

# Biochemical tests

Pre-analytical phase  
Quantification errors  
Quantification properties  
Validation/Verification  
Reference materials  
Reference values  
Predictive values  
Diagnostic effectivity





# Laboratory automation and consolidation





# Standalone analyzers



# POCT – way of the future?





# POCT

**ABR, glucometers, coaguchecks, CRP measurement, cardiac markers, atherosclerosis markers**



# What use are laboratory tests

- Screening
- Diagnosis
- Monitoring
- Response to therapy
- Hormonal disbalance
- Markers of anemie
- Markers sepsis
- Tumormarkers
- Cardial markers
- TDM
- Renal functions
- Liver functions
- .....

Doctor



Preanalytical phase  
(patient preparation, sample collection, transport, preparation)



Analytical phase  
(proper instrumentation, calibration, proper dilution...)



Post analytical phase  
(result supervision, comparison to patient history  
and diagnosis...)

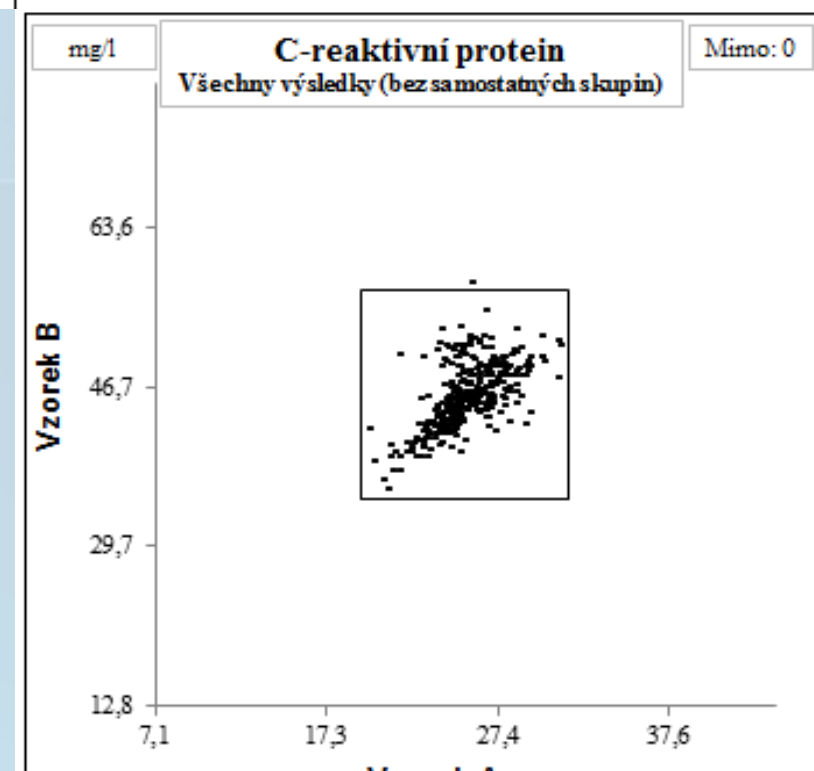
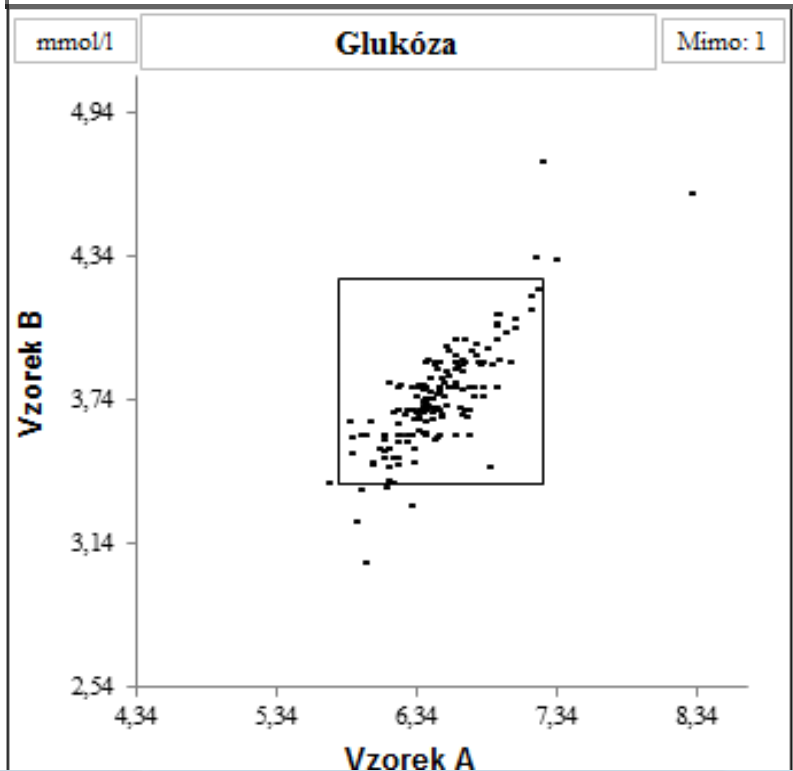
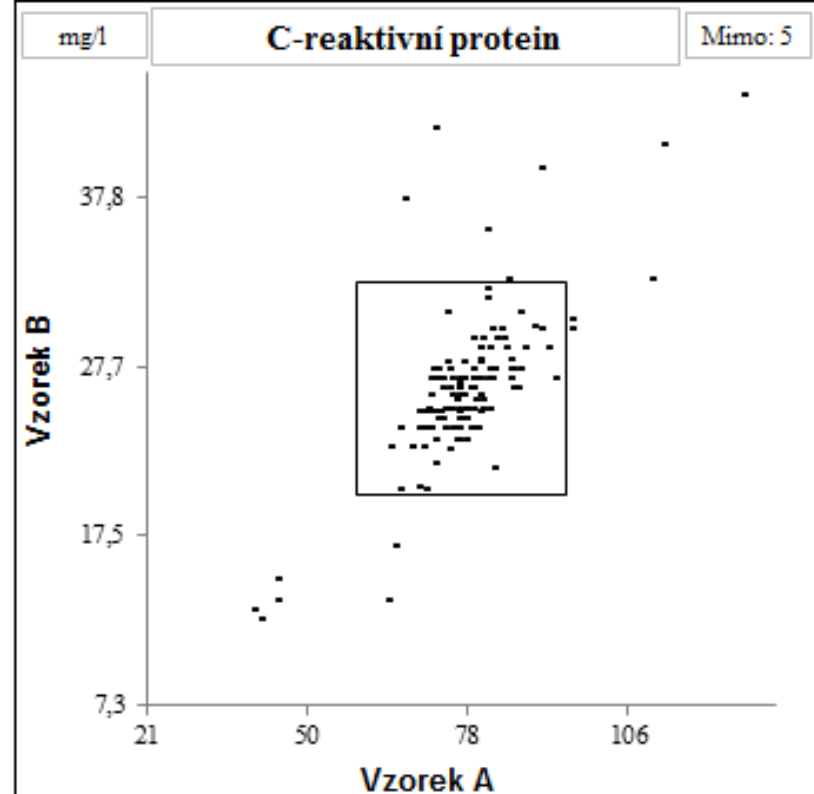
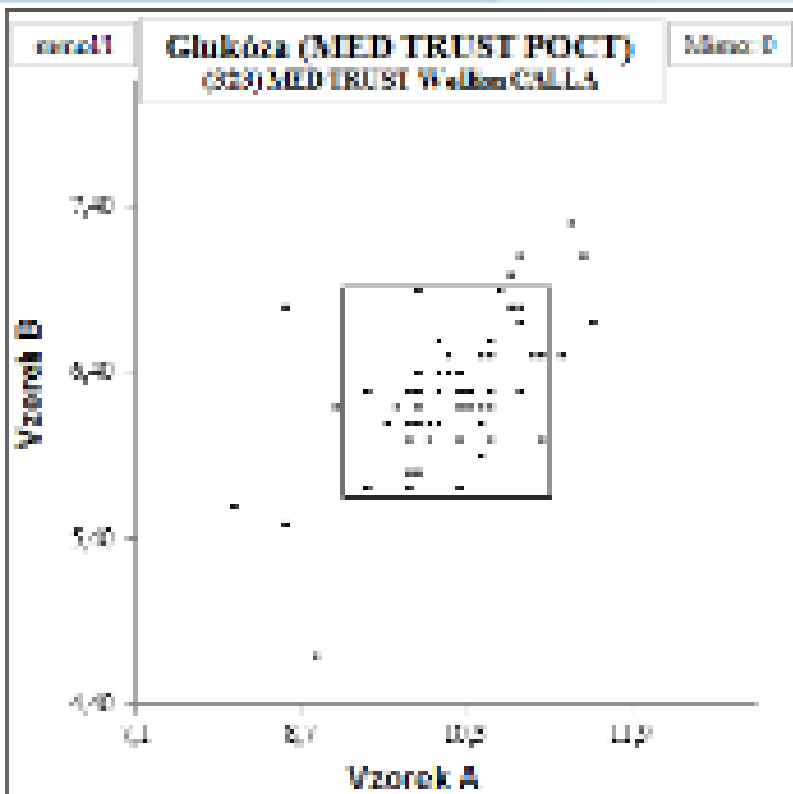


Doctor



How much trust do you put to laboratory results?

