Basics in laboratory medicine:  
Samples, Reference intervals and  
Quality assurance  

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Laboratory medicine  

- clinical chemistry (and laboratory hematology)  
- clinical microbiology  
- clinical physiology and nuclear medicine  
- clinical neurophysiology  
- clinical genetics  
- pathology  

HUSLAB:  
-comprises the six clinical special fields + primary health care lab services
Clinical Chemistry Unit

University of Helsinki > Faculty of Medicine

Teaching activities:
- Basic training in clinical chemistry for medical and dental students
- Specialist training in clinical chemistry for medical doctors and clinical chemists
- Clinical chemistry training in The Master’s Degree Programme in Translational Medicine (Transmed) programme
- Scientific research training / Postgraduate research education

Research

Clinical Chemistry Unit

- The department provides a unique academic research environment that encourages interaction between the different fields (clinic and labs)
- Research projects:
  - Exocytosis, neurotransmission
  - Modelling of the mechanisms of schizophrenia, chronic pain and prostatic cancer
  - Prostatic cancer
  - Treatment of prostatic cancer
  - Chorionic gonadotropin (hCG) in pregnancy and cancer diagnostics
Clinical utility of laboratory tests

- Primary diagnosis: confirming a clinical suspicion
- Screening for disease
  - hypercholesterolemia
  - cancer screening
    - prostatic cancer, PSA
- Establishing risk factors
  - cholesterol
  - blood sugar
- Providing a prognosis
  - breast cancer, leukemia
- Excluding a diagnosis
- Monitorig of treatment

Laboratory testing

- contributes to ca. 70% of all clinical decisions
- 4% of total health care expenditures
- high impact on patient care
Finnish health care system

- municipal health care system
  - basic health care provided by health care centers
  - high-calibre specialist medical care provided by 20 hospital districts throughout the province of Uusimaa
- private health care services

Laboratory services in Finland I

- Production of ca. 10 diagnostic tests / individual / year ~ 50€
- ca. 30% in basic health care and ca. 55% in specialist care
- if less than 100 diagnostic tests / year -> worth buying the respective test
PAP

- Finland’s first large-scale protein production unit
- Crystallization
- Three-Dimensional structure

The Nobel Prize Poster in Physiology / Medicine 1999

PNAS 90:799-803, 1993
EMBO J 12:2609-15, 1993
Nature Biotechnology 13:1230-34, 1995

Content

- Specimen collection
- Types of specimens: whole blood, serum, plasma, urine, feces, spinal, bone marrow, puncture samples, various types of solid tissue
- Preanalytical variables
- Reference intervals
- Quality control

Types of specimens

- Use of abbreviation system for laboratory tests
- Nomenclature maintained by the Finnish Local and Regional Authorities
  http://www.kuntaliitto.fi/soster/luokitukset
- examples: 1471 P-Gluk, P-Glukoosi, mmol/l
- 1-2 character abbreviation system preceding the abbreviated laboratory test contains information on sample type (B, P, S)
- B = whole blood, P = plasma, S = serum, U = urine
- f-character preceding the type of specimen refers to fasting sample, e.g. fS = fasting serum
Blood samples

Composition:
• Blood cells (red blood cells, white blood cells and platelets) and their properties
• Proteins
• Substances carried in blood

• What blood test measures:
  ◆ the levels or properties of different factors that have a specific role in the blood
  ◆ the levels of substances transported through the blood stream to another part of the body

Blood test measures substance produced by the body or substances which originate outside the body (nutrients, drugs, toxins, pathogens, etc.)

DISEASE-RELATED BIOMARKERS IN BLOOD

• When tissues are damaged by injury or disease, they release into the bloodstream e.g. structural proteins and enzymes, which are measured in blood in order to detect and monitor the tissue damage and also assess the extent of the damage
  ◆ Markers of cardiac injury (e.g. cardiac troponin T in myocardial infarction)
  ◆ Hepatocellular injury (ALAT, GT)
  ◆ Acute pancreatitis (amylase)
• Tumor markers (e.g. PSA)
PATHOGEN DETECTION FROM BLOOD SAMPLES

- Bacteria, viruses, yeasts, molds, protozoa
- Detection based on
  - Culture media
  - Antigen detection
  - Staining + microscopy
  - PCR-technic

Venipuncture

- Venous blood is usually the specimen of choice in adults
- Most commonly obtained from the superficial veins on the anterior forearm:
  - v.intermedia cubiti
  - v.basilica
  - vcephalica
  - Venous occlusion (=blood pressure cuff or a tourniquet applied 10 to 15 cm above the intended puncture site)
WHOLE BLOOD (B)

