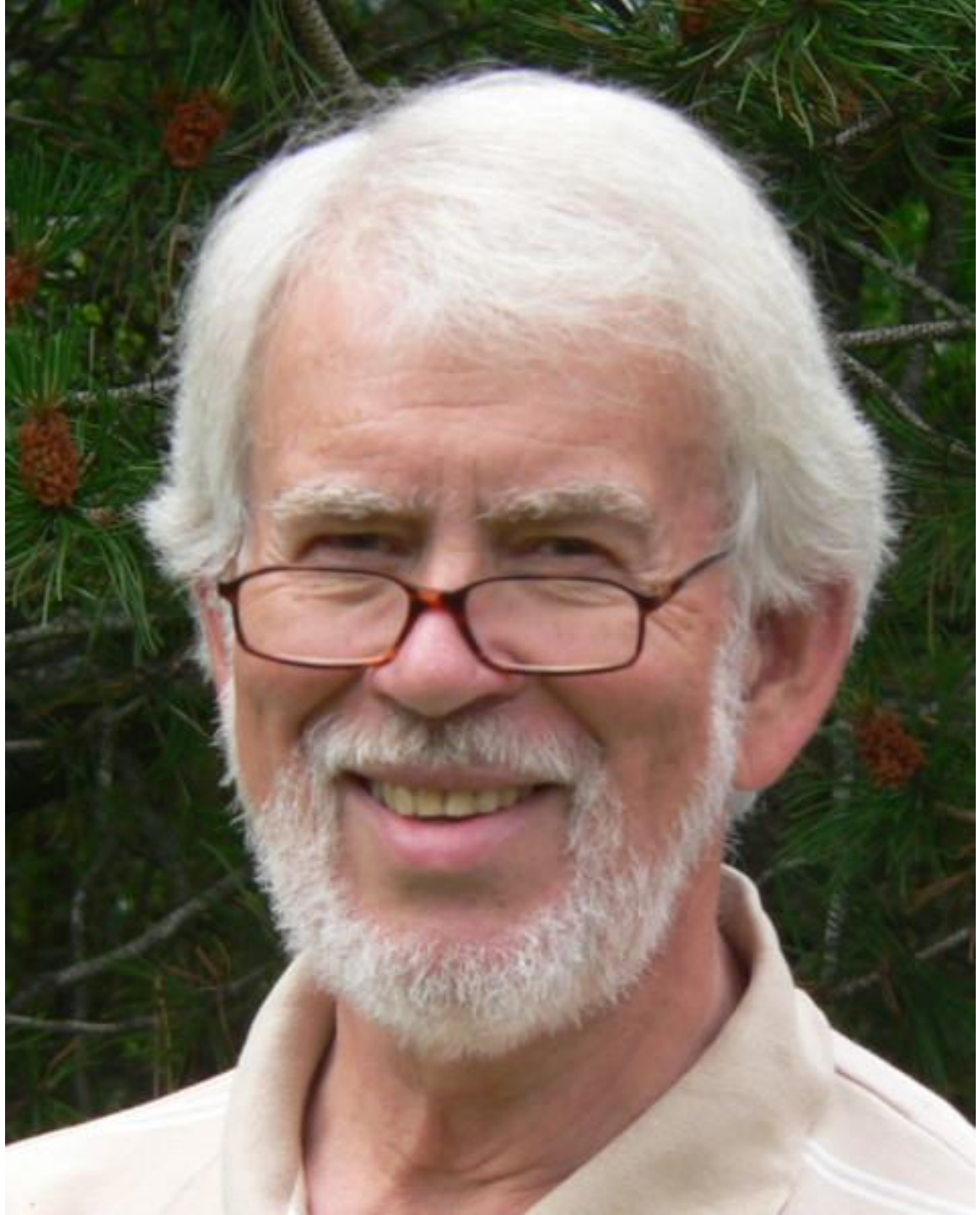
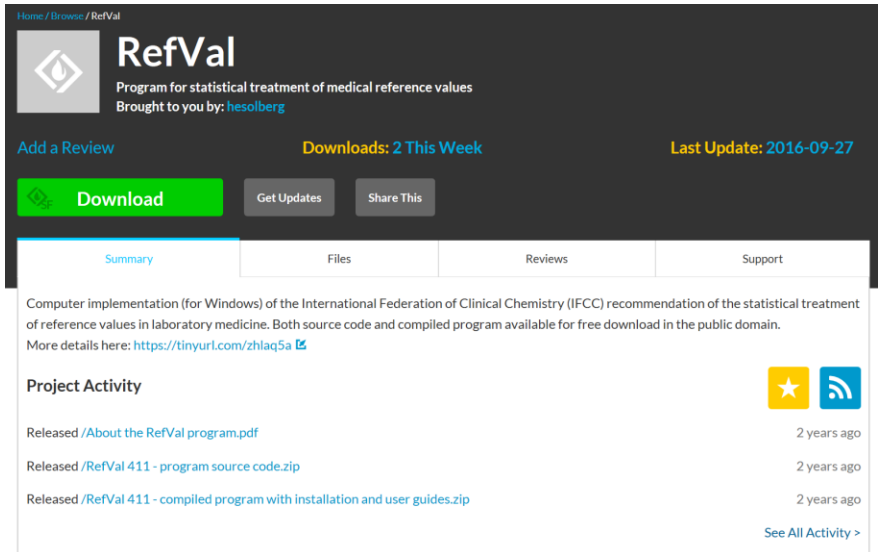
Reference intervals

Elvar Theodorsson



Helge Erik Solberg

- Born 1937
- Rikshospitalet – Klinisk biokjemi
- Retired 2005
- RefVal https://sourceforge.net/projects/refval/



“Normal intervals” and reference intervals

- Population
- Healthy/sick

”Normal”

- In the statistical sense
  - Normal/Gaussian distribution
- In the epidemiological sense
  - Normal in this sense should be replaced e.g. by the terms common, frequent, habitual, usual, and typical
- In the clinical sense
  - Absense of disease
  - Absense of risk for disease
- Gräsbäck & Saris

“Reference **Interval**” or “reference range”

- Range
  - A **single** figure
  - Upper minus lower
  - Can be anywhere in the measurable interval
- Interval
  - **Two** figures – a lower and an upper – reference limits

Reference individual

- An individual selected for comparison using defined criteria

Reference value

- A value obtained by observation or measurement of a particular type of quantity on a reference individual

Clinical decision limits

- Separation of values/concentrations according to clinical categories/clinical outcomes
  - Based on outcome studies
  - Focused on risks
- E.g.
  - Diabetes
  - Hyperlipidemia
- Subject-based
- Population-based

Number of reference individuals needed

- In the **parametric method** 40 observations are needed to establish the 0.025 and 0.975 limits
- In the **nonparametric method** a sample sise of at least 120 is needed in order to be able to determine the confidence limits of the reference limits
  - 0.025*(n+1) to 0.975(n+1)
  - In a data set of 120, these are the 3rd and 118th ranked results
- At least 40 observations are needed for the **resampling method**