What would you do when you doubt the results validity?









# How to standardize lab results?

## Follow instructions!

### Lab. příručka OKB

- A [Obsah a úvod](#)
- B [Info o laboratoři](#)
- C [Manuál pro odběry](#)
- D [Preanalytické procesy](#)
- E [Vydávání výsledků](#)
- F [Seznam vyš. OKB](#)
  -  [FN Brno](#)
  -  [Bohunice](#)
  -  [Dětská nem.](#)
  -  [Porodnice](#)
- G [Pokyny pro pacienty](#)
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- J [POCT](#)
- K [Revize příručky](#)
- [Archiv](#)



The screenshot shows the website of the Česká společnost klinické biochemie (Czech Society of Clinical Biochemistry). The header features the society's name in large blue text, its logo (a stylized blue and red 'A' shape), and the text 'Česká lékařská společnost Jana Evangelisty Purkyně'. Below the header is a navigation menu with items: Časopisy, **Doporučení** (highlighted in red), Stanoviska, Spolupráce, Sekce laborantů, Kvalita, Legislativa, and Odkazy. The main content area displays the EFLM logo (EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE) and a search bar. Below the EFLM logo is a navigation menu with items: WHO WE ARE, CONGRESS CALENDAR, EFLM PUBLICATIONS, EFLM MEMBERS, EXECUTIVE BOARD, and GENERAL INFORMATION. The footer features the IFCC logo (International Federation of Clinical Chemistry and Laboratory Medicine) and the tagline 'Advancing excellence in laboratory medicine for better healthcare worldwide'. The bottom navigation menu includes: Executive Board and Council, Scientific Division, Education and Management, **Communications and Publications** (highlighted in blue), and Congresses and Conferences.



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### Mobile app available



**\*\*FREE\*\***

iPhone/iPad  
Android

### Our Commitment



We comply with the HONCode Standard for trustworthy health information.  
**Verify compliance.**

### Our Health Network



## Lab Tests Online-UK

Lab Tests Online-UK is written by practising laboratory doctors and scientists to help you understand the many clinical laboratory tests that are used in diagnosis, monitoring and treatment of disease. The [about this site](#) page describes how the site can help you. Search under [conditions](#) and [diseases](#) and find information on laboratory tests used for particular diagnosis and/or management or alternatively, if you know the test name, just search under tests.

Let us know what you think of the site and how we might improve it. If you found Lab Tests Online-UK useful, please spread the word!

## Topics in the News

### Viral DNA test successfully screened those at high risk of nasopharyngeal cancer

*22 September 2017*

Cancer of the nasopharynx (that part of the throat between the back of the nose and the back of the mouth) is prevalent in Southeast Asia. It often causes no symptoms until locally advanced. In a study published in *The New England Journal of Medicine* on 10 August 2017, blood plasma samples from more than 20,000 men of Chinese descent aged 40 to 62 living in Hong Kong were tested for circulating DNA fragments of the Epstein-Barr virus. There were persistently positive results in 309 men who were then offered examination of their nasopharynx with an endoscope and by MRI scanning. Of the 300 men examined 34 (11%) were found to have nasopharyngeal cancer, and it was at an early and potentially curable stage in 16 of them.

### Lithium present in tap water may protect against dementia

*14 September 2017*

A recent publication published in *JAMA Psychiatry* suggests that the lithium present naturally in small quantities in tap water may have a protective effect against dementia.

### MHRA seizes HIV home-test kits over false result risk

Use the search box and menus below to quickly navigate Lab Tests Online

**SEARCH**

### Tests

List of all tests and acronyms  
Test not listed?   
5-HIAA  
17-Hydroxyprogesterone  
ACE  
Acetylcholine Receptor (AChf   
ACR

### Conditions/Diseases

List of all conditions/diseases  
Acidosis/Alkalosis   
Acromegaly  
Addison's disease  
Adrenal Insufficiency  
AIDS   
Alcoholism

### Screening

List of screening tests  
Newborns   
Infants  
Children  
Young Adults  
Adults   
Adults 50+

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# Laboratorní vyšetření

název vyšetření	Amoniak P-NH3 NH3	
pracoviště	OKB Bohunice / rutinní tel. 3168, statim+pohot. 3057	
materiál		plazma
odezva - RUTINA - STATIM	denně 60-120 minut	
odb. nádoba RUTINA	Sarstedt	Multivette: EDTA K 600ul - červená
odb. nádoba STATIM	Sarstedt	Multivette: EDTA K 600ul - červená
pokyny k odběru	ihned uzavřít, promíchat, transport v ledové tříšti! plazmu je nutno separovat do 30min. po odběru!	
stabilita před přijetím	čas teplota	30min 18 - 25 °C
stabilita v laboratoři	čas teplota	3h 2 - 8 °C
jednotka	umol/l	( molární koncentrace )
přepočet jednotek	ug/dl x 0,587 = umol/l	
analytická nejistota měření	8,6%	
metoda	fotometrie, enzymaticky	
SOP - číslo - název	37421159 Stanovení amoniaku (analyzátor Cobas- Roche)	
klíč NČLP	10849	
vykazování pro pojišťovnu RUTINA STATIM	výkon 81341 81119	body 81 87

GIGO => „Garbage in...

Pre-analytical error



Analytical error



Post-analytical error

Increasing total error

GIGO => ...garbage out“



# Pre-analytical phase

- The time period between the physician's indication and the laboratory analysis
- Proper pre-analytical procedures are necessary for accurate results (46-68 % of erroneous results are caused by faulty pre-analytics)
- Instructions are provided by laboratory

# Pre-analytical phase

- **Biological variability** of measured parameters require patients and medical personnel to mind several factors before taking the sample:

- **Controlable factors**

timing / exclusion of medication with interfering properties / diet / physical and psychological stress, smoking, alcohol / patient position during sample taking / sample container

- **Uncontrollable factors**

age, gender, race, gravidity

# Pre-analytical phase

- **Intra-individual variability**

Time-dependent parameter changing widening the interval of physiological values

- **Inter-individual variability**

Time-independent parameter value variability in a healthy population (genetic, environmental factors)



