8th Congress of the Croatian society of medical biochemistry and laboratory medicine
22 – 26 September 2015, Rijeka

Biological variation remains relevant

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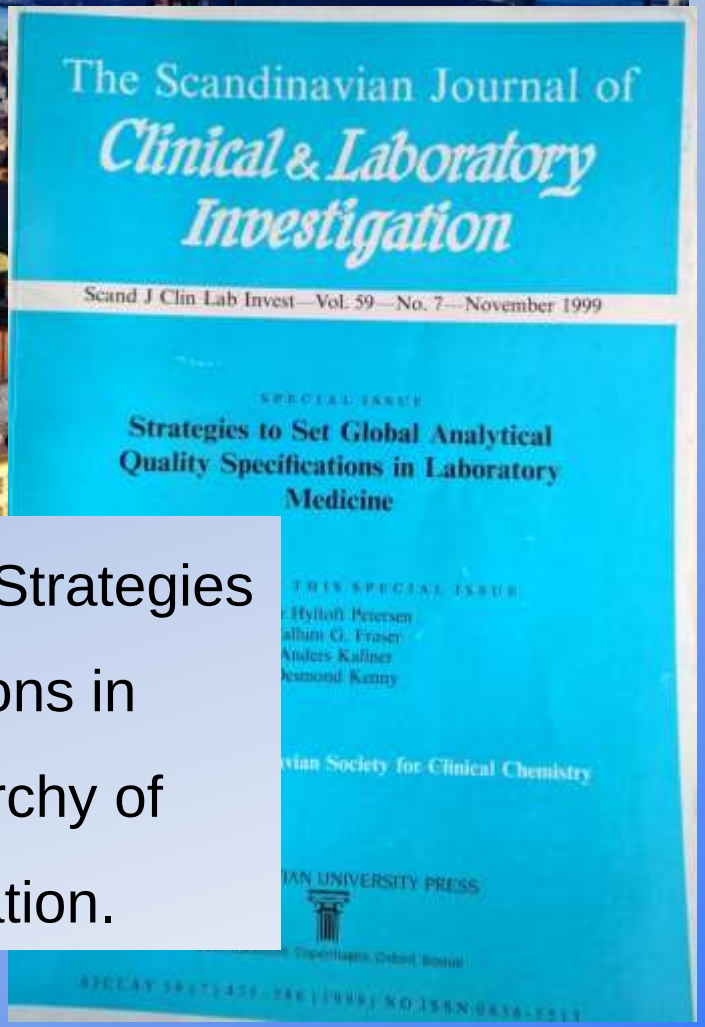
Member of Task and Finish Group on Biological Variation Database

"It is impossible, and rather not-productive, to discuss quality in laboratory medicine unless analytical quality specifications (analytical goal) are set a priori"

(Kallner A, Scand J Clin Chem Lab Invest 1999;59:475-6)

"It is recognised that objective analytical quality specifications must be attained so that adequate patient care is provided"

(Fraser CG, Scand J Clin Chem Lab Invest 1999;59:477-8)



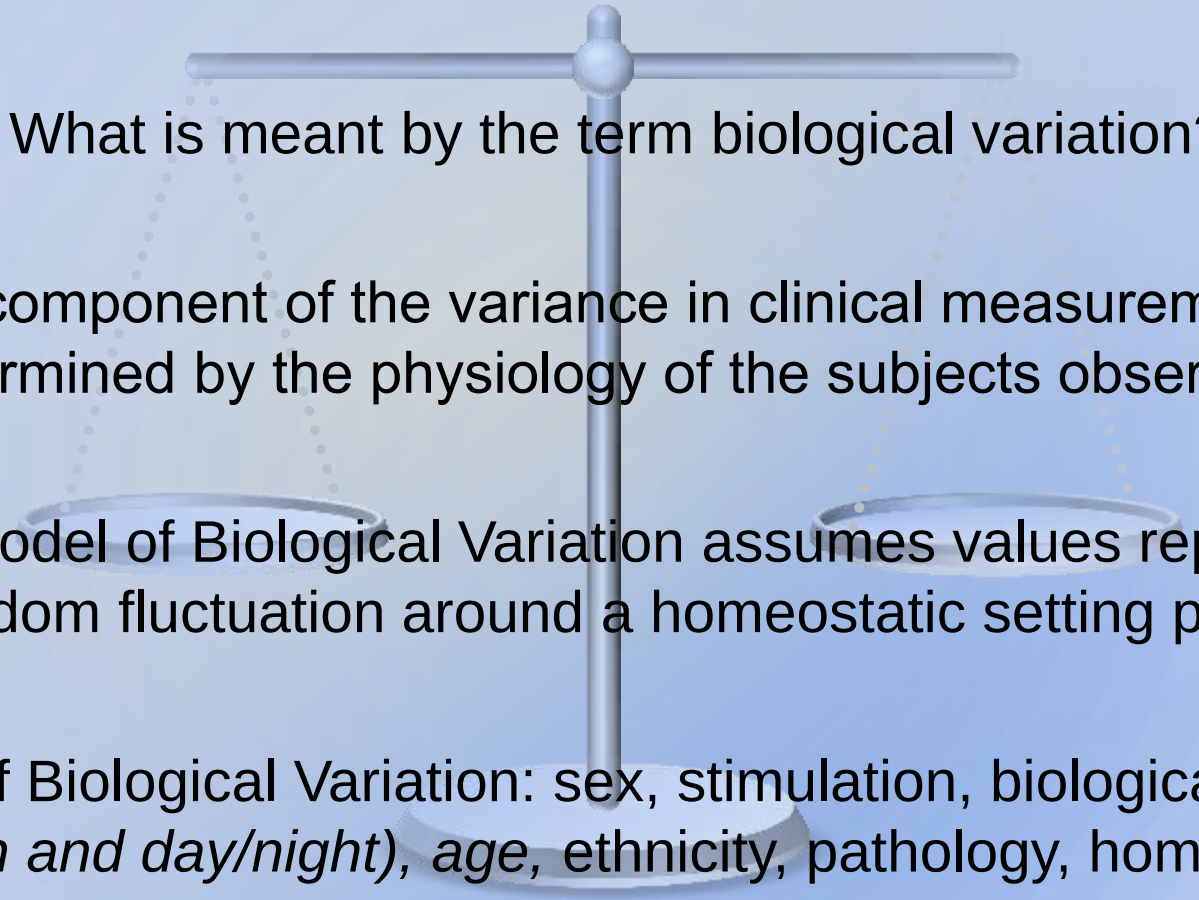
The Stockholm conference held in 1999 on «Strategies to set global analytical quality specifications in laboratory medicine», established a hierarchy of models to set analytical quality specification.

Hierarchy of models in order to set quality specifications

(Stockholm conference, April 1999)

1. Evaluation of the effect of analytical performance on clinical outcomes in specific settings;
2. Evaluation of the effect of analytical performance on clinical decisions in general using a) data based on components of biological variation, or b) analysis of clinicians' opinion;
3. Published professional recommendation from a) national and international expert bodies, or b) expert local groups or individuals;
4. Performance goals set by a) regulatory bodies, or b) organisers of EQAS;
5. Goals based on the current state of the art as a) demonstrated by data from EQA or proficiency testing scheme, or b) found in current pubblications or methodology.

Nature of Biological Variation



What is meant by the term biological variation?

"A component of the variance in clinical measurements determined by the physiology of the subjects observed."

The model of Biological Variation assumes values represent random fluctuation around a homeostatic setting point.

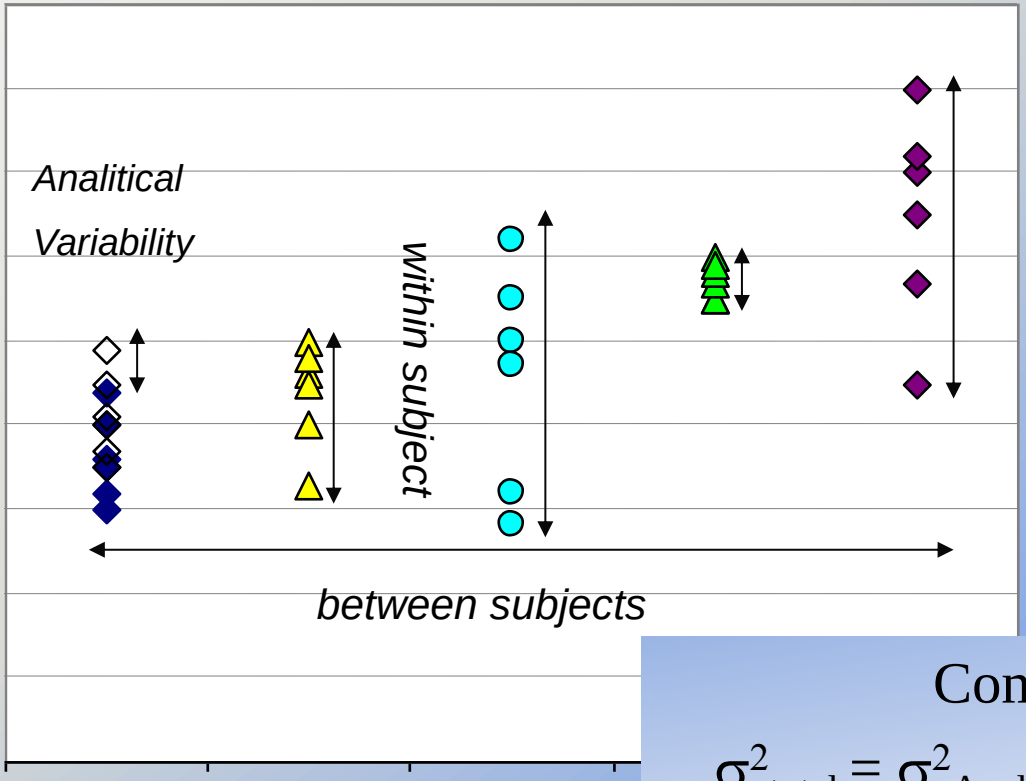
Source of Biological Variation: sex, stimulation, biological rhythms *(season and day/night), age,* ethnicity, pathology, homeostasis

Importance of Biological Variation

- All clinical measurements change with time;
- Knowledge of temporal changes useful in diagnosis, interpretation and monitoring;
- Rate of change may be useful in prognosis;
- Understanding of the sources of biological variation in non-diseased subjects is fundamental to the development of reference data.

Components of Variability in Clinical Measurements

- Analytical variability
- Within Subject biological variability
- Between Subject biological variability



Components of variance:

$$\sigma^2_{total} = \sigma^2_{Analytical} + \sigma^2_{intra\text{-}subject} + \sigma^2_{inter\text{-}subjects}$$

Current application of VB data

- ✓ Setting of analytical goals (CV_{goal}).
- ✓ Quality specifications for:
  - total allowable error (TE_A)
  - Bias (B_A)
- ✓ Evaluating the significance of change in serial results (RCV).
- ✓ Assessing the utility of reference intervals (Index of Individuality).
- ✓ Assessing number of specimens required to estimate homeostatic set points.
- ✓ Timing of specimens.