Selection of reference individuals

- Type of sampling
  - **Direct** (selected from the population according to certain criteria)
  - **Indirect** (using samples collected for other purpose, e.g. blood donors)
- A priori vs a posteriori
  - Individuals selected for sampling in case they fulfil specified criteria)
  - Use of a database of results from healthy individuals or patients meeting certain inclusion criteria
- Random vs non-random
  - Process of selection giving each individual in the population equal probability of being selected or not

Indirect sampling

- Blood donors
- Individuals undergoing routine physical examinations for periodic health screening
- Patients undergoing minor surgical procedures
- Individuals undergoing genetic screening (unaffected relatives)Dixon

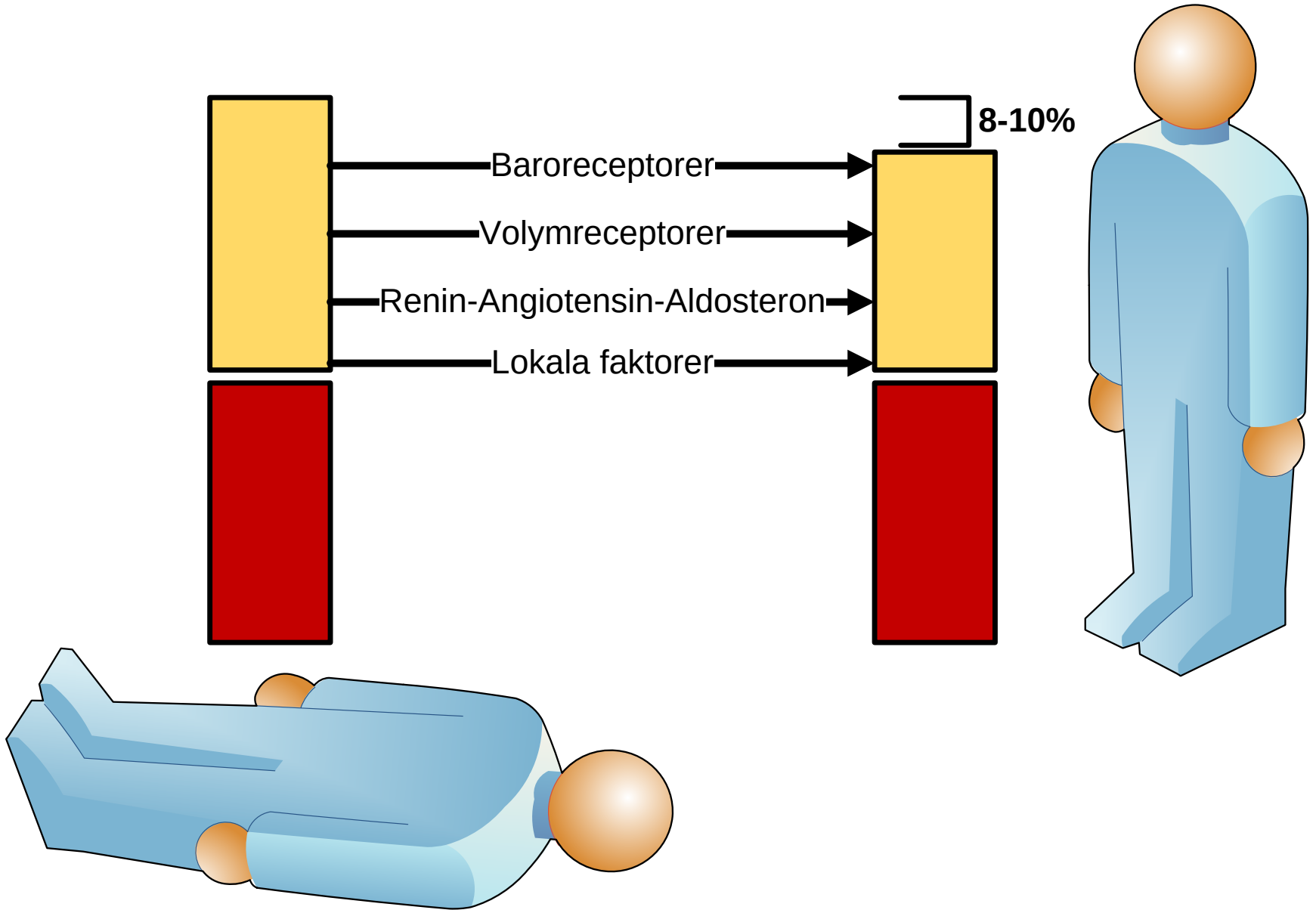
Exclusion criteria

- Age
- Sex
- Hypertension
- Pregnancy
- Oral contraceptives
- Recent blood donation
- Recent intensive exercise
- Recent intensive fasting
- Recent transfusion
- Prescription drugs
- Drugs of abuse
- Excessive alcohol intake
- Genetic factors
- Illness

Standardisation of preanalytical conditions

- Make it as simple as possible – but not simpler (Einstein)
- The same rules should be used as in the clinical situation
  - Food and beverage restrictions
  - Exercise restrictions
  - Time sitting or lying down before phlebotomy
  - Torniquet time

Standard-ising preana-lytics



Partitioning of the reference individuals

- Age
- Sex
- Menstrual cycle

Reference intervals for large geographic areas

- Skåne
- NORIP
- Commutable calibrators and calibration

The NORIP project – Paul Rustad - Fürst

- The year 2000
- The origin of many of the reference intervals used in laboratories is commonly obscure.
- Using commutable normal serum in external quality assurance programs it was noticed, and especially in Norway documented, that the reference intervals used within the country for the same quantity in the same age and gender groups varied more than the corresponding analytical deviations could account for. It was believed that the populations in the Nordic countries were too homogenous to make room for a biological explanation.
- Supported by the Nordic Society of Clinical Chemistry (NFKK) a Nordic project was launched on March the 27th 1998 in Oslo based on a decentralized design from Denmark. A subproject for routine hematology with origin in Finland was added later.
- The project was performed mainly during 2000 and 2001 in the five Nordic countries according to a common protocol.