$\mu\text{g}/\text{dl}$

300

250

200

150

100

50

0

**cortisol**

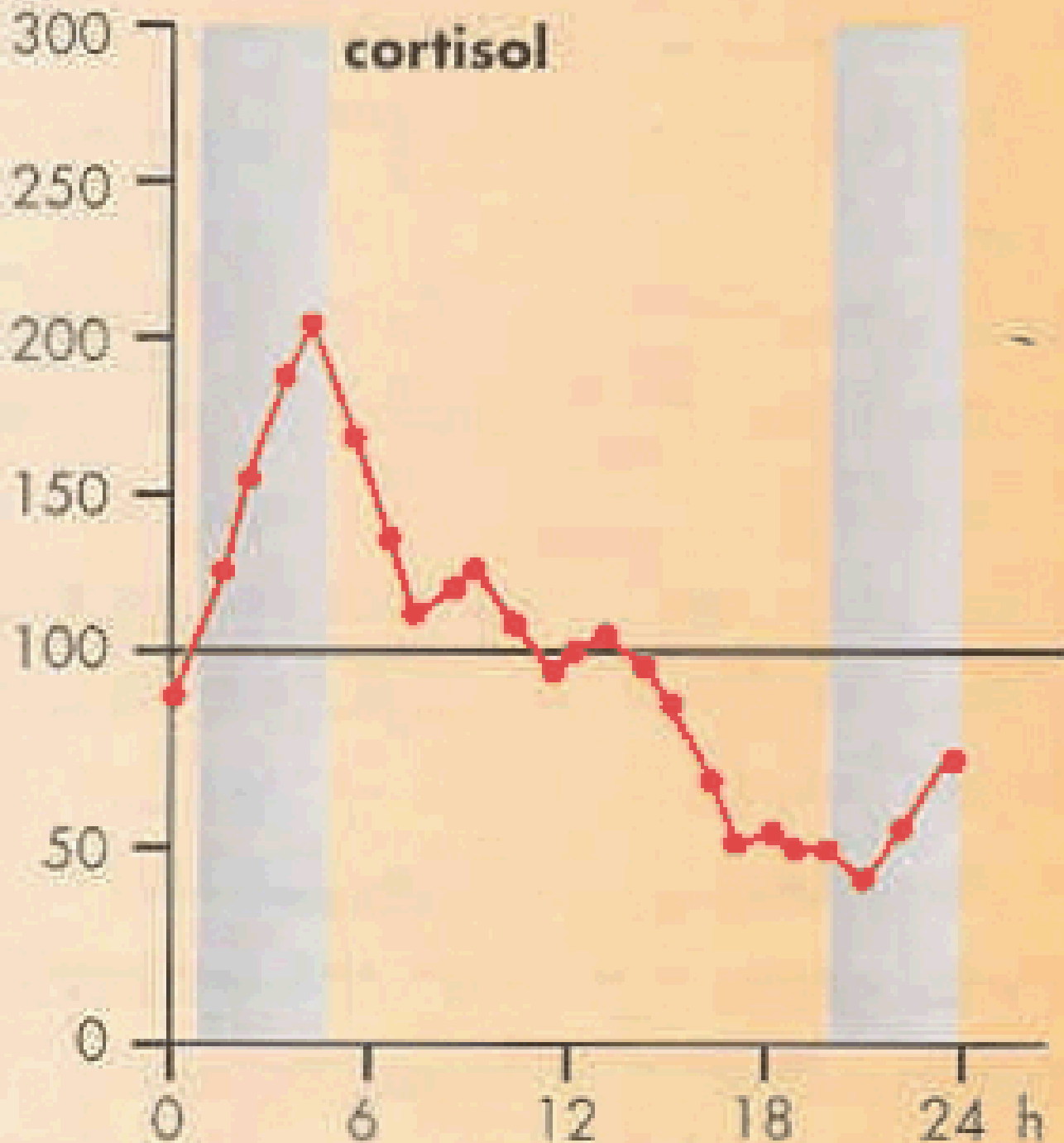
0

6

12

18

24 h



# Analysis

- Analysis is a process where values of examined biological material parameters are determined
- Analyzed parameter is referred to as an analyte determined in a process called quantification
- Accuracy of analysis is limited by measurement errors, results are limited by value uncertainty

# Quantification error

- The term error is understood as a difference between measured value and referent value (not to be confused with a mistake)
- Errors may be divided in groups of **random, systematic and gross errors**

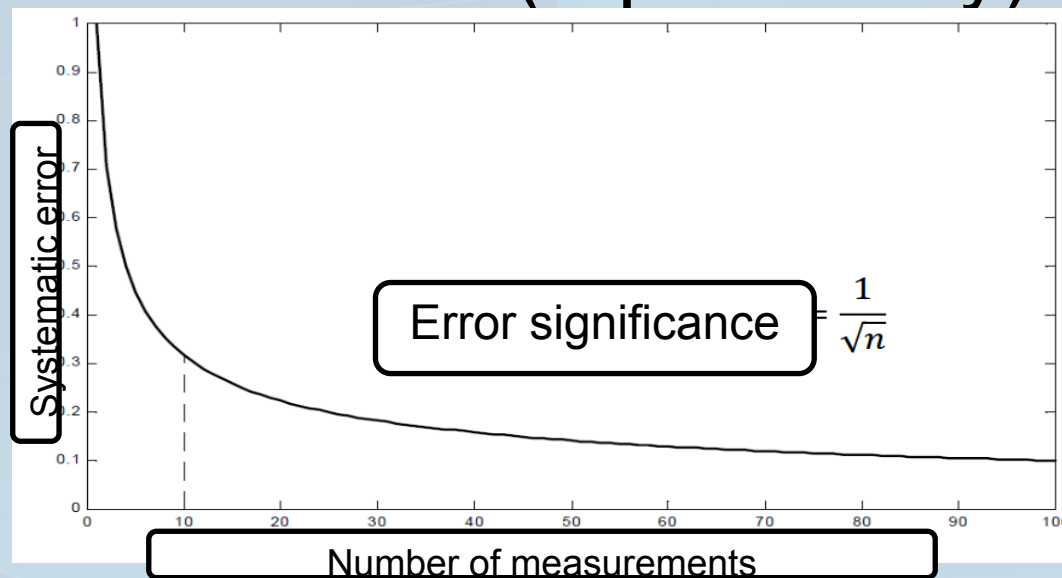


# Gross errors

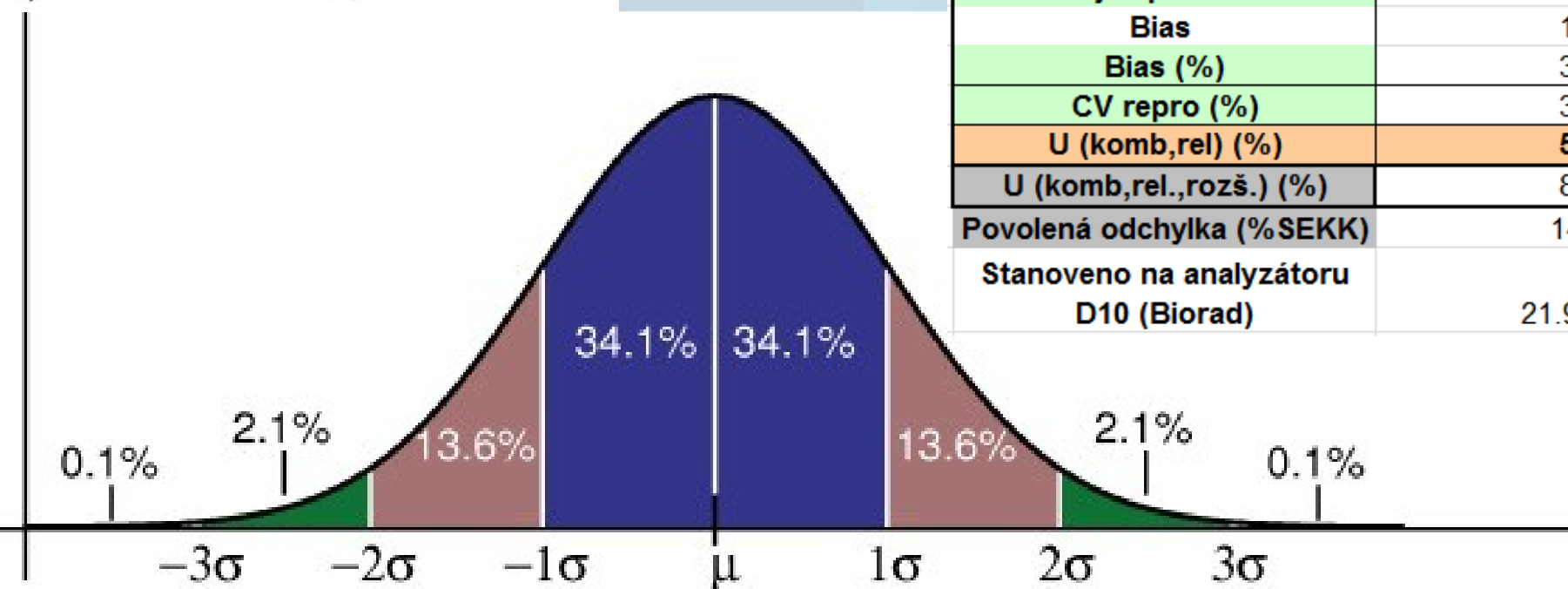
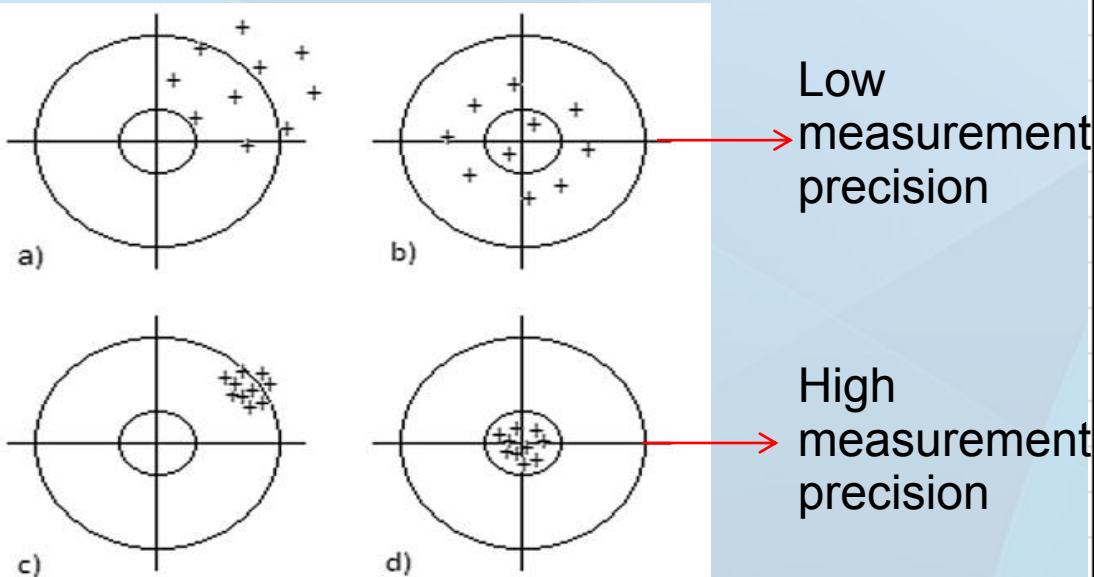
- Caused by faulty methodology, fatigued personnel, analyzer malfunction
- May be uncovered only through test repeat
- Such errors register as outliers on plots and may be tested and removed from data sheet
- Q-test, Grubbs, Cochran C, z-test