Contains plasma and blood cells = Cells + proteins + water + soluble substances
- Sample is drawn into tubes containing anticoagulant (EDTA, heparine, citrate or oxalate)
- F.ex.:
  - Blood count:
    - 2474 PVKT, Basic blood count
    - 2475 PVK+TKD Full blood count
  - Some point-of-care tests

PLASMA (P)

- Contains all plasma proteins, including clotting factors, but no cells.
- Sample is drawn into an anticoagulant containing tube, the cells are centrifuged to the bottom of the tube and plasma is separated.
- Faster to produce than serum
- More suitable for assessing the body in vivo properties
- Most chemical analyses are performed from plasma samples, e.g.
  - electrolytes
  - CRP, INR, liver enzymes, creatinine...
SERUM (S)

- no cells, devoid of fibrinogen and some other clotting factors.
- Sample is drawn into:
  - A tube without additives
  - A tube with procoagulant
  - A tube with procoagulant and gel
- A clot is allowed to form, then centrifuged to the bottom of the tube and serum is separated
- Can be used in most chemical analysis, but takes more time compared to plasma
- e.g. serum protein electrophoresis, some endocrinological and immunological analyses

Skin puncture

- Previously known as capillary blood sample (cB-)
- Contains a mixture of capillary, arterial and venous blood (=from small arterioles and venules) with small amounts of tissue fluid (interstitial fluid) and intracellular fluid
- Used in several so called point-of-care testing (POCT) bed side/near patient -tests (f.ex. Hb or gluc).
- Can sometimes replace venous sample especially in infants
- Composition of the capillary sample is varying, but is usually closer to arterial than venous blood
Urine samples

- Most common analyses:
  - Urine basic tests: 1881 U-KemSeul, U-chemical screening of urine ja 20033 UCells, U-urine particle analyses, automated
  - Urine culture, bacterial: 1155 U-BaktVI, U-Bakterial culture

- Indications:
  - Renal or urinary tract disease
  - To measure the levels of substances passed into urine from blood (or their metabolites)
  - Variety of substances relating to drug abuse

- **U** = single urine sample, **dU** = Timed collection specimen = daily urine sample (collection time can be shorter, f.ex. 4 h or 12 h)

- Preferably standardized sampling, laboratory instructions to the patient (sample container and sample tubes)
  - Recommended: first morning specimen
  - At least 4-6 h from previous urination
  - Most common sampling method: Midstream Clean Catch Specimen
Urine samples transported as soon as possible to the lab

Other types of laboratory samples

Other types of laboratory samples
• Puncture samples
  ❖ E.g. Pleural fluid, ascites, amniotic fluid, synovial fluid
• Liquor samples (cerebrospinal fluid, CSF)
  ❖ Central nervous system (CNS) disease may change the composition of CSF
  ❖ E.g. subarachnoid haemorrhage (blood), meningitis (bacteria, leukocytes), multiple sclerosis (MS; immunoglobulins)
• Bone marrow— to examine the source of the blood cells
  ❖ Suspcion of haematopoietic disease (eg, leukemia)
  ❖ Two types: aspiration or biopsy
  ❖ Examination of the hematopoietic tissue cell components number, morphology and their properties, including chromosomal/molecular genetic characteristics
Preanalytical phase

Process approach in HUSLAB

- **Customer/Information Management**
  - **Pre-pre-analytic**
  - **Pre-analytic**
  - **Analytic**
    - Guiding patients and patient preparation
  - **Quality control**
  - **Post-analytic**
    - Report generated
    - Results conveyed to clinicians
    - Samples storage
    - Laboratory consultation
    - Additional order placed

- **Laboratory/Information Management**
  - **Pre-analytic**
  - **Analytic**
    - Patient identification
    - Specimen obtained
    - Tube handling, storage, transportation
    - Specimen separation and analyzed
    - Verification of the results/reassessment
  - **Quality control**
  - **Post-analytic**
Preanalytical Variables

- account for 32-75% of laboratory errors -

- There are many factors that contribute to accurate test results in the medical laboratory
  - Patient Preparation
    - Fasting
    - Postprandial
    - Posture during blood sampling
    - Smoking, alcohol
    - Before or after medication dosage
    - Physical activity, exercise
    - Physiologic factor, stress
  - Sample collection, Proper Tube Handling and Specimen Processing