The “Tryding” dilemma

- In the process of creating common reference intervals in Skåne

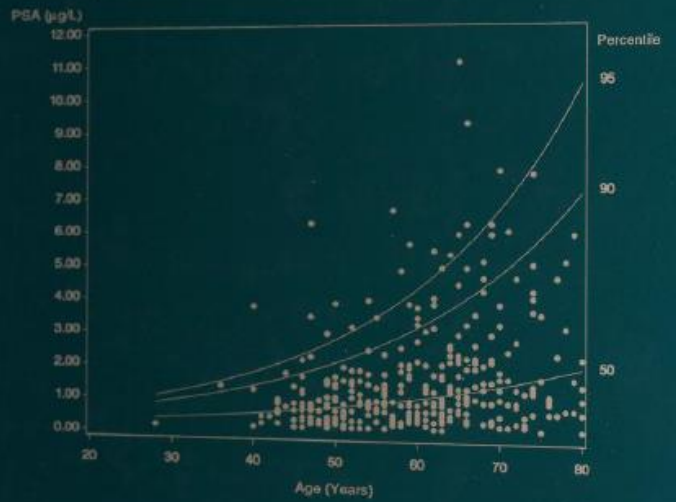
October 2010

EP28-A3c

Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition

STATISTICS: textbooks and monographs

STATISTICAL BASES OF REFERENCE VALUES IN LABORATORY MEDICINE



EUGENE K. HARRIS
JAMES C. BOYD

Reference Intervals

A USER'S GUIDE

PAUL S. HORN
AMADEO J. PESCE

This book describes reference intervals and their use in the clinical laboratory setting. Intended for those who work in the area of laboratory medicine, *Reference Intervals* addresses the common methods and limitations of deriving and interpreting reference intervals and covers the following topics:

- History and use of the reference interval
- Description of the current standards
- Real-world constraints and solutions
- Statistical modeling of reference intervals
- How to deal with outliers and/or small samples
- Description of robust reference intervals
- The transference problem

The accompanying CD-ROM contains Microsoft Word® files with gender-age-ethnic group-specific reference intervals based on physician-determined healthy individuals from the Third National Health and Nutrition Examination Survey (NHANES III). Also included: SAS® programs that derive traditional and robust reference intervals with outliers removed.

System Requirements for the CD-ROM:

- Microsoft Windows ® 98 or above and Microsoft Word ® 97 or above to view the NHANES reference intervals
- SAS ® Version 8.2 or above to derive reference intervals from raw data

ISBN 1-59425-035-9
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Reference Intervals A USER'S GUIDE HORN & PESCE

Reference Intervals

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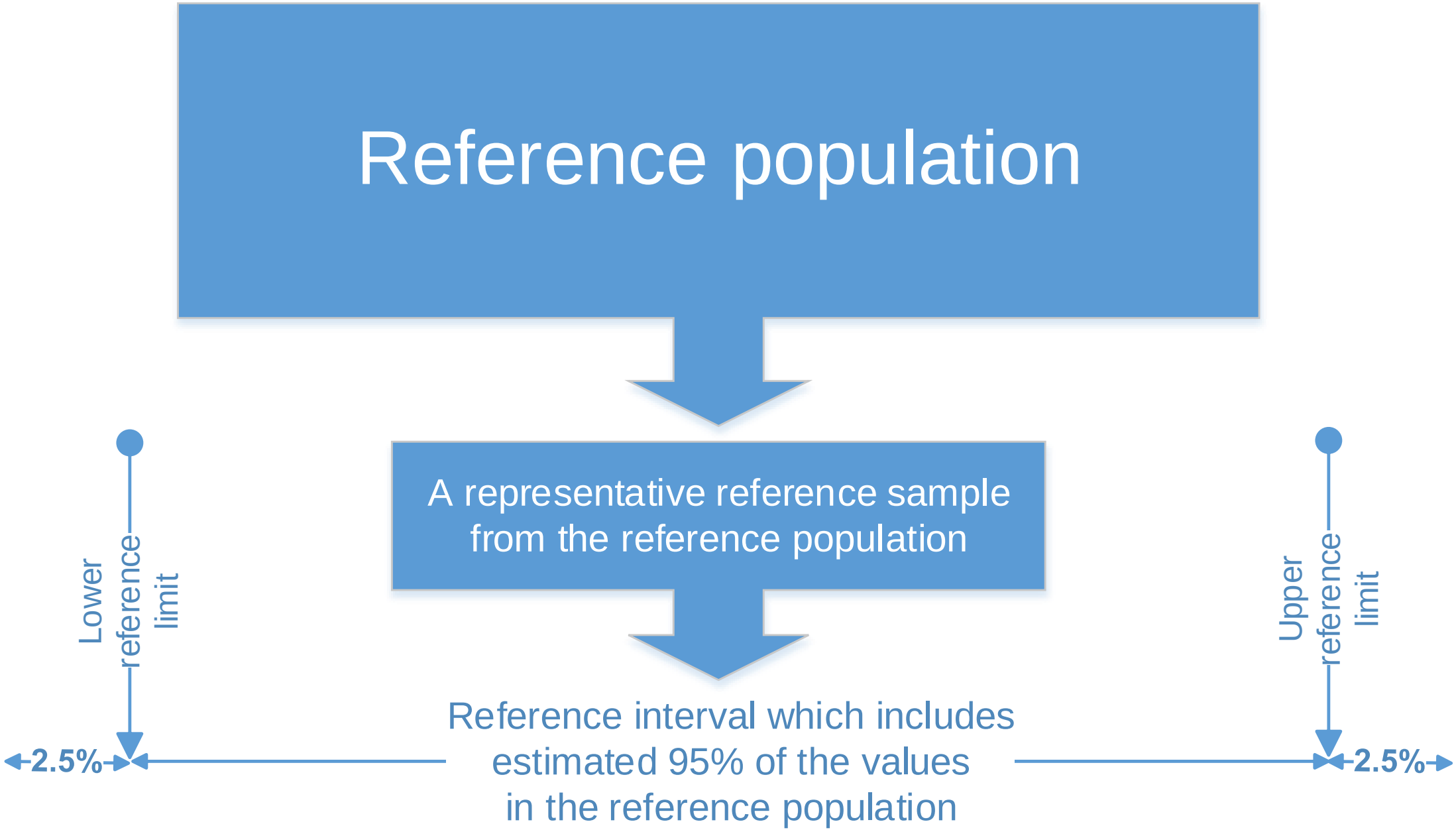
AACCPress


Reference population
A representative reference sample from the reference population
Lower reference limit
Upper reference limit
2.5%
Reference interval which includes estimated 95% of the values in the reference population
2.5%

Reference interval

- Estimating reference intervals means dealing with uncertainties and probabilities. All probabilistic methods are based on assumptions about a **theoretical distribution** which fundamentally determines the conclusion that can be drawn from the data.
- The most commonly used probabilistic methods are the **parametric** methods that assume that observations in the population are distributed according to the Gaussian/Normal distribution.
- These are the methods of choice if the data are Gaussian or can be transformed to that distribution since the data themselves with the added knowledge of the distribution of the data in the sample and population enables the user of the data to draw firmer conclusions than if only the are known.

