efficiency [%]:

The diagnostic efficiency can be calculated according to two formulas:

$$\text{Efficiency} = \frac{\text{Number of correct positive} + \text{Number of correct negative results}}{\text{Total number of results}}$$

$$\text{Efficiency} = \text{Prevalence} \bullet \text{Sensitivity} + (1 - \text{Prevalence}) \bullet \text{Specificity}$$

Mortality and morbidity - I

Mortality:

Number of individuals in a population dying from a disease within an observation period of 1 year. The mortality is based on 100.000 individuals.

Prevalence:

Number of individuals in a population suffering from a disease within an observation period of 1 year. The prevalence is based on 100.000 individuals.

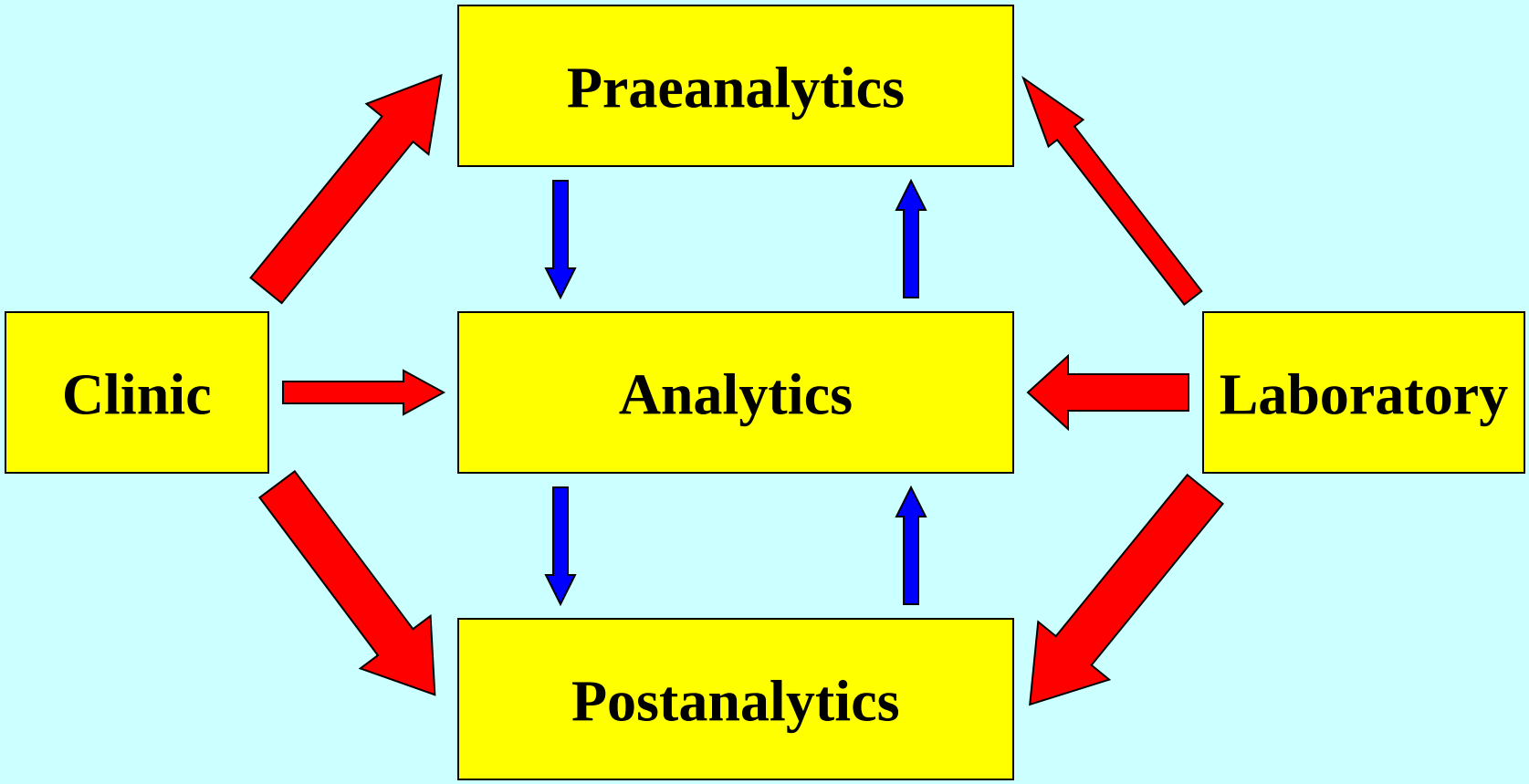
Mortality and morbidity - II

Examples of mortality and morbidity:

Mortality and morbidity of Creutzfeld-Jacob disease (CJD) are about 1/1000000. Both values are very similar because the time course of the lethal disease is only few months.

