Sample materials for in-vitro diagnostics

Blood
Serum/plasma
Urine
Feces
Cerebrospinal fluid
Saliva
Sweat
Punctuation 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 immune complexes
- 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
- Positive 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 represents the population?
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:

$y = 0.911 \cdot x + 0.215$

$r_s = 0.913; p < 0.001$

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

$n = 130$ samples

Linear regression according to Passing and Bablock:

$y = 0.865 \cdot x + 0.501$

$r_s = 0.859; p < 0.001$

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

$n = 130$ samples

Linear regression according to Passing and Bablock:

$y = 0.947 \cdot x + 0.319$

$r_s = 0.843; p < 0.001$
Sensitivity and specificity of laboratory tests - I

Cut off

Controls

Patients

Relative frequency [%]

High sensitivity
Low specificity

Low sensitivity
High specificity

Value
Sensitivity and specificity of laboratory tests - II

Healthy population

Decision criterion

1: Increase of sensitivity
Decrease of specificity

2: Decrease of sensitivity
Increase of specificity

Patient population

False negative
False positive
Result height
Sensitivity and specificity of laboratory tests - III

**Diagnostic sensitivity:**

Correct positive \[\frac{\text{Correct positive}}{\text{Correct positive + false negative}}\] \(\bullet\) 100 %

**Diagnostic specificity:**

Correct negative \[\frac{\text{Correct negative}}{\text{Correct negative + false positive}}\] \(\bullet\) 100 %

Values of diagnostic sensitivity and specificity are given in [%]
Sensitivity and specificity of laboratory tests - IV

ROC curves of CDT and $\gamma$-GT

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

Positive predictive value:

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

$^\#$) correct and false positive results

Negative predictive value:

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

$^\#$) 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:

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

Negative predictive value:

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

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

\[
\text{Efficiency} = \text{Prevalence} \times \text{Sensitivity} + (1 - \text{Prevalence}) \times \text{Specificity}
\]
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

- Clinic
- Praeanalytics
- Analytics
- Postanalytics
- Laboratory
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 laboratoriumsmmedizinischer Untersuchungen (RiliBAEK)

5 spezielle Teile, die Details zur regelmäßigen internen Qualitätssicherung und zur Teilnahme an Ring-versuchen für folgende Bereiche regeln:
– B 1 „Quantitative laboratoriumsmmedizinische Untersuchungen“ (in Kraft seit 1.4.2008)
– B 2 „Qualitative laboratoriumsmmedizinische 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 laboratoriumsmmedizinische 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 laboratoriumsmmedizinische 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):

\[
\text{CV-value} = \frac{\text{Standard deviation}}{\text{Mean value}} \cdot 100\% 
\]

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.
Mean value and SDs of the control material are specified by the manufacturer.
Control cards - III

Well

All results below the mean

One result >3SD

7 consecutive results biased in one way
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Parameter</th>
<th>Target value(^1)</th>
<th>Maximum imprecision</th>
<th>Maximum incorrectness</th>
<th>Maximum deviation of single value</th>
<th>Analyte concentration range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Mass concentration</td>
<td>RMV</td>
<td>6 %</td>
<td>11 %</td>
<td>23 %</td>
<td></td>
</tr>
<tr>
<td>Aldosteron</td>
<td>Amount of substance Mass concentration</td>
<td>RMV</td>
<td>14 %</td>
<td>16 %</td>
<td>44 %</td>
<td></td>
</tr>
<tr>
<td>Alkalische Phosphatase (EC 3.1.3.1)</td>
<td>Enzyme activity</td>
<td>RMV</td>
<td>7 %</td>
<td>11 %</td>
<td>25 %</td>
<td></td>
</tr>
<tr>
<td>Bilirubin total</td>
<td>Amount of substance Mass concentration</td>
<td>RMV/SV</td>
<td>7 %</td>
<td>12 %</td>
<td>26 %</td>
<td>≥1.5 mg/dl</td>
</tr>
<tr>
<td>Calcium</td>
<td>Amount of substance</td>
<td>RMV</td>
<td>3 %</td>
<td>5 %</td>
<td>11 %</td>
<td>&lt;1.5 mg/dl</td>
</tr>
<tr>
<td>Carbamazepin</td>
<td>Mass concentration</td>
<td>SV</td>
<td>7 %</td>
<td>10 %</td>
<td>24 %</td>
<td></td>
</tr>
<tr>
<td>Chlorid</td>
<td>Amount of substance</td>
<td>RMV</td>
<td>2.5 %</td>
<td>4 %</td>
<td>9 %</td>
<td></td>
</tr>
<tr>
<td>Cholesterin total</td>
<td>Amount of substance Mass concentration</td>
<td>RMV</td>
<td>4 %</td>
<td>6 %</td>
<td>14 %</td>
<td></td>
</tr>
<tr>
<td>Choline-esterase</td>
<td>Enzyme activity</td>
<td>RMV</td>
<td>6 %</td>
<td>6 %</td>
<td>18 %</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) 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 Medizinproduktesgesetzes (Medizinproduktesgesetz - MPG)

