Pre-requisite for use of common reference intervals

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Reference Intervals: interested parties

1. Manufactures

DIRECTIVE 98/79/EC on *in vitro* diagnostic medical devices

ANNEX I

ESSENTIAL REQUIREMENTS

B. DESIGN AND MANUFACTURING REQUIREMENTS

8. Information supplied by the manufacturer

8.7. Where appropriate, the instructions for use must contain the following particulars:

(l) the reference intervals for the quantities being determined, including a description of the appropriate reference population;

Reference Intervals: interested parties

2. Clinical Laboratories

ISO 15189 Medical laboratories — Particular requirements for quality and competence

5 Technical requirements

5.5.5 Biological reference intervals shall be periodically reviewed. If the laboratory has reason to believe that a particular interval is no longer appropriate for the reference population, then an investigation shall be undertaken, followed, if necessary, by corrective action. A review of biological reference intervals shall also take place when the laboratory changes an examination procedure or pre-examination procedure, if appropriate.

Reference Intervals: interested parties

3. Clinicians

⇒ different reference intervals from different laboratories;

⇒ confusion between Reference Intervals and Decision Limits.

4. Patients

⇒ same value can be considered “normal” or “abnormal” in different laboratories.

R.I. Traditional Method

1) Determine biological variables & analytical interferences
2) Determine selection/exclusion/partitioning criteria
3) Obtain written consent and completed questionnaire
4) Categorize reference individuals
5) Exclude individuals as determined a priori
6) Insure an adequate number of reference individuals
7) Prepare reference individuals for sample collection
8) Collect samples
9) Analyze samples
10) Inspect frequency distribution of data
11) Identify data errors and outliers
12) Determine reference intervals (and confidence limits)
13) Document all of the previously mentioned steps and procedures.

Number Needed

- for non-parametric technique,
  - n = (100/P)-1
  - for p = 2.5% (central 95%), n = (100/2.5) - 1 = 39
- problem with using n = 39 is two-fold:
  - Impossible to define confidence intervals
  - The lower and the upper values represent 2.5 and 97.5 percentile: overly sensitive to extreme values
- To calculate confidence intervals, minimum n is ***<u>120</u>*** *(per partition)*

Possible solution

- **Common Reference Intervals** defined internationally through multicenter trials

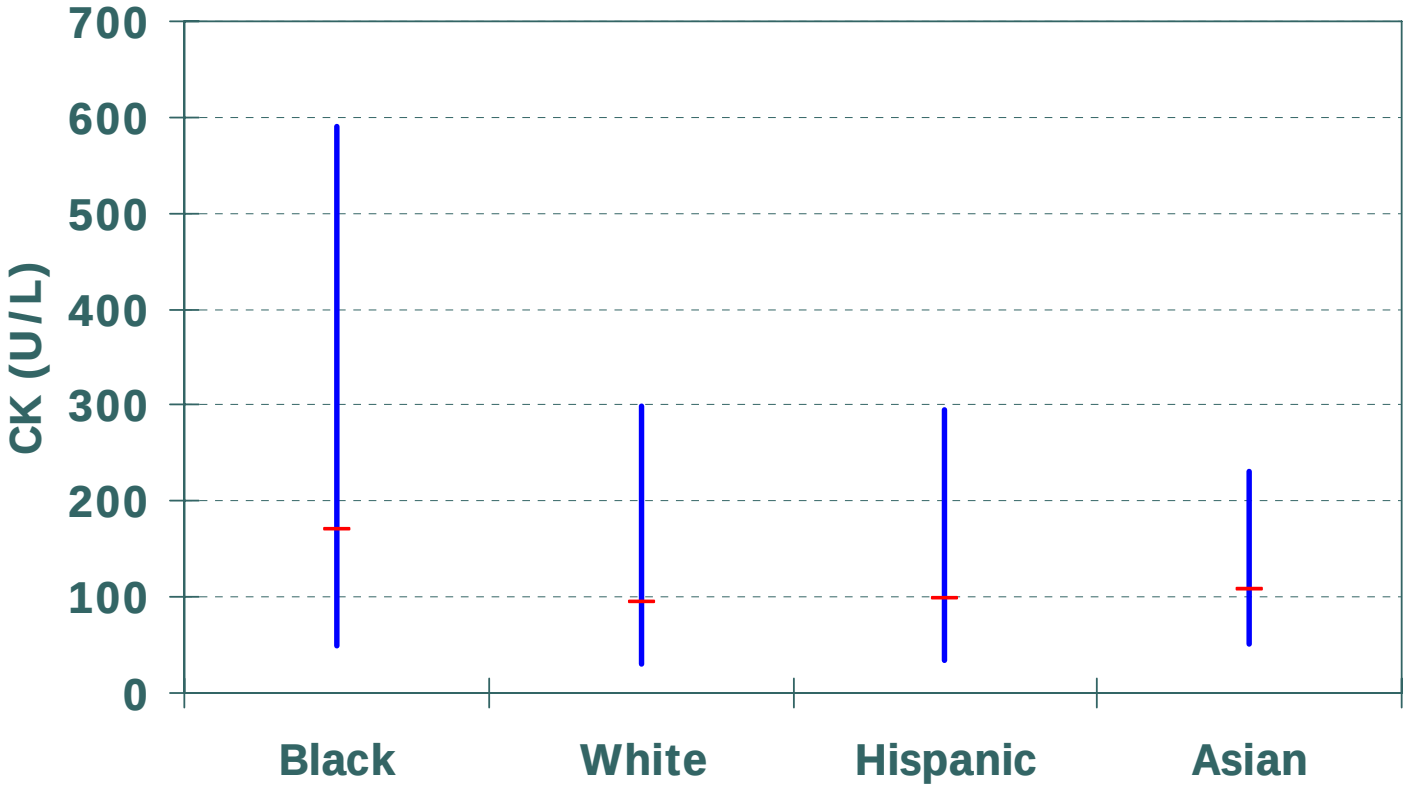
Are common R.I. applicable?