Desirable quality specification

Desirable quality specification for analytical imprecision (CV_{goal}):

$$CV_{goal} = 0.5\ CV_I$$

calculated as half the within-subject variation
[optimum CV = 0.25 CV_I ; minimum CV= 0.75 CV_I]

Desirable quality specification for analytical bias (B_A):

$$B_A = 0.25\sqrt{CV_I^2 + CVG^2}$$

[Optimum 0.125 $\sqrt{CV_I^2 + CVG^2}$; Minimum 0.375 $\sqrt{CV_I^2 + CVG^2}$]

Desirable quality specification for total allowable error (TE_A):

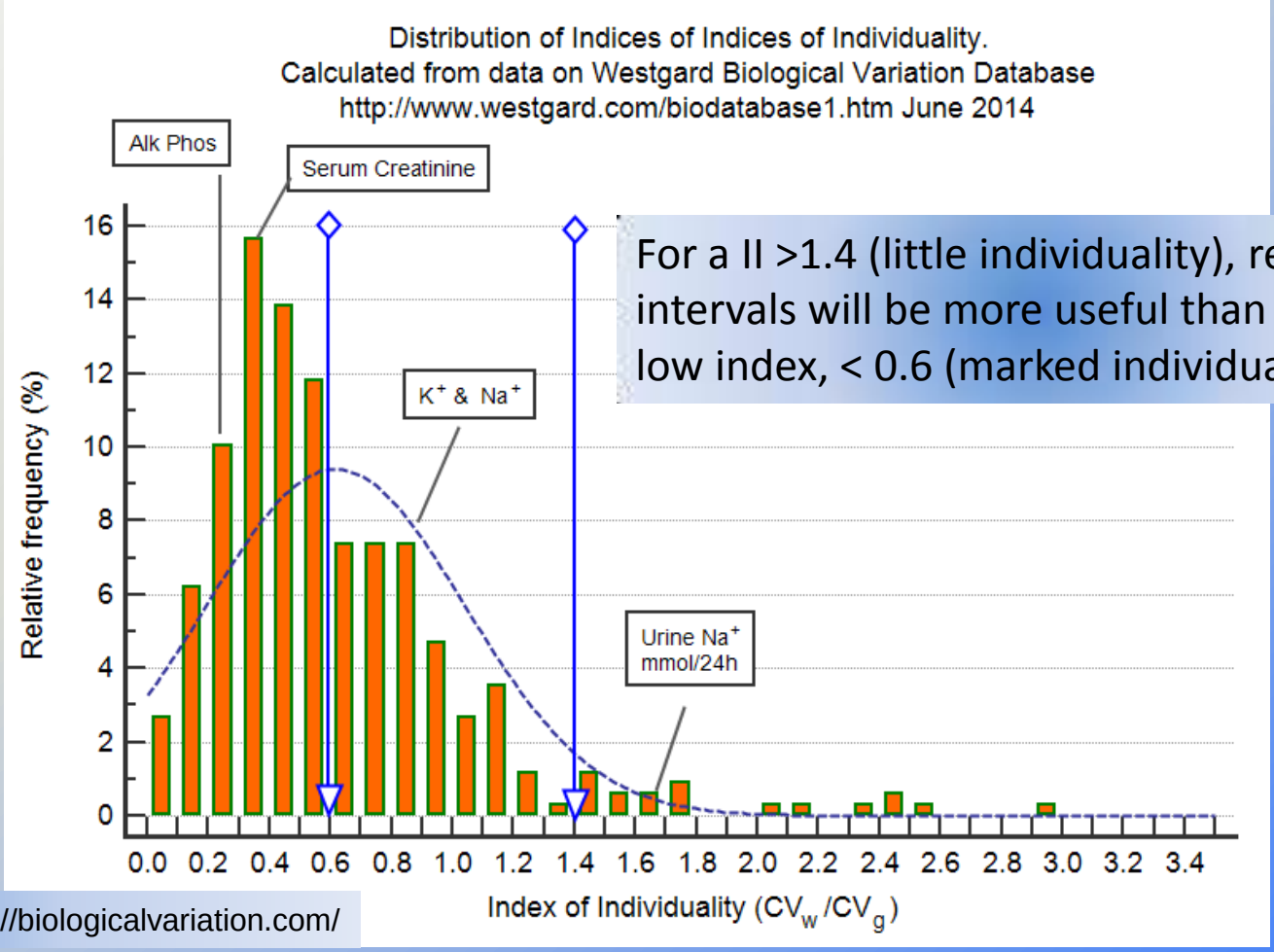
$$TE_A = B_A + 1.65\ CV_{goal}$$

Index of individuality (II)

Ratio of Within to Between subject variances.

$II = \sqrt{CV_A^2 + CVI^2} / CV_G$

$\approx CV_I / CV_G$ (close approximation if $CV_A <= CV_I$)



For a II >1.4 (little individuality), reference intervals will be more useful than for a low index, < 0.6 (marked individuality)

Bill Bartlett, http://biologicalvariation.com/

Reference Change Values (RCV)

Where individuality is marked the individual is the best point of reference.

RCV is widely used by clinical laboratories to identify changes that are statistically significant based on the dispersion of two results, usually at a 95% confidence level

$$RCV = z \cdot \sqrt{2} \cdot CV_T$$

Where $CV_T = \sqrt{CV_A^2 + CVI^2}$

$$RCV = z \cdot \sqrt{2} \cdot \sqrt{CV_A^2 + CVI^2}$$

The Z score determines the level of significance of the change

(e.g 2 tailed 95% = 1.96)

How to obtain Biological Variation data?

In 1989 Fraser and Harris published:

"Generation and application of data on biological variation in clinical chemistry" Crit Rev Clin Lab Sci 1989;27:409-37

This review proposed a standard approach to the definition and analysis of BV:

- **Selection of Subjects**: They should be "reference individuals", apparently healthy (inclusion and exclusion criteria);
- **Sample collection, handling and storage:** taking samples at the same time of day (usually early morning), under the same conditions, by the same phlebotomist, into tubes of the same lot number, freezing the samples to do the measurements in the same analytical run (if possible);
- **Analysis:** keeping analytical variation as low as possible (one instrument, one operator, one set of calibrators, one reagent..);
- **The best experimental design: sample measurements in duplicate in a single analytical run;**
- **Distributional assumptions** (homogeneity and normality) distribution of the data (if not normal, a transformation procedure (often logarithms) should been done;
- **Statistical treatment of raw data**: detection of outliers at **three different steps** (set of duplicate results, results for each subject, subject in a group);
- Once we have detected and eliminated outliers, and once we have verified our distribution, we can then estimate components of Biological Variation (with **ANOVA**).

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HOME › CLIA & QUALITY › QUALITY REQUIREMENTS › DESIRABLE BIOLOGICAL VARIATION DATABASE SPECIF

DESIRABLE BIOLOGICAL VARIATION DATABASE SPECIFICATIONS

Biological variation database, and quality specifications for imprecision, bias and total error (desirable and minimum). The 2014 update

Joana Minchinela[1,2], Carmen Ricós[1], Carmen Perich* [1,3], Pilar Fernández-Calle[1,4], Virtudes Alvarez[1,5], Mariví Domenech[1,6], Margarita Simón[1,7], Carmen Biosca[1,8], Beatriz Boned[1,9], Fernando Cava[1,10], José-Vicente García -Lario[1,11], Mª Pilar Fernández-Fernández[1]

	Analyte	Number of Papers	Biological Variation		Desirable specification		
			CV_I	CVg	I(%)	B(%)	TE (%)
S-	11-Desoxycortisol	2	21.3	31.5	10.7	9.5	27.1
S-	17-Hydroxyprogesterone	2	19.6	50.4	9.8	13.5	29.7
U-	4-hydroxy-3-methoximandelate (VMA)	1	22.2	47.0	11.1	13.0	31.3
S-	5' Nucleotidase	2	23.2	19.9	11.6	7.6	26.8

EFLM
EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

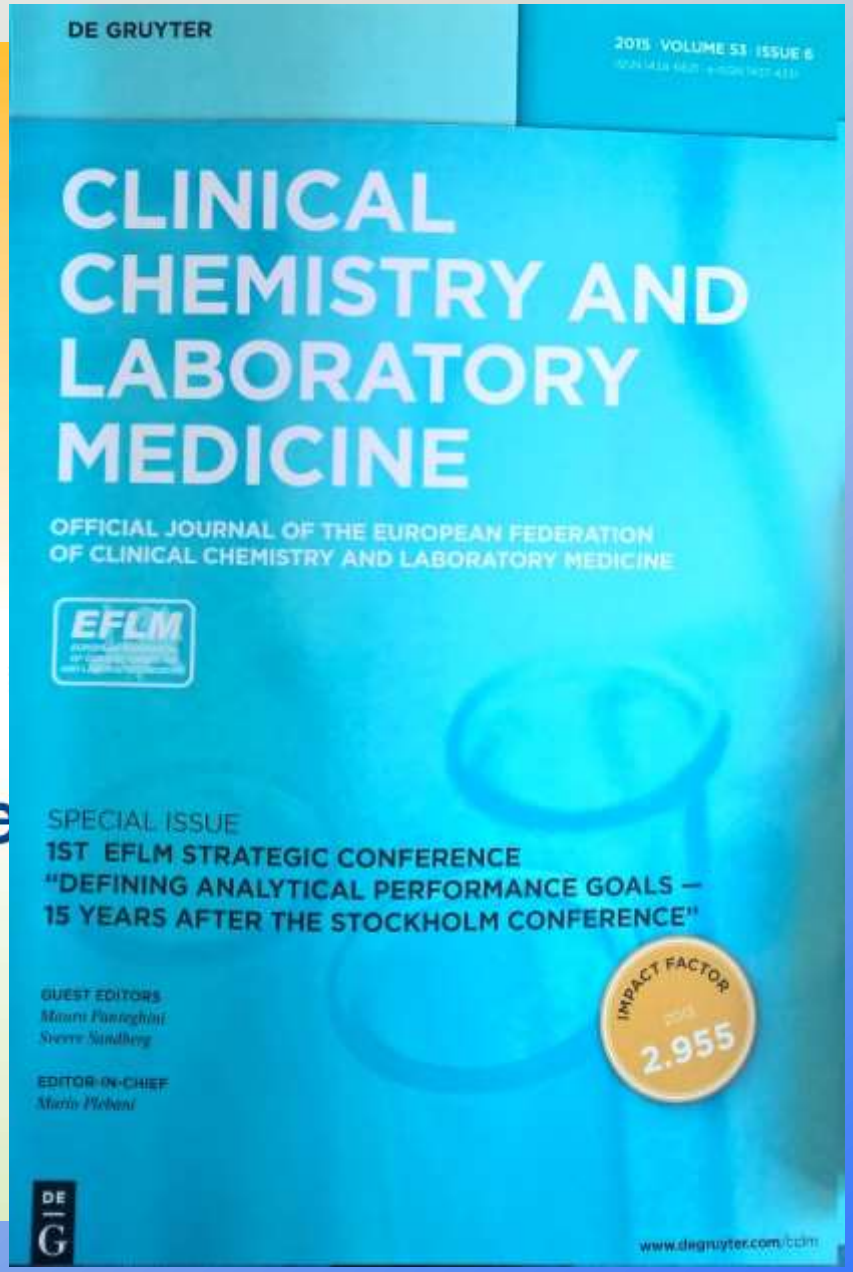
European Commission
Joint Research Centre
IRMM
Institute for Reference Materials and Measurements

1st EFLM Strategic Conference

Defining analytical performance goals 15 years after the Stockholm Conference

8th CIRME International Scientific Meeting

Milan (IT)
24-25 November 2014



DE GRUYTER Clin Chem Lab Med 2015; 53(6): 833–835

Consensus Statement

Sverre Sandberg*, Callum G. Fraser, Andrea Rita Horvath, Rob Jansen, Graham Jones, Wytze Oosterhuis, Per Hyltoft Petersen, Heinz Schimmel, Ken Sikaris and Mauro Panteghini

Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine

- Model 1. Based on the effect of analytical performance on clinical outcomes
  - Direct outcome studies
  - Indirect outcome studies (by simulation or decision analysis)

Disadvantage: it is only useful if the links between the test and clinical outcomes are strong (only for few tests)

- Model 2. Based on components of biological variation of the measurand

Disadvantage: need to carefully assess the relevance and validity of the biological variation data

- Model 3. Based on state-of-the-art

Disadvantage: there may be no relationship between state-of-the-art and what is needed to obtain an improved clinical outome

BV remains relevant !

