Clinical Laboratory Investigations

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Definitions

- **Analysis of samples obtained from the human body**

Methodology

Spectrophotometry (absorption, emission)

Luminescence (photo - chemi)

Electrometry

Immune chemistry

Separation techniques

Cell counting and identification (flow cytometry)

Microbiology

The paradox of the course

Human body: approx. 70kg - 10^{15} living cells

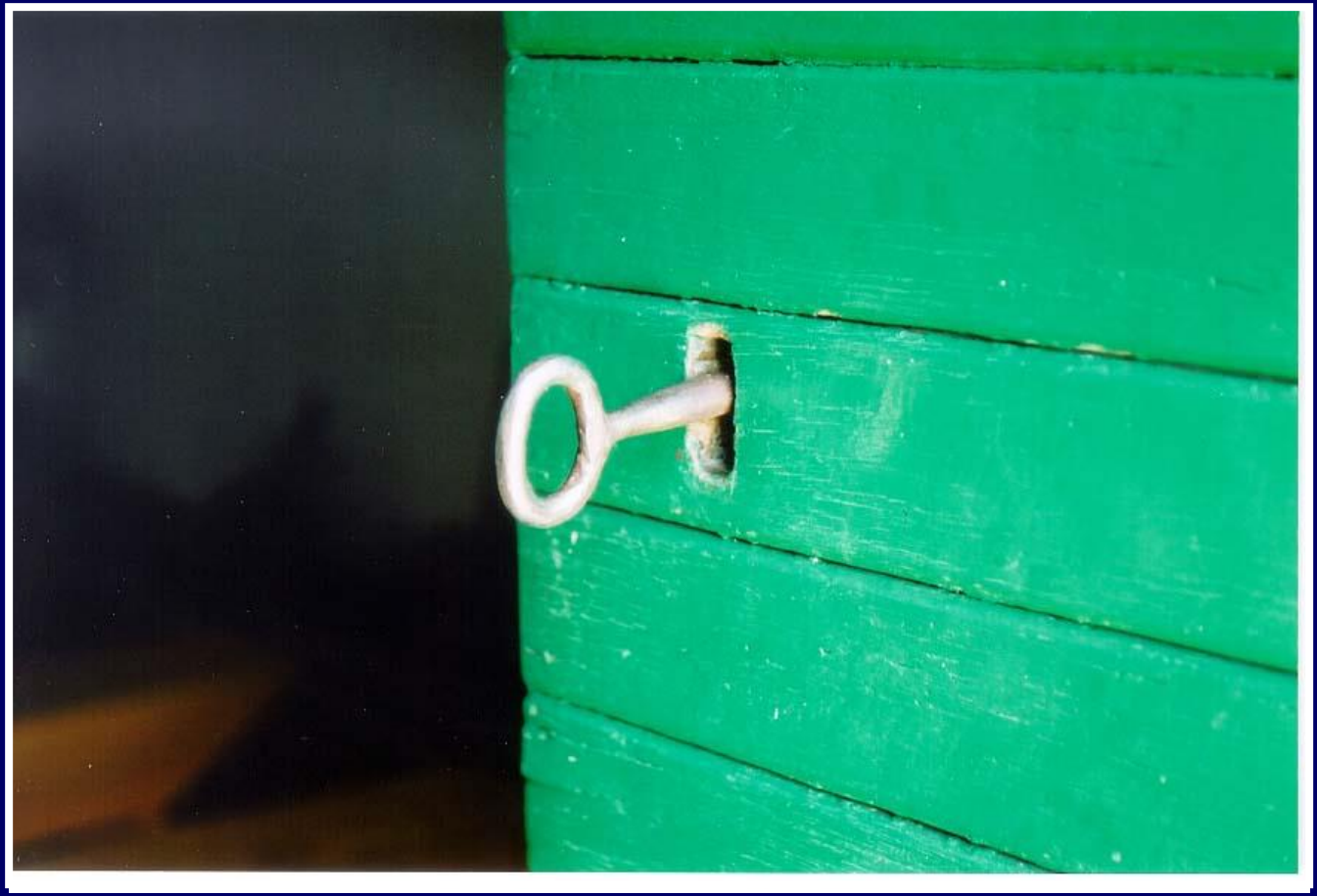
The paradox of the course

Sampling:

Extracellular space (60% water, 5 l of blood)