About 17 million Americans are believed to have diabetes mellitus. About one third of those do not know they have it. This is a morbidity of about 4 %. Diabetes is the direct or indirect cause of at least 200.000 deaths per year. Because of the relatively low mortality of the disease the values of morbidity and mortality are different.

Phases in laboratory analytics



What is analytics?

Analytics includes all steps of sample measurement

It includes the estimation of precision and accuracy of the results

It is in the responsibility of the physician or clinical chemist performing the analysis

It is subject of laboratory organisation

It is subject of internal and external quality controls

Legal guidelines

Richtlinie der Bundesärztekammer zur Qualitätssicherung quantitativer laboratoriumsmedizinischer Untersuchungen (RiliBAEK)

5 spezielle Teile, die Details zur regelmäßigen internen Qualitätssicherung und zur Teilnahme an Ringversuchen für folgende Bereiche regeln:

- B 1 „Quantitative laboratoriumsmedizinische Untersuchungen“ (in Kraft seit 1.4.2008)
- B 2 „Qualitative laboratoriumsmedizinische Untersuchungen“ (in Kraft seit 1.7.2011)
- B 3 „Direkter Nachweis und Charakterisierung von Infektionserregern“ (In Kraft seit 1.4.2013)
- B 4 „Ejakulatuntersuchungen“ (in Kraft seit 1.1.2011)
- B 5 „Molekular- und zytogenetische laboratoriumsmedizinische Untersuchungen“ (in Kraft seit 1.10. 2011)

(Deutsches Ärzteblatt 98, 42 (19.10. 2001), Seite A 2747-2759 + Deutsches Ärzteblatt 99, 17 (26.04.2002), Seite A 1187, + Deutsches Ärzteblatt 100, 50 (12.12.2003), Seite A 3335 - A 3338)

Qualitätssicherungsregelungen für laboratoriumsmedizinische Untersuchungen komplett Dtsch Arztebl 2013; 110(12): A-575 / B-511 / C-511

Richtlinie der Bundesärztekammer zur Qualitätssicherung quantitativer laboratoriumsmedizinischer Untersuchungen (RiliBAeK) - I

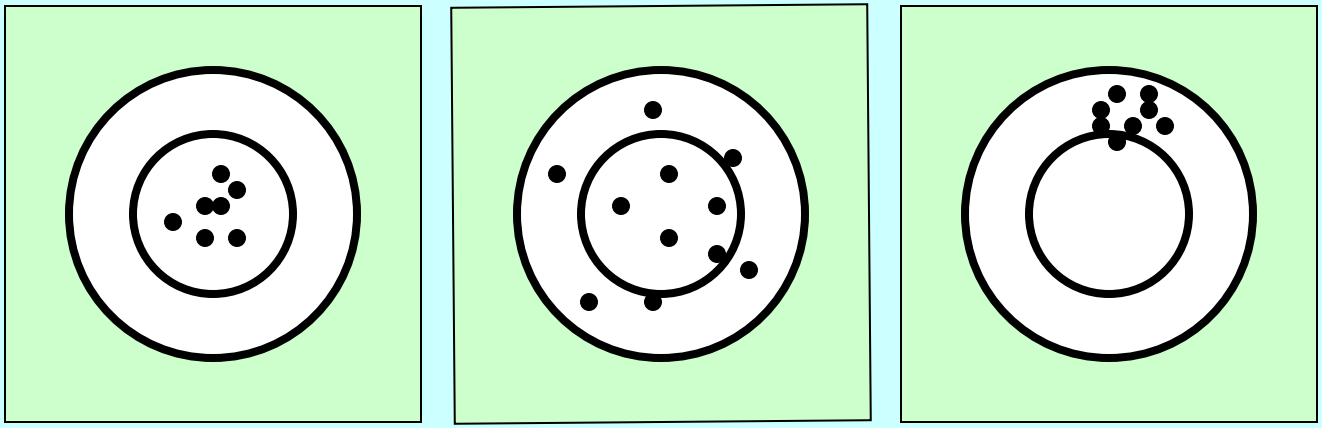
Defines terms in laboratory analysis (e. g. analytical series, expected value, control cycle, analytical method, reference method, precision, imprecision, correctness, true value, method dependent target value).