# Random errors

- Unpredictably occurring value offset participating in the total error of quantification.
- Gaussian distribution with null average value and standard deviation.
- Size of error can be decreased by higher number of run tests (repeatability).



# Random errors



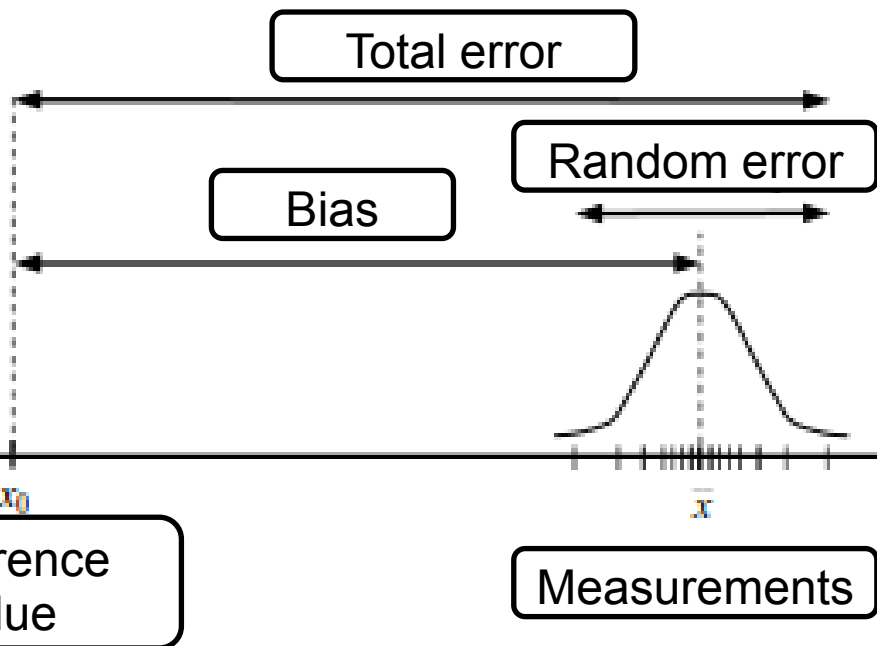
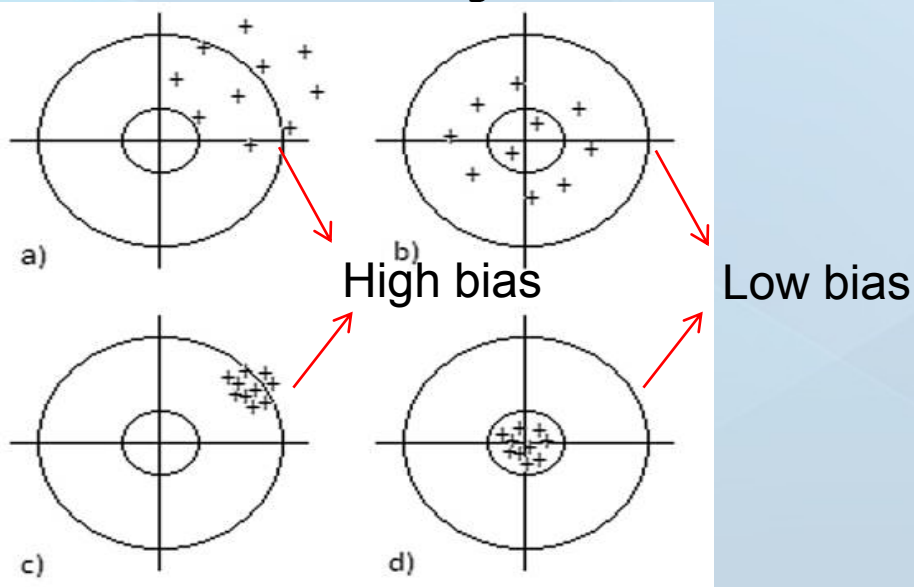
analyt	HbA1c
materiál	KD1/17 A (5019)
jednotky	[mmol/mol]
Target Value	42,8
nejist.TV (%)	1,3
1	45
2	44
3	44
4	44
5	44
6	44
7	44
8	45
9	46
10	45
průměr	44,5
sm.odch.opakovatelnosti	0,67
CV opak (%)	1,51
nejist.průměru	0,48
Bias	1,70
Bias (%)	3,97
CV repro (%)	3,15
U (komb,rel) (%)	5,26
U (komb,rel.,rozš.) (%)	8,44
Povolená odchylka (%SEKK)	14,00
Stanoveno na analyzátoru D10 (Biorad)	21.9.2017



# Systematic errors

- Predictably occurring value offset participating in the total error of quantification.
- The cause of a systematic error may be known or not. The error may be compensated by applying a correction factor.

# Quantification error - systematic



analyt	HbA1c
materiál	KD1/17 A (5019)
jednotky	[mmol/mol]
Target Value	42,8
nejist.TV (%)	1,3
1	45
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# Quantification properties