5-15 ml sample

The paradox of the course



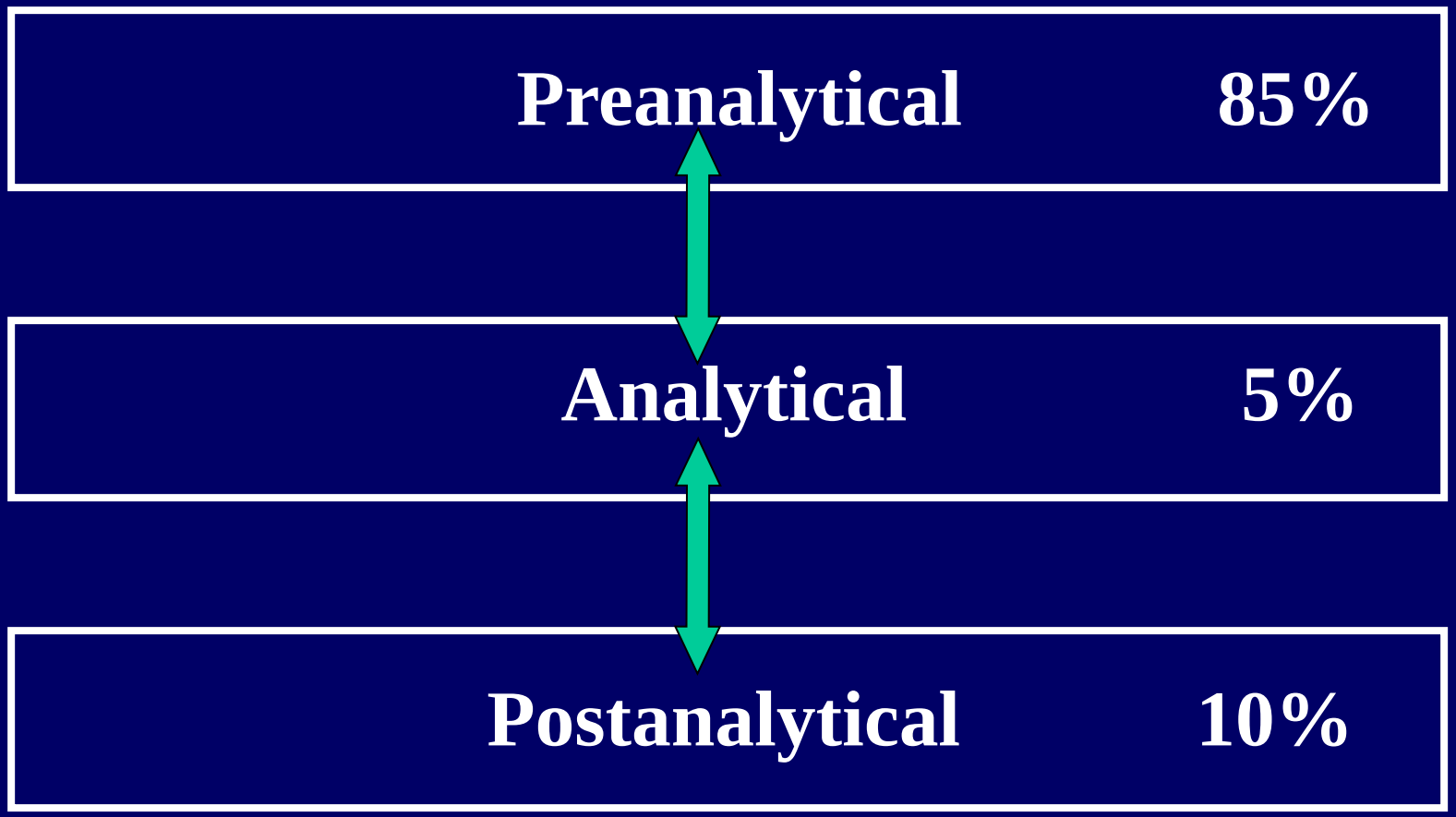
Analysis of sample: 2 - 100µl/test

Medical decision making: ~ 80%

Major goals of the course

- **Following the underlying biochemical processes in pathological conditions**
- **Introduction into instrumental analysis**
- **Sources of errors during the laboratory processes**
- **Quality control**
- **Interpretation of test results**
- **Examples and case discussions**

The phases of laboratory analysis, sources of errors



Preanalytical phase

- Patient preparation
- Test request
- Sample collection
- Sample identification
- Storage of samples
- Sending of samples to the lab
- Acceptance of samples (laboratory)

Patient preparation related to different lab tests

- Diet, 12h fasting
- Drug consumption?
- Smoking, alcohol consumption