Defines the principles and the requirements for internal (analysis of control materials purchased from manufacturers; concentrations are published prior to analysis) and external (analyses of external control quality control (e. g. from Instand e. V.), concentrations published weeks after the analysis).

Defines reference laboratories for external quality control.

Defines target values for the precision of numerous analytes.

Precision and accuracy



Precision	Well	Bad	Well
Accuracy	Well	Well	Bad
Evaluation	Optimal	Random error	Systematical error

Within-batch and between-batch variability

The precision of analytical methods cannot be determined.

Therefore the „imprecision“ is determined by calculation of the coefficient of variation (CV-value):

$$\text{CV-value} = \frac{\text{Standard deviation}}{\text{Mean value}} \bullet 100\ \%$$

Two distinct CV-values are established to describe the precision of an analytical method:

Within-batch variability: Repeated measurement of one sample (e. g. control material) in one analytical series (e. g. 10 times).

Between-batch variability: Measurement of one sample (e. g. control material at consecutive days (e. g. 10 days).

Typically the between-batch variability is higher than the within-batch variability.

Control cards - I

Control sera/control materials must be measured in every analytical series to get information about the validity of the obtained results

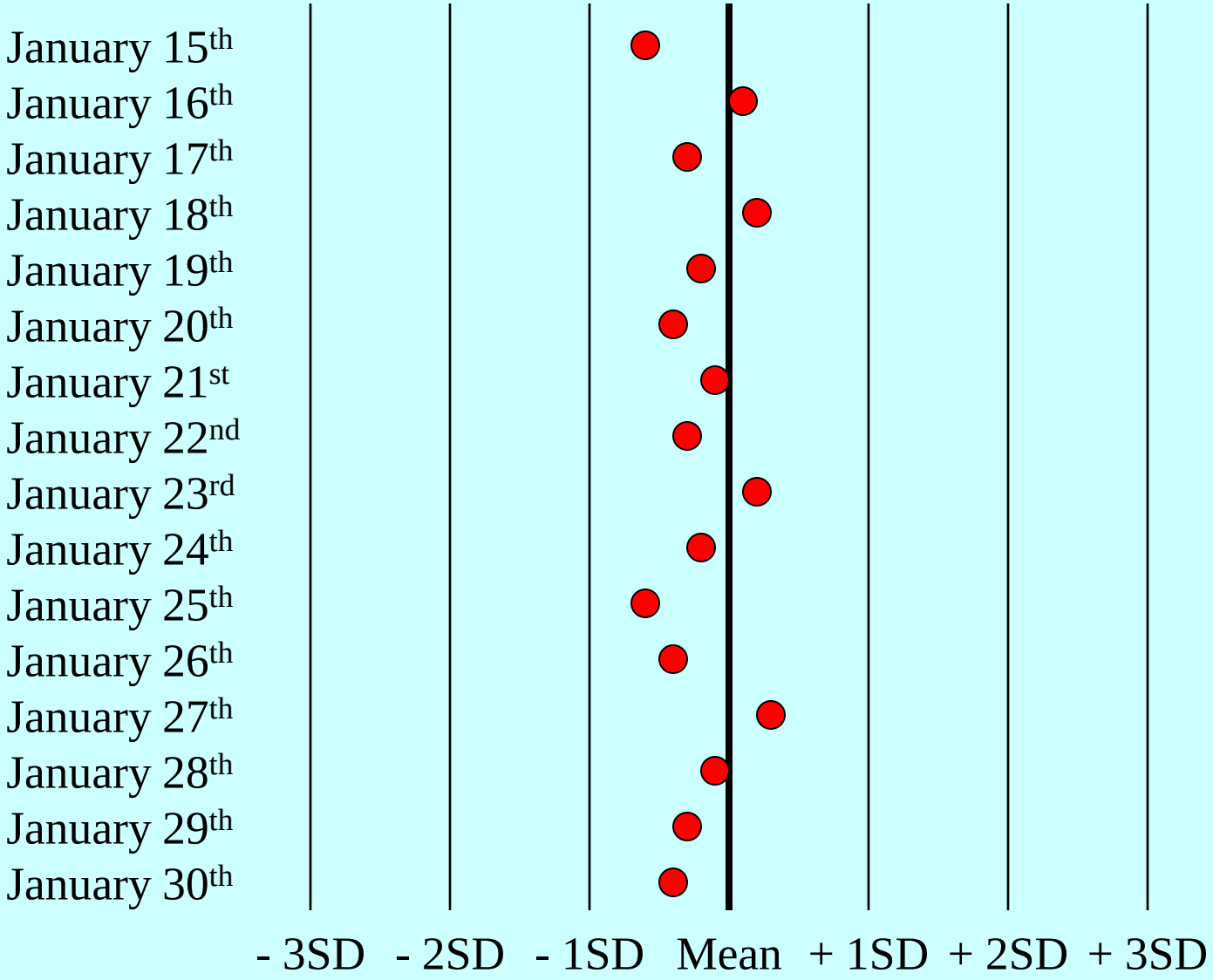
The obtained values are continuously recorded and compiled on control cards