But… with some limitations

DE GRUYTER

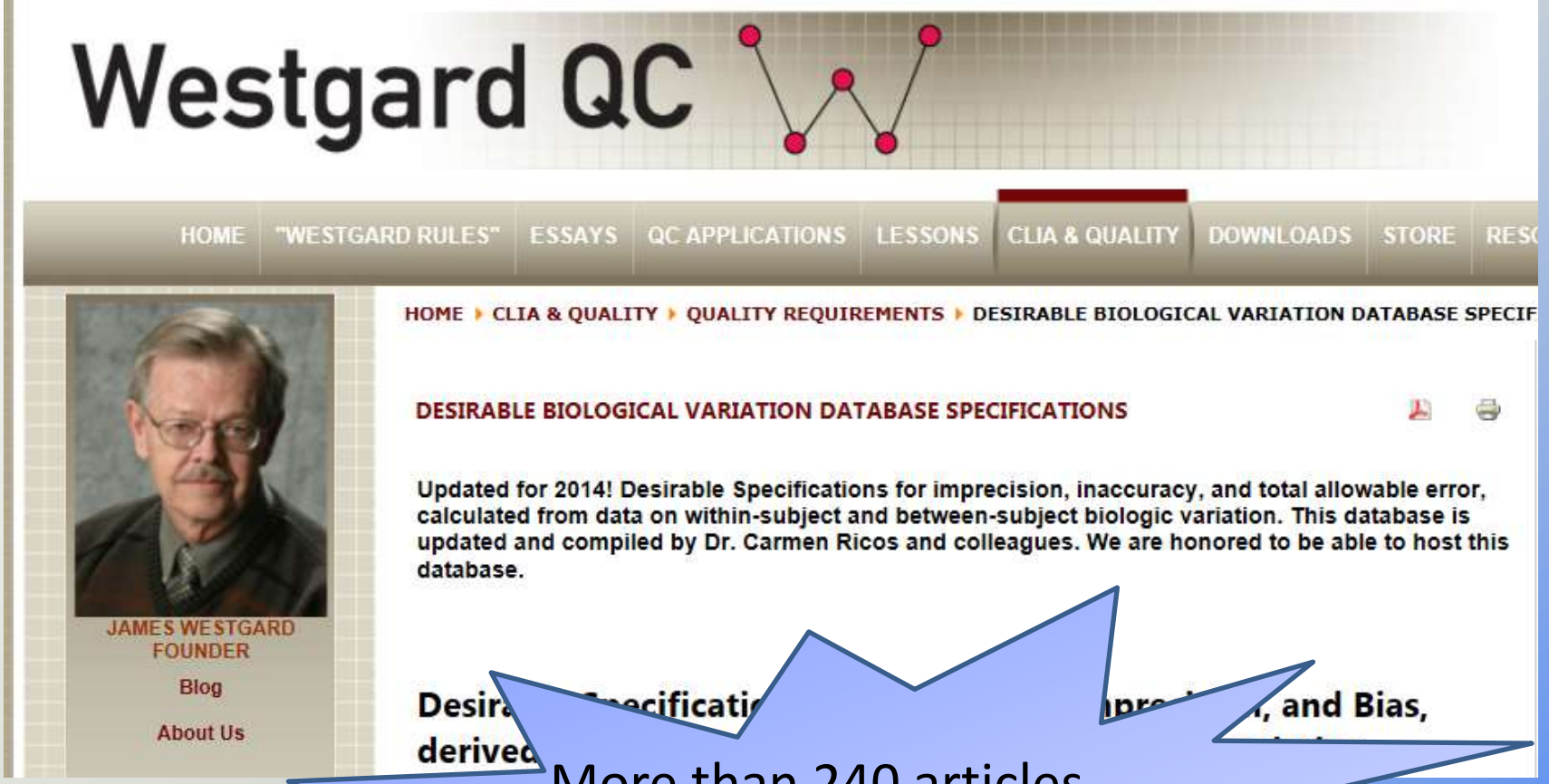
Clin Chem Lab Med 2015; 53(6): 871–877

Opinion Paper

Anna Carobene*

Reliability of biological variation data available in an online database: need for improvement

Let us check the available BV data

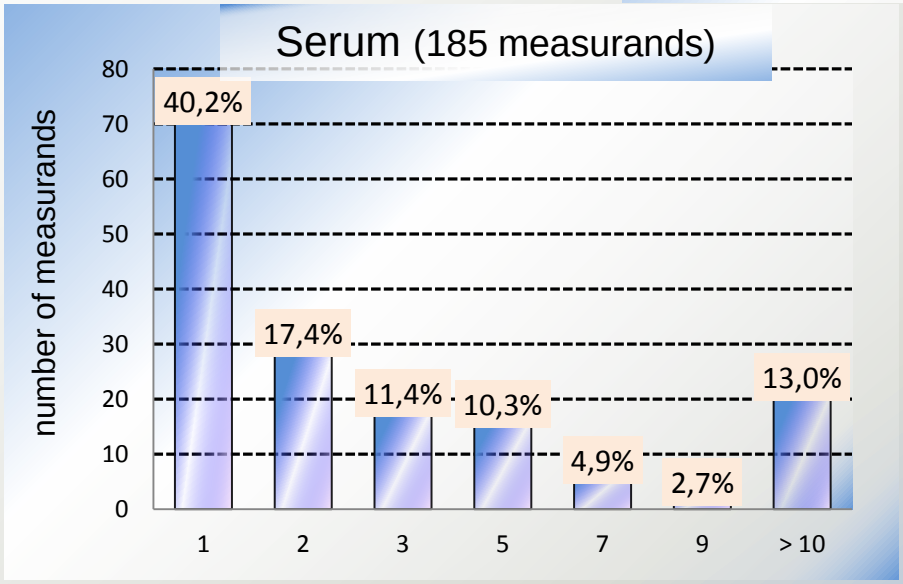


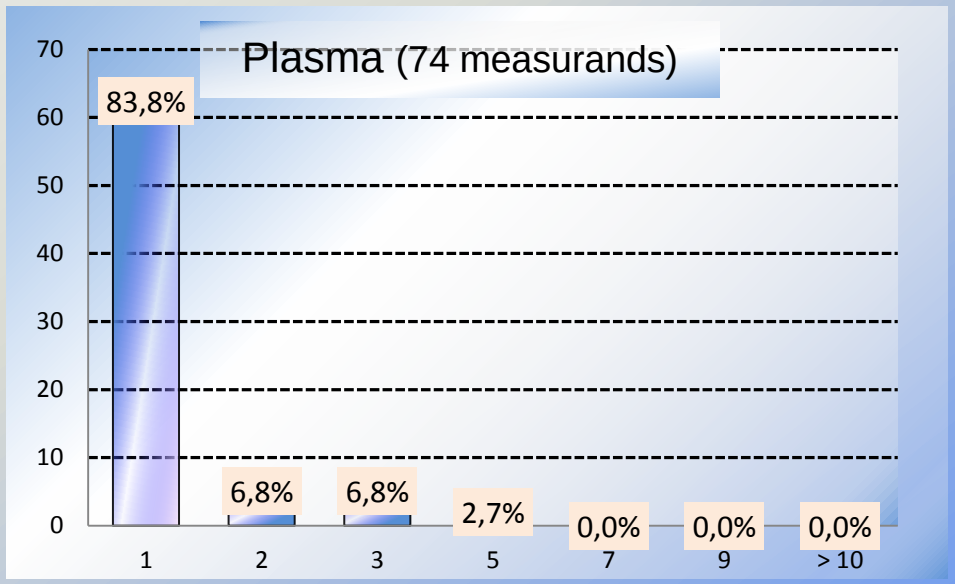
More than 240 articles

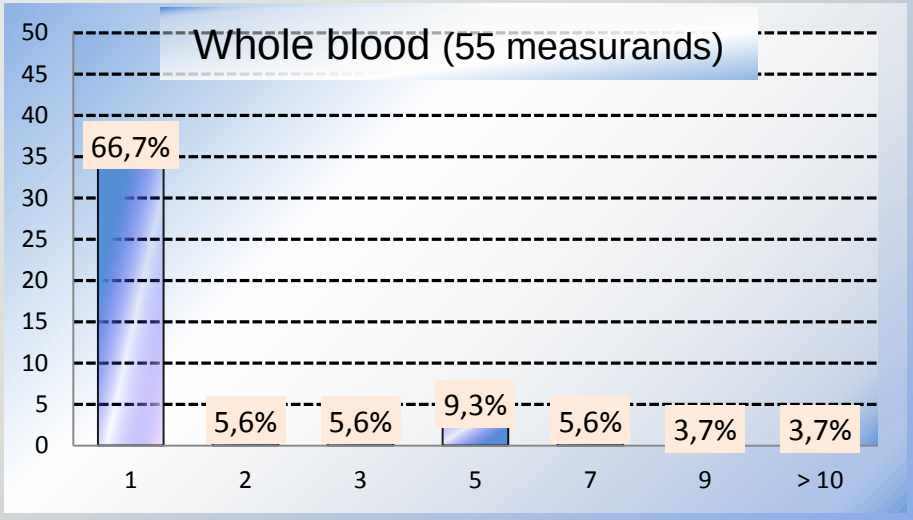
More than 350 measurands

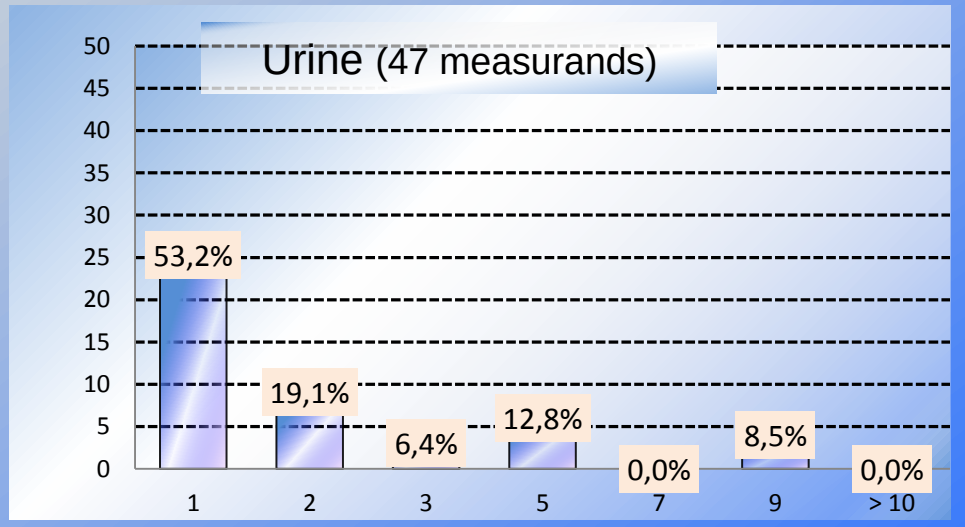
n° papers/measurand

(access June 2015)