Limitations

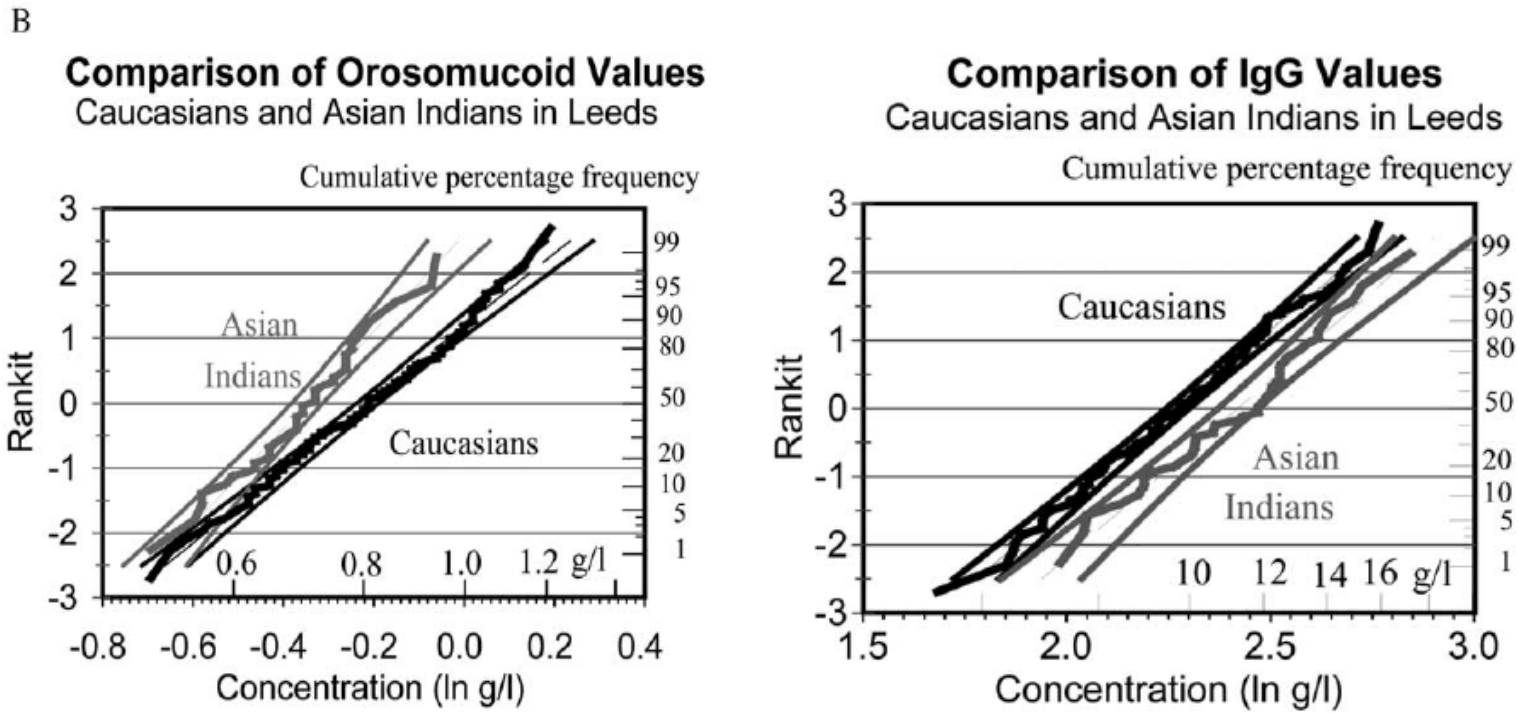
- **Biology**: population differences
  - Do they exist?
  - How big are the effects due to ethnicity or different life habits?
- Pre-analytical and **analytical**
  - Standardization
  - Traceability

