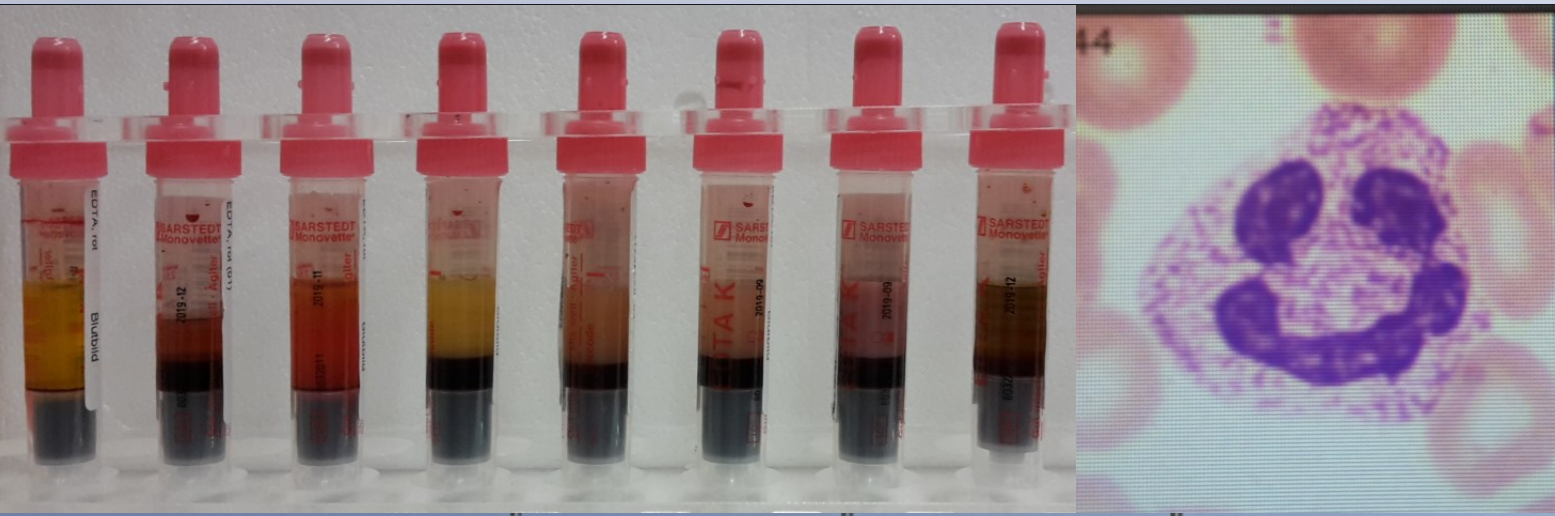




Course:

Biomedical Technologies

Lecture series:

**Biomedical Technologies
in Diagnostic and Therapy I**

Insights into Laboratory Medicine

What is measured in Laboratory Medicine?

From a **principal** point of view:

- blood samples
 - whole blood
 - serum
 - plasma
 - capillary blood
- aspirate samples
 - ascites aspirates
 - pleurocentesis aspirates
 - arthrocentesis aspirates
 - abscess aspirates
- liquor samples
- urine samples
- stool samples
- nasal discharges, sudor samples ...

What is measured in Laboratory Medicine?

From a **medical**
point of view:

In screenings:

Evidences for ...

- ruling out
- (early) diagnosis of
... a disease.

In the event of illness:

Evidences for ...

- confirmation
- disproof
- differential diagnosis
... of a suspected diagnosis.

What is measured in Laboratory Medicine?

From a **medical**
point of view:

In screenings:

Evidences for ...

- ruling out
- (early) diagnosis of
... a disease.

In the event of illness:

Evidences for ...

- confirmation
- disproof
- differential diagnosis
... of a suspected diagnosis.

By the way ...

What means „healthy“?

What means „sick“?

NORMAL DISTRIBUTION:

$$\mu \pm 1 \cdot \sigma \rightarrow \approx 68.27 \%$$

$$\mu \pm 2 \cdot \sigma \rightarrow \approx 95.45 \%$$

$$\mu \pm 3 \cdot \sigma \rightarrow \approx 99.73 \%$$

What is measured in Laboratory Medicine?

