Performance criteria based on biological variation

Reliability of available biological variation information: need for improvement

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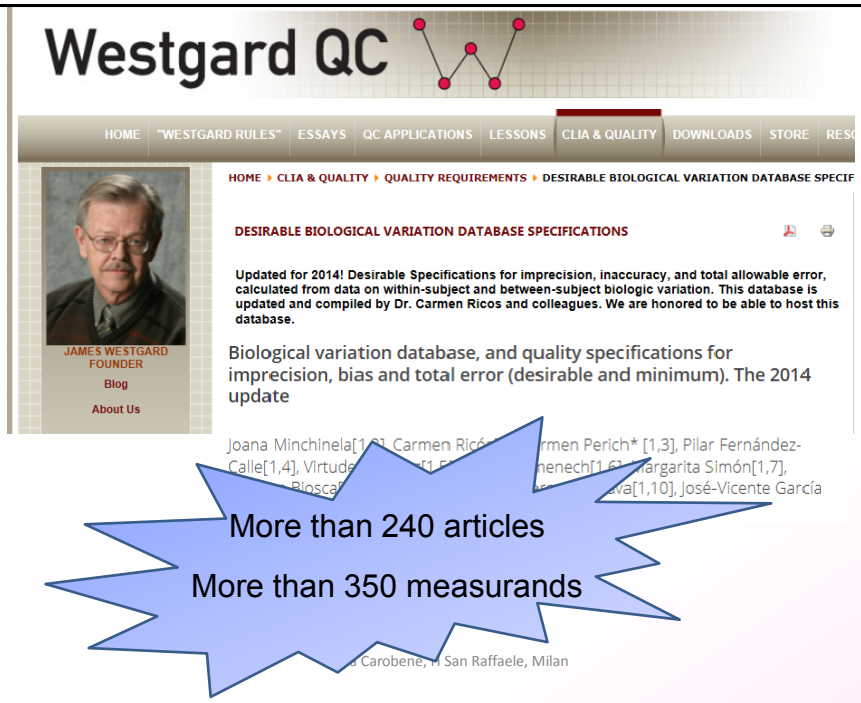
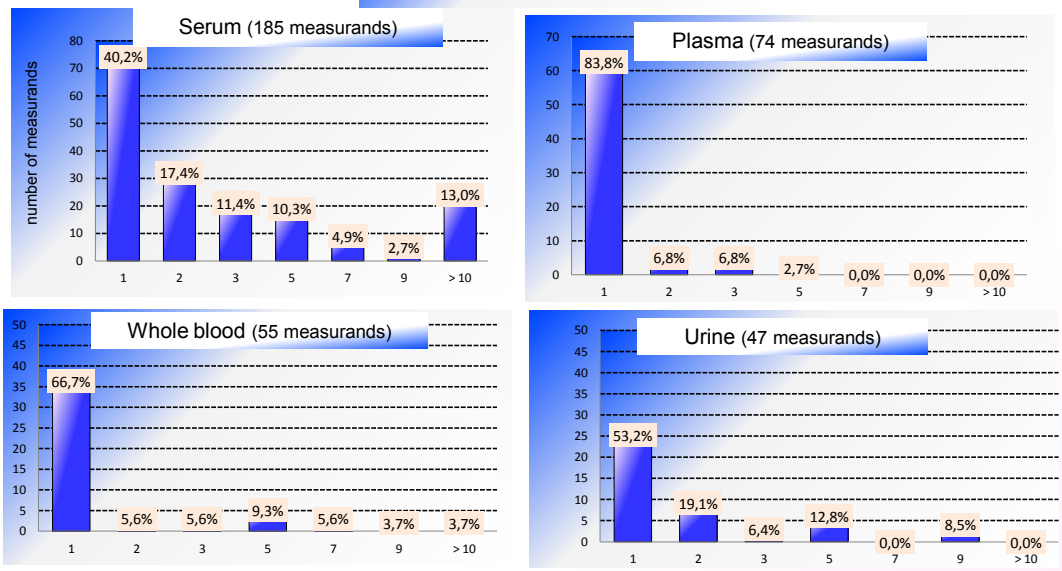
Member of EFLM Biological Variation WG