Values of the between-batch variability allow the estimation of accuracy and precision

At least two control materials (normal and pathological) are analyzed

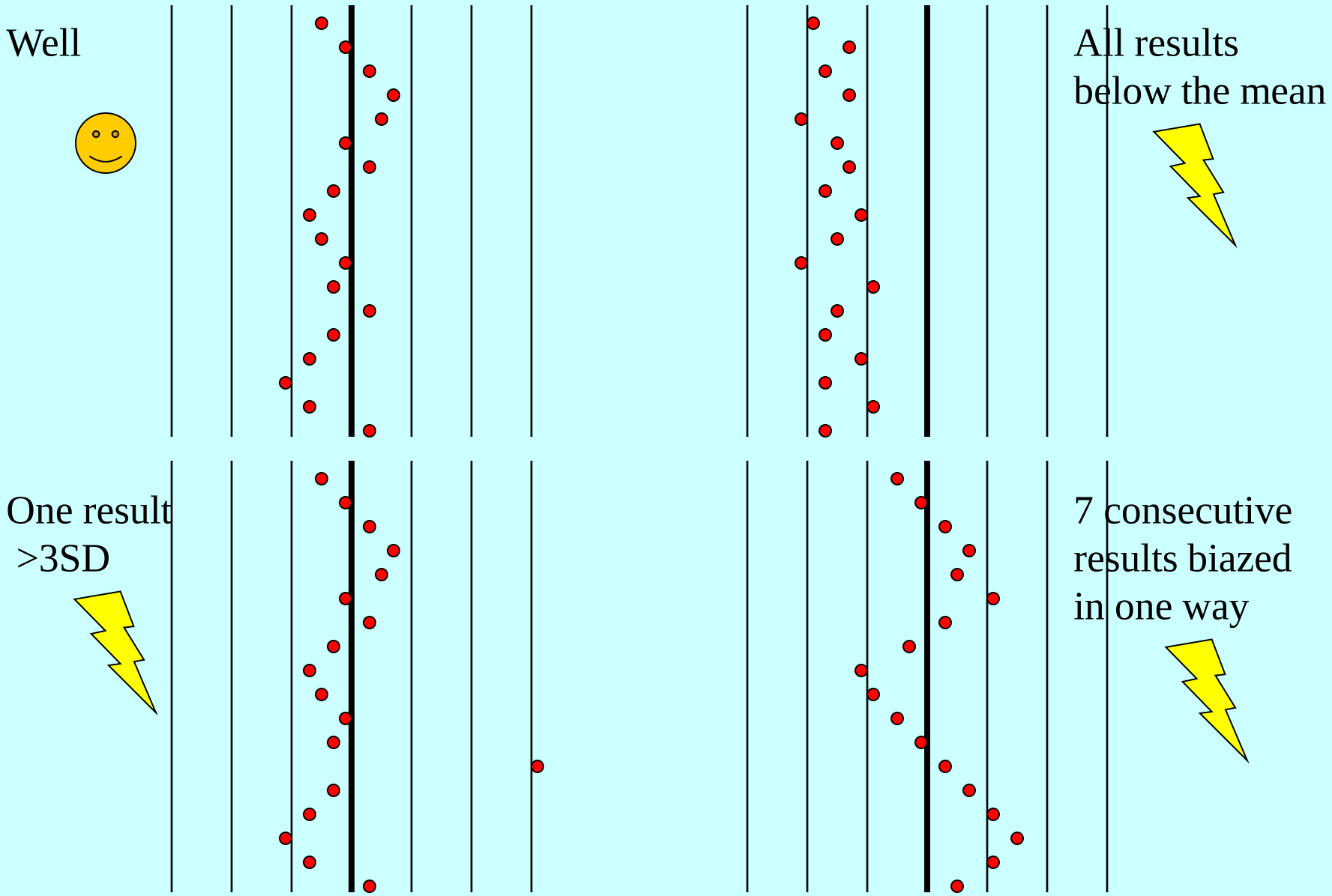
Often three control materials (low, normal, high) are analyzed