Ethnicity of CK reference values

Harris EK et al **Clin Chem** 1991;**37:**1580-2



Specific protein



Myron Johnson A et al. Clin Chem Lab Med 2004;42:792-9

Potassium

Table 1. Average Blood Potassium Concentration Values in Men Living in Different Cities

	No. of subjects	K^+, mmol/L Mean	K^+, mmol/L SD	No. (%) <3.5 mmol/L
Serum				
Shanghai, China	53	3.82	0.27	5(9)
Salvador, Brazil	100	4.06	0.29	3(3)
Salzburg, Austria	30	4.14	0.44	1(3)
Philadelphia, PA	51	4.31	0.32	0(0)
Newark, NJ	52	4.45	0.41	1(2)
Santo Domingo, Dominican Republic	38	4.37	0.33	0(0)
Plasma				
Ibadan, Nigeria[a]	82	3.85	0.67	17(21)

[a] The value for the Nigerians averages less than for serum samples because the potassium in platelets is not released into plasma but is released into serum.

Reidenberg MM et al. Clin Chem 1993;39:72-5

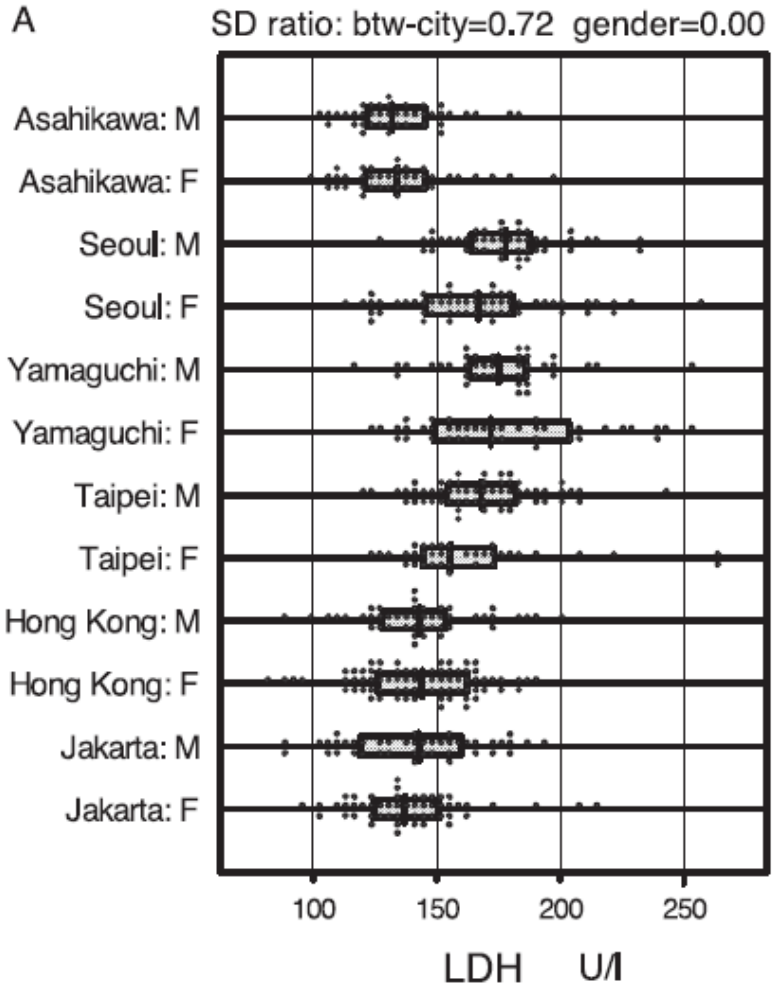
Insufficient n° of subject

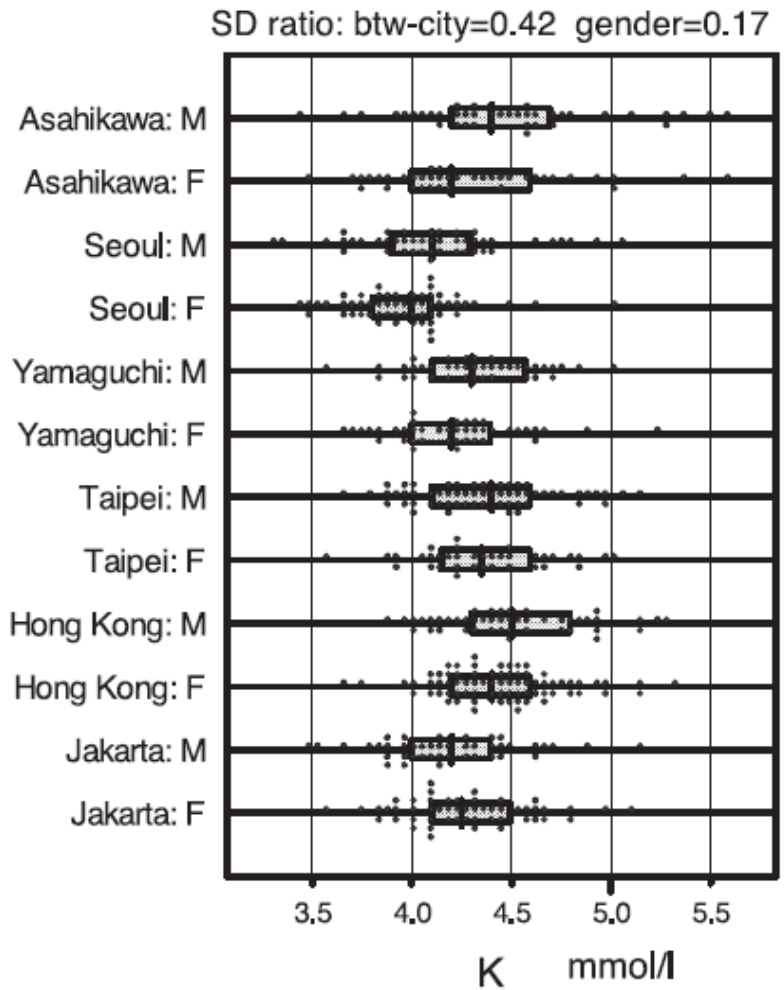
Insufficient standardization

Different matrices

Can we rely upon similar data?

Analytes with geographical (ethnical?) differences





Ichihara K. et al. Clin Chem 2008;54:356

Analytes with and without geographical differences

With

- LDH, Total protein, Globulin, Urea, C3, C4, IgG, CRP, Na, K, Cl, Inorganic phosphate