- Physical exercise
- Stress
- Instructions for the patients
  (e.g. collection of urine)

Test requests regarding the type of analysis

- **Screening tests: 1 patient/many tests**
  large population/few tests

- **Discretionary tests**
- **Monitoring tests**

- **Urgent tests (always discretionary, often monitoring type)**

Sampling – sample types

- **Blood:** venous, capillary, arterial
  whole blood, fractions of blood
  native – anticoagulated blood

  Anticoagulants: heparin
  EDTA, citrate
  defibrination

  Enzyme inhibitors: NaF, iodoacetamide
- **Standardization, sources of errors (closed sampling system, e.g. Vacutainer)**

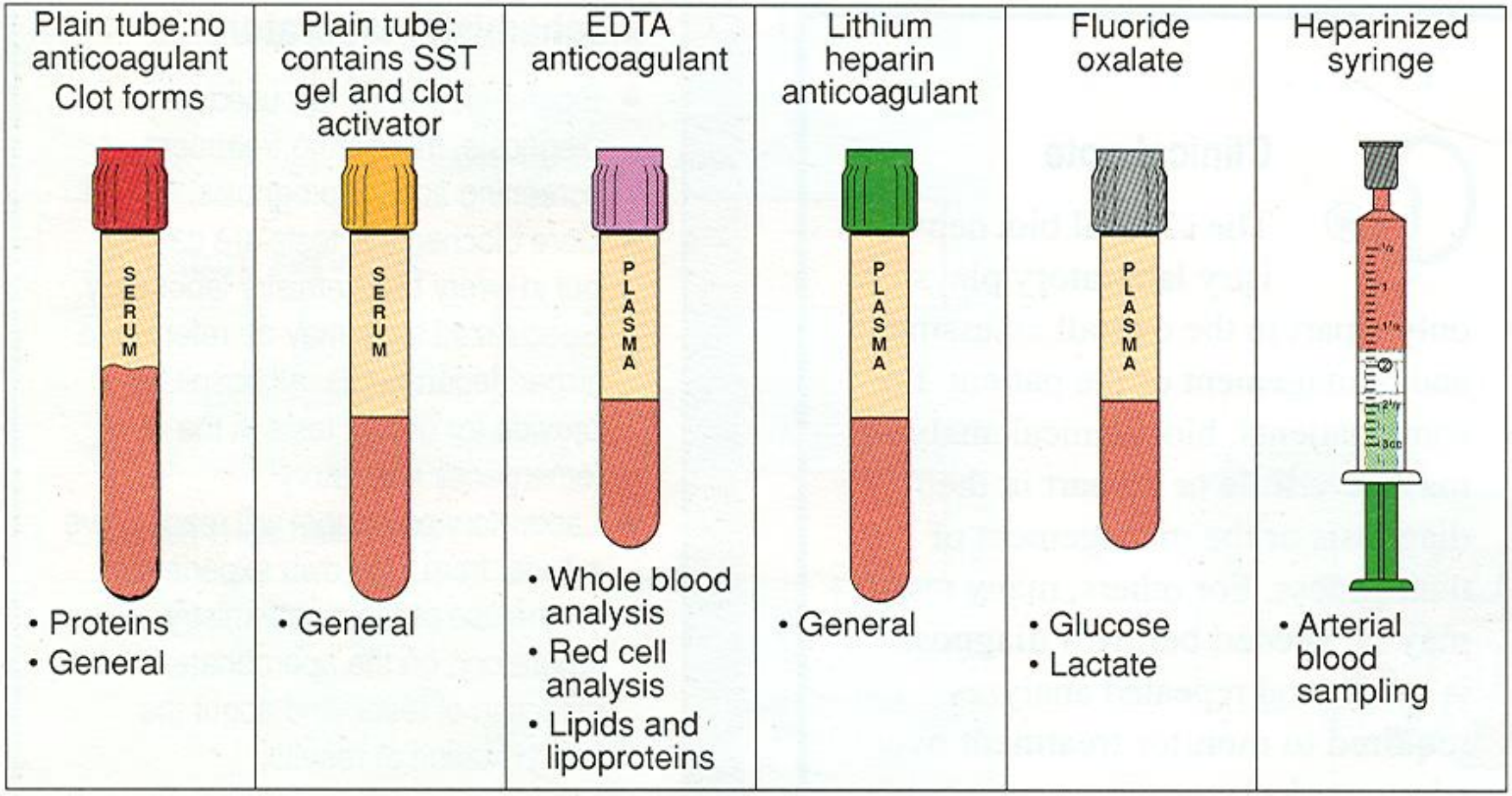


Fig. 1 **Blood specimen tubes for specific biochemical tests.** The colour-coded tubes are the vacutainers in use in the authors' hospital and laboratory.