n° papers/measurand

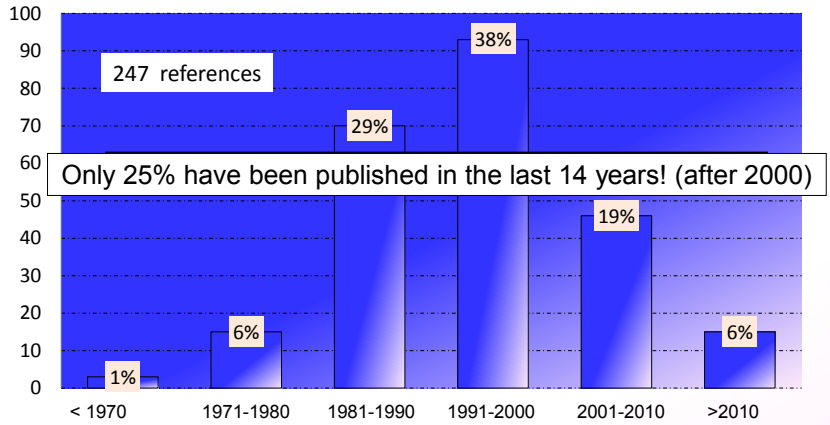
(access September 2014)



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

References: years of publication

(access September 2014)



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

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**)

✓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?

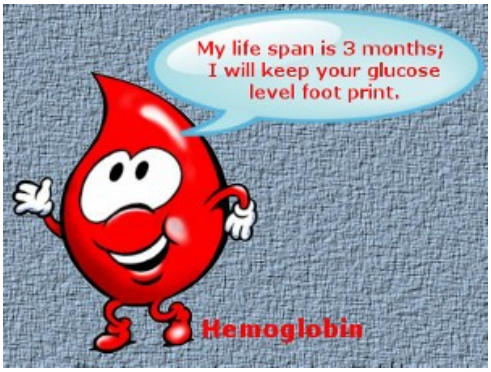
✓These data do not have any information about their Confidence Interval (CI). So, how can we know if they are from the same population to combine them?

✓We should calculate CI around these BV data; what do we need to calculate them?

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

✓Some examples: HbA1c, CRP, GA, ALT, AST, γGT

Glycated hemoglobin - HbA1c



HbA1c

Clinica Chimica Acta 411 (2010) 1606–1610

Conclusions:

✓A clear message from this systematic review of available literature is the obvious lack of robust data on biological variability of HbA1c concentration in blood

✓ Values on biological variability components for HbA1c listed in this database are apparently obtained from the (weighed?) mean of results available in the literature….

✓ It is however impossible to understand the criterion (if one) of study evaluation and selection of data so that some doubts appear justified

Table 4
Summary of the chara
this systematic revie

Study no.	Method sp as per HbA measuranc definition				
1	No				
2	No				
3	No				
4	±				
5	±				
6	±	Yes (F only)	Yes	±	Yes
7	Yes	No	No	No	No
8	No	Yes (M only)	Yes	No	Yes
9	±	No	No	No	Yes

review of available literature
biological variability of HbA_{1c}
ite all limitations and remarks
gical variability of biochemical

and hematological analytes, employed in daily practice worldwide, are those compiled by Ricòs et al., freely available at www.westgard.com/biodatabase1.htm. Values of biological variability components for HbA_{1c} listed in this database (CV_I 3.4%, CV_G 5.1%) are apparently obtained from the (weighed?) mean of results available in the literature, taken as valid by the authors of the compilation. It is, however, impossible to understand the criterion (if one) of study evaluation and selection of data, so that some doubts appear justified.