Without

- AST, ALT, GGT, CK, Amylase, Albumin, Creatinine, Uric acid, Ca, IgA, IgM

From Ichihara K. et al. Clin Chem 2008;54:356

Serum Creatinine reference intervals

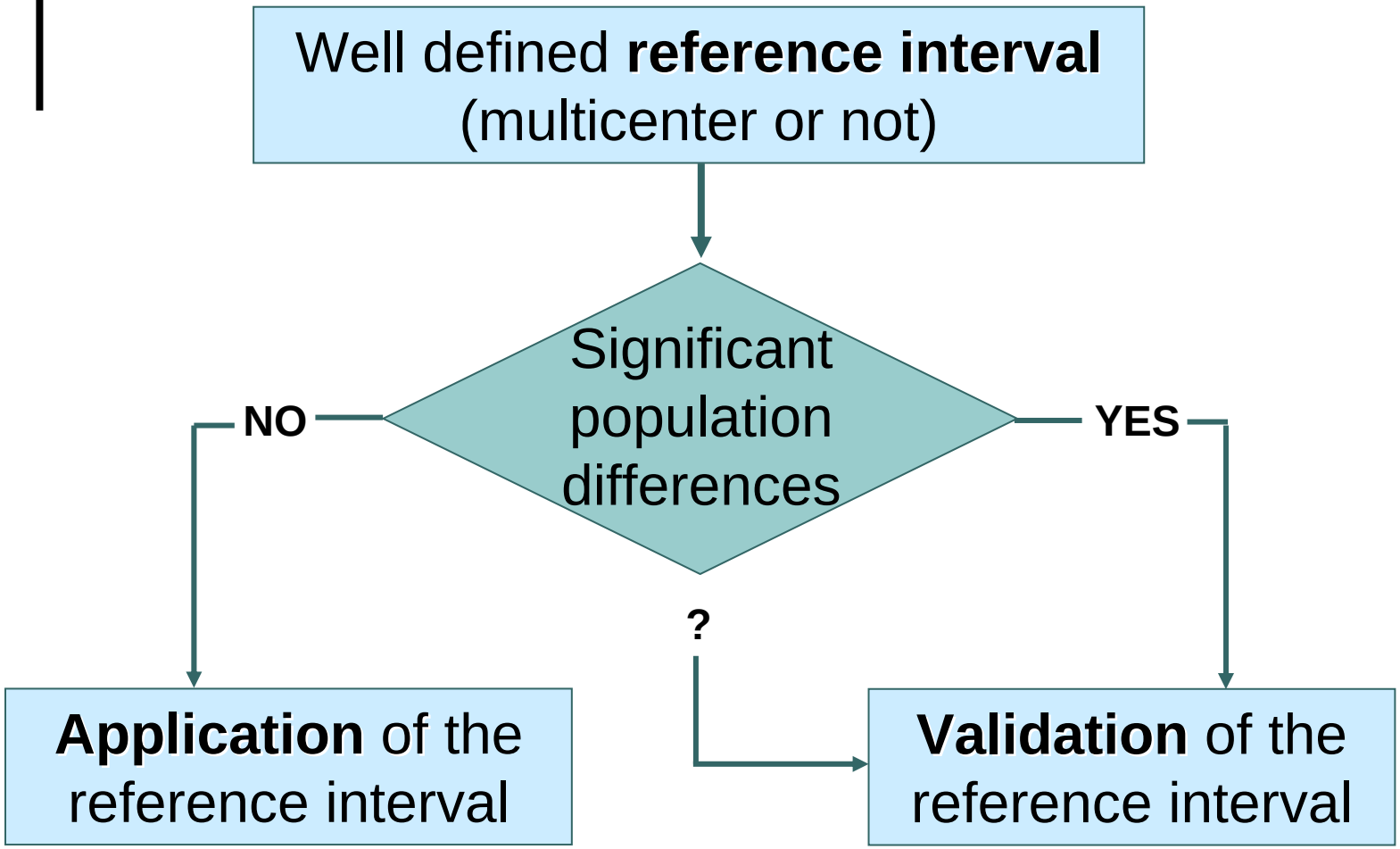
	Australia	Northern Europe	Germany	Spain
Method	Enz	Various	Enz	Jaffe
R.I. males (µmol/L)	59 - 104	60 - 105	64 - 104	64 - 106
R.I females (µmol/L)	45 – 84	45 – 90	49 - 90	52 - 85

Analytical requirements for the use of common R.I.

Requisite	Responsibility
Existence of a Reference Measurement System (accuracy base)	IFCC, JCTLM, National and international Metrological Institutes
Existence of field methods producing traceable results	Manufacturers
Correct implementation of the methods in the clinical laboratories	Clinical Laboratories
Control of the performances of the field methods to keep them within stated limits for imprecision and bias	Clinical Laboratories, EQAS organisers

Clinical requirements for the use of common R.I.

Requisite	Responsibility
Accurate definition of reference intervals providing information on the influence of biological and environmental factors	Joint effort IFCC – manufactures – clinical laboratories
Compatible pre-analytical phase	Clinical laboratories
Validation of the applicability of the common reference intervals to the laboratory's own population	Clinical laboratories


Well defined **reference interval** (multicenter or not)
Significant population differences
NO
YES
?
Application of the reference interval
Validation of the reference interval

Validation of an existing R.I.

- A priori selection of 20 reference subjects
- Formal outlier test
- Apply one of the following techniques:
  - Binomial test (insensitive if skewed population)
  - Kolmogorov-Smirnov test (but you need all the data)
  - Use Horn and Pesce robust technique to calculate R.I, if the proposed R.I. are inside the confidence limits you can accept them

Conclusions

- The concept of Common Reference Intervals is valid, but its application has to be done carefully
- A large amount of data is still missing, but efforts are in place to close these gaps, at least for the more common analytes

Thank you!