Vacutainer tools

Technique for venous blood collection

Sampling – sample types

- **Urine:** **spontaneously voided**
  (first morning, midstream)

  collected
  (4h, 12h, 24h, stabilizers)

- **Standardization, sources of errors**

Sources of error in sampling

- **Urine in general**

  chemical cleanliness, sterility (for microbiological analysis)
- **Spontaneous urine sample**

  first morning specimen, midstream
- **Collected urine sample**

  24h, 12h, volume, mixing, additives, light sensitivity

  cooling

Identification, storage and sending of samples

- **Identification: bar code! – printed request form (LIS, HIS)**
- **Storage: Samples that can not be stored!**

  Serum, plasma, +4°C, -20°C

- **Sending: Immediately, frozen, on ice**
- **Accepting: Identification (demographical data, test requests**

Sampling related to the biological half-life

- **Monitoring tests in acute cases:**

  4 minutes - PTH

  several hours - proinflammatory mediators

  1-2 days - prothrombin
- **Monitoring tests in chronic cases:**

  2 weeks: platelets

  3 weeks: albumin

  1- 3 months: tumor markers, HbA1c

Analytical phase – precision and accuracy

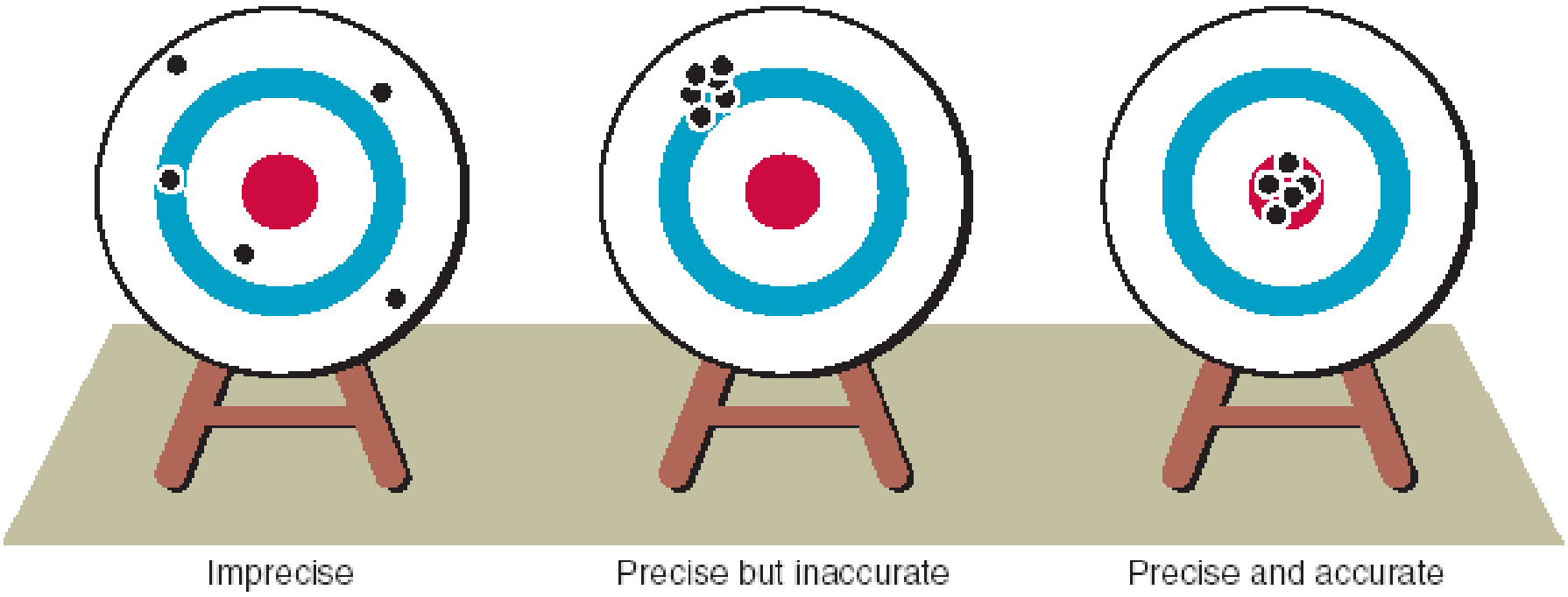
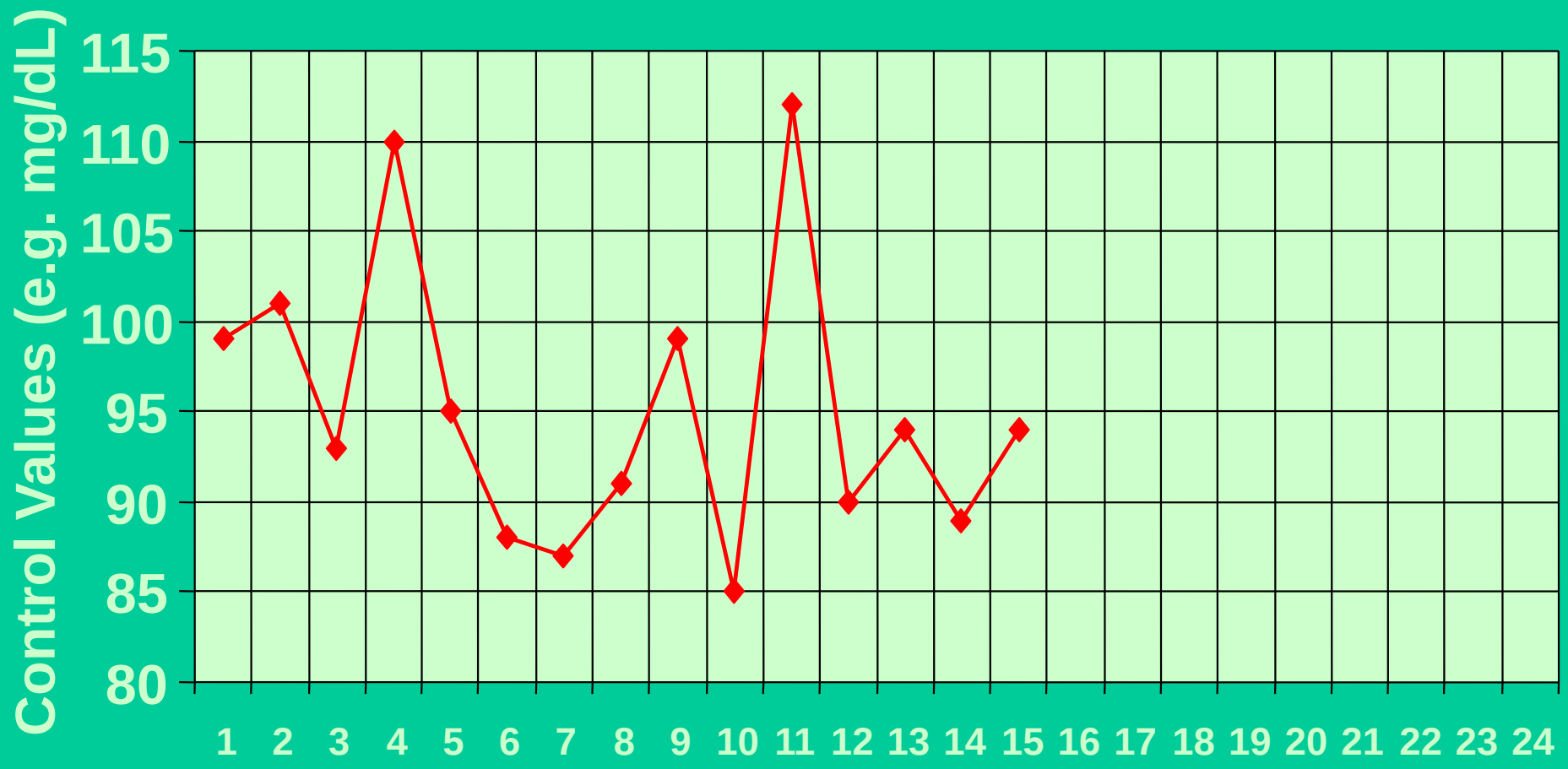