- **Result uncertainty**

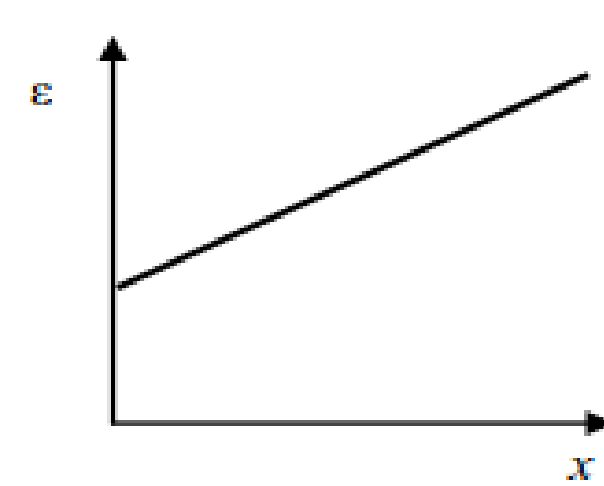
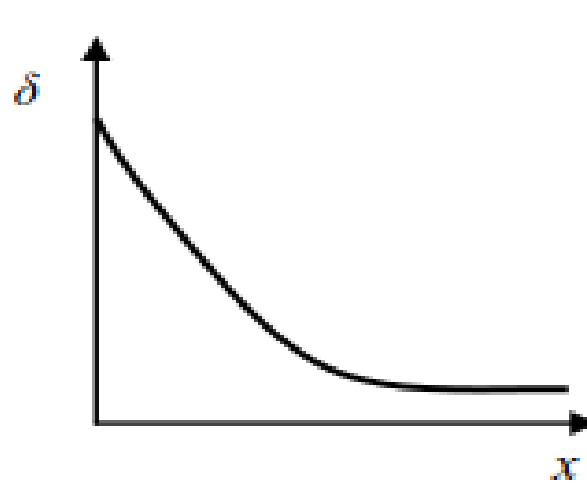
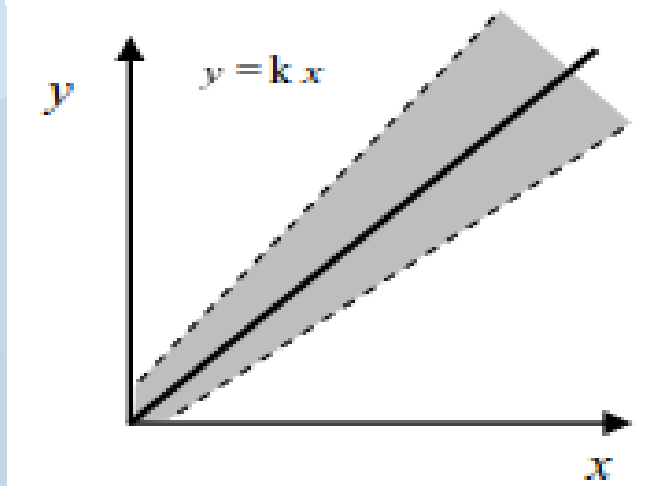
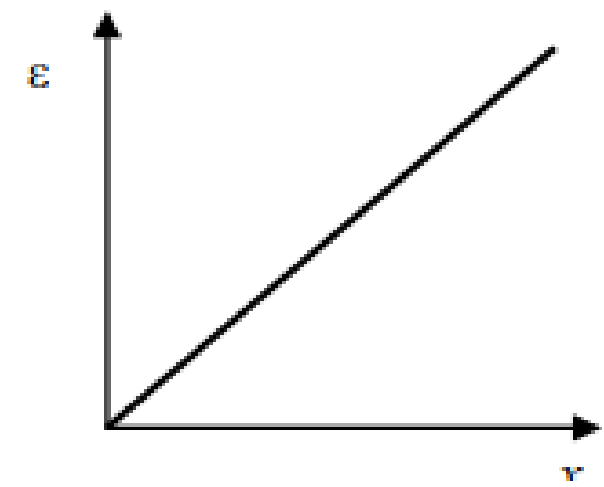
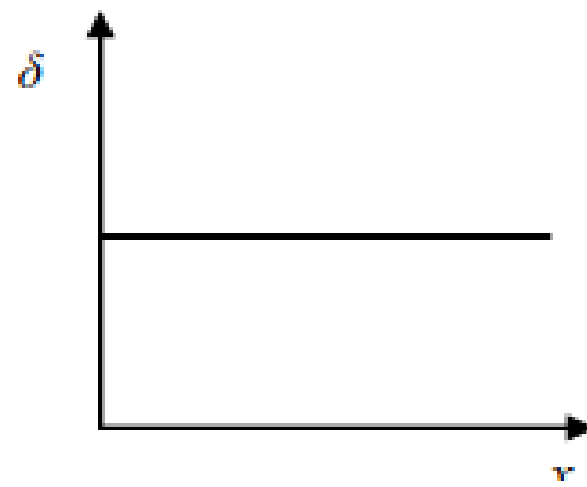
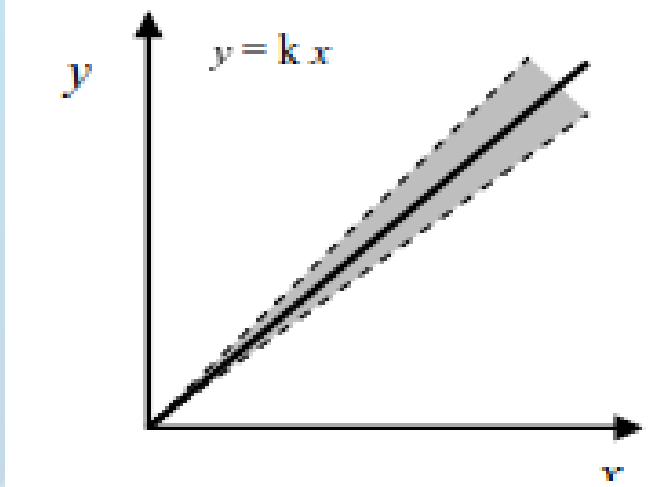
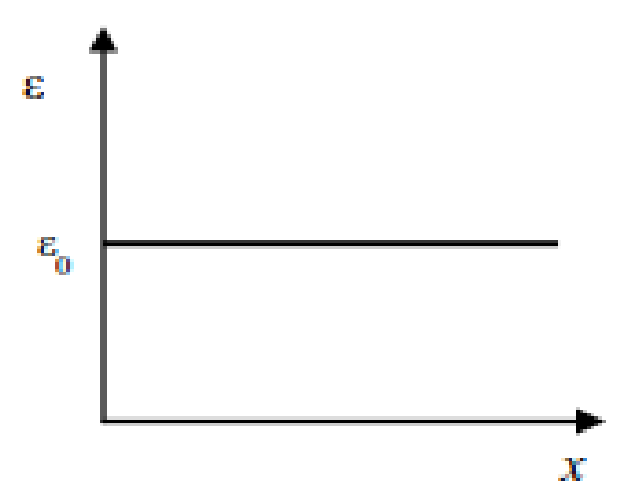
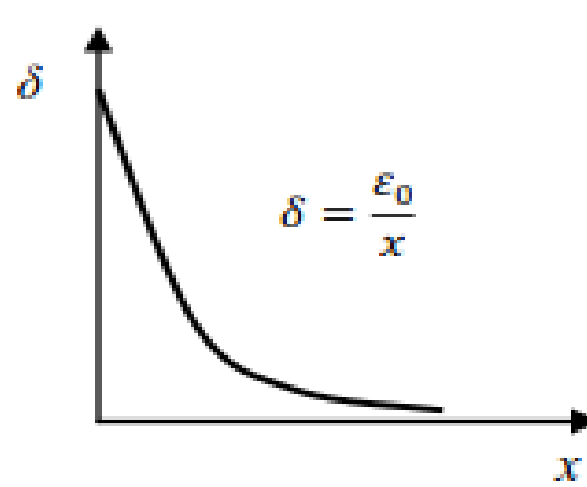
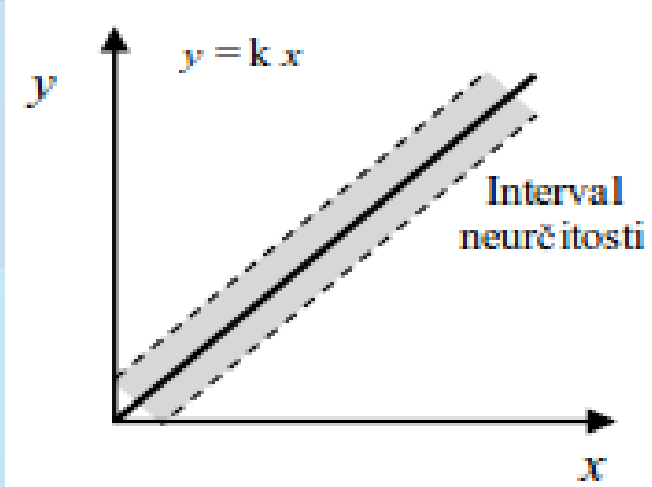
Characterizing the distribution of values around the result with normal probability of occurrence

Standard deviations or variation coefficients are generally used for standard uncertainty assessment

Combined standard uncertainty is calculated after determination of all uncertainty sources

- **Expanded combined uncertainty** is calculated from combined standard uncertainty ( $k=2$ )

$$U_c = k \cdot u_c \text{ (} k=2 \text{ for 95\% probability range)}$$



# Quantification properties

- **Limit of detection (LoD)**

The minimal amount of analyte in sample to register a significant signal

(3s)

- **Limit of quantification (LoQ)**

The minimal amount of analyte in sample that can be quantified with appointed result uncertainty

(10s)