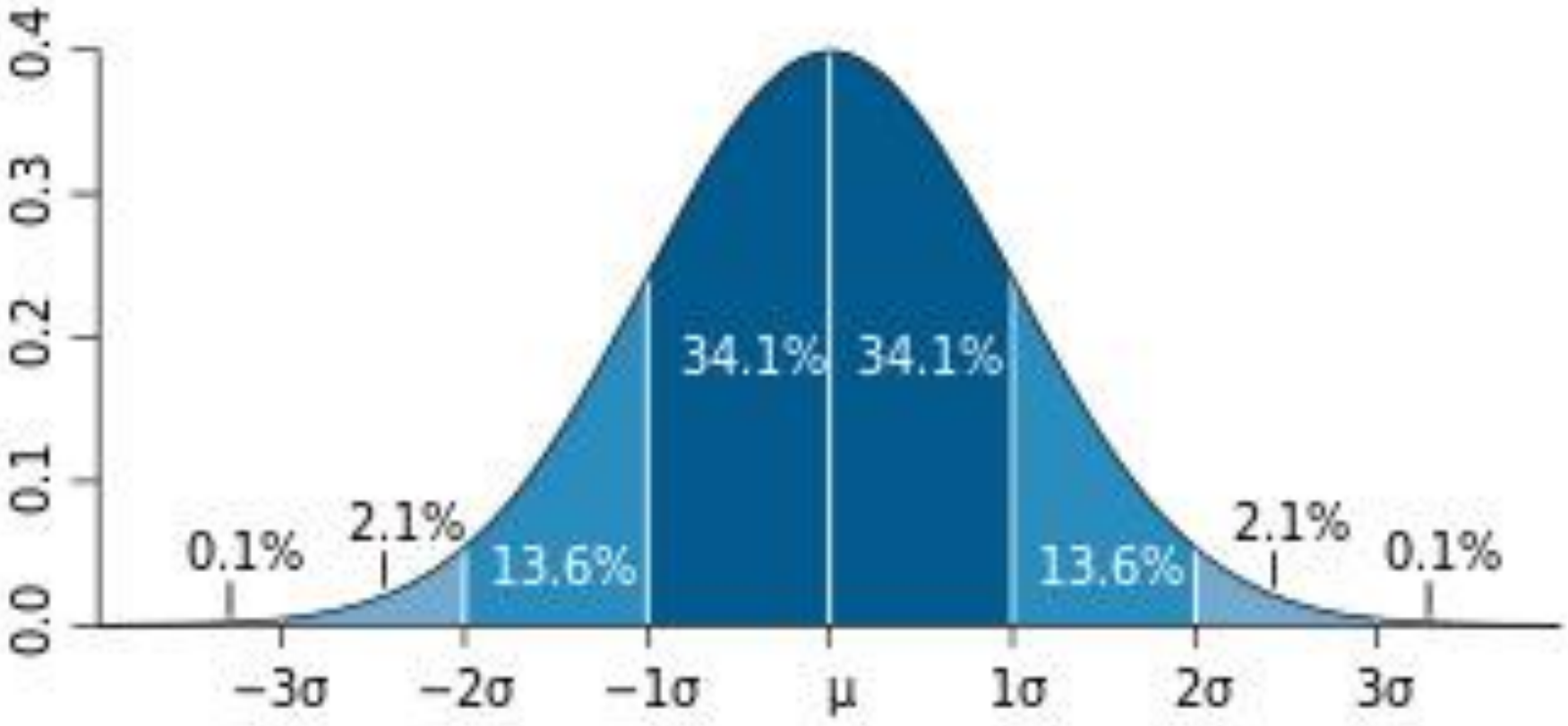
Reference interval

- To estimate the **2.5th percentile** distinct from the 5th percentile, or the 95th percentile distinct from the 97.5th (ie, P = 2.5), a minimum of **39 measurements** is required [39 = (100/2.5) − 1]. The smallest observation in the sample is the nonparametric estimate of the 2.5th percentile of the population, while the largest observation estimates the 97.5th percentile.
- Clinical and Laboratory Standards Institute (CLSI **EP28-A3c**) recommend the following formulas: lower limit has the rank number 0.025 x (n+1) and the upper limit the rank number 0.975 x (n+1). This method is practicable mainly when the number of reference samples is **120** or more.

Calculation

- Grubbs test for outliers
- Anderson-Darling test for normality
- Box-Cox transformation

Normal distribution



Detection of outliers

- Dixon test (base on the range) $Q = \frac{\text{gap}}{\text{range}}$
- Grubbs test (based on the SD/Z-score) $G = \frac{Y_{\max} - \bar{Y}}{s}$