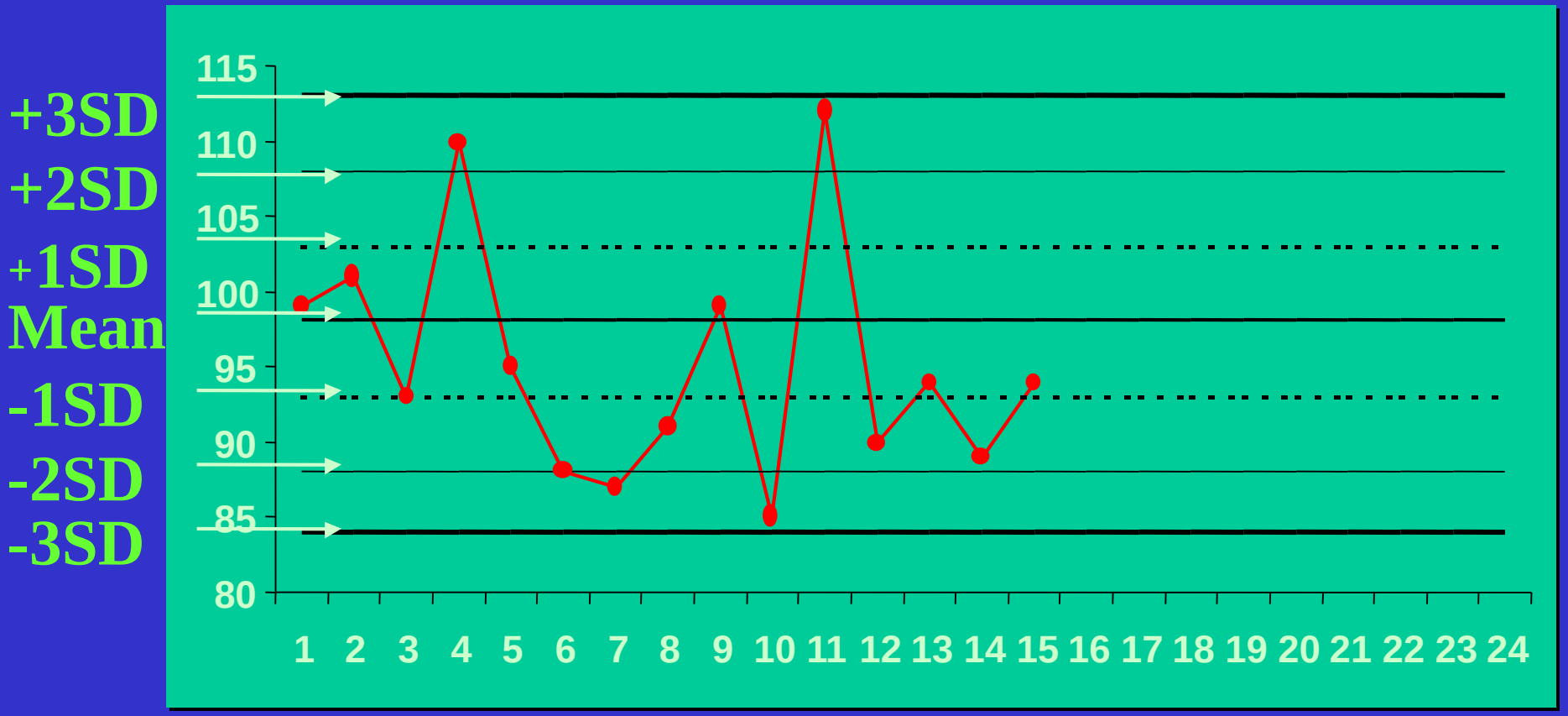
Fig. 2 **Precision and accuracy.**

Quality control samples with declared values

Quality control card
Levey-Jennings chart



Quality control card
Levey-Jennings chart

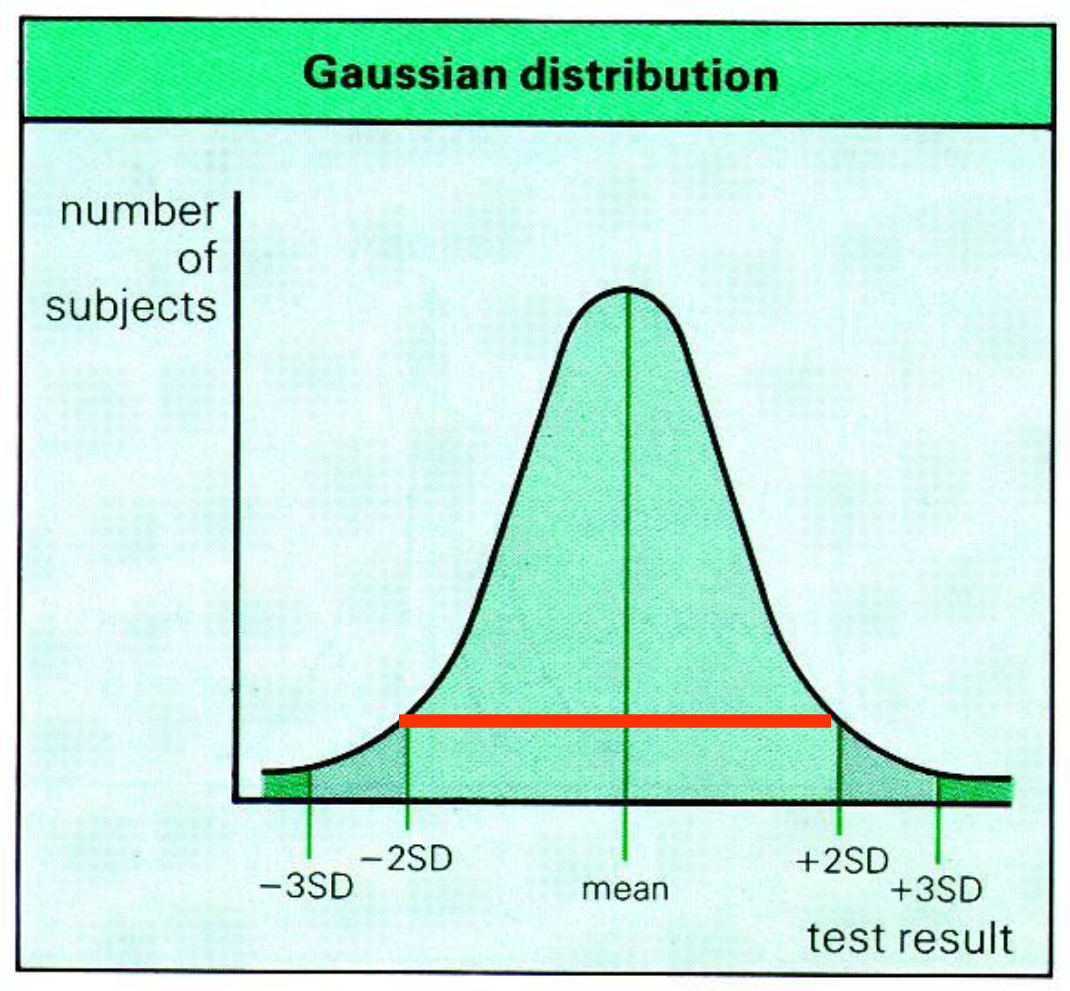


Postanalytical phase – test report and interpretation

- On line report
- Report of urgent tests (time factor)
- „Flags”, suggestions
- Consultation with the physician

- Interpretation of test results in view of the patient
  (pathological-normal, plausible)

Postanalytical phase - test report and interpretation



Reference range

- **representative population**
- **large number of test**
- **instrument, method**