From a **medical**
point of view:

In screenings:

Evidences for ...

- ruling out
- (early) diagnosis of
... a disease.

In the event of illness:

Evidences for ...

- confirmation
 - disproof
 - differential diagnosis
- ... of a suspected diagnosis.

By the way ...

What means „healthy“?

What means „sick“?

REFERENCE INTERVAL:

$$\mu \pm 1.96 \cdot \sigma \rightarrow 95 \%$$

What is measured in Laboratory Medicine?

From a **medical**
point of view:

In screenings:

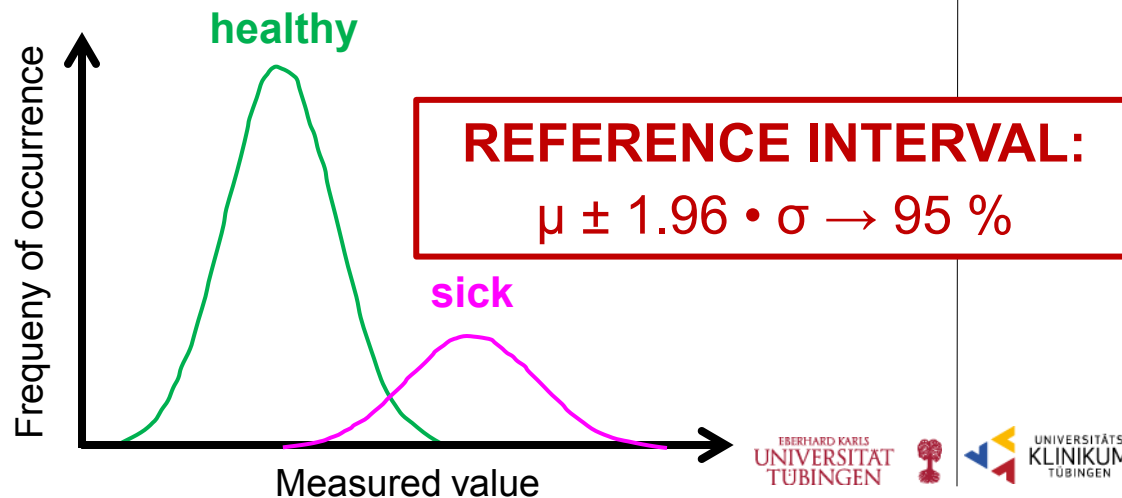
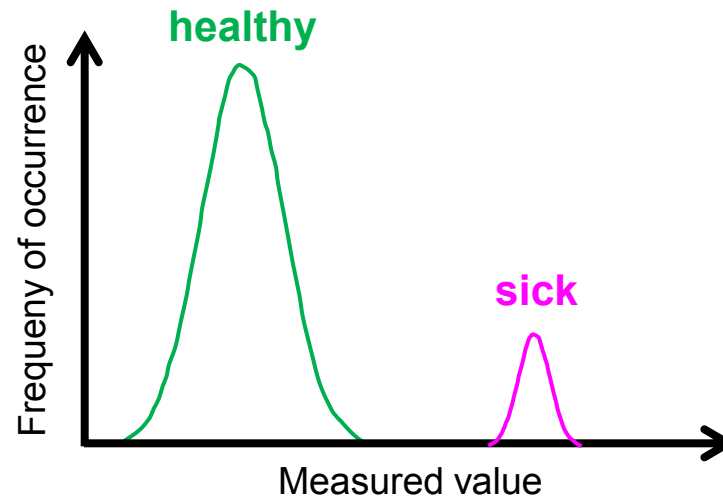
Evidences for ...

- ruling out
- (early) diagnosis of ... a disease.

In the event of illness:

Evidences for ...

- confirmation
 - disproof
 - differential diagnosis
- ... of a suspected diagnosis.



What is measured in Laboratory Medicine?

From a **medical**
point of view:

In screenings:

Evidences for ...