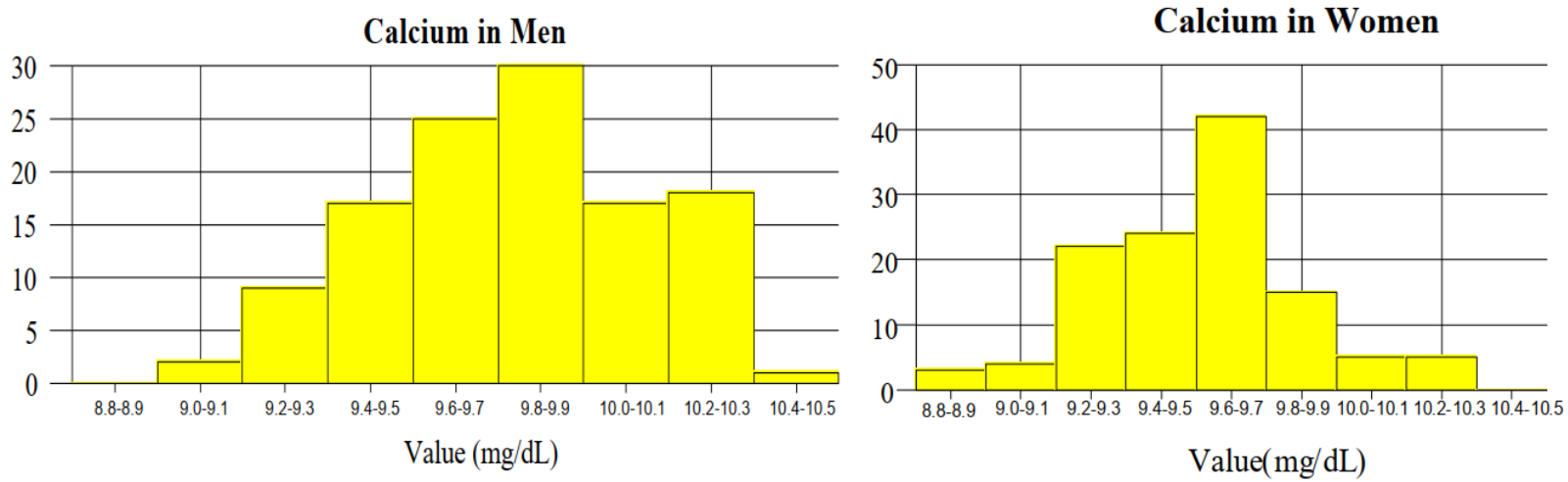


Figure 2. Calcium Histograms

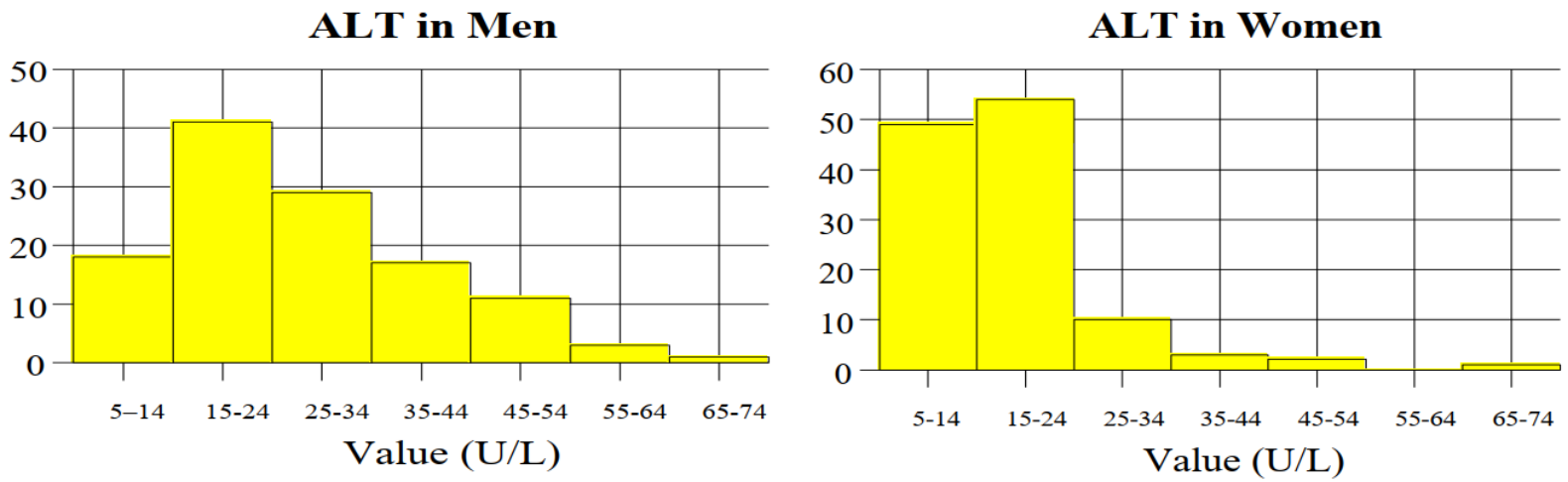
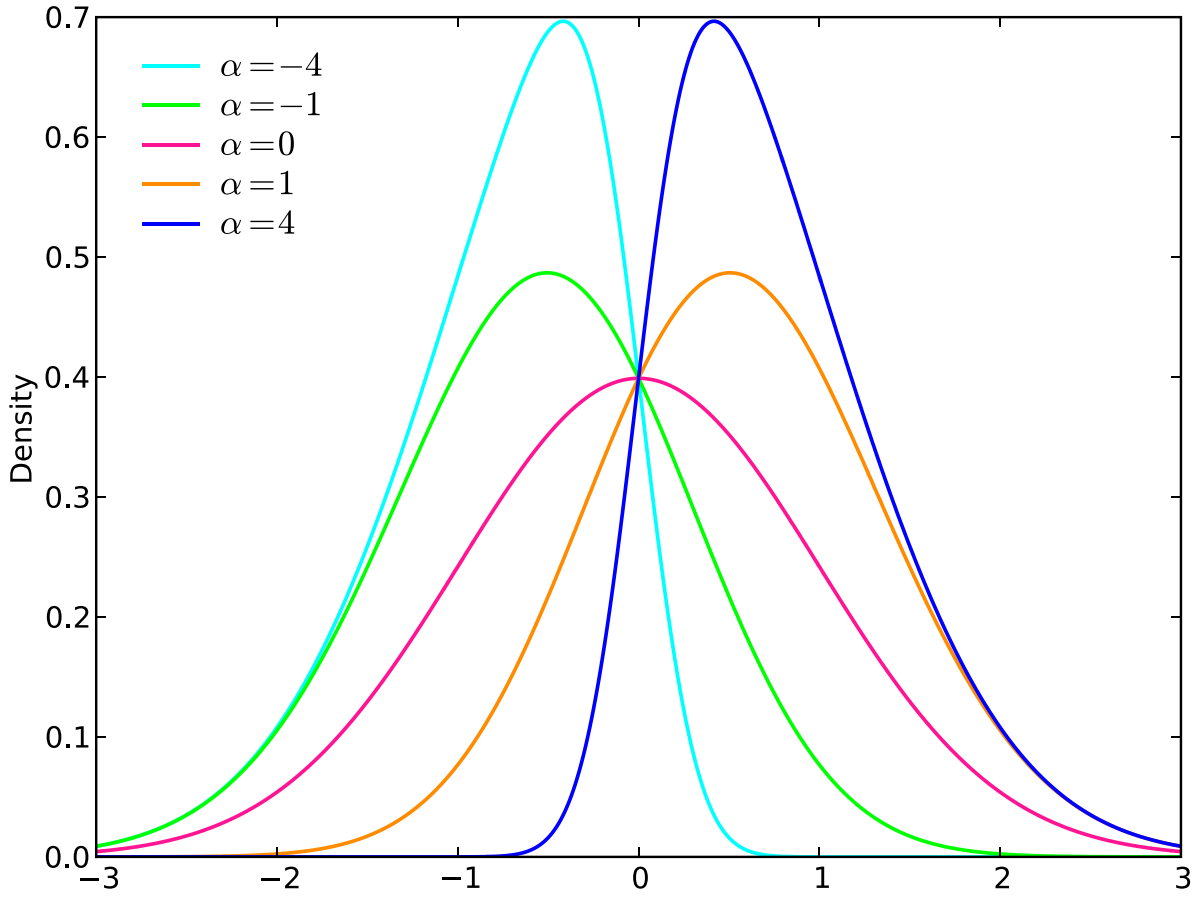
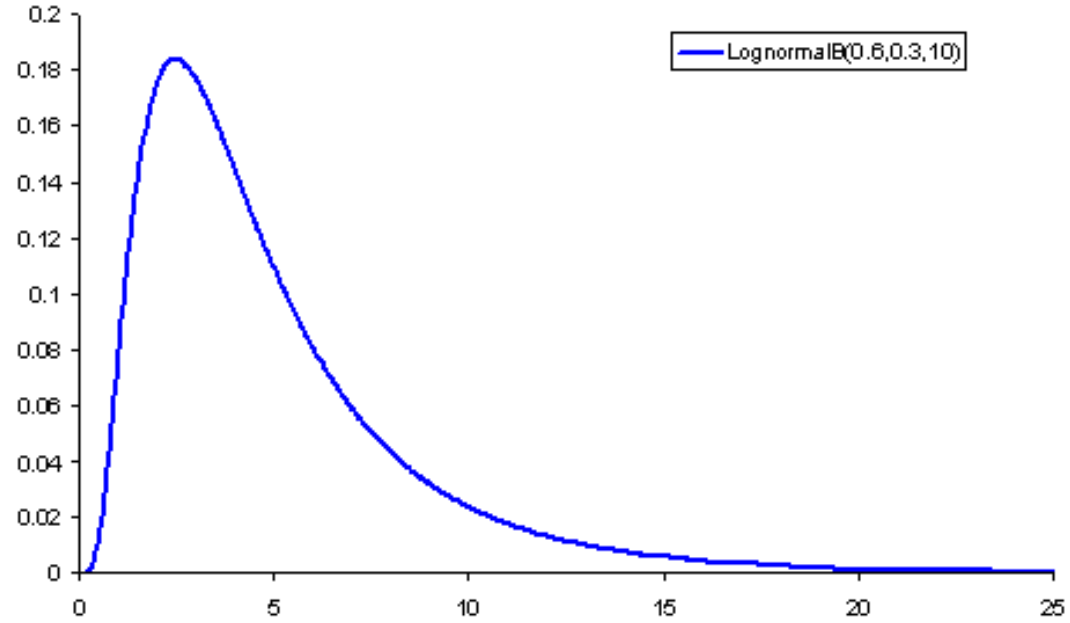