German Law on Medical Devices

Medizinprodukte-Sicherheitsplanverordnung - MPSV

Ordinance on the Medical Devices Vigilance System
Competent Authority responsible in charge according to MPSV

Medical devices

In-vitro diagnostics

Federal Institute for Drugs and Medical Devices (BfArM)

Paul Ehrlich Institute (PEI)

*) Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM)
<table>
<thead>
<tr>
<th>Products for immune hematological testing and tissue typing:</th>
<th>Annex of Directive 98/79/EC</th>
<th>Responsibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood groups of the AB0 system(^1,2)</td>
<td>IIa</td>
<td>PEI</td>
</tr>
<tr>
<td>Blood groups of the Rhesus system (C, c, D, E, e)(^1,2)</td>
<td>IIa</td>
<td>PEI</td>
</tr>
<tr>
<td>Blood groups of the Kell system(^1,2)</td>
<td>IIa</td>
<td>PEI</td>
</tr>
<tr>
<td>Blood groups of the Duffy and the Kidd system(^1,2)</td>
<td>IIb</td>
<td>PEI</td>
</tr>
<tr>
<td>Irregular anti-erythrocyte antibodies(^1,2)</td>
<td>IIb</td>
<td>PEI</td>
</tr>
<tr>
<td>Markers for HLA(^3) typing, markers DR, A and B(^1,2)</td>
<td>IIb</td>
<td>PEI</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
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<tr>
<td>Markers of HIV(^4) infection (HIV-1 and HIV-2)(^1,2)</td>
<td>IIa</td>
<td>PEI</td>
</tr>
<tr>
<td>HTLV-I(^5) und HTLV-II(^1,2)</td>
<td>IIa</td>
<td>PEI</td>
</tr>
<tr>
<td>Hepatitis B, C und D(^1,2)</td>
<td>IIa</td>
<td>PEI</td>
</tr>
<tr>
<td>Congenital infection with rubella(^1,2)</td>
<td>IIb</td>
<td>PEI</td>
</tr>
<tr>
<td>Congenital infection with toxoplasma(^1,2)</td>
<td>IIb</td>
<td>PEI</td>
</tr>
<tr>
<td>Cytomegalovirus (CMV)(^1,2)</td>
<td>IIb</td>
<td>PEI</td>
</tr>
<tr>
<td>Chlamydia(^1,2)</td>
<td>IIb</td>
<td>PEI</td>
</tr>
</tbody>
</table>

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<tr>
<th>Other products:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor marker PSA(^1,6)</td>
<td>IIb</td>
<td>BfArM</td>
</tr>
<tr>
<td>Hereditary diseases phenylketonuria and Down syndrome (trisomia 21, including software)(^1)</td>
<td>IIb</td>
<td>BfArM</td>
</tr>
</tbody>
</table>

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<tr>
<th>Products for self testing:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Systems for measurement of blood glucose(^1)</td>
<td>IIb</td>
<td>BfArM</td>
</tr>
</tbody>
</table>

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\(^1\) 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.