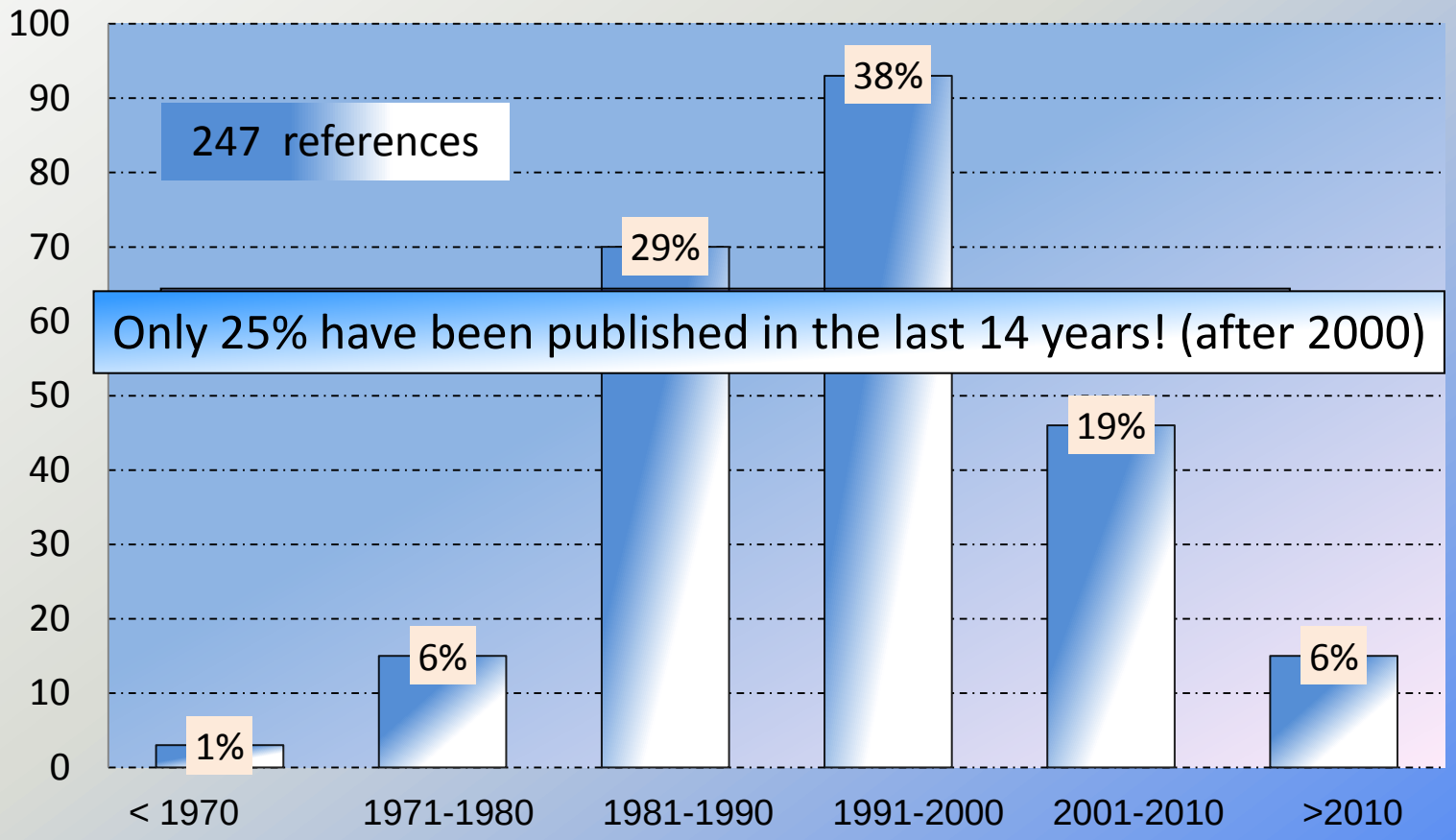




http://www.westgard.com/optimal-biodatabase1htm.htm

References: years of publication

(access June 2015)



http://www.westgard.com/optimal-biodatabase1htm.htm

✓The majority of publications are very dated (1980s and 1990s), before the Fraser and Harris paper: what kind of protocol was used?

✓Most BV values come from just one paper, or from very few. Was the protocol followed? Are they reliable data?

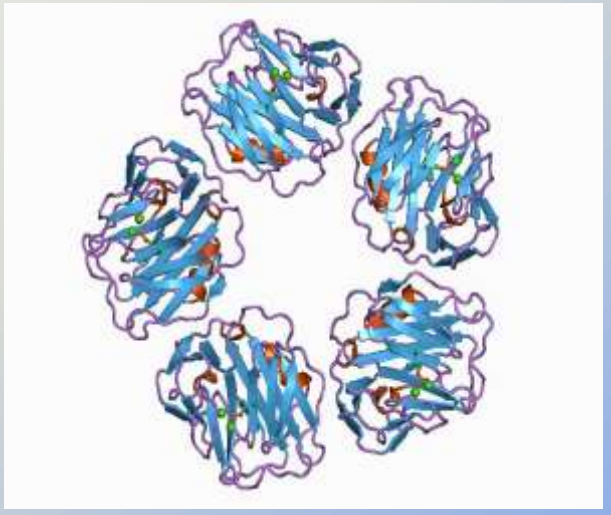
✓For the BV values that come from more than one paper, was the protocol followed? Do these papers agree with each other?

✓These data do not have any information about BV Confidence Interval (CI). So, if they come from more papers, how can we know if they are from the same population to combine them?

✓Only after answering can we say if BV values are reliable

✓Some examples: CRP, ALT, AST, γGT

C- Reactive Protein



Clinica Chimica Acta 413 (2012) 1179–1183

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Invited critical review

Biologic variability of C-reactive protein: Is the available information reliable?

Federica Braga *, Mauro Panteghini

11 papers, found in literature (published from 1993-2010), were evaluated on the basis of:

- Number and type of enrolled subjects;
- Duration of the study;
- Frequence of sample collection;
- Sample type;
- Sample storage;
- Analytical methodology;
- Assay sensitivity;
- Statistical analysis

Conclusions:

"among the eleven studies analyzed in this systematic review, only one appeared to fulfill all major pre-analytical, analytical and post analytical requirements….

It is obvious that additional well defined studies are needed to define reliable values "

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http://www.westgard.com/biodatabase1.h

It is impossible to have CV values greater than 33.3%.

Any CV >33% means that the distributions are not Gaussian (normal) and the statistical handling must be done another way (e.g. non parametric elaboration or logarithmic transformation)

	Analyte
S-	C reactive protein

	Analyte	
S-	C Reactive Protein	24, 100, 197

24. Clark GH, Fraser CG. Biological variation of acute phase proteins. Ann Clin Biochem 1993; 30: 373-376

serum samples | CV_I = 63%; | CV_G 76.3%

100. Macy EM, Hayes TE, Tracy RP. Variability in the measurements of C- reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 1997; 43: 52-58

plasma samples | CV_I = 42.2%; | CV_G 92.5%

197. Cho Li Wei, Jayagopal V, Kilpatrick ES, Atkin SL. The biological variation of C-reactive protein in polycystic ovarian syndrome. Clin Chem 2005;51:1905-1907 (not 2006)

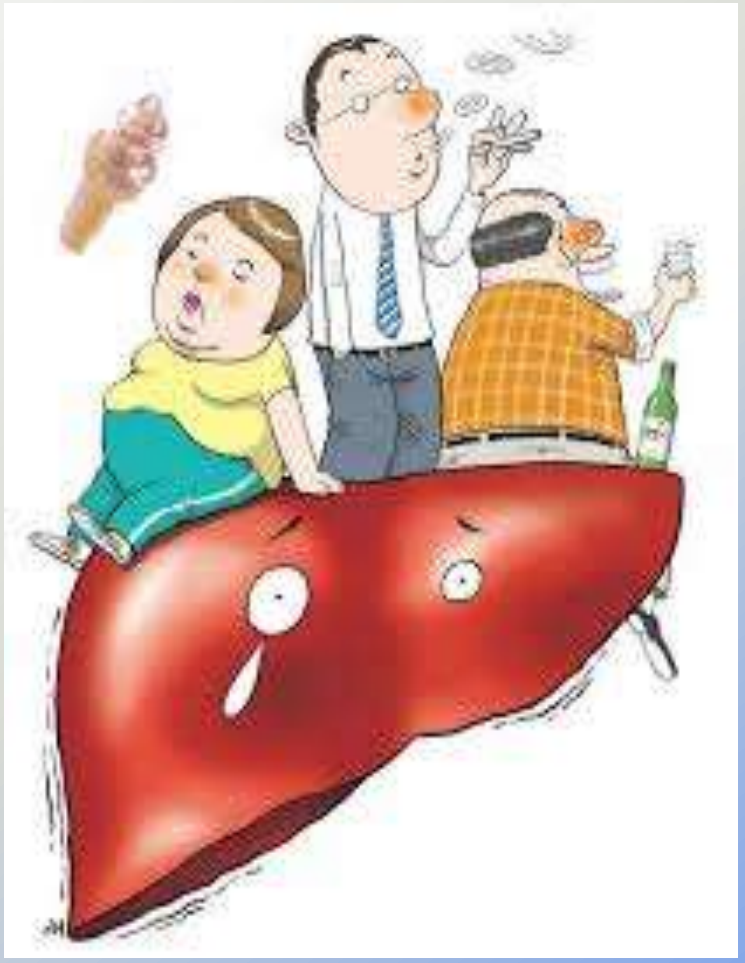
serum samples | CV_I = 36,8%; | CV_G 62.2%