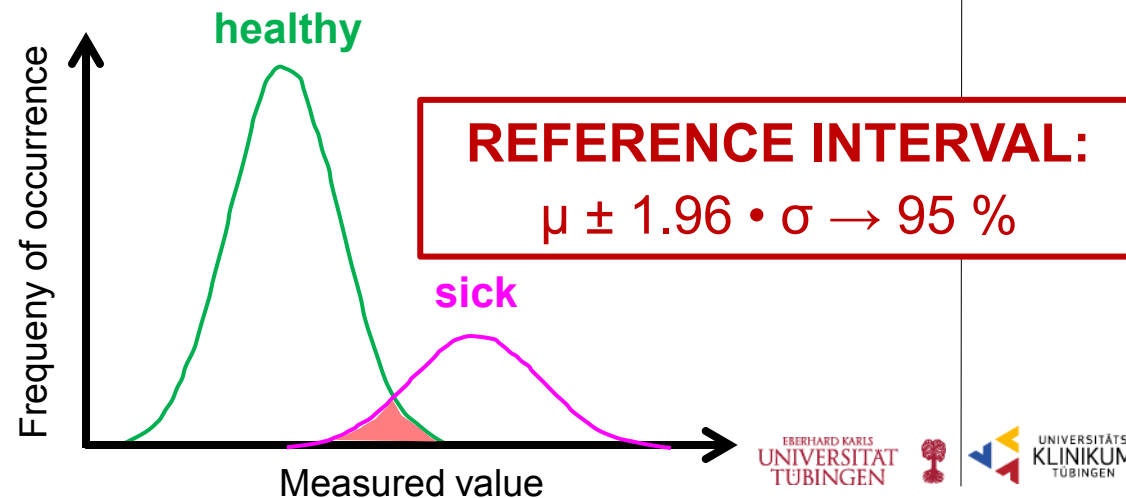
- ruling out
- (early) diagnosis of ... a disease.

Test result	healthy	sick	
„positive“	false positive	true positive	total positive results
„negative“	true negative	false negative	total negative results
	total healthy subjects	total sick subjects	total number

In the event of illness:

Evidences for ...

- confirmation
 - disproof
 - differential diagnosis
- ... of a suspected diagnosis.



What is measured in Laboratory Medicine?

From a **medical**
point of view:

In screenings:

Evidences for ...

- ruling out
- (early) diagnosis of ... a disease.

Test result	healthy	sick	
„positive“	false positive	true positive	total positive results
„negative“	true negative	false negative	total negative results
	total healthy subjects	total sick subjects	total number

DIAGNOSTIC PERFORMANCE:

Diagnostic SENSITIVITY = true positives/total sick subjects

„chance that a sick patient indeed receives a positive result“

Diagnostic SPECIFICITY = true negatives/total healthy subjects

„chance that a healthy patient indeed receives a negative result“

POSITIVE PREDICTIVE VALUE = true positives/total positive results

„chance that a positive result indeed identifies a sick patient“

NEGATIVE PREDICTIVE VALUE = true negatives/total negative results

„chance that a negative result indeed identifies a healthy patient“

What is measured in Laboratory Medicine?

From a **medical**
point of view:

In screenings:

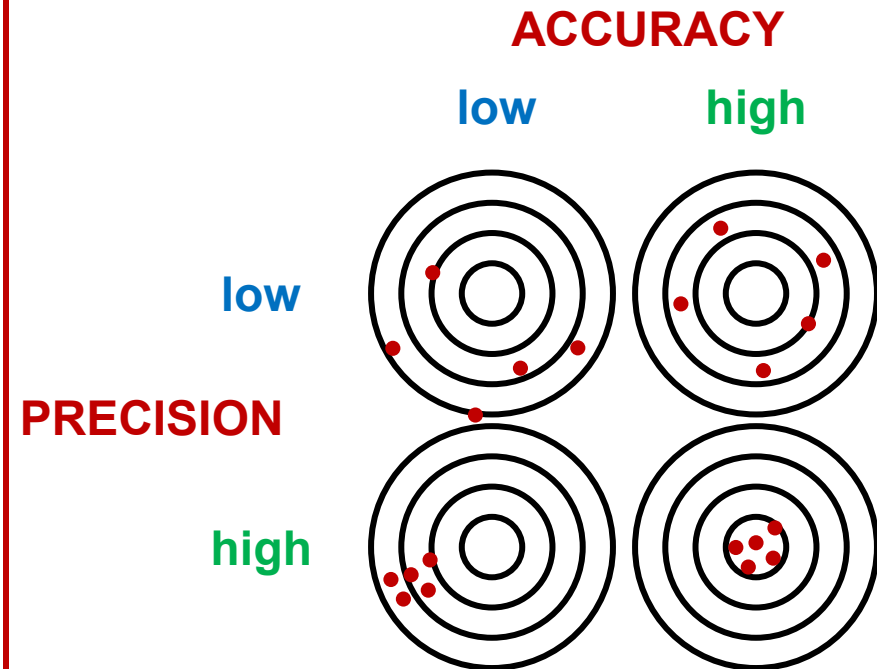
Evidences for ...

- ruling out
- (early) diagnosis of
... a disease.

Precision: variation of coefficient (CV)

Accuracy: deviation from target value

ANALYTICAL PERFORMANCE:



What is measured in Laboratory Medicine?

From a **medical** point of view:

In screenings:

Evidences for ...

- ruling out
- (early) diagnosis of ... a disease.

In the event of illness:

Evidences for ...

- confirmation
- disproof
- differential diagnosis ... of a suspected diagnosis.

From a **biochemical** point of view:

- cell counts
 - erythrocytes
 - leukocytes
 - granulocytes
 - lymphocytes
 - monocytes
 - thrombocytes
- concentrations
 - metabolites
 - toxic substances
 - drugs
- enzyme activities

What is measured in Laboratory Medicine?

From a **medical** point of view:

In screenings:

Evidences for ...

- ruling out
- (early) diagnosis of ... a disease.

In the event of illness:

Evidences for ...

- confirmation
- disproof
- differential diagnosis ... of a suspected diagnosis.

From a **biochemical** point of view:

- cell counts
 - erythrocytes
 - leukocytes
 - granulocytes
 - lymphocytes
 - monocytes
 - thrombocytes
- concentrations
 - metabolites
 - toxic substances
 - drugs
- enzyme activities

From a **physiological** point of view:

Adaptations, changes or disturbances of the body (function) in consequence of pathologic conditions.

What is measured in Laboratory Medicine?

From a **medical** point of view:

In screenings:

Evidences for ...

- ruling out
- (early) diagnosis of ... a disease.

In the event of illness:

Evidences for ...

- confirmation
- disproof
- differential diagnosis ... of a suspected diagnosis.

From a **biochemical** point of view:

- cell counts
 - erythrocytes
 - leukocytes
 - granulocytes
 - lymphocytes
 - monocytes
 - thrombocytes
- concentrations
 - metabolites
 - toxic substances
 - drugs
- enzyme activities

From an **analytical** point of view:

- artefacts
- influencing factors
 - age
 - gender
 - nutrition
- interference factors
 - hemolysis
 - lipemia
 - icterus
- preanalytical errors
 - instability of analytes

What is measured in Laboratory Medicine?

From a **medical** point of view:

In screenings:

Evidences for ...

- ruling out
- (early) diagnosis of ... a disease.

In the event of illness:

Evidences for ...

- confirmation
- disproof
- differential diagnosis ... of a suspected diagnosis.

From a **biochemical** point of view:

- cell counts
 - erythrocytes
 - leukocytes
 - granulocytes
 - lymphocytes
 - monocytes
 - thrombocytes
- concentrations
 - metabolites
 - toxic substances
 - drugs
- enzyme activities

From a **physiological** point of view:

Adaptations, changes or disturbances of the body (function) in consequence of pathologic conditions.

From a **physical** point of view:

- light intensities
- light scattering
- electrochemical potentials
- impedance
- mechanical resistance

Which methodologies are used?