HbA1c

Westgard QC Access September 2014

	Analyte	Number of papers	Biological Variation		Desirable specification		
			CVw	CVg	I(%)	B(%)	TE (%)
B-	Hemoglobin A1 C	8	1.9	5.7	0.9	1.5	3.0

✓60. Godslang IF. Intra-individual variation: significant changes in parameters of lipid and carbohydrate metabolism in the individual and intra-individual variation in different test populations. Ann Clin Biochem 1985; 22: 618:624

✓173. Garde AH, Hansen AM, Sk… riation of blood concentrations of total cholesterol … prolactin and free testosterone in healthy women. C…

✓176. Kikpatrick ES, Maylor PV, Keevil BG. Biological variation of glycated hemoglobin. Diabetes care 1998;21:251-264

✓186. Rohlfing C, Wiedmeyer HM, Little R, Grota L, Tennill A, England J, Madsen R, Goldstein D. Biological variaiton of glycohemoglobin. Clin Chem 2002;48:1116-1118

✓226. Carlsen S, Petersen PH, Skeie S, Skadberg O, Sandberg S. Within subject biological variation of glucose and HbA1c in healthy persons and in type 1 diabetes patients. CCLM 2011; 49(9):1501-1507

✓229. Braga F, Dolci A, Montagnar… idi GC, Panteghini M. Revaluation of biological variation of glycated hemoglobin (HbA1c) us… rotocol and an assay traceable to the IFCC reference system. CCA 2011;412:1412-1416

2 out of 8 are from diabetic patients

Only two recent papers

HbA1c

Paper	…	…	…	CV_G % (CI)
…	…	…	…	… (5.2 – 10.7)
…	…	…	…	…
Ric…	…	…	…	1.9

From these studies we have to conclude that Prof Panteghini and Prof Sandberg do not agree!!!

Why these differences? Can we make some hypothesis? Different number of patients? Gender? Different number of samples/subject?

HbA1c

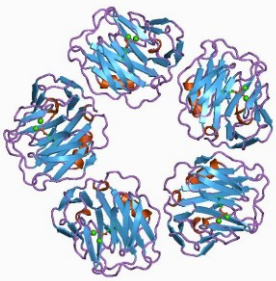
Paper	N° subject	Samples/subject	Unit	CV_A %	CV_I %	CV_G %
Braga … Panteghini	18 (9M, 9F)	5 samples, once …rtnight	mmol/mol	2.4	2.5 (1.9 - 3.2)	7.1 (5.2 – 10.7)
		…, once …rtnight	%	1.4	1.4 (1.1 - 1.8)	4.5 (3.3 – 6.8)
Sandberg		a week				- 9.0)
Ricos			?		1.9	5.7

My previous conclusions were wrong! Fortunately Prof Panteghini and Prof Sandberg do agree!!!

CVs in % units are lower than in IFCC units (mmol/mol) and therefore cannot be directly combined

But that value is out from both CIs values reported! Is it reliable?

C- Reactive Protein



CRP

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 sistematic 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 "

CRP

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

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 — CVw = 63%; CVg 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 — CVw = 42.2%; CVg 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 — CVw = 36,8%; CVg 62.2%

Glycated Albumin

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Glycated Albumin

	Analyte	Number of papers	Biological Variation		Desirable specification		
			CVw	CVg	I(%)	B(%)	TE (%)
S-	Albumin, glycated	3	5.2	10.3	2.6	2.9	7.2

	Analyte	Reference
S-	Albumin, glycated	31, 60, 129

31. Davie SJ, Whiting KL, Gould BJ. Biological variation in glycated proteins. Ann Clin Biochem 1993; 30: 260-264

Values reported are the same as in the database: CV_I =5.2, CV_G=10.3
They come from 10 subiects, 5 samples/sub, but just 1 replicate.
⇒ It is not possible to calculate CI around the BV values obtained!!

60. Godslang IF. Intra-individual variation: significant changes in parameters of lipid and carbohydrate metabolism in the individual andintra-individual variation in different test populations. Ann Clin Biochem 1985; 22: 618:624

Measurements of triglycerides, HDL Chol, Glucose, insulin and haemoglobin A1 in volunteers
⇒ No value for glycated albumin!