Figure 3. Alanine Aminotransferase Histograms

Skew distributions, e.g. lognormal





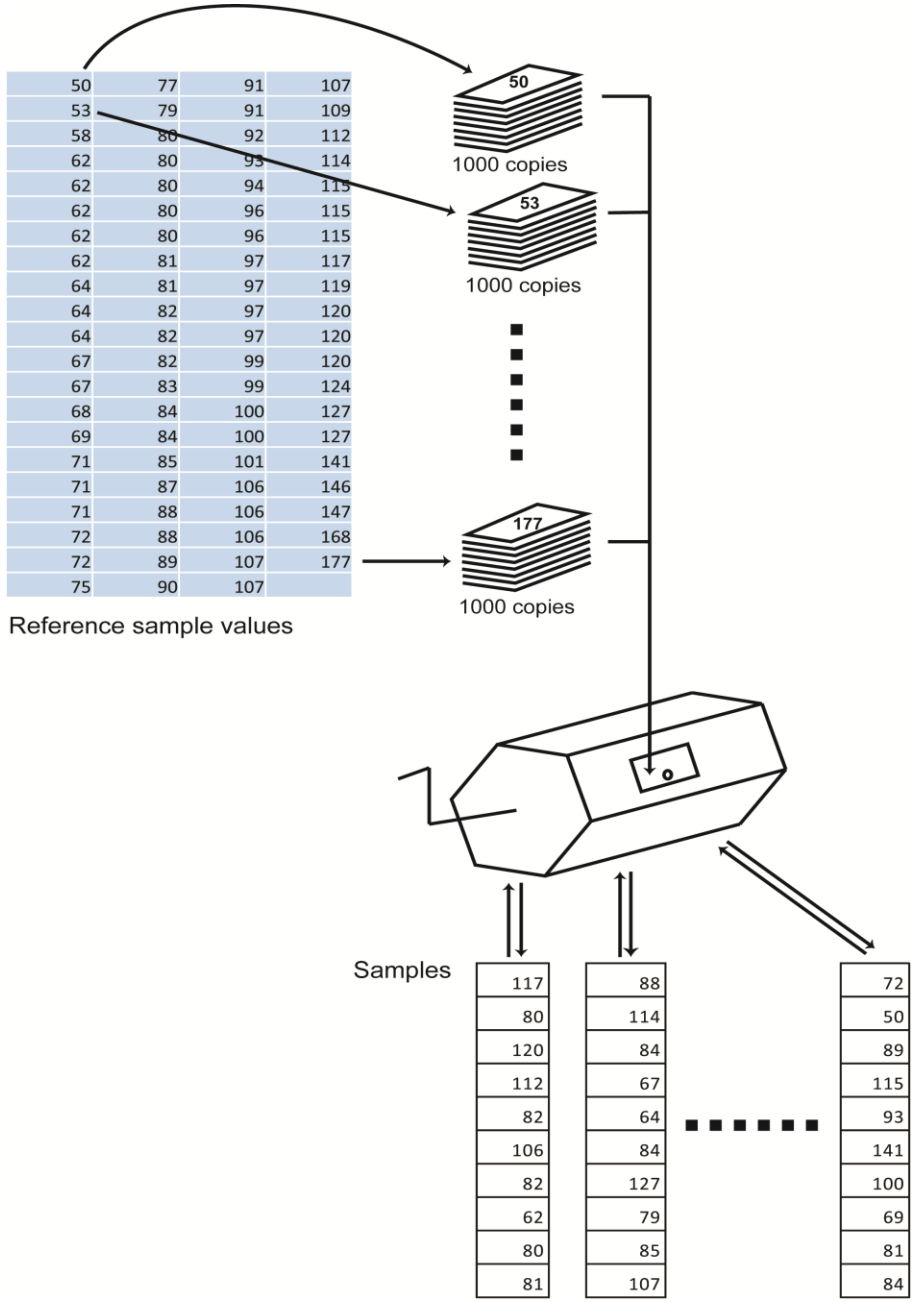
Tests for normality

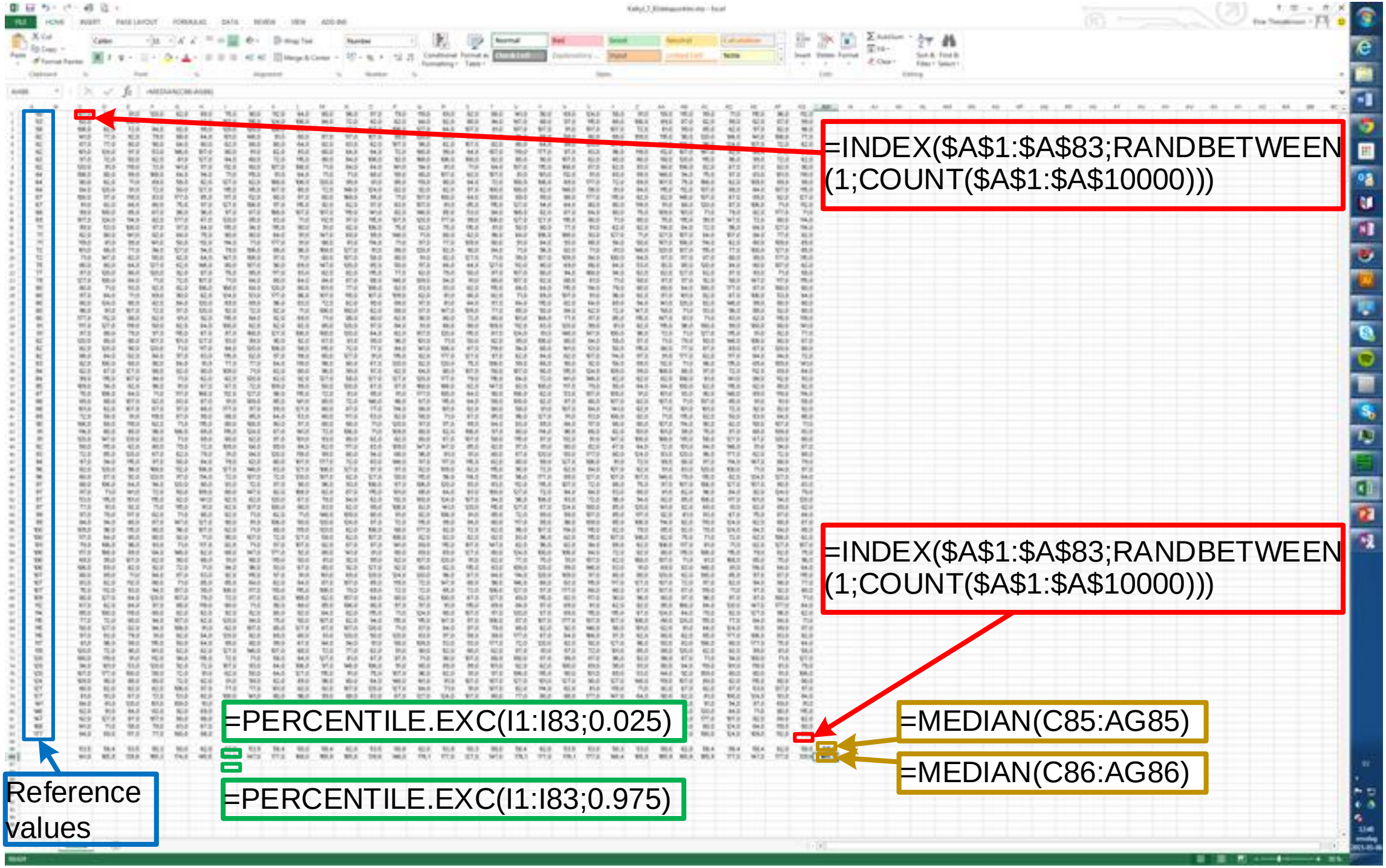
- Anderson – Darling
- Komolgorov - Smirnov

