Biological Variation Working Group

Biological Variation Database, time for an update?

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www.biologicalvariation.com

Current Applications of BV 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.**
- **Choice of specimen type.**
- **Timing of specimens.**

Analytical Performance Specifications

Stockholm Hierarchy 1999 and EFLM Strategic conference 2014 advocate use of biological variation data.

Understand and Characterise Biological variation and aim to: -

"minimize the ratio of 'analytical noise' to the biological signal"

Quality Specifications

Desirable

$CV_A < 0.5 \times CV_I$

$B_A < 0.25 \times (CV_I^2 + CV_G^2)^{0.5}$

$Tea < 1.65 \times 0.5 \times CV_I + 0.25 \times (CV_I^2 + CV_G^2)^{0.5}$

Optimum

$CV_A < 0.25 \times CV_I$

$B_A < 0.125 \times (CV_I^2 + CVG^2)^{0.5}$

$Tea < 1.65 \times 0.5 \times CV_I + 0.125 \times (CV_I^2 + CV_G^2)^{0.5}$

Minimum

$CV_A < 0.75 \times CV_I$

$B_A < 0.375 \times (CV_I^2 + CVG^2)^{0.5}$

$Tea < 1.65 \times 0.5 \times CV_I + 0.375 \times (CV_I^2 + CV_G^2)^{0.5}$

Consensus Statement EFLM Strategic Conference Milan 2014.

Sandberg *et al Clin Chem Lab Med 2015;53(6):833-5*

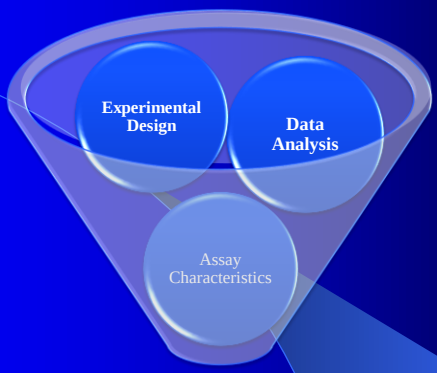
"There are limitations to this approach, including the need to carefully assess the relevance and validity of the biological variation data….."

Challenge to users of BV data?

- Identification of data that are: -
  - robust.
  - have characteristics that are concordant with the population to which the measurement procedure is to be applied.
  - method specific?

NB! These data are **reference data** and are often poorly characterised

Fundamental Questions?



What is the uncertainty surrounding these data?

What are the quality standards for BV Data?

Are they applicable to my practice?

Limitations of existing BV data?

50 years of data

- Do the data travel through time?
- Impact of method developments?

Quality

- Enough reported detail?
- Good design?
- Inconsistent terminology

Transportable

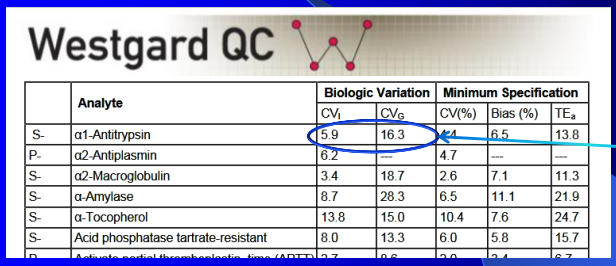
- Population demographics.
- Healthy?
- Diseased?

Translated into databases

- Excellent Resources
- Granular enough?
- Data archetype required?

Ricos *et al* Database

https://www.westgard.com/biodatabase-2014-update.htm



Westgard QC

	Analyte	Biologic Variation		Minimum Specification		
		CV_I	CV_G	CV(%)	Bias (%)	TE_a
S-	α1-Antitrypsin	5.9	16.3	4.4	6.5	13.8
P-	α2-Antiplasmin	6.2	---	4.7	---	---
S-	α2-Macroglobulin	3.4	18.7	2.6	7.1	11.3
S-	α-Amylase	8.7	28.3	6.5	11.1	21.9
S-	α-Tocopherol	13.8	15.0	10.4	7.6	24.7
S-	Acid phosphatase tartrate-resistant	8.0	13.3	6.0	5.8	15.7

Median Values of Published Data

Biological variation database: structure and criteria used for generation and update Perich et al CCLM 2014

Biological variation database: structure and criteria used for generation and update.