129. Phillipou George, and Phillips Patrick. Intraindividual Variation of Glycohemoglobin: Implications for Interpretation and Analytical Goals. Clin Chem 1993;39:2305-2308

Measurements of glycohemoglobin (GHb) in diabetic patients
⇒ No value for glycated albumin!

Clinica Chimica Acta 423 (2013) 1–4

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

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

Glycated Albumin

paper still missing from the database

Evaluation of biological variation of glycated albumin (GA) and fructosamine in healthy subjects

Martina Montagnana *, Renata Paleari, Elisa Danese, Gian Luca Salvagno, Giuseppe Lippi, Gian Cesare Guidi, Andrea Mosca

[a] Sezione di Biochimica Clinica, Dipartimento di Scienze della Vita e della Riproduzione, Università degli Studi di Verona, Italy
[b] Centro per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME). Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti. Università degli Studi di Milano. Milano, Italy
[c] L

Table 1

Mean values of GA, albumin and fructosamine[illegible]ted average variance components.

Analyte	Group	Mean	CV_A, %	CV_W, %	CD, %
Glycated albumin, %	All	11.4	1.7	2.1	10.6
	Men	10.9		1.4	11.2
	Women	11.9		2.5	8.2

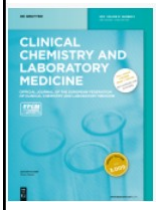
it is out from CI! Is it reliable?

From
- number of subjects,
- number of samples,
- numbers of replicates,
- and CV_A, CI were calculated

	CV_I (CI)	CV_G (CI)
Ricos'	5.2	10.3
All:	2.1 (1.7 - 2.6)	10.6 (7.9 -15.9)
Men:	1.4 (0.7- 2.1)	11.2 (7.5 - 21.5)
Women:	2.5 (1.9 - 3.4)	8.2 (5.5 - 15.8)

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

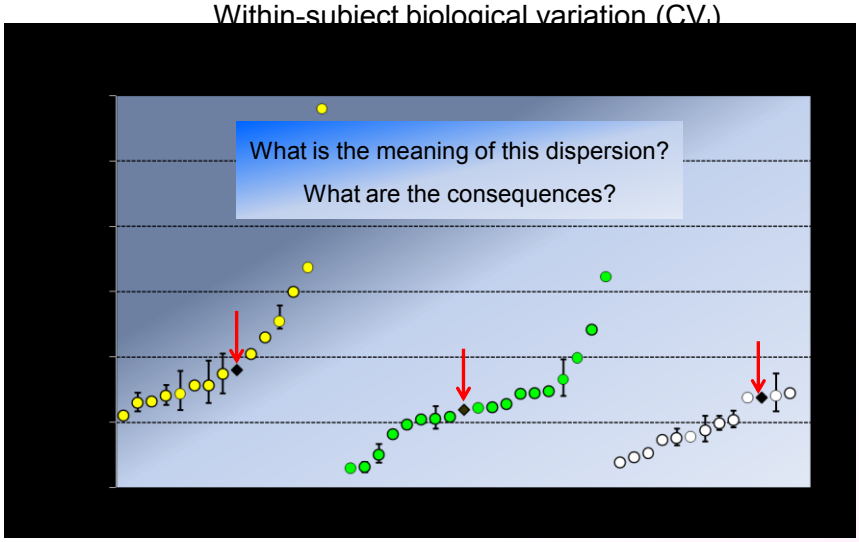
IFCC 2002/5

[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)



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_{aqs}	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

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, September 2014

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 of plas

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

90. Juan-Pereira L. Variabilitat biologica intrain es.. Doctoral Thesis, Barcelona University 1989.

138. Ricós C, Codina R. La variabilidad biológica i l 1989; 38: 34-36

139. Ricos C, García-Arumí E, Rodriguiez-Rubio R, Sc ad. 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 J, Rodelgo-Jin Gómez Serranillos M. Biological variation and reference Change Value laboratory analytes in the eldery population. Clin Chem Lab Med 2013;

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

Reliability of available BV data

Since the true value cannot be absolutely determined, an estimate of the measurement uncertainty (CI) must be given.
Moreover, without CI values it is difficult to compare the estimates from different papers

Confidence Intervals (CI): How to calculate them?