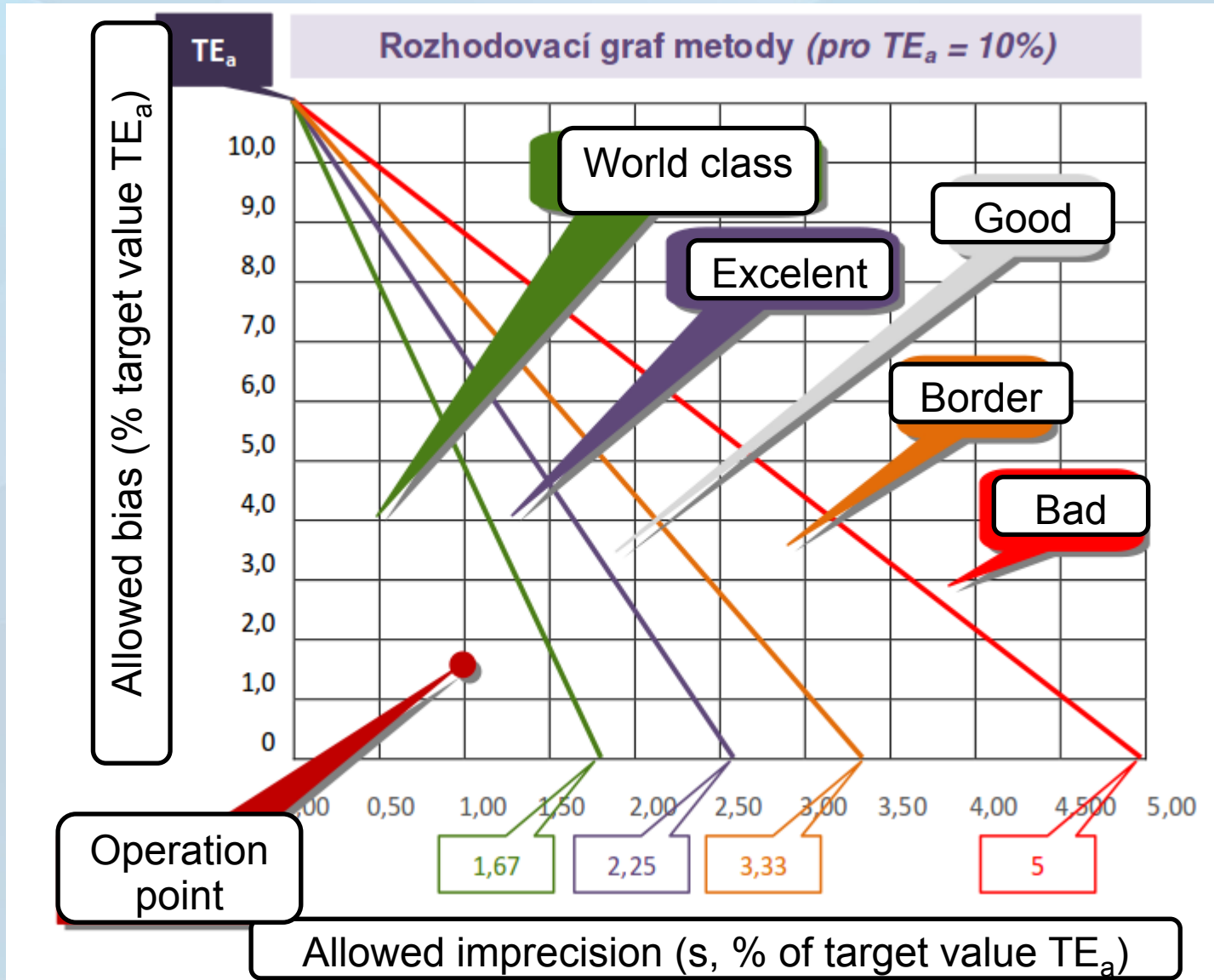
- **Working range**

Range of analyte concentrations between the minimal and maximal limits of quantification



# Total quantification error

$$TE_a (\%) \leq 1,65 \cdot \frac{2}{8} \cdot CV_i + \frac{1}{8} \sqrt{CV_i^2 + CV_g^2}$$



# Laboratorní vyšetření

\*A - akreditováno

název vyšetření	Albumin S/P-Alb	
pracoviště	OKB Bohunice / rutinní tel. 3168, statim+pohot. 3057	
materiál		sérum/plazma
odezva - RUTINA - STATIM	denně 60-120 minut	
odb. nádoba RUTINA	Sarstedt	Monovette: Serum Gel 4.9ml - hnědá
odb. nádoba STATIM	Sarstedt	Monovette: Li Heparin 4.9/5.5ml - oranžová
pokyny k odběru	běžný odběr - bez zvláštních opatření	
stabilita před přijetím	čas teplota	°C
stabilita v laboratoři	čas teplota	5 měsíců 2 - 8 °C
jednotka	g/l	( váhová koncentrace )
přepočít jednotek		
analytická nejistota měření	5,2%	
metoda	fotometrie endpoint, s bromkrezolovou zelení	
SOP - číslo - název	37421805 Stanovení albuminu fotometricky (analyzátor Cobas Roche)	
klíč NČLP	00507	
vykazování pro pojišťovnu RUTINA STATIM	výkon 81329 81115	body 14 22

# Validation/Verification

- Adopting new method of quantification requires either validation or verification

- **Validation** is a process of method authentication and checking if the parameters qualify for its designed use

Precision, trueness, LoD, LoQ, selectivity, specificity, working range, linearity, robustness, repeatability  
reproducibility

- **Verification** is a process of values proofing stated in validation, and the methods specifications meet its requirements

# Reference materials & calibrations

- Comparison of results with referent materials values for the assessment of quantification trueness
- Calibration materials are derived from materials with higher specification called traceability via hierarchy of referent materials