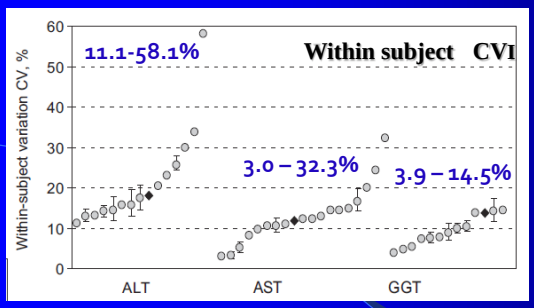
Perich et al Clin Chem Lab Med 2014

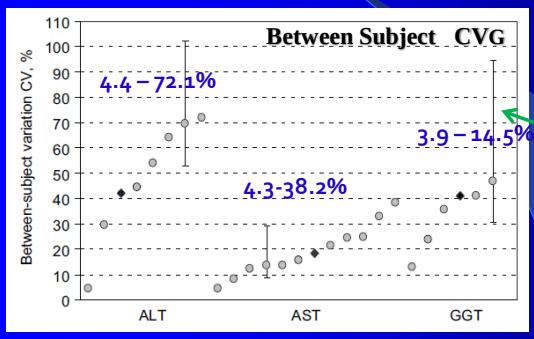
Version 8 2014

	No of Analytes	Number of Publications	Reliable estimates of CV_I	Score
	27	10+	33%	5 or 6 (PI +MM)
	129	2 - 9	36%	PI+2 MM= 3 or 4
	202	1 only	55%	5 or 6 (PI +MM)
Total	358			

Performance Index:
PI = CVA/0.5*CVI,
i) Score 2: PI < 1;
ii) Score 1: PI between 1 and 2;
iii) Score 0: PI > 2 or unknown.

Mathematical model (MM) used by the authors to calculate CVI and CVG:
i) Score 4: ANOVA;
ii) Score 3: model described by Fraser and Harris [1, 9] ;
iii) Score 2: unclear model;
iv) Score 1: not described model.





Systematic Review of Data on Biological variation of ALT, AST and GGT.

Confidence Intervals

Historical Application. Carobene et al Clin Chem Lab Med 2013;51:1997–2007

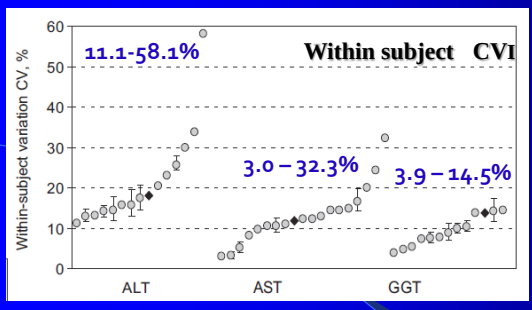
Confidence Intervals and Power Calculations for Within-Person Biological Variation: Effect of Analytical Imprecision, Number of Replicates, Number of Samples, and Number of Individuals

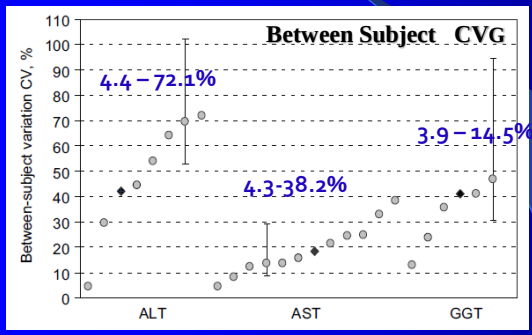
Thomas Røraas, Per H. Petersen, and Sverre Sandberg

Clinical Chemistry 58:91306–1313 (2012)

- design of an experiment to estimate biological variation should take into account the analytical imprecision.
- Estimates of biological variation should always be reported with confidence intervals (CIs)

What are the potential impacts of variation in the BV data?





Systematic Review of Data on Biological variation of ALT, AST and GGT.