Transformation

- Logarithm
- Square root
- Box-Cox-Transformation

Resampling techniques




=INDEX($A$1:$A$83;RANDBETWEEN(1;COUNT($A$1:$A$10000)))
=INDEX($A$1:$A$83;RANDBETWEEN(1;COUNT($A$1:$A$10000)))
=PERCENTILE.EXC(I1:I83;0.025)
=PERCENTILE.EXC(I1:I83;0.975)
=MEDIAN(C85:AG85)
=MEDIAN(C86:AG86)
Reference values

Verifying reference intervals

- Reference intervals calculated from results of analytes measured in presumably healthy patients, e.g. Patients visiting primary care or blood donors
- Use of resampling techniques – requiring in the order of 40 results

Diskusionspunkter - gruppearbeid

1. Hvilke rutiner har dere for å verifisere et referanseområde?
    1. Når?
    2. Hvordan gjøre dere det?
2. Brukes andre metoder for å verifisere referanseområder hos barn enn hos voksne? I så fall hvorfor?
3. Vi snakker iblant om beslutningsgrenser og iblant om referanseområder når vi vil tolke laboratorieresultat. Når er det hensiktsmessig å bruke det ene eller det andre og hvorfor?
4. Da vi som oftest bruker friske/normale personer som referansepersoner – hvorfor kaller vi ikke det vi får frem for «normalområde» istedenfor «referanseområde»?