Postanalytical phase - test report and interpretation

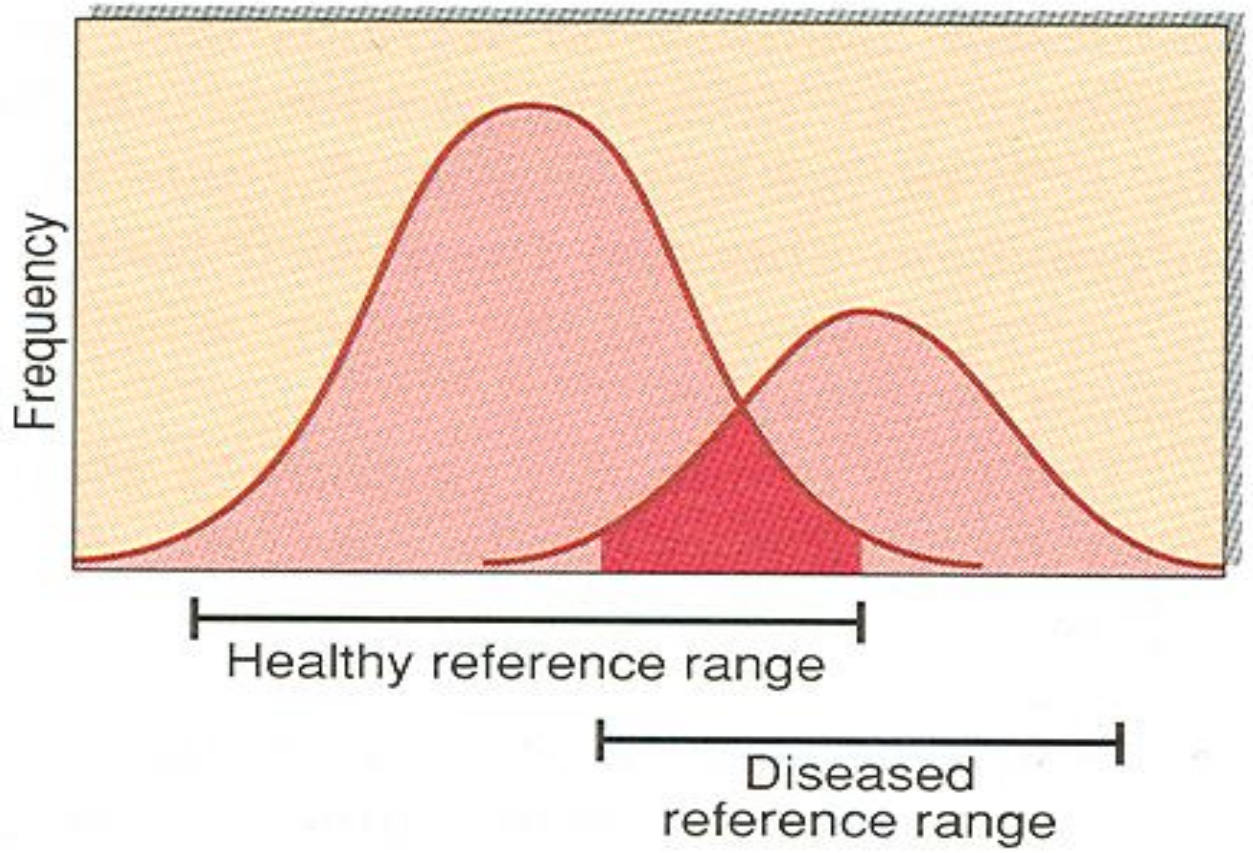
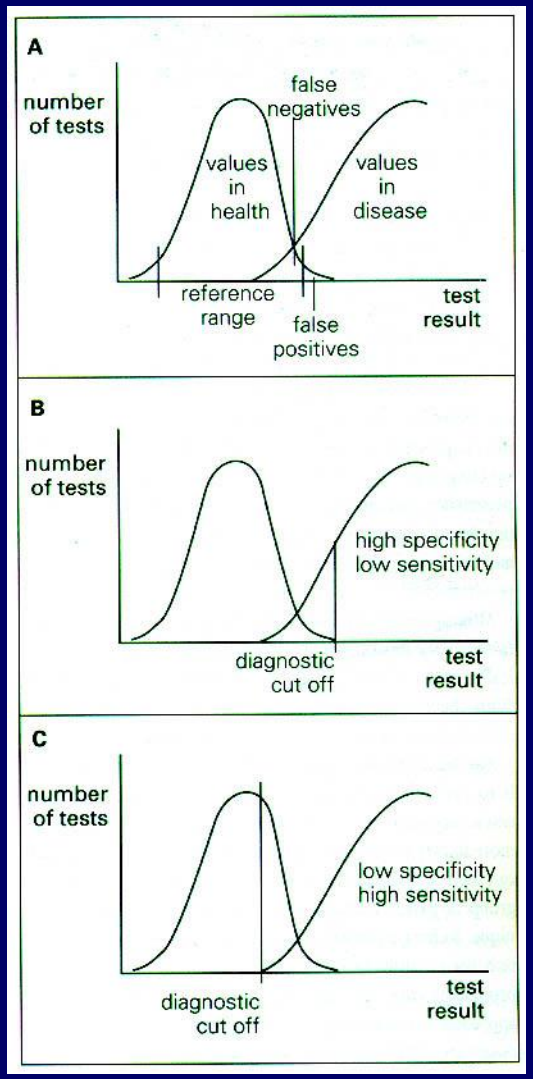