Historical Application. Carobene et al Clin Chem Lab Med 2013;51:1997–2007

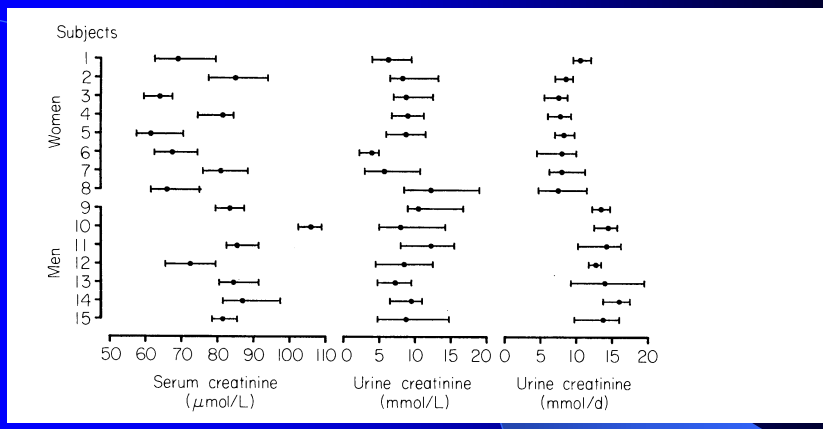
Derived quality specifications and derived indices at the maximum and minimum values of within-subject BV published for ALT, AST and GGT and in Ricos et al. database (shaded area)

Table 4 Derived quality specifications and derived indices at the maximum and minimum values of within-subject BV published for ALT, AST and GGT (Tables 1–3) and in Ricos et al. database (shaded area) [6].

	Biological variation, %		Derived quality specifications			Significance of change, RCV, %[a]	
	Within-subject	Between-subject	Imprecision[b]	Bias[c]	Allowable error[d]	Probability level	
	CV_w	CV_b	CV_{aqs}	B_A	TE_A	0.05	0.01
ALT	11.0	16.9[e]	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
GGT	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

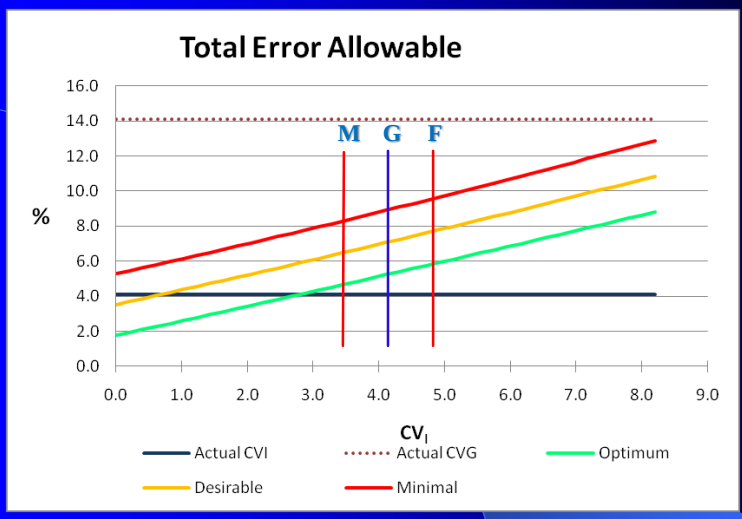
[b]Desirable quality specification for analytical imprecision (CV_{aqs}) calculated as half the within-subject variation; [c]Desirable quality specification for analytical bias, $B_A=0.25(CV_w^2+CV_b^2)^{1/2}$; [d]Desirable quality specification for total allowable error, $TE_A=B_A+1.65\ CV_{aqs}$; [e]No CV_b reported in the same study of minimum CV_w value, therefore CV_b as quoted on the Ricos et al. database was used. CV_a was set at 4.0% in all cases to enable comparison.

Creatinine Biological Variation



Biological Variation Serum Creatinine: Average within subject (CVI) = 4.1%
Gowans & Fraser. Ann Clin Biochem 1988:25:259-263

	CV_I	CV_G	CV_A	RCV 0.95	RCV 0.99	B_A	Te_a	Number of Samples to predict set point 5%	Number of Samples to predict set point 10%
Fraser									
Male	3.4	6.8	1.5	10.3	13.6	1.9	4.7	2	1
Female	4.9	11.8	1.5	14.2	18.7	3.2	7.2	4	1
Whole	4.1	14.1	1.5	12.1	15.9	3.7	7.1	3	1
Ricos Database	5.3	14.2	1.5	15.3	20.1	3.8	8.2	5	1
Reinhard et al	4.7	14.4	1.5	13.7	18.0	3.8	7.7	4	1



www.biologicalvariation.com/tools
Biological Variation Data Simulation Package V3.2 Excel

Urinary Albumin Excretion.