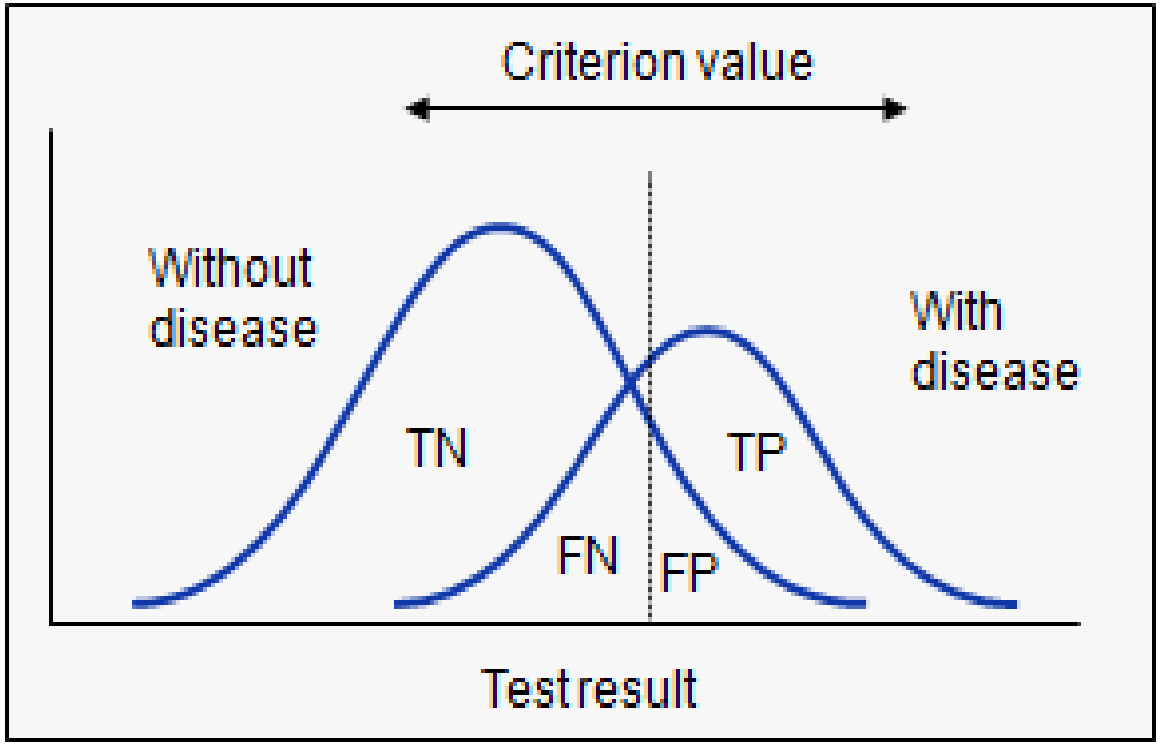
Diskusionspunkter - gruppearbeid

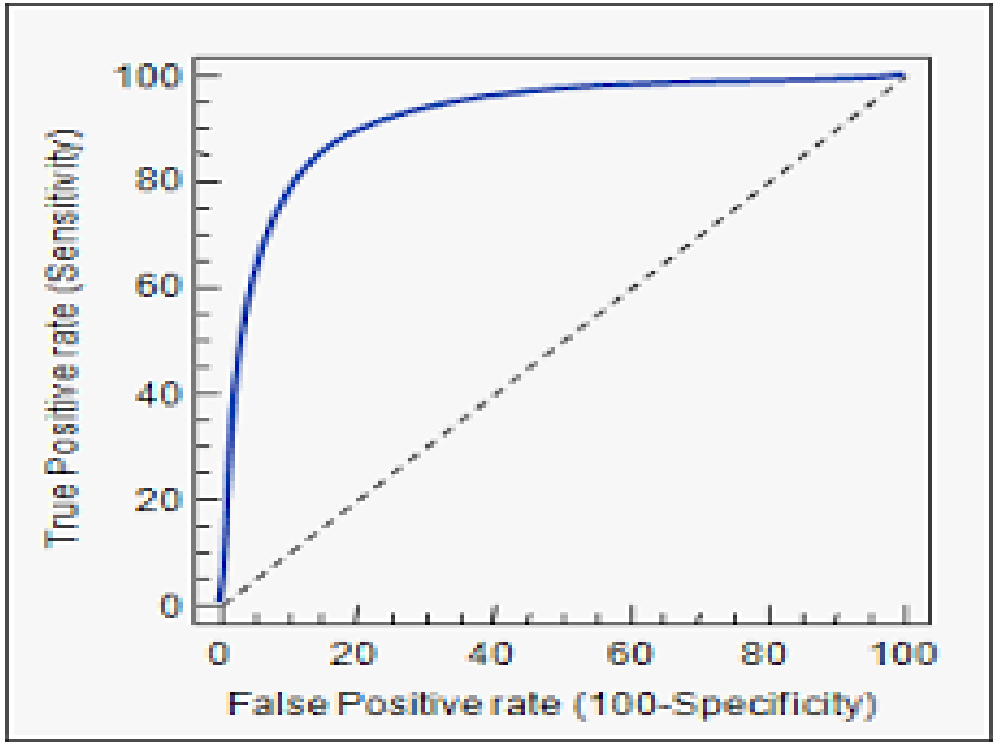
5. Forklar hvorfor vi ber referansepersoner sitte 20-30 min før prøvetakning og hvilken betydning det har når vi vil benytte referanseområdene for å tolke pasientresultat.
6. Hva mener vi med ekstremverdier/outliers når vi vurderer hvilke resultater som skal inngå i beregningsgrunnlaget for et referanseområde?
7. Hvorfor kan man ikke uten videre ta vekk ekstremverdier?
8. Det anbefales å basere referanseområde på resultat fra minst 120 individer – hvorfor?
9. Hvordan kan man vite om en referanseperson er frisk?

Diskusionspunkter - gruppearbeid

10. Å velge ut sykehuspersonell, brannmenn, politimenn eller studenter forenkler håndteringen, men finnes det noen ulemper med det?
11. Kurver som de over brukes ofte som en hjelp i beslutningen om en pasient er frisk eller syk. Har sånne kurver forbindelse med referanseintervaller? I så fall hvilken forbindelse?

Diskusionspunkter - gruppearbeid





Questions to discuss

Normal interval vs reference interval

- We commonly employ healthy individuals when establishing reference intervals.
- Why not use the concept of “normal interval” instead of reference interval.

Decision limits vs reference intervals

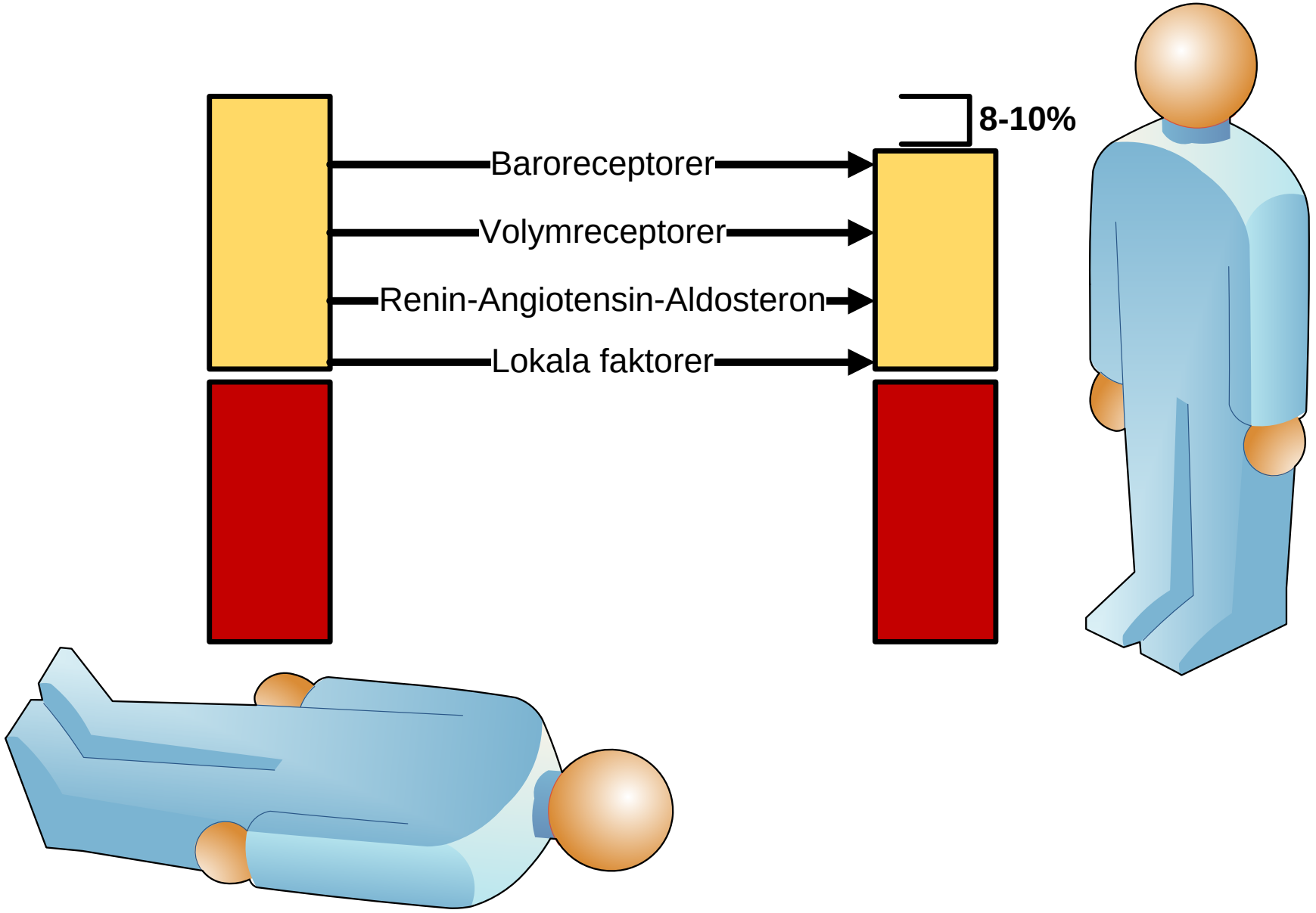
- When we are interpreting laboratory results we sometimes talk about **reference intervals** and sometimes about **decision limits**
- When should we appropriately use the one or the other?
- Why

Reference interval vs reference range

- We are advised to say “reference interval” and not “reference range”
- Why?

Sitting 20-30 minutes before taking sample

- Explain why!


8-10%
Baroreceptorer
Volymreceptorer
Renin-Angiotensin-Aldosteron
Lokala faktorer

Outliers – what are they

- What do we mean by the concept of “outliers” when converting reference values to reference interval.
- Why not just remove ”obvious” outliers right away?

What is so “magic” about 120

- At least 120 reference individuals – why?

Who is healthy?

- How can we know whether a reference person is healthy?

Choice of reference persons

- Are there any disadvangages of choosing healthcare workers, firemen, police or students as reference persons?

Children vs grownups

- Are different methods used for establishing reference intervals in children compared to adults. In that case why?

ROC vs reference intervals

- ROC curves are commonly used for support when discering between healthy and sick. Are there any relations between ROC curves and reference intervals?