Enzymes:

alanine aminotrasferase (ALT)

aspartate aminotransferase (AST)

and γ- glutamyl transferase (γGT)



DE GRUYTER DOI 10.1515/cclm-2013-0096 — Clin Chem Lab Med 2013; aop

Anna Carobene*, Federica Braga, Thomas Roraas, Sverre Sandberg and William A. Bartlett

A systematic review of data on biological variation for alanine aminotransferase, aspartate aminotransefrase and γ-glutamyl transferase

The following characteristics of studies on BV were compared:

- Year of publication
- Number and type of subject (gender and health status)
- Number of samples and frequency
- Number of replicates
- Type of samples
- Sample storage
- Analytical method
- CV_A
- BV data (CV_I and CV_G)
- CI (if possible to calculate)

Clin Chem Lab Med 2002; 40(7):718–724 © 2002 by Walter de Gruyter · Berlin · New York

IFCC 2002/5

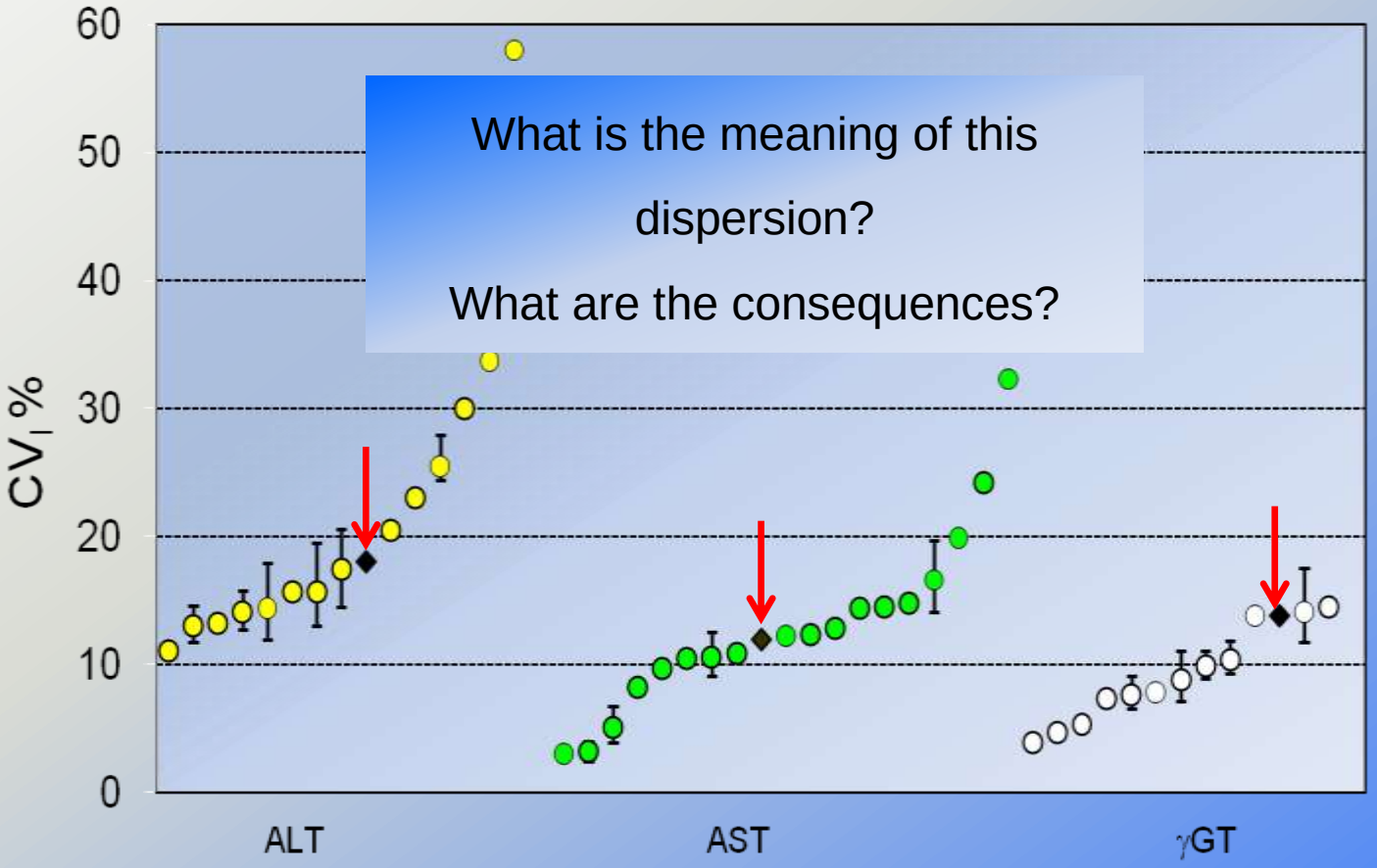
IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 °C

[23]	1975	11 healthy (M)	21–27	15 (1/3 days)	2	S	−20°C	AutoChemist Multi_ Channel Analytical System	16.7	17.4 (14.5–20.6)	NC
[24]	1976	14 healthy (8M, 6F)	25–40	6 (1/day)	2	S	−20°C	Routine method not defined at 30°C	ND	13.2 (NC)	29.6 (NC)
[25] (a)	1978	10 healthy (5M, 5F)	23–51	16 (4/week)	1	S	−20°C	Abbott analyser ABA-100	18.6	20.5 (NC)	54.1 (NC)
[25] (b)	1978	10 healthy (5M, 5F)	23–51	16 (4/week)	1	S	−20°C	Perkin Elmer KA150 enzyme analyzer	37.7	33.7 (NC)	63.6 (NC)
[25] (c)	1978	10 healthy (5M, 5F)	23–51	16 (4/week)	1	S	−20°C and −70°C	Technicon SMAC system	27.1	58.1 (NC)	72.1 (NC)
[26]	1983	20 patients with uncomplicated myocardial infarction (ND)	ND	14 (1/week)	2	P	−70°C	Cobas Roche	5.3	25.5 (23.4–27.9)	69.7 (52.8–102.1)
[27]	1985	274 healthy (148M, 126F)	18–63	6 (1/month)	1	S	−80°C	ND	0.9	30.0 (NC)	NC
[21]	1986	10 healthy (ND)	25–40	5 (1/day)	1	S	−25°C	ND	5.7	23.0 (NC)	4.4 (NC)
[28] (a)	1987	20 patients with chronic liver disease (10M, 10F)	36–73	7 (1/4 days)	2	S	−196°C	ND	5.0	11.1 (9.6–13.0)	NC
[28] (b)	1987	20 healthy (10M, 10F)	20–44	8 (1/week)	2	S	−196°C	ND	5.0	F:15.7 (13.0–19.5); M:14.4 (11.9–17.9)	NC
[17]	1987	27 IDDM (16M, 11F)	18–52	8 (1/week)	2	S	−196°C	ND	5.0	F:14.1 (12.7–15.8); M:13.0 (11.7–14.6)	NC
[20]	1992	10 healthy (5M, 5F)	25–30	10 (1/week)	2	S	−20°C	DAX 96 Bayer Diagnostici Milano	2.1	15.7 (13.4–18.8)	NC

CI, confidence interval at 95%; CV_a analytical variation; CV_w, within-subject variation; CV_b, between-subject variation; F, females; IDMM, insulin dependent diabetes mellitus; M, males; NC, not calculated; ND, not documented; NP, not performed; P, plasma; S, serum.

ALT, AST and γGT

Within-subject biological variation (CV_I)
from the papers found ± CI (where available).



The arrows show the values currently used from the database of Ricos et al.

Derived quality specifications and derived indices at the maximum and minimum values of BV data for ALT, AST and γGT in Ricos et al. database (shaded area)

	Biological variation (%)		Derived quality specifications			Significance of change, RCV (%)	
	Within-subject	Between-subject	Imprecision	Bias	Allowable Error	Probability Level	
	CV_I	CV_G	CV_{ags}	B_A	TE_A	0.05	0.01
ALT	11.0	16.9	5.5	5.2	14.3	34.8	45.8
	18.0	42.0	9.0	11.4	26.3	51.1	67.3
	58.0	72.0	29.0	23.1	71.0	161.1	212.1
AST	3.0	4.3	1.5	1.3	3.8	13.9	18.2
	11.9	16.9	6.0	5.4	15.2	34.8	45.8
	32.0	38.0	16.0	12.4	38.8	89.4	117.7
γGT	3.9	23.8	2.0	5.8	9.0	15.5	20.4
	13.8	14.1	6.9	5.4	16.8	34.8	45.8
	14.5	41.0	7.3	10.9	22.9	41.7	54.9

Reference change value (RCV) calculated as 2 tailed values at levels of probability of significant changed set at 0.95 RCV = [2.77(CV a 2 + CV w 2) 1/2] and 0.99 RCV = [3.65(CV a 2 + CV w 2) 1/2]; CV_A was set at 4.0% in all cases to enable comparison

	Analyte	Number of Papers	Biological Variation		Desirable specification		
			CVw	CVg	I(%)	B(%)	TE (%)
S-	Alanine aminotransferase (ALT)	9	19.40	41.6	9.7	11.48	27.48

S-	Alanine aminotransferase (ALT)	27,46,71,90,138,139, 153,161, 246

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Access, June 2015

27. Costongs GMPJ, Janson PCW, Bas BM. Short-term and long-term intra-individual variations and critical differences of clinical chemical laboratory parameters. J Clin Chem Clin Biochem 1985; 23: 7-16.

46. Fraser CG, Williams P . Short-term biological variation 1983;29:508-510

71. Hölzel WGE. Intra-individual variation of analytes in se seases. Clin Chem 1987; 33: 1133-1136

90. Juan-Pereira L. Variabilitat biologica ir cions cliniques.. Doctoral Thesis, Barcelona Uni

138. Ricós C, Codina R. La variabilidad bioló Rev Diag Biol 1989; 38: 34-36

139. Ricos C, García-Arumí E, Rodriguiez-Rubi ntrolde calidad. Quim Clin 1986; 5: 159-165

153. Statland BE, Winkel P and Killingsworth LM. Factors Contributing to Intra-Individual Variation of Serum Constituents: 6. Physiological Day-to-Day Variation in Concentrations of 10 Specific Proteins in Sera of Healthy Subjects. Clin Chem 1976; 22: 1635-1638

161. Van Steirteghem AC, Robertson EA and Young DS. Variance Components of Serum Constituents in Healthy Individuals. Clin Chem 1978; 24: 212-222

246. Pineda-Tenor E, Laserna-Mendieta EJ, Timón Zapata d Gómez Serranillos M. Biological variation and reference laboratory analytes in the eldery population. Clin Chem

Plasma analytes in renal disease

Chronic liver disease

Written in Spanish, not available

Written in Spanish, not available

Written in Spanish, not available

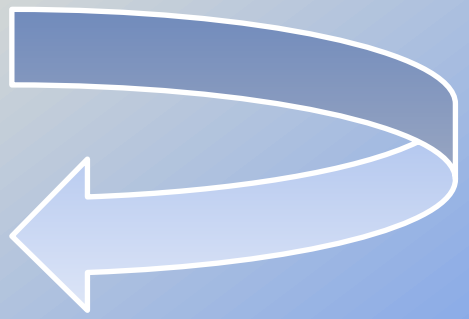
Paper added in the last update:
4 samples /subject immediately assayed by routine method in single

On the other hand...