Miller *et al* Clin Chem 2009;55:24-38

CV_I 4% to 103% with central tertile 28% to 48%

40 studies with confounding factors: -

- Time period over which samples were collected
- Study design
- Type of sample and concentration range studied
- Population studied and state of health
- Preanalytical factors
- Poorly described statistical methods

Glycated Haemoglobin

Braga et al Clinica Chimica Acta 2010;411:1606-1610.

- Highlights the need for a structured approach

"Nine recruited studies were limited by choice of analytic methodology, population selection, protocol application and statistical analysis"

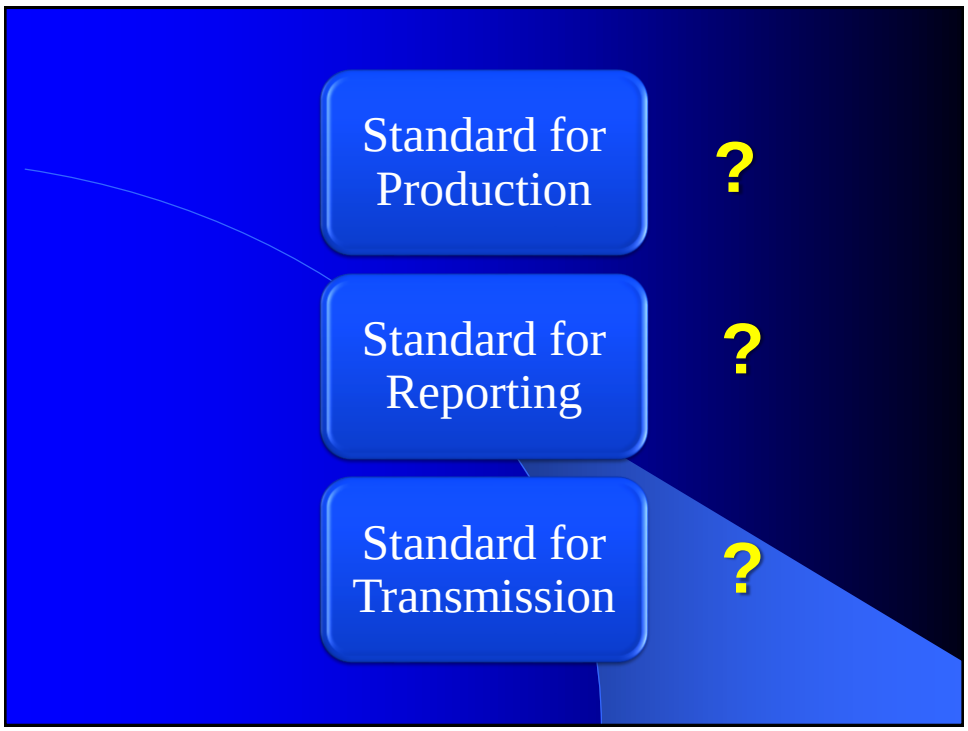
Issues: -

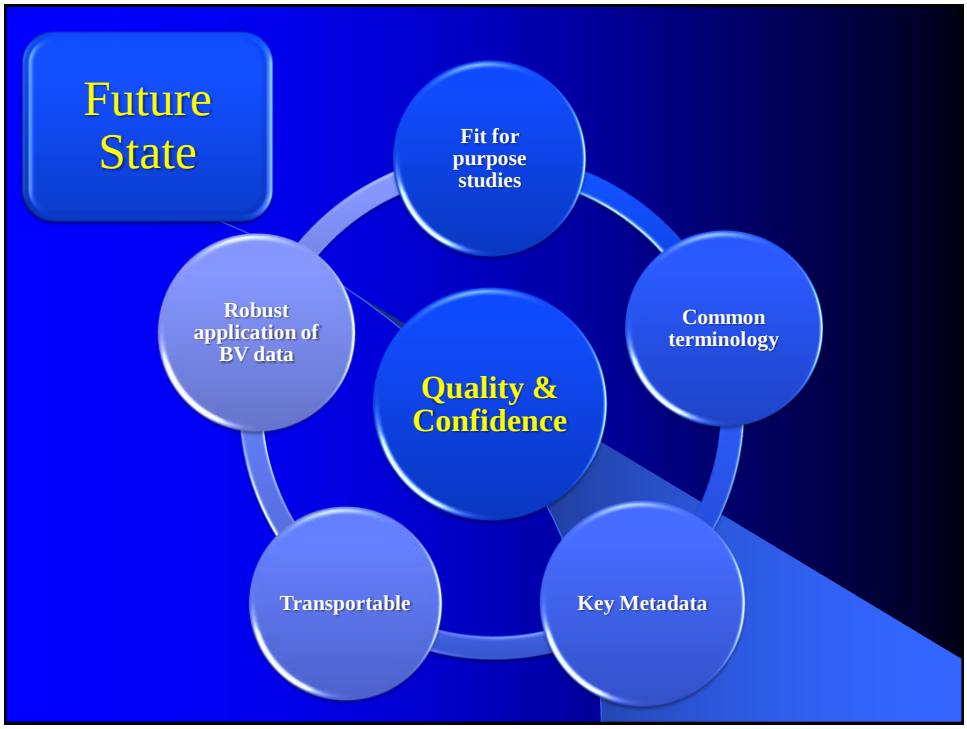
- Heterogeneity in experimental model
- Length of study inappropriate (3 days to 6 months)
- Methods with differing specificities
- Statistical methods not specified