Fig. 4 **Overlap of biochemical results in health and disease.**

Interpretation - specificity, sensitivity

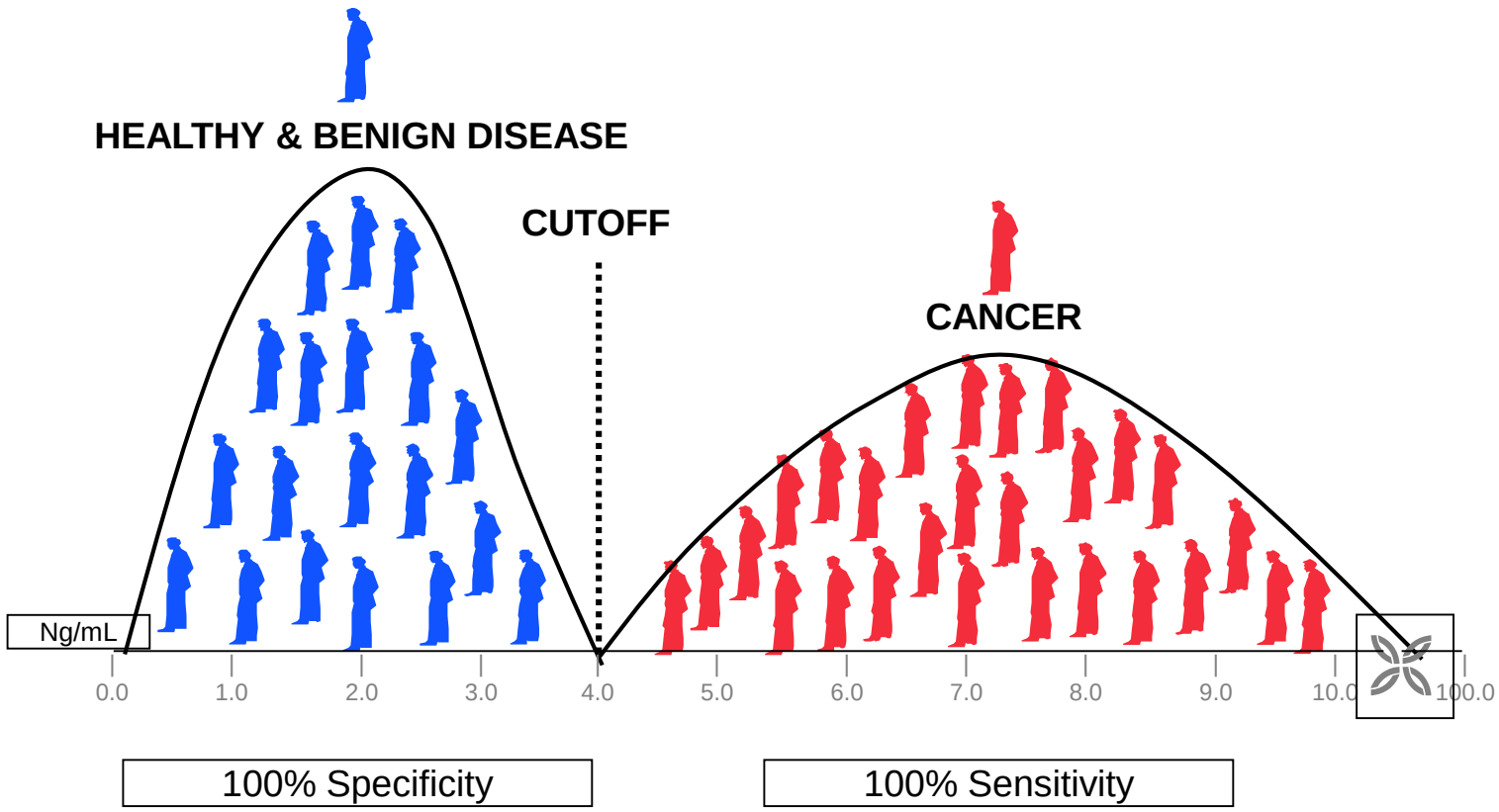


Sensitivity (%): $\frac{TP}{\text{all patients } (TP+FN)*100}$

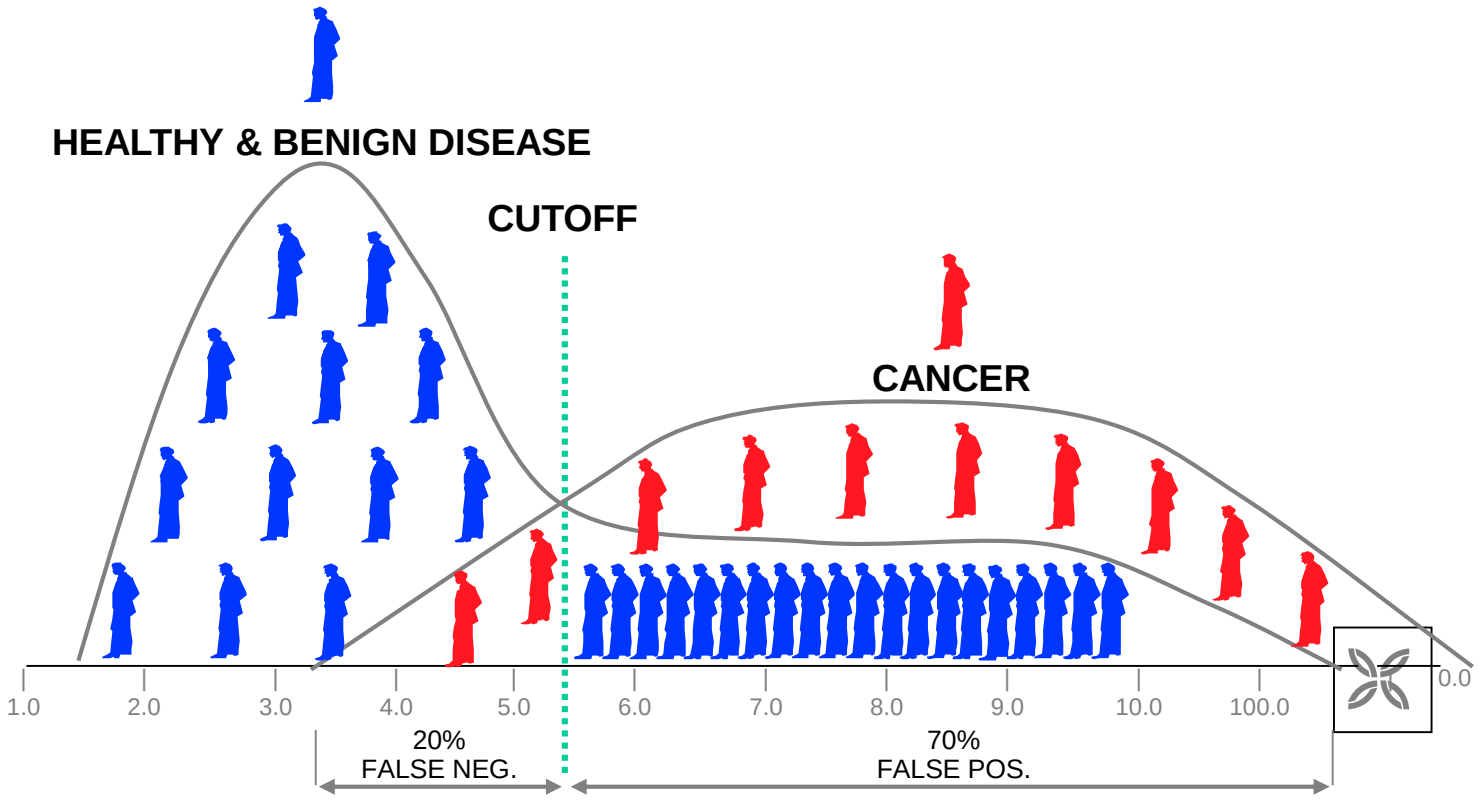
Specificity (%): $\frac{TN}{\text{all healthy ind. } (TN+FP)*100}$

Diagnostic cutoff!