1.) For separation or isolation

- **Mechanical (acceleration)**

- centrifugation
- flow cytometry

LIQUID chromatography (solid/liquid)

- thin layer chromatography (TLC)
- high performance liquid chromatography (HPLC)

GAS chromatography (liquid/gaseous)

- **Physicochemical**

→ **Chromatography:** differential partitioning between a stationary phase and a mobile phase based on

- **molecular size**

- size-exclusion chromatography
- gel permeation chromatography

- **polarity/ionic charge (density)**

- ion exchange chromatography (IEC)

- **solubility**

- (reversed-phase) partition chromat.

- **chemical binding affinity (strength)**

- affinity chromatography

- **adsorbability**

- adsorption chromatography

- **Electrochemical**

→ **Electrophoresis:** differential motion of dispersed molecules in an electric field based on

- **molecular size**

- SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis)

- **molecular charge density**

- lipid electrophoresis
- serum protein electrophoresis

GEL electrophoresis

CAPILLARY electrophoresis

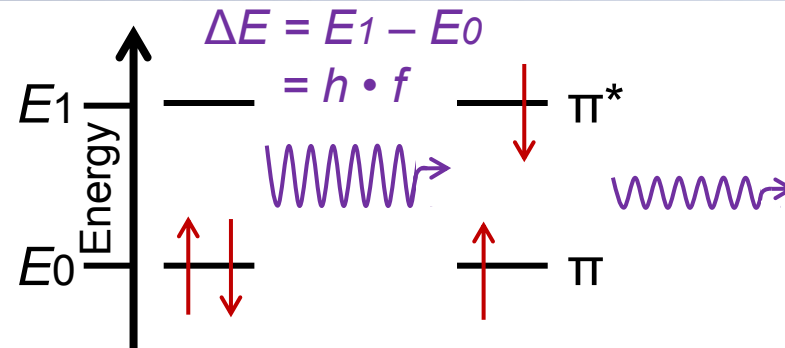
Which methodologies are used?

2.) For detection or measurement

- **Optical measurements**

- **light absorption**

- photometry
 - atomic absorption spectroscopy



Which methodologies are used?

2.) For detection or measurement

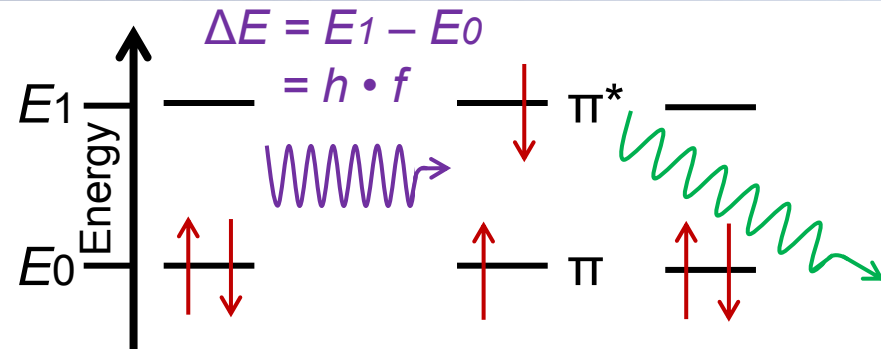
• Optical measurements

• light absorption

- photometry
- atomic absorption spectroscopy

• light emission

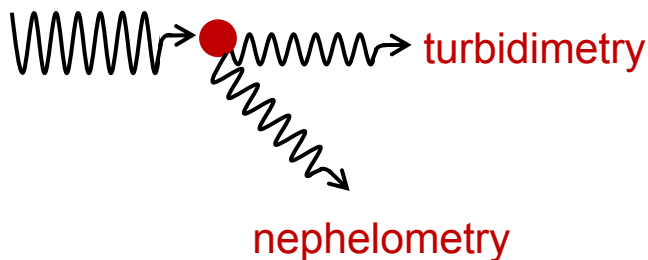
- luminescence measurements
- fluorometry (e.g. FRET)
- atomic emission spectroscopy



Which methodologies are used?