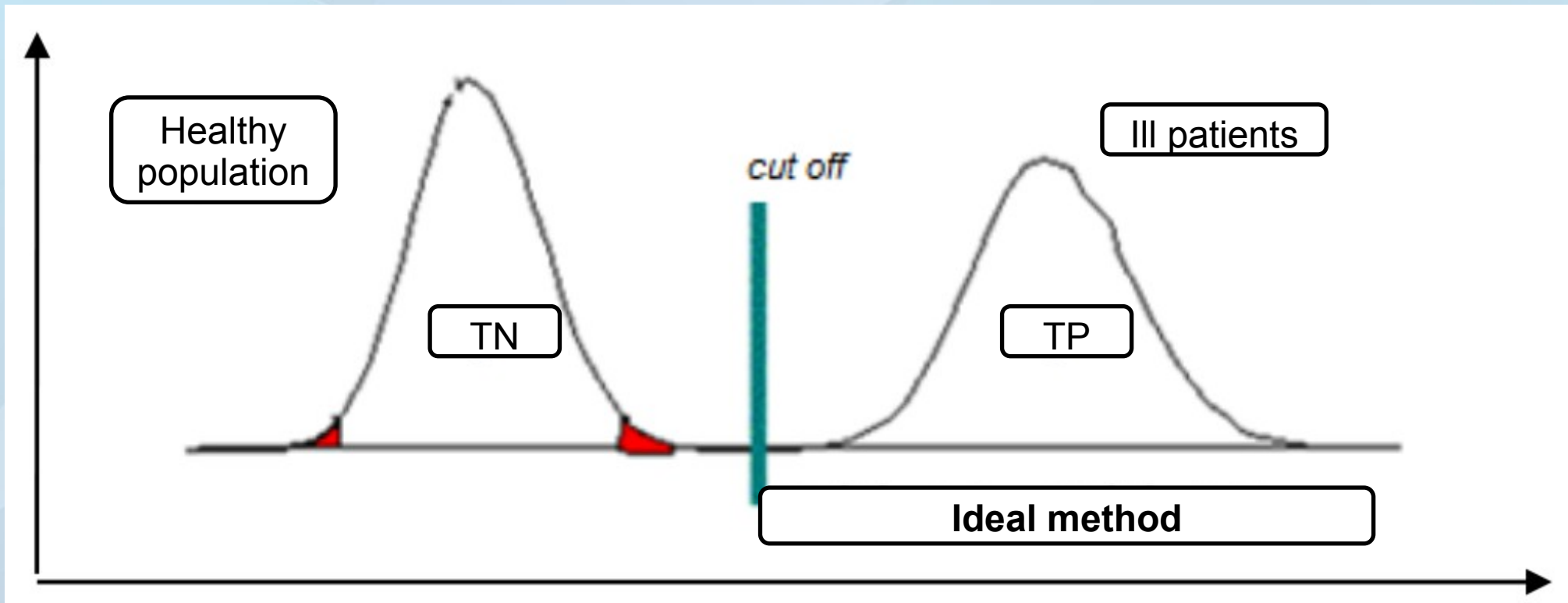
# Reference values

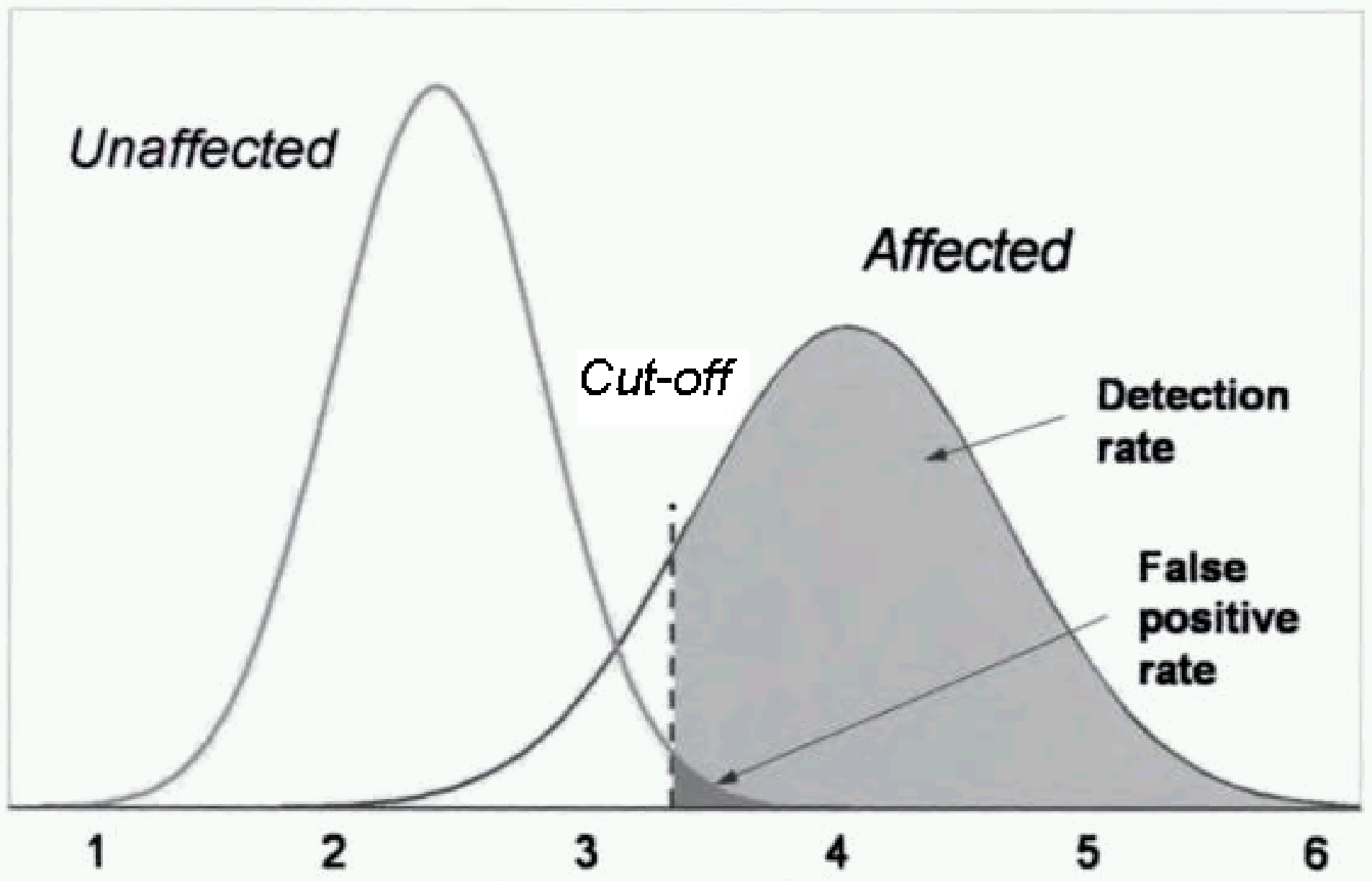




# Reference values

- May be obtained by analysis of healthy population with high standard specification of individuals – such population is called reference population
- Literature, data from quantification assay supplier, consensus of experts





# Quantification diagnostic effectivity

## – Specificity

Measure of avoiding cross-reactivity in negative samples

$$\text{Diagnostic specificity} = \frac{\text{Healthy with negative test}}{\text{All healthy}} = \frac{SN}{SN + FP}$$

Valued especially in tests verifying the final diagnosis and with serious diseases (intervention seriously damages patient)

Study sample	Disease	Healthy	Total
Positive test	TP	FP	TP + FP
Negative test	FN	TN	FN + TN
Total	TP + FN	FP + TN	TP + FP + FN + TN

# Quantification diagnostic effectivity

## – Sensitivity

Measure of detecting low analyte concentrations in positive samples

$$\text{Diagnostic sensitivity} = \frac{\text{ill with positive test}}{\text{All ill patients}} = \frac{SP}{SP + FN}$$

Valued especially in screening tests for early disease detection

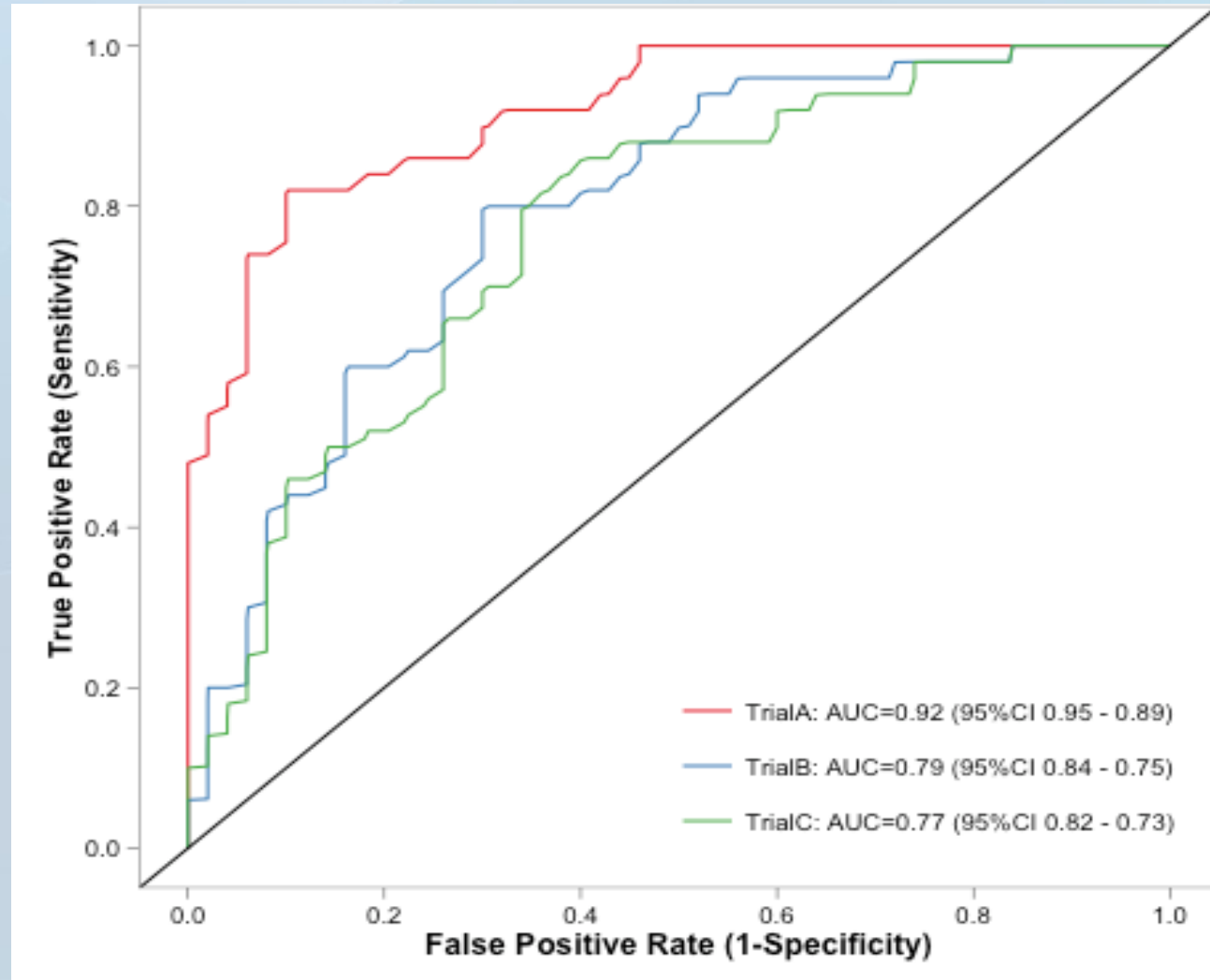
Study sample	Disease	Healthy	Total
Positive test	TP	FP	TP + FP
Negative test	FN	TN	FN + TN
Total	TP + FN	FP + TN	TP + FP + FN + TN