- Biological variations; intra-individual variability, partially also chronobiological variables (rhythmic variability within the 24-hour day)
- Physical activity
  - Strenuous exercise induces stress reaction, followed by increased stress hormone release
    - Increased Cortisol, Adrenalin, Glucose levels
    - Overactivation of the renin-angiotensin system, increased aldosteron levels
    - First decreased fatty acid levels, later levels increase
    - Increased apolipoprotein AI and HDL-C levels; decreased LDL-C, apolipoprotein B, and triglyceride levels with long-term strenuous exercise
  - Increased leukocyte numbers (eosinophils)
- Long-term exercise increases the levels of muscle isoenzymes
  - CK (creatine kinase), LDH (lactate dehydrogenase), AST (aspartate transaminase)
Fasting

- The effects of diet on analyte concentrations
  - Blood glucose elevated
  - Triglycerides elevated
    - Lipemia of the blood sample, ‘cloudy sample’
    - Lipemia interferes with nearly all photometric measurement by light scattering and absorption
- The effect of prolonged fasting
  - Decreased glucose and insulin levels
- (10) 12-h overnight fast recommended, since increase in triglycerides persist up to 9 h after fatty meal
- After 48-h fast, 240% increase in bilirubin level; decreased levels of prealbumin, albumin, and C3.
- Caffeine: adrenalin and noradrenalin release ↑, cortisol and free fatty acids ↑, gastric acid, pepsin and gastrin ↑

Posture during blood sampling

- All blood samples should be drawn with the subject in a sitting position preceded by a 10-15 min rest
- Change in posture from a supine (lying down with the face up) to upright position or sitting position can result in a shift in body water from the intravascular to the interstitial compartment
- As a result, the concentration of large molecules (urea, albumin etc.) that are not filterable is increased
- In supine position the plasma volume increases
- Hemoglobin decreases 5-15%
  - Physiological phenomena when the patient comes to the lab
  - Must be considered when suspecting bleeding
- Small molecules are not much affected by posture during sampling
Smoking, ethanol

- Smoking
  - Increased levels of plasma epinephrine, aldosterone, cortisol, free fatty acids, and free glycerol within 1h of smoking

- Ethanol
  - Short- vs long-term effect
  - Decreased glucose levels
  - Moderate intake increases levels of HDL and triglyceride
  - Abundant intake increases the levels of liver enzymes
    - GGT (Gamma glutamyl transpeptidase)
    - AST (Aspartate transaminase)
    - APL (alkaline phosphatase)
  - Elevated MCV values

Effect of medication:
Hormones, oral contraceptives, postmenopausal estrogen replacement therapy

- Suppression of gonadotropin secretion (LH, FSH)
- Suppression of gonadal function
  - Decreased estrogen and androgen production
- Increases the level of steroid hormone-binding proteins
  - SHBG (estradiol, testosteron)
  - TBB (tyroksin, T4, and trijodtyronin, T3)
  - CBG (kortisol)
  - The levels of protein bound hormone increase

- The above medication does not affect free hormone levels
Effect of medication:
Effects of cytotoxic agents chemotherapy)

- Blood count
  - Leukopenia
  - Trombocytopenia
  - Anemia
- Suppression of Gonadal function
  - Decreased estrogen release in women
  - Decreased androgen production in men
  - Increased gonadotropin release (lack of negative feedback)
  - LH, FSH, hCG

  Increased levels of hCG due to hypogonadism must be considered when using hCG as a tumor marker

Reference values
Reference values

- It is a basis for a physician or other health professional to interpret a set of results in a particular patient
- Internationally recognized in the 70's
- It replaces the term “normal value”
- Importance of high quality reference values in clinical decision making

Reference and normal values

- “Normal values” is almost the same, but it has not been clearly defined
- “Normal” in medicine means in general healthy
- The definition for health in the constitution of the World Health Organization (WHO):
  - “A state of complete physical, mental, social wellbeing, and not merely absence of disease or infirmity”
- This definition has been criticized as unrealistic
- As defined by the International Federation of Clinical Chemistry (IFCC), the terms “reference range” or “interval of reference” (IR) mean a range of values obtained from individuals (usually, but not necessarily healthy) randomly chosen, but appropriately selected in order to satisfy suitably defined criteria
Concept and terminology of reference values as recommended by the IFCC

- As an example, determination of B-Hb levels in healthy male
- Sample: should be a random sample of all the individuals in the parent population (at least 120 individuals)
- Reference values
  - Subgrouping should be considered before population sampling = reference group values
- Reference limits
  - Determined from a set of reference values
  - The term traditionally means the range of values that includes 95% of the test results observed in the reference population
  - This range excludes the highest 2.5% and the lowest 2.5% of the results
  - Closely approximates the mean ± 2 standard deviation (SD)
  - The basic assumption is that the values fit a Gaussian distribution