It is necessary to know:

- SD_A and SD_I (to calculate the ratio SD_A/SD_I)
- Number of replicates (at least two)
- Number of samples
- Number of individuals

Clinical Chemistry 58:9
1306–1313 (2012)

Informatics and Statistics

Confidence Intervals and Power Calculations for Within-Person Biological Variation: Effect of Analytical Imprecision, Number of Replicates, Number of Samples, and Number of Individuals

Thomas Røraas,[1*] Per H. Petersen,[1] and Sverre Sandberg[2,3]

From this paper,

to publish BV data without their CI values is unacceptable!

(or at least without the possibility to calculate them)

Expected width of CI95 for the SD_I (in % of the SD_I) with varying number of individuals, samples and replicates for different ratios between the SD_A and SD_I

		0.25			0.5			0.75			1			1.5			2		
		Replicates			Replicates			Replicates			Replicates			Replicates			Replicates		
Individuals	Samples	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4
10	2	109	108	107	119	114	112	138	125	119	177	141	131	233	215	167	269	242	227
	4	55	55	55	61	58	57	71	64	61	87	73	67	164	100	86	185	169	115
	6	42	41	41	46	44	43	53	48	46	65	55	51	107	75	65	165	109	86
	8	35	35	35	38	37	36	45	41	39	54	46	43	87	63	54	155	89	71
	10	31	30	30	34	32	32	39	36	34	47	40	37	75	55	48	140	77	63

Thomas Roraas et al.

Fraser and Harris *(Crit Rev Clin Lab Sci 1989;27:409-37)* stated that:

"the components of variation can be obtained from a relatively small number of specimens collected from a small group of subjects …"

But now we know that:

Study design, number of replicates, number of samples, and number of subjects have a great impact on the reliability with which we can estimate the SD_I of an analyte

The number of samples collected per subject is more important than the number of subjects examined when the SD_I is estimated

The analytical imprecision must be taken into consideration in order to obtain good estimates

It is time for improvement!

Biological Variation EFLM WG

(in collaboration with Ricos's group):

new projects

Theoretical approach:
Critical appraisal checklist for papers on biological variation
(next presentation)

Practical approach:
Samples collection from healthy volunteers for BV Values Update

Samples collection from healthy volunteers for BV Values Update

- Multicenter study involving seven European Laboratories
  1. S. Raffaele Hospital Milan, Italy - Anna Carobene (coordinator); Ferruccio Ceriotti (promotor)
  2. Blood Sciences Ninewells Hospital, Dundee – UK - Bill Bartlett
  3. Haukeland University Hospital Bergen – Norway – Swerre Sandberg
  4. Hospital Universitario La Paz Madrid – Spain - Pilar Fernández-Calle
  5. Dept. of Laboratory Medicine University Hospital, Padova –Italy – Mario Plebani
  6. Acibadem University, Gülsuyu, Maltepe Istanbul – Turkey - Abdurrahman Coskun
  7. Wilhelmina Ziekenhuis Assen Europaweg-Zuid 1, Assen, the Netherlands - Niels Jonker
- 105 healthy volunteers will be enrolled (15 subjects/lab):
  - 49 men between 18 and 50 years old (7/lab)
  - 42 women between 18 and 50 years old (6/lab)
  - 14 women > 60 years old (2/lab)
- 10 collections /subject; once a week
  - total of 120 aliquotes of serum/ subject,
  - total of 40 plasma EDTA / subject
  - total of 40 plasma citrate / subject
- Subject samples will be stored at -80°C, delivered and measured in a single lab

We would like to thank SIBioC
and Becton Dickinson
whose fundings have made possible
the kick-off of the project

Thank you for your attention

But the best is yet to come!