More than 240 articles

More than 350 measurands

An immense amount of work of a huge value!!!



And even if for some analytes the BV data are not reliable for several reasons (poor adherence to the theoretical protocol, often obsolete papers…)
for many of them we have really robust BV data,
both for numbers of papers
and for well followed protocol!

So it is important to add the information in the database about the “quality” of the BV data published

It is time for improvement!

1st

New EFLM TFG on BV data base: evaluation of papers already published

2nd

EFLM BV-WG project: new experimental BV data

DE GRUYTER Clin Chem Lab Med 2015; 53(6): 829–832

Editorial

Mauro Panteghini and Sverre Sandberg

Defining analytical performance specifications 15 years after the Stockholm conference

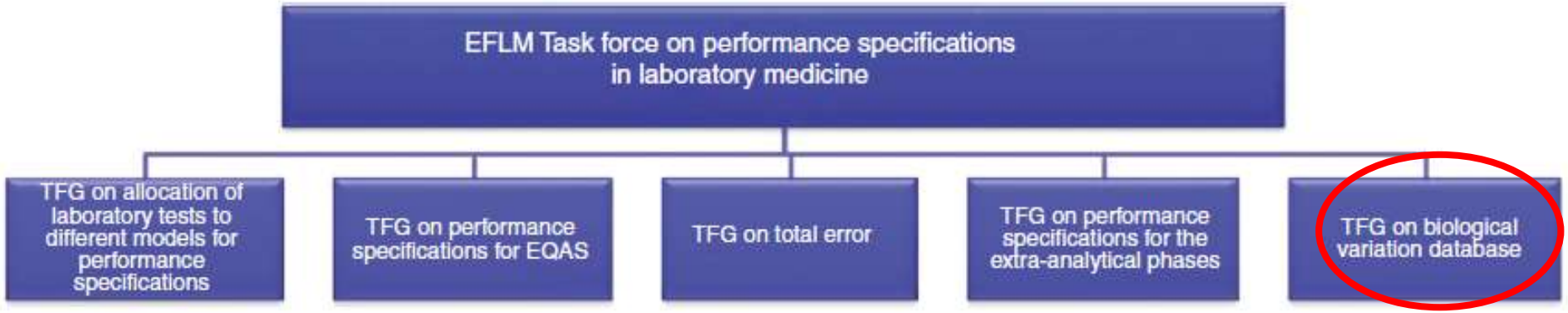


Figure 1: Structure of the EFLM task force on performance specifications in laboratory medicine.

The main outcome of the conference has been the creation of an EFLM Task Force (TF) on Performance Specifications in Laboratory Medicine (TF-PS).

Under the TF-PS five Tash and Finish Groups (TFG) have been established dealing with the main topic of the conference

1st

The EFLM Task and Finish Group Biological variation database (TFG-BVD) has developed a quality checklist for judging articles, based on the Standard for Reporting of Studies of Biological Variation.

DE GRUYTER Clin Chem Lab Med 2015; 53(6): 879–885

Opinion Paper

William A. Bartlett*, Federica Braga, Anna Carobene, Abdurrahman Coşkun, Richard Prusa, Pilar Fernandez-Calle, Thomas Røraas, Neils Jonker and Sverre Sandberg, on behalf of the Biological Variation Working Group, European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

A checklist for critical appraisal of studies of biological variation

The checklist identifies key elements to be reported in studies to enable safe accurate and effective transport of biological variation data sets.

1st

Table 1. Biological Variation Data Reporting Checklist.

Section and Topic\	Item # (MDS Domain Mapping: A-F)	
Title/ Abstract /keywords	1	The title should indicate that the content relates to a study of biological variation, the subject of the study, the sample matrix, and the population studied.
Abstract	1.1	As a minimum it should contain the headline BV data, the major characteristics of the population studied, clearly identify the analyte and measurand/s studied, the statistical approach taken, the duration of the study and the geographical location of the study.
Introduction	2	Introduction should clearly identify the context and aims of the study and cite any previous relevant studies of biological variability of the target analyte
Methods	3	Described in enough detail. BV data produced are effectively reference data
Analyte/ Measurand	3.1 (A)	The described study should clearly identify the target analyte and measurand/s.
Subjects	3.2 (B)	The description of the subjects and population studied should be carefully detailed. This should include number of subjects studied, age, gender, and state of well being.
Measurement Procedure	3.3 (A)	A clear description of the analytical methodology used should form part of the metadata. Standardisation and traceability should be clearly identified.
Length of Study	3.4 (C)	Length of the study periods should be clearly identified.
Sampling	3.5 (C)	Sampling protocols that minimise pre-analytical variation should be adequately described. Numbers of samples taken should be sufficient to deliver the required power to the study.
Samples	3.6 (C)	Recorded details should include the beginning and end date of the study and timings of sampling. Sampling conditions and sample type should be described in detail. Pre-analytical storage conditions of samples should be described.
Conditions for analysis	3.7 (C)	A description of conditions under which the samples were analysed. Analytical protocols should be designed to minimise sources of analytical variation

1st

Section and Topic	Item # (MDS Domain Mapping: A-F)	
Data Analysis	4	Data analysis techniques should be described. The power of the study to Identify indices of biological variation should be calculated and presented.
Outlier analysis	4.1 (C)	Outliers should be excluded from the final analysis of the data. Test for outliers should be applied to all levels of data (between replicate analysis, between samples within subject, between subjects). The numbers of outliers and reasons for their exclusion must be given.
Heterogeneity of variance.	4.2 (C)	Subjects with outlying within subject variance should be rejected from calculations used to determine an estimate of common true variance. The numbers of outliers and reasons for their exclusion must be given.
Statistical methods	4.3 (C)	Statistical methods used should be appropriately identified. Data that do not conform to a normal distribution should be appropriately transformed.
Results	5	Unified terminology should be used and appropriately defined metadata clearly presented to enable understanding of the data through time and across health care systems.
Terminology	5.1 (D)	Terms and symbols should be used to describe biological variation should conform standards identified by Simundic et al.
Results clearly presented and managed	5.2 (D)	BV data, with derived indices, should be tabulated in a format that enables extraction of the key data unambiguously associated with a minimum data set to enable their transportability. Power of the study and confidence limits around estimates of BV data should be presented. The results section should clearly identify the results of outlier analysis undertaken and confirm homogeneity of the data sets. If data are stratified the variables used should be clearly characterised.
Discussion	6	The discussion of the data should clearly include a focus on factors that impact on the transportability of the data to other settings. Limitations and strengths of the study should be addressed. If the data are used to set analytical performance specifications, derive reference change values and study individuality, the recommendations of Simundic et al. should be followed.

1st

Articles must be reviewed and judged prior to their use as bases for estimates of BV in the future BV database that is to be published on the EFLM webpage.

Grading system:

- A– GOOD: there is full compliance with all items on the checklist as shown below present.
- B – ACCEPTABLE: article scored with at least one “B”, but no “C” or “D”
- C – LOW QUALITY: article scored with at least one “C”, but no “D”
- D – POOR: article scored with one or more “D”

Effects of grading:

- Articles with scoring C will only be included if no articles of higher quality are available.
- Articles with scoring D will not be included in the database.
- A list of articles with scoring C not included in the database and articles with scoring D will be made available on the webpage.

1st

“Samples collection from healthy volunteers for biological variation values update”

A new project presented by BV Working Group established by European Federation of Clinical Chemistry and Laboratory Medicine (EFLM).

A multicenter study involving 6 European laboratories

[3] Haukeland University Hospital, Bergen, Norway.

[2] Wilhelmina Ziekenhuis Assen Europaweg-Zuid 1, 9401 RK Assen, the Netherlands.

[5] Dept. of Laboratory Medicine University Hospital, Padova –Italy.

[4] Hospital Universitario La Paz, Madrid, Spain, and Quality Analytical Commission of Spanish Society of Clinical Chemistry (SEQC).

[1] Servizio Medicina di Laboratorio, Ospedale San Raffaele, Milano, Italy.

[6] Acibadem University, School of Medicine, Atasehir, Istanbul, Turkey.

2nd

Project supported by Italian Society of Clinical Chemistry (SIBioC) and by Becton Dickinson (BD)

Samples collection BV Project: subjects enrolled

The samples collection, between April and June 2015, has been made from 98 volunteers: 44 men (mean= 34; range 21-59), 44 women (mean 34; range 21-49), 10 women>50 (mean 61; range 55-69) years old.

	Men (20-60)	Age (mean and range)	Women (20-50)	Age (mean and range)	Women (50-70)	Age (mean and range)
ITALY - MI (20 subjects)	10	38 (24-59)	7	34 (24-48)	3	57
ITALY - PD (16 subjects)	7	31 (23-37)	8	37 (27-49)	1	69
Norwey (15 subjects)	7	36 (28-42)	6	41 (32-48)	2	63
The Netherland (13 subjects)	5	36 (23-45)	6	39 (29-49)	2	60
Spain (16 subjects)	7	37 (26-54)	7	30 (24-48)	2	60
Turkey (18 subjects)	8	26 (21-35)	10	32 (21-38)	/	/
TOTAL	44	34 (21-59)	44	34 (21-49)	10	61 (55-69)

2nd

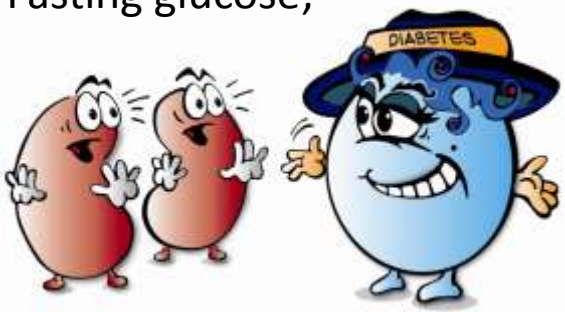
Samples collection BV Project: subjects enrolment

Because “good Health” is a relative concept, the enrolment has to be done with a **detailed questionnaire** in order to collect information not only on "quantitative characteristics" but also on the habits and on life style of the subjects.