Gaussian or normal distribution

The parametric method is based on the determination of ±1.96 SD limits from the mean of the reference distribution. In a Gaussian distribution, these limits correspond to the 0.025 and 0.975 fractiles.
Determination of reference values

- Manufacturers produce reference values.
- Each laboratory must establish its own reference values using data from its own equipment and methods and compare it to the reference values provided by the manufacturer.
- Pre-analytical conditions must be considered:
  - Harmonized guidelines for blood sampling and patient preparation (fasting, no physical stain, morning blood sampling, blood sampling without venous stasis or short-term venous stasis).
- Sample size:
  - The number of reference values in each subclass should be sufficient, at least 120 individuals.
- Stratification according to age and gender, if there are women pregnant or taking any anti-conceptional drug may be of value for some quantities.

Reference values: General drawbacks and limitations

- The reference group differs from the patient group:
  - The reference range consists of medical staff samples.
- The use of different methods in determining reference values:
  - Instruments and lab techniques used, or how the measurements are interpreted by observers.
  - Unsatisfactory method standardization.
- Determinants such as age, diet, etc. that are not compensated for.
- Pregnancy:
  - Blood volume increases (plasma).
  - Hemoglobin decreased; hemodilution.
  - The levels of several hormones increased.
- In children unnecessary blood sampling must be considered (suspicion of the disease).
Age and sex-dependent values of carbohydrate antigen 19-9 (CA 19-9)

Assay-based reference values

- Only slight age and sex dependent changes
  - Electrolytes (Na, K, Ca)
- Moderate age and sex dependent changes
  - Plasma proteins, blood cells
- Highly age and sex dependent changes
  - Hormones
Intra-individual variation

- Intra-individual biological variation factors, diet, physical and mental conditions, sampling and variations related to patient preparation
- Chronobiologic reference values - circadian rhythms and circannual rhythms
- Biological variation and analytical interferences for different analytes
- In the figure, variation of the levels of APL in a few weeks time period
- Ideally, the best reference interval for an analyte would be individual specific. The value for the analyte, determined when the individual is ill, could be compared with the limits for this analyte, established on this same individual, when he or she was healthy or without the illness.

Decision limit

- A test score used as the criterion for a "positive test." All test scores at or beyond this test score are considered to be "positive"; those not at or beyond the score are considered to be "negative."
- Clinical laboratory can participate in the estimation of decision limits by offering and treating reference data
- Recommendations about decision limits are based on clinical research
- Different decision limit choices will yield different sensitivity and specificity estimates
- Diagnostic accuracy: the extent to which a measurement is close to the true value
- LR = directly determine positive predictive value, likelihood ratio, Odds

\[
\text{Sensitivity} = \frac{TP}{(TP + FN)} \quad \text{Specificity} = \frac{TN}{(TN + FP)}
\]
Sensitivity and specificity

Are characteristics of the test. The population does not affect the results.

\[
\text{Sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}
\]

= probability of a positive test given that the patient is ill

\[
\text{Specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}
\]

= probability of a negative test given that the patient is well

Predictive value and efficiency

- Clinical sensitivity and specificity are terms used to evaluate a clinical test
- In general, the higher the sensitivity, the lower the specificity, and vice versa
- Positive and negative predictive values are influenced by the prevalence of disease in the population that is being tested. If we test in a high prevalence setting, it is more likely that persons who test positive truly have disease than if the test is performed in a population with low prevalence
- Predictive value = A value that predicts the likelihood that a result from a test reflects the presence or absence of a disease
- PPV = positive predictive value = the probability that a patient with a positive test result really does have the condition for which the test was conducted
- Clinical efficiency = Efficiency is the proportion of correctly classified cases at the considered cutoff limit (proportion of the sum of true positives and true negatives of the whole population)
- Efficiency is used to define the best decision limit for diagnosis of the disease
The fecal occult blood (FOB) screen test was used in 2030 people to look for bowel cancer:

<table>
<thead>
<tr>
<th>Fecal Occult Blood Screen Test Outcome</th>
<th>Test Outcome Positive</th>
<th>Test Outcome Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition Positive</td>
<td>True Positive (TP) = 20</td>
<td>False Negative (FN) = 10</td>
</tr>
<tr>
<td>Condition Negative</td>
<td>False Positive (FP) = 180</td>
<td>True Negative (TN) = 1820</td>
</tr>
</tbody>
</table>