Control cards - II



Mean value and SDs of the control material are specified by the manufacturer

Control cards - III



RiliBAeK - II

Analyte	Parameter	Target value[1)]	Maximum imprecision	Maximum incorrectness	Maximum deviation of single value	Analyte concentration range
Albumin	Mass concentration	RMV	6 %	11 %	23 %	
Aldosteron	Amount of substance Mass concentration	RMV	14 %	16 %	44 %	
Alkalische Phosphatase (EC 3.1.3.1)	Enzyme activity	RMV	7 %	11 %	25 %	
Bilirubin total	Amount of substance Mass concentration	RMV/SV	7 % 0.1 mg/dl	12 % 0.2 mg/dl	26 % 0.4 mg/dl	≥1.5 mg/dl <1.5 mg/dl
Calcium	Amount of substance	RMV	3 %	5 %	11 %	
Carbamazepin	Mass concentration	SV	7 %	10 %	24 %	
Chlorid	Amount of substance	RMV	2.5 %	4 %	9 %	
Cholesterin total	Amount of substance Mass concentration	RMV	4 %	6 %	14 %	
Choline-esterase (EC 3.1.1.8)	Enzyme activity	RMV	6 %	6 %	18 %	

[1)] RMV: Reference method value; SV: Specific method dependent value

(Deutsches Ärzteblatt 98, 42 (19.10. 2001), Seite A 2747-2759 + Deutsches Ärzteblatt 99, 17 (26.04.2002), Seite A 1187, + Deutsches Ärzteblatt 100, 50 (12.12.2003), Seite A 3335 - A 3338)

European Directive 98/79/EC on In-vitro Diagnostics

Gesetz zur Änderung des Medizinproduktegesetzes (Medizinproduktegesetz - MPG)

German Law on Medical Devices

Medizinprodukte-Sicherheitsplanverordnung - MPSV

Ordinance on the Medical Devices Vigilance System

Competent Autority responsible in charge according to MPSV

Medical devices

Federal Institute for Drugs and Medical Devices (BfArM)[#]

In-vitro diagnostics

Paul Ehrlich Institute (PEI)

[#]) Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM)

	Annex of Directive 98/79/EC	Responsibility
Products for immune hematological testing and tissue typing:		
Blood groups of the AB0 system[1, 2]	IIa	PEI
Blood groups of the Rhesus system (C, c, D, E, e)[1, 2]	IIa	PEI
Blood groups of the Kell system[1, 2]	IIa	PEI
Blood groups of the Duffy and the Kidd system[1, 2]	IIb	PEI
Irregular anti-erythrocyte antibodies[1, 2]	IIb	PEI
Markers for HLA[3)] typing, markers DR, A and B[1, 2]	IIb	PEI
Products for infection testing:		
Markers of HIV[4)] infection (HIV-1 and HIV-2)[1, 2]	IIa	PEI
HTLV-I[5)] und HTLV-II[1, 2]	IIa	PEI
Hepatitis B, C und D[1, 2]	IIa	PEI
Congentital infection with rubella[1, 2]	IIb	PEI
Congenital infection with toxoplasma[1, 2]	IIb	PEI
Cytomegalovirus (CMV)[1, 2]	IIb	PEI
Chlamydia[1, 2]	IIb	PEI
Other products:		
Tumor marker PSA[1, 6]	IIb	BfArM
Hereditary diseases phenylketonuria and Down syndrome (trisomia 21, including software)[1]	IIb	BfArM
Products for self testing:		
Systems for measurement of blood glucose[1]	IIb	BfArM

[1]Reagents and reagent products for detection, confirmation and quantification;
[2]Analyzers on which these tests are performed are in the responsibility of the BfArM;
[3]HLA: Human leukocyte antigen;
[4]HIV: Human immune deficiency virus;
[5]HTLV: Human T-cell leukemia virus;
[6]PSA: Prostate specific antigen.

Responsibilities of BfArM/PEI according Annex II Directive 98/79/EC