# ROC

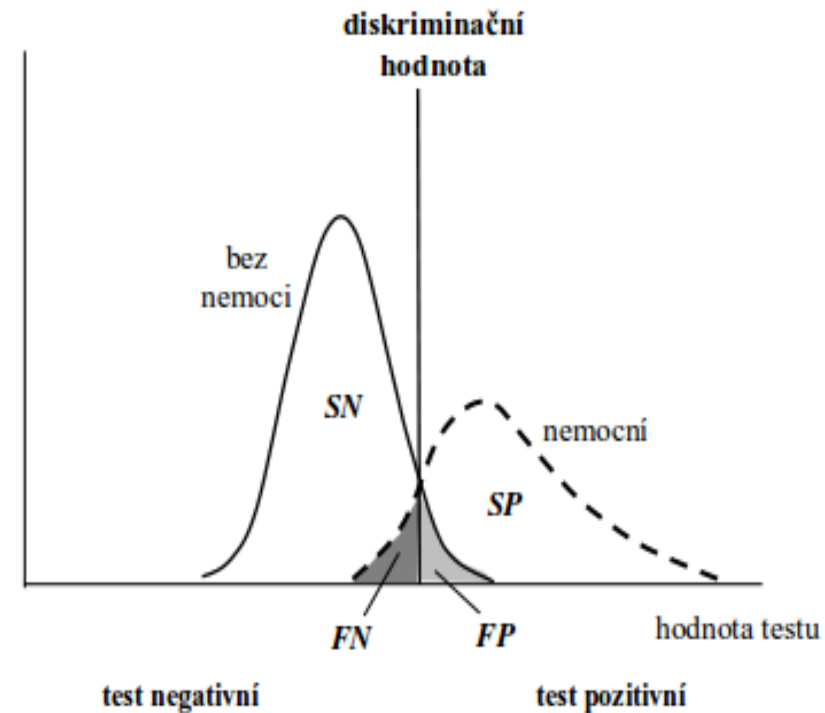
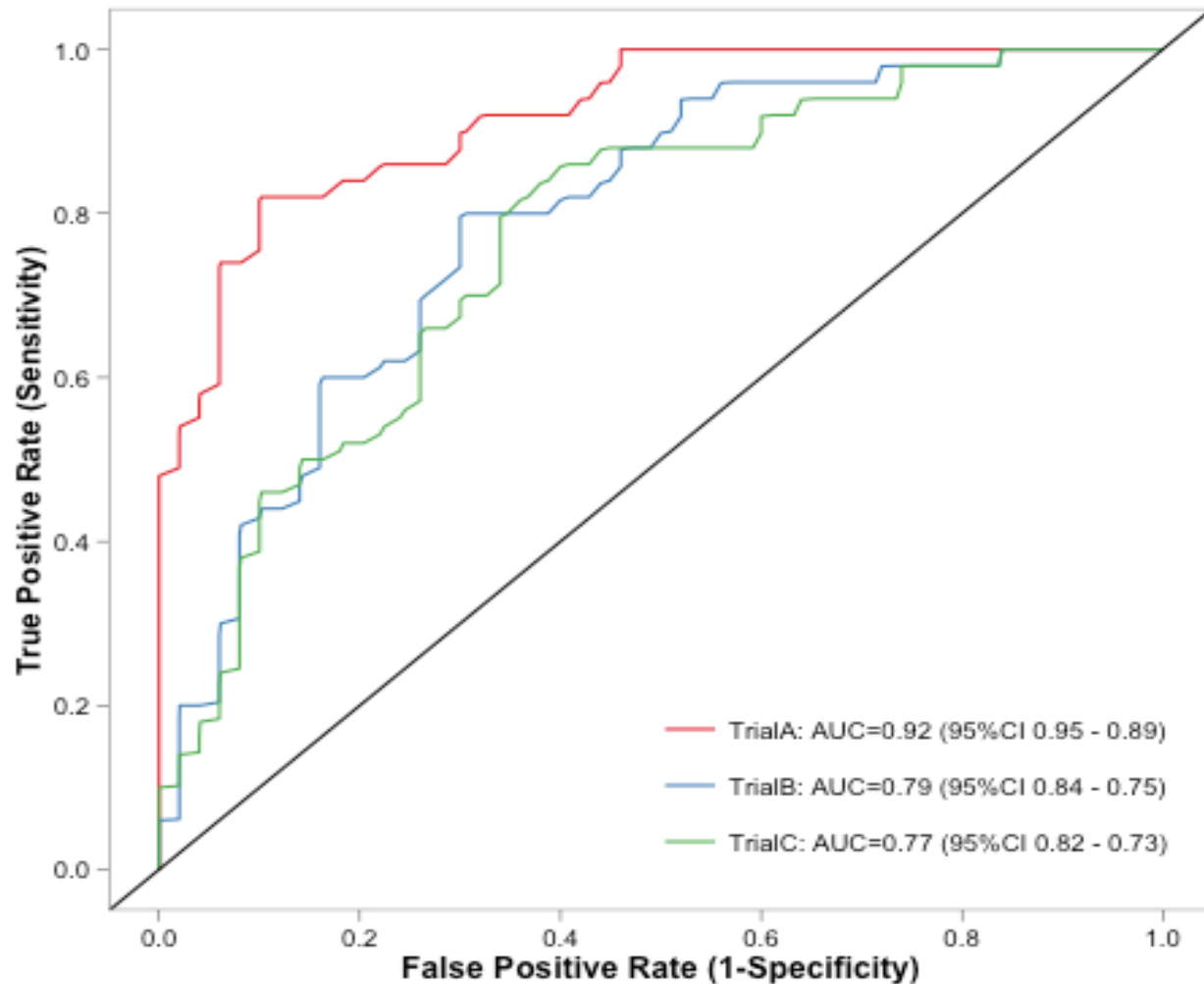
(Receiver operating characteristic plot)

- Graphical evaluation of test discriminatory capabilities
- The y axis comprises of the test sensitivity and x axis comprises of unspecificity for all cut-off values



# ROC

(Receiver operating characteristic plot)



- AUC quantifies diagnostic effectivity of the test

- sensitivity, specificity, positive and negative predictive values,

# Predictive values

- Positive predictive value

Probability that positive test means ill individual

$$PV^+ = \frac{\text{Ill with positive test}}{\text{All with positive test}} = \frac{TP}{TP + FP}$$

- Negative predictive value

Probability that negative test means healthy individual

$$PV^- = \frac{\text{Healthy with negative test}}{\text{All with negative test}} = \frac{TN}{TN + FN}$$

# Predictive values

- Prevalence

Rate of representation among population

$$\text{Prevalence} = \frac{\text{III}}{\text{The whole sample}} = \frac{\text{TP} + \text{FN}}{\text{TP} + \text{FN} + \text{FP} + \text{TN}}$$

- Odds ratio

Ratio of positive to negative tests, assesses the test information yield

$$OR = \frac{\text{TP} \times \text{TN}}{\text{FP} \times \text{FN}}$$

HEALTHY

DISEASED

99800

200

Screening test

Specificity - 94,3 %

Sensitivity - 95 %

TN = 94111

FN = 10

FP = 5689

TP = 190

OAPR<sub>1</sub> = 190:5689 = 1:30

Diagnostics test

Specificity - 99,5 %

Sensitivity - 99 %

TN = 5661

FN = 2

FP = 28

TP = 188

OAPR<sub>2</sub> = 188:28 = 7:1



**Interpretation of laboratory results must go hand in hand with the clinical state of the patient**

**When suspicious of erroneous results, contact the laboratory, measurements may be repeated from the same sample or you can perform new sample collection**

**Be mindful of proper preanalytics – „Trash in -> Trash out“**

**Always incorporate POCT systems in cooperation with laboratory**

**Don't forget about the false positive/false negative areas for tests. Reference values and cut-offs are not everything)**

**Thank you for attention**