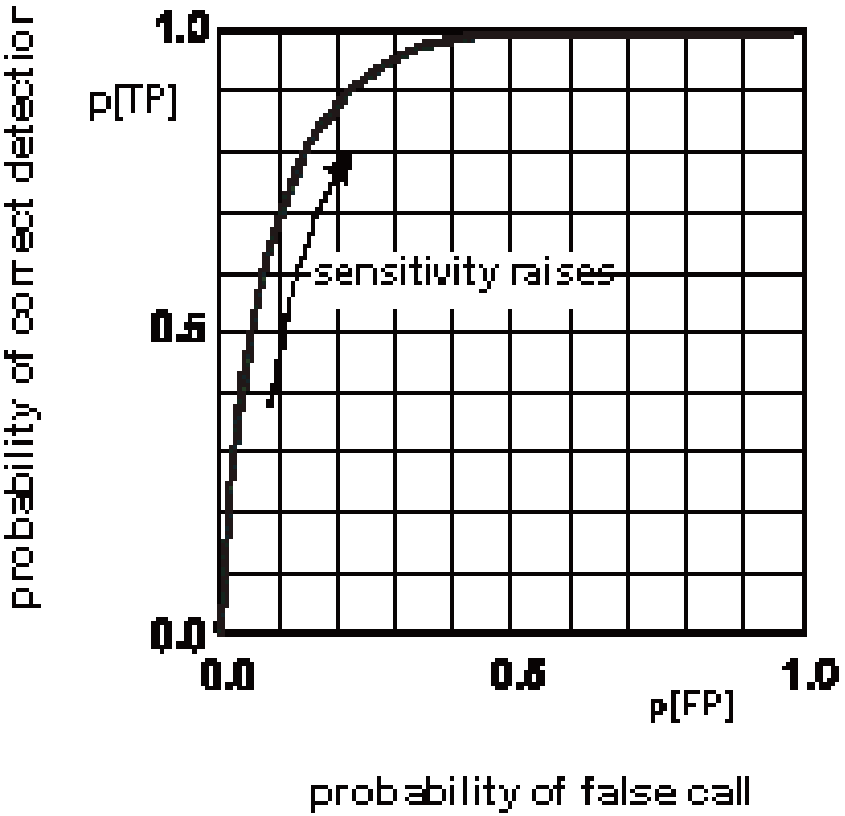
IIdeal biomarker



Ideal marker in reality

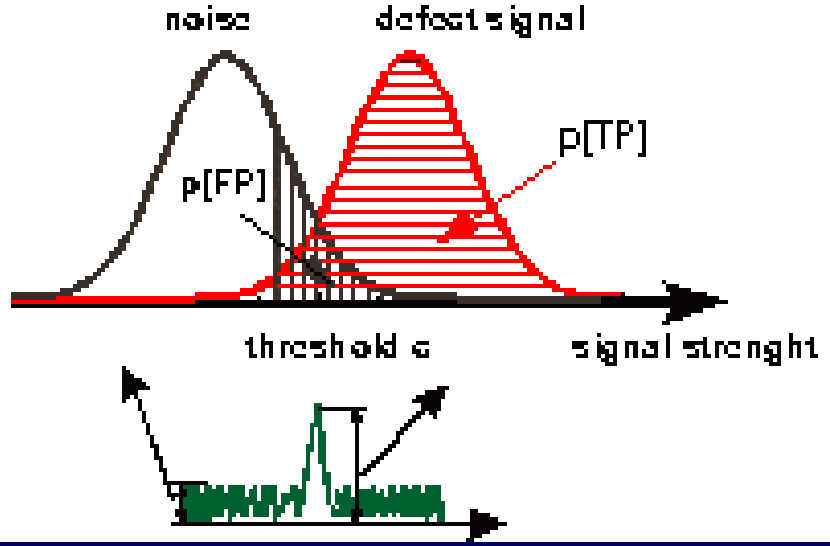


Receiver operating characteristics (ROC) curve

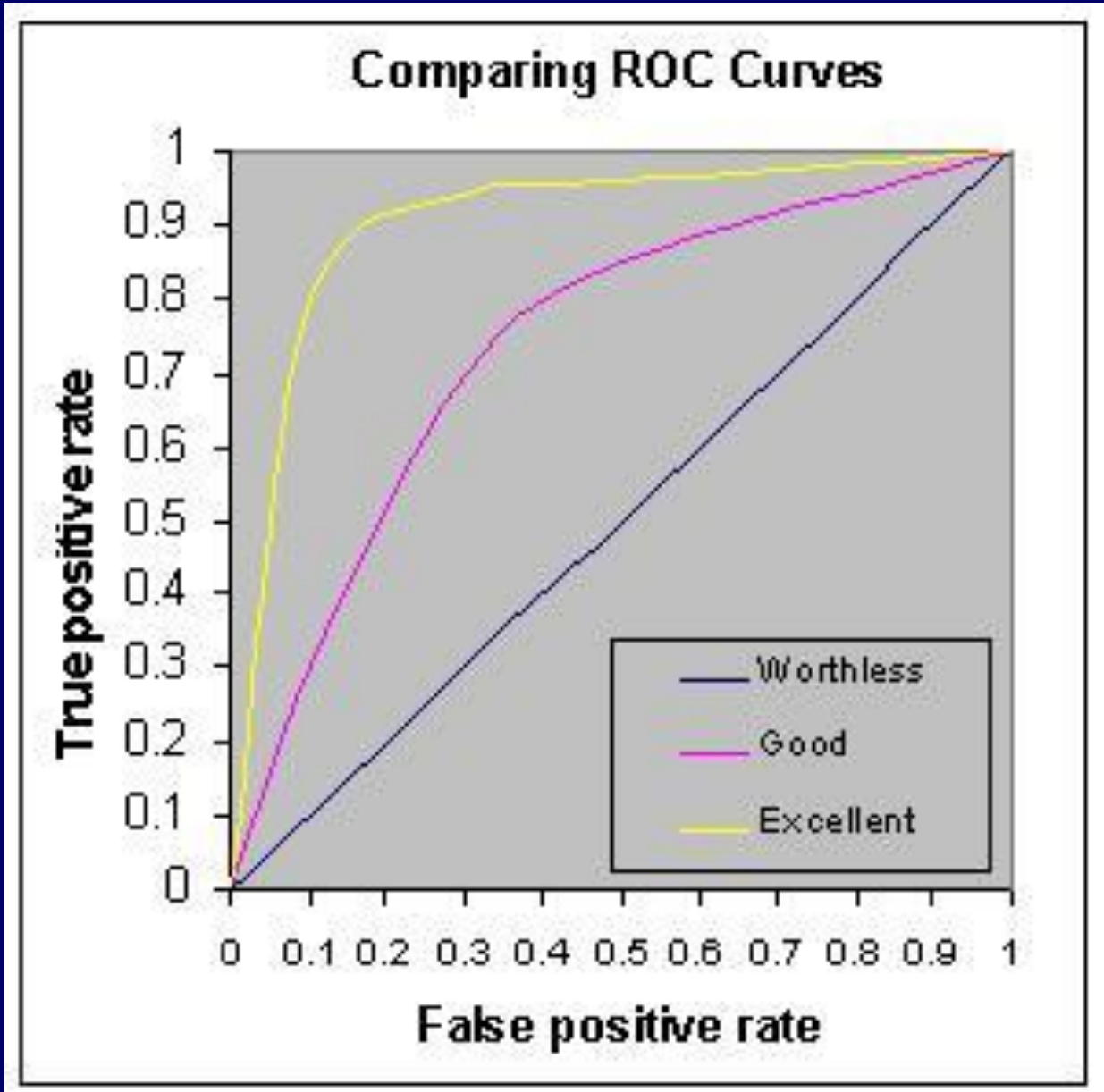


ROC

(Receiver Operating Characteristic)
as reliability curve



ROC curves


Comparing ROC Curves
True positive rate
False positive rate
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Worthless
Good
Excellent

Postanalytics - test reports and interpretation

- On line reports (phone!)
- Urgent test reports within 1h
- „Flag” reports, suggestions
- Consultation with physicians

- Interpretation considering the patient (abnormal-normal, plausibility)

How to increase diagnostic value?

- **Repeated analysis - monitoring**
- **Additional tests**

  („organ panels”, negative and/or confirmatory data)
- **Proper timing of the tests – biological half-life**