Positive predictive value = \( \frac{TP}{TP + FP} \) = \( \frac{20}{20 + 180} \) = 10%

Negative predictive value = \( \frac{TN}{FN + TN} \) = \( \frac{1820}{10 + 1820} \) ~ 99.5%

Sensitivity = \( \frac{TP}{TP + FN} \) = \( \frac{20}{20 + 10} \) ~ 67%

Specificity = \( \frac{TN}{FP + TN} \) = \( \frac{1820}{180 + 1820} \) = 91%

Incorrect laboratory results

- Nowadays, the probability of an analytical error is rather small due to quality management system
- Most errors occur in the preanalytical phase
- Analytical errors: 1) random caused by by timing, temperature, or pipetting variations during the measurement process or 2) systematic caused by change in instrument calibration
- Postanalytical errors:
  - Transcription errors
  - Production of reference values (wrong value / wrong units)?
  - Age and sex dependent reference ranges?
  - Effect of medication?
  - Pregnancy?
  - Daytime variation
  - Use of accurate and reliable result
Quality Control

Definitions

- **Quality Control** - QC refers to the measures that must be included during each assay run to verify that the test is working properly.

- **Quality Assurance** - QA is defined as the overall program that ensures that the final results reported by the laboratory are correct.

- “The aim of quality control is simply to ensure that the results generated by the test are correct. However, quality assurance is concerned with much more: that the right test is carried out on the right specimen, and that the right result and right interpretation is delivered to the right person at the right time”
Quality control (QC)

- **Internal quality control**
  - used to establish whether a series of techniques and procedures are performing consistently over a period of time
    - A QC material with a reduced, normal and high level should be included at the beginning and end of each series
    - Intra- and inter-unit determinations
    - Results not answered, if the control not precise
    - Ensure that the value is stable

- **External quality control**
  - used to identify the degree of agreement between one laboratory’s results and those obtained by other centres
    - Control samples analyzed weekly/monthly
    - Results reported to QC-organizers
    - Reports from QC-organizers

Measurement uncertainty = imprecision

- The discrepancy between the result of a measurement and the true (or accepted true) value

- Analytical errors in measurements:
  - The magnitude of an analytical error depends on the method/assay and instrument in use
  - Consist of random errors, which determine the precision or repeatability of measurement and systematic errors, which determine the accuracy or bias of measurement
  - Systematic errors can be assessed by comparing the results to international reference material concentration values
  - Total measurement uncertainty: includes all possible error factors, it can be expressed as percentage
  - Reference method: an analytical method sufficiently free of random or systematic error to make it useful for validating a new analytical procedure for the analyte; possible true positive results + specific for the proposed analyte. Not always suitable for routine use.
Quality control

- CV% = coefficient of variation, the ratio of the standard deviation to the mean
- Bias = deviation

Process approach in HUSLAB
Quality assessment

- Technical and analytical quality
- Display validity
- Effective process
- Appropriate test requisition
- Preanalytical error prevention
- Harmonized analyzing system
- Appropriate Analytical quality
- Continual method development
- Supportive IT-solutions
- Appropriate reporting
- Reliability of the result
- Accuracy of the test results
- Timeliness of the results
- Access to laboratory results
- Service orientation
- Deliberating effective Customer service
- Expert personnel support and solution
- Availability of Professional systems
- Availability of lab Analytics (emergency)
- Study scope and range according to the clinical need
- Quality of functioning/management
- Quality of functioning/management
- Objective and on-line assessment of the operational system
- Solveig Linko

Standardization - Objectives

- True results
- Different methods produce same results
- Does not guarantee the repeatability of the method
- Harmonisation
  - Different methods produce same results
  - The obtained result may be incorrect
Requirements for standardization

• Standards
  – Pure standards; calibration by direct weighing
    Unit mol/L or g/L
  – Not totally pure standard
    • Biological or arbitrary calibration
    • WHO standard for hormone peptides and proteins
    • Unit IU/L or U/L

• Reference method
  – Available only for a part of the measurements
    • For example mass spectrometry

Standardization problems

• Unspecific measurement procedures
  – The assay measures something else than the analyte
    • Similar hormones
      – Estradiol and estron
    • Metabolites
      – Drugs
  – Variables that affect the quality of the results
    • Turbidity of the sample, lipemia
    • Antibodies interference in immune methods

• Incorrect calibration of the measurement procedure
  – Value transfer from reference method to routine

• Commercial interests
  – Calibration of the new method considered to be expensive
HUSSLABin ohjekirja

http://huslab.fi/ohjekirja/index.html