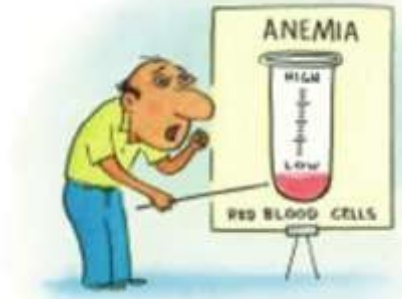
Each volunteer has been subjected to a blood sampling in order to verify:

BMI

Fasting glucose;

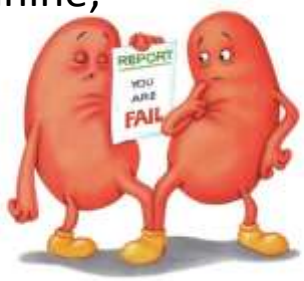


Cell blood count



Total Cholesterol; Triglycerides; γGT; ALT; CK; CRP

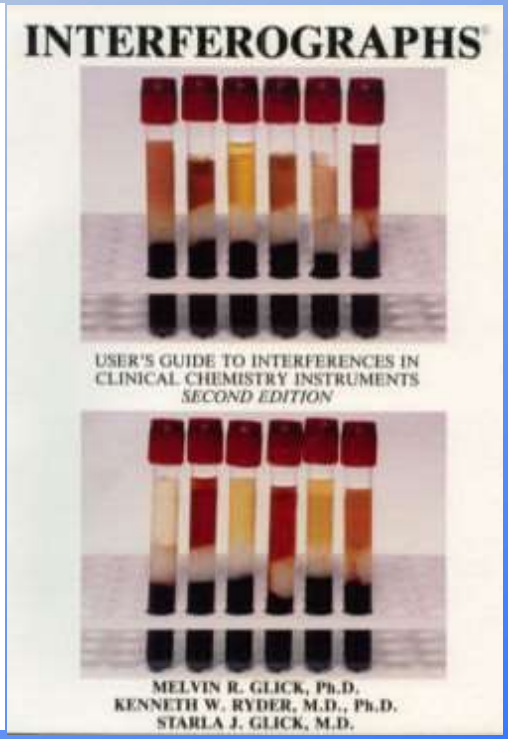
Creatinine;



2nd

As called “volunters”

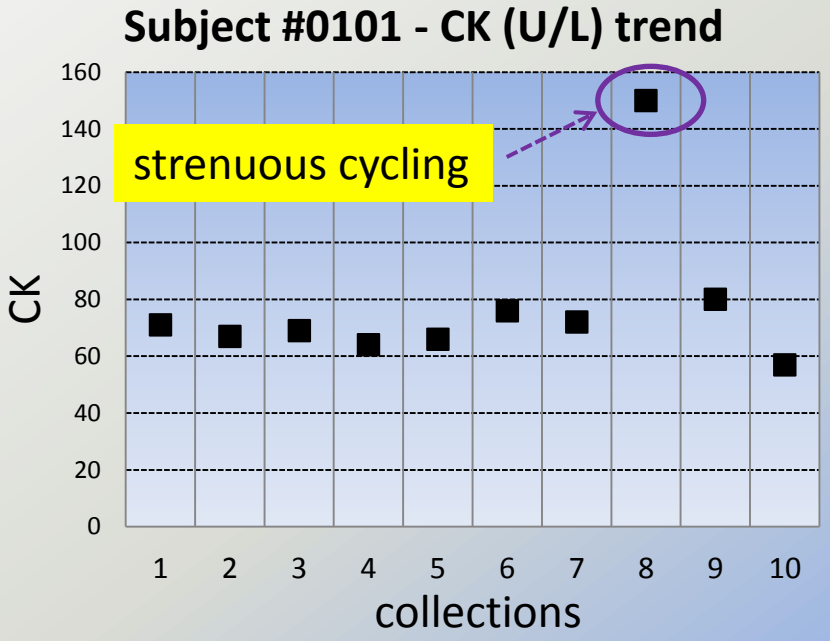
Once a week for 10 weeks, 1 venipuncture per subject have been made to collect serum, plasma EDTA and plasma citrate samples. The serum indexes of *lipemia*, *hemolysis* and *icterus* have been also measured to guarantee the acceptability of the samples.

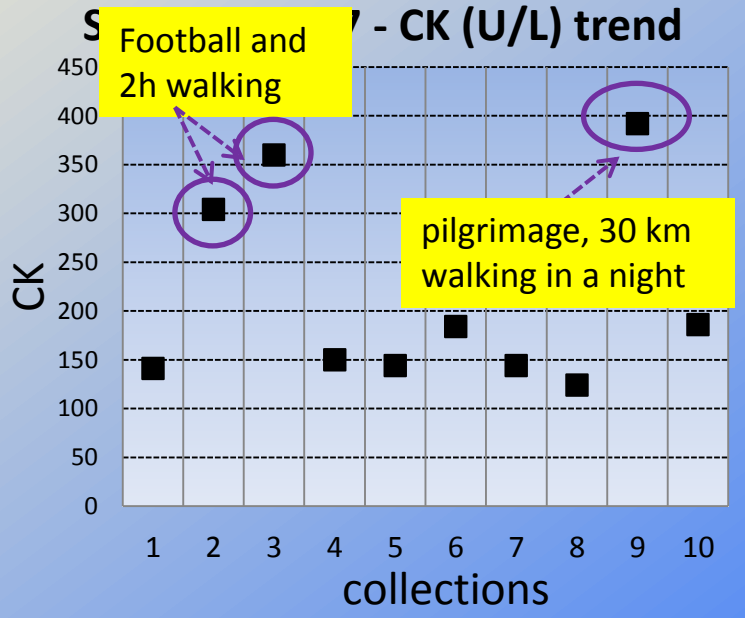


2nd

A shorter health questionnaire has been also completed and some biochemical tests have been performed at each sampling… why? Some examples