2.) For detection or measurement

- **Optical measurements**
 - **light absorption**
 - photometry
 - atomic absorption spectroscopy
 - **light emission**
 - luminescence measurements
 - fluorometry (e.g. FRET)
 - atomic emission spectroscopy
 - **light scattering**
 - flow cytometry (FSC, SSC)
 - nephelometry
 - turbidimetry



Which methodologies are used?

2.) For detection or measurement

- **Optical measurements**
 - **light absorption**
 - photometry
 - atomic absorption spectroscopy
 - **light emission**
 - luminescence measurements
 - fluorometry (e.g. FRET)
 - atomic emission spectroscopy
 - **light scattering**
 - flow cytometry (FSC, SSC)
 - nephelometry
 - turbidimetry
 - **microscopy**
 - analog/digital microscopy
 - light/electron microscopy

Which methodologies are used?

2.) For detection or measurement

- **Optical measurements**
 - **light absorption**
 - photometry
 - atomic absorption spectroscopy
 - **light emission**
 - luminescence measurements
 - fluorometry (e.g. FRET)
 - atomic emission spectroscopy
 - **light scattering**
 - flow cytometry (FSC, SSC)
 - nephelometry
 - turbidimetry
 - **microscopy**
 - analog/digital microscopy
 - light/electron microscopy
- **Mechanical measurements**
 - mass spectrometry
 - coagulometry
 - radioactivity measurements
- **Electrochemical measurements**
 - **electric voltage**
 - potentiometry
 - ion-selective electrodes (ISE)
 - **(changes in) electric current**
 - amperometry (Clark electrode)
 - **electric charge quantity**
 - coulometry
 - **electrical impedance**
 - flow cytometry
 - **electrochemical charge density**
 - electrophoresis
- **Calorimetric measurements**
 - osmometry
- **Immunological methodologies**
 - antigen-antibody agglutination
 - complement-binding reaction

Which methodologies are used?

2.) For detection or measurement

- **Optical measurements**
 - **light absorption**
 - photometry (EIA, e.g. ELISA)
 - atomic absorption spectroscopy
 - **light emission**
 - immunoluminescence (LIA)
 - immunofluorescence (FIA, FACS)
 - atomic emission spectroscopy
 - **light scattering**
 - flow cytometry (FSC, SSC)
 - immunonephelometry
 - immunoturbidimetry
 - **microscopy**
 - analog/digital microscopy
 - light/electron microscopy
- **Mechanical measurements**
 - mass spectrometry
 - coagulometry
 - radioactivity measurements (RIA)
- **Electrochemical measurements**
 - **electric voltage**
 - potentiometry
 - ion-selective electrodes (ISE)
 - **(changes in) electric current**
 - amperometry (Clark electrode)
 - **electric charge quantity**
 - coulometry
 - **electrical impedance**
 - flow cytometry
 - **electrochemical charge density**
 - immunoelectrophoresis (IES)
 - immunofixation electrophoresis
 - immunoblot (Western blot)
- **Calorimetric measurements**
 - osmometry
- **Immunological methodologies**
 - immunodiffusion

What is important to know in Laboratory Medicine?

- results of medical measurements are interpreted in the context of reference intervals covering $\approx 95\%$ of the subjects in a „healthy“ cohort
 - results outside of the reference interval are **not** necessarily the proof of a pathological circumstance
 - sick patients do **not** necessarily present with results outside of the reference interval
 - the **clinical performance** of a diagnostic test can be described with the features **sensitivity**, **specificity**, **positive predictive value** and **negative predictive value**
 - the **analytical performance** of a diagnostic test can be described with the features **coefficient of variation** (indicating the **precision**) and **deviation of the mean from the target value** (indicating the **accuracy**)
- many different biochemical and physicochemical methodologies are applied in Clinical Chemistry
 - optical methodologies are based on light absorption (**photometry**), light emission (**luminescence**, **fluorescence**) and light scattering (**nephelometry**, **turbidimetry**)
 - blood counts are based on **flow cytometry** with FSC and SSC

Universitätsklinikum Tübingen

**Kompetenz
mit Herz**

www.uniklinikum-tuebingen.de



Vielen Dank
für Ihre Aufmerksamkeit!