Example # 01: CK

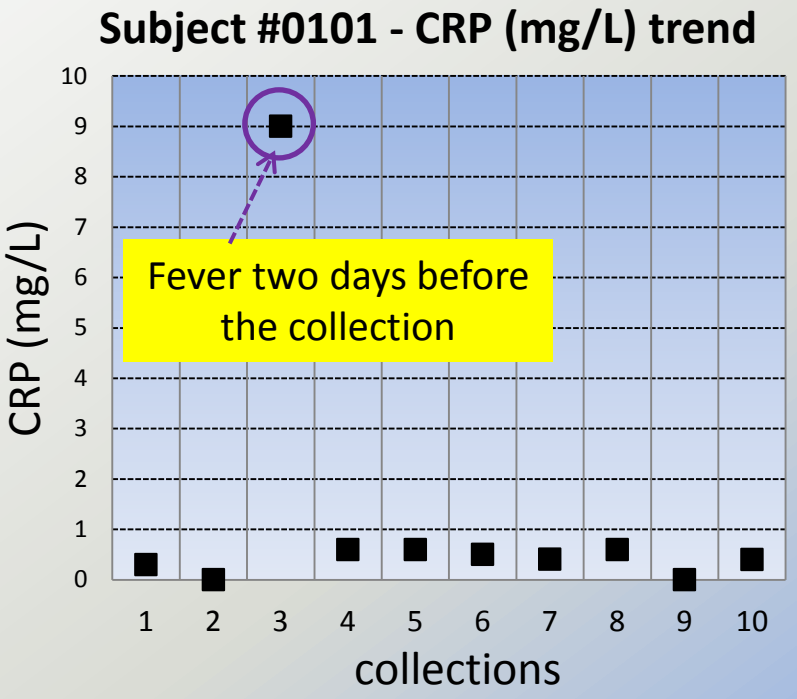


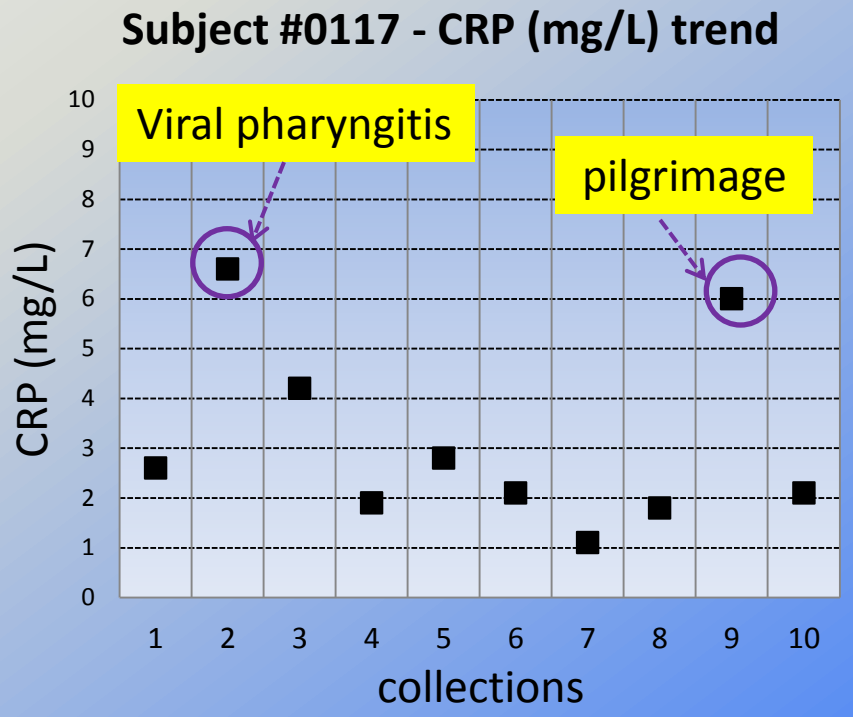


Real data Lab#01

2nd

Example # 02: C- Reactive Protein



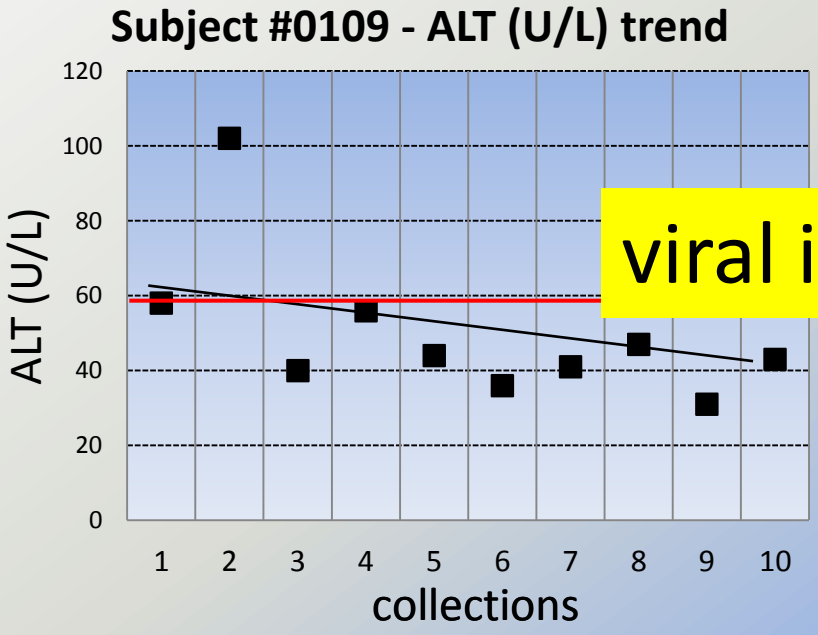


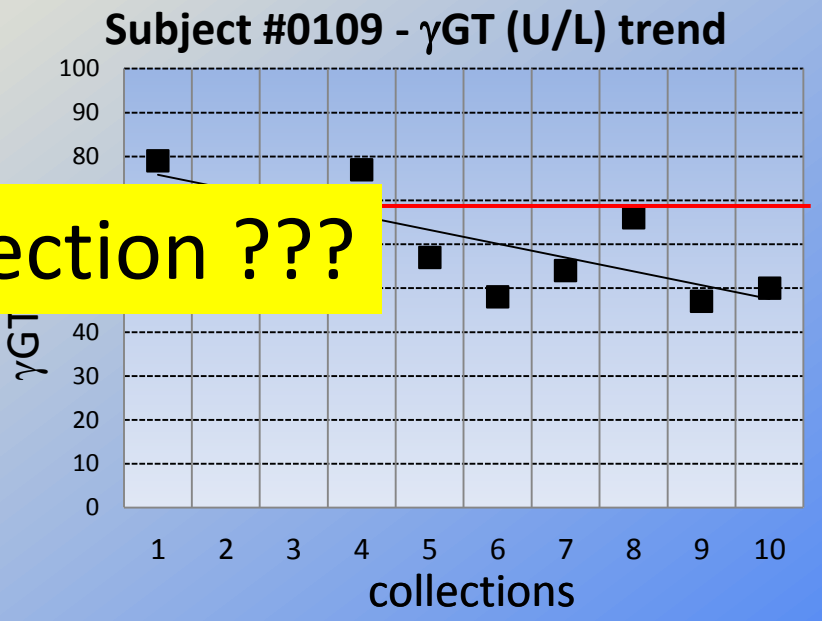
Real data Lab#01

2nd

… but sometimes from the questionnaires, we are not able to read the results

EXAMPLE #03: ALT and γGT



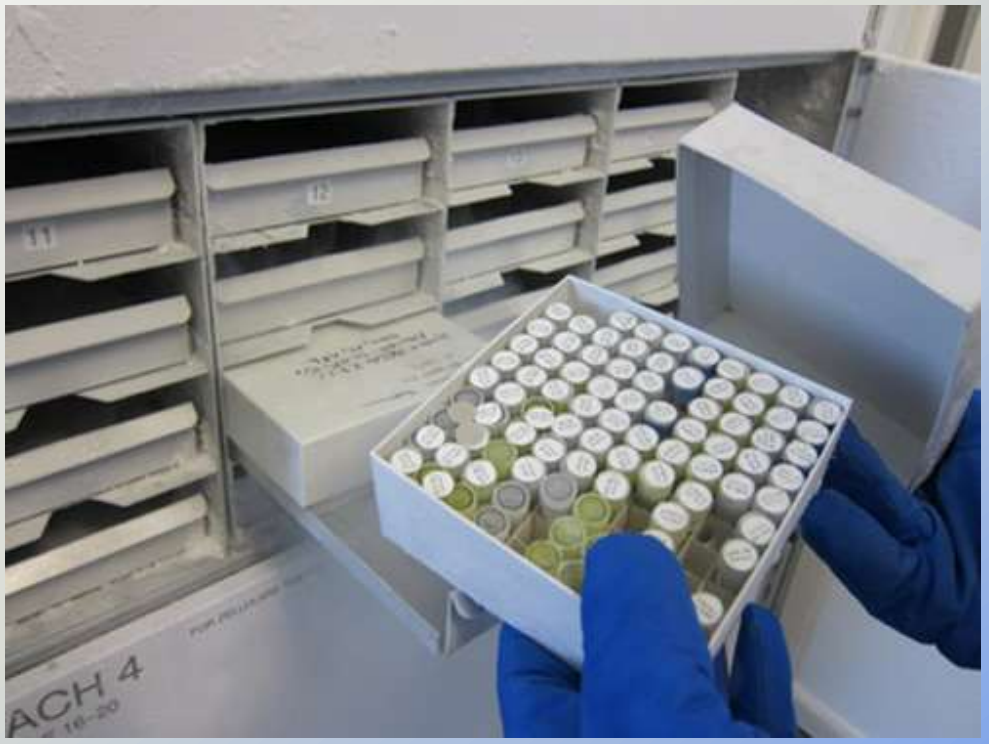


viral infection ???

Real data Lab#01

2nd

Each lab has processed and frozen the specimens strictly in agreement with the procedure reported in the protocol. It is very important to follow exactly step to step the protocol in order to avoid pre-analytical variability, one of the major source of heterogeneity in available literature.



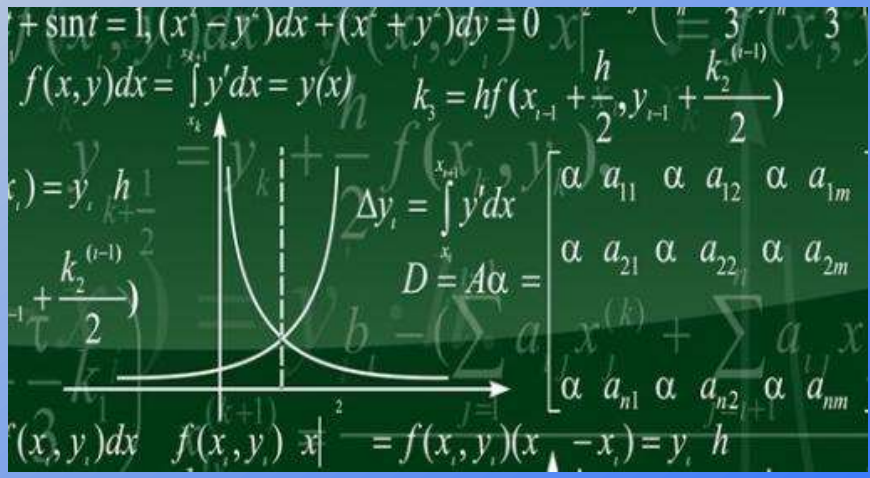
Results: A total of about 19.500 aliquots have been collected: 120 aliquots of serum, 40 of plasma EDTA, and 40 of plasma citrate for each subject. The samples have stored at -80 ºC until the delivery to the coordinating laboratory (Milan, Italy), where they will be stored in a dedicated freezer at -80°C until the measurements [samples storage is another source of variability].

2nd

The data will be treated according to recommended procedures [Fraser & Harris] for calculating BV including: outliers analysis at three different levels (replicate, samples and subjects), homoscedasticity of variances, normality of the distribution, and a nested ANOVA.

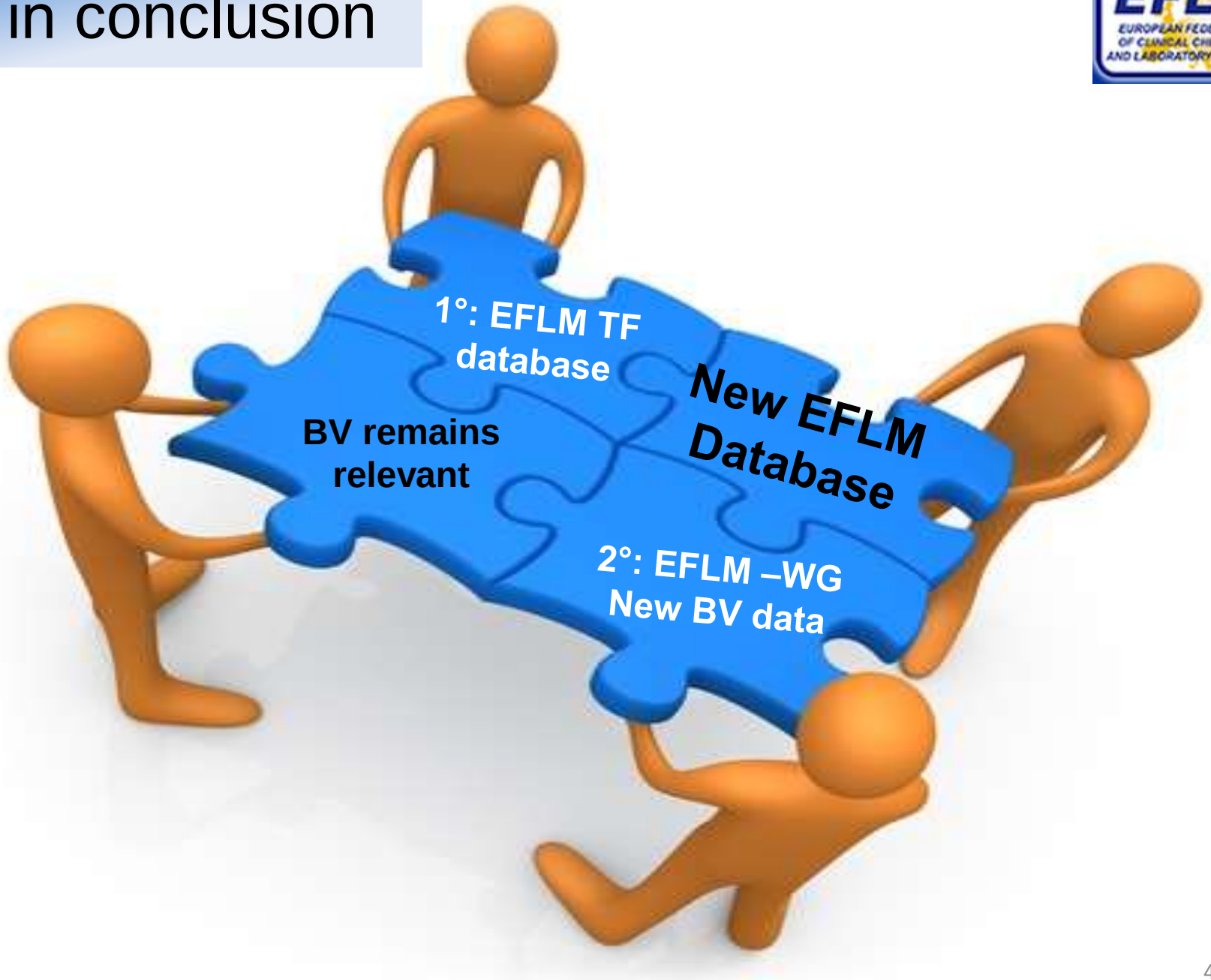
Future work:

A large number of tests, including enzymes, substrates, proteins, electrolytes, hormones, vitamins, tumor markers and coagulation tests will be performed in duplicate in the same analytical run, as suggested by the Fraser & Harris protocol



2nd

… in conclusion



The team - *From left:* Jorge Díaz-Garzón, Beatriz Boned, Abdurrahman Coşkun, Mariví Doménech, Pilar Fernández-Calle, Niels Jonker, Virtudes Alvarez, Carmen Perich, Carmen Biosca, Thomas Røraas, Margarita Simón, Elisabet González, Pilar Fernández-Fernández, Anna Carobene, Carrmen Ricós, Federica Braga, Bill Bartlett, Aasne K. Aarsand and Sverre Sandberg.
Not in the picture: Fernando Cava and Joana Minchinella
Not attending the meeting in Barcelona: Mauro Panteghini, Per Hyltoft Petersen and Callum Fraser

Thank you for your attention

… and the best is yet to come!