Within subject Biological variation in disease: collated data and clinical consequences.

Ricos et al Ann Clin Biochem 2007:44:343-352

- 66 quantities 34 disease with 45 references.
- "For the majority of quantities studied CV_I of same order as diseased. "
- Disease specific RCVs may be necessary in some cases.
- Effect of variability in variability not quantitatively studied.
- "Heterogeneity in study designs and methods compiled"





EFLM Biological Variation Working Group & Collaborators

- Federica Braga
- Anna Carobene
- Abdurrhaman Coskun
- Niels Jonkers
- Irini Leimoni
- Richard Prusa
- Pilar Fernandez-Calle
- Thomas Røraas
- Sverre Sandberg

Biological Variation Working Group

Critical Appraisal Checklist

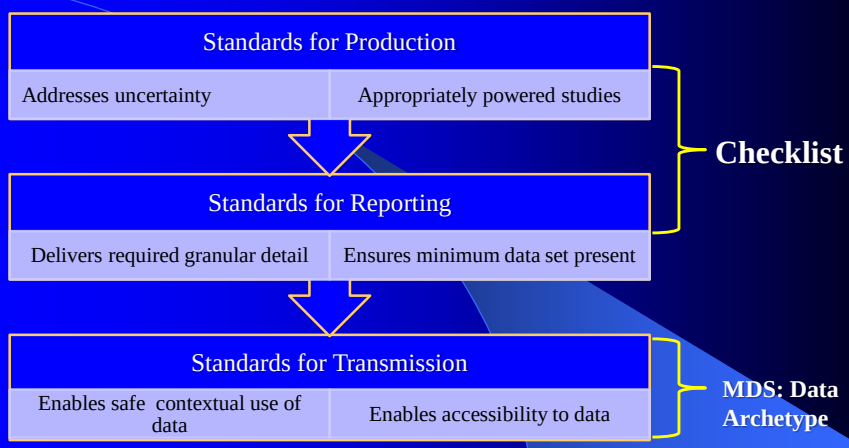
A checklist for critical appraisal of studies of biological variation.

Bartlett WA, Braga F, Carobene A, Coşkun A, Prusa R, Fernandez-Calle P, Røraas T, Jonker N, Sandberg S; Biological Variation Working Group, European Federation of Clinical Chemistry and Laboratory Medicine (EFLM).

Clin Chem Lab Med. 2015;53(6):879-85.

Opinion Paper

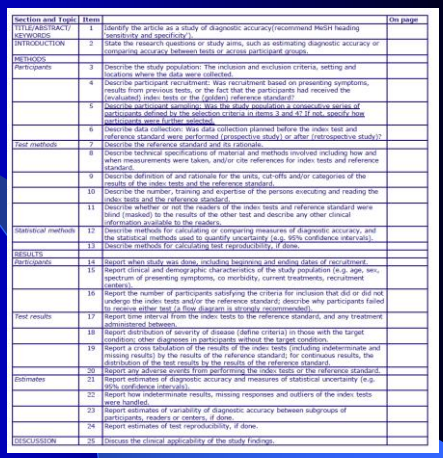
Quality and Confidence



Safe accurate and effective application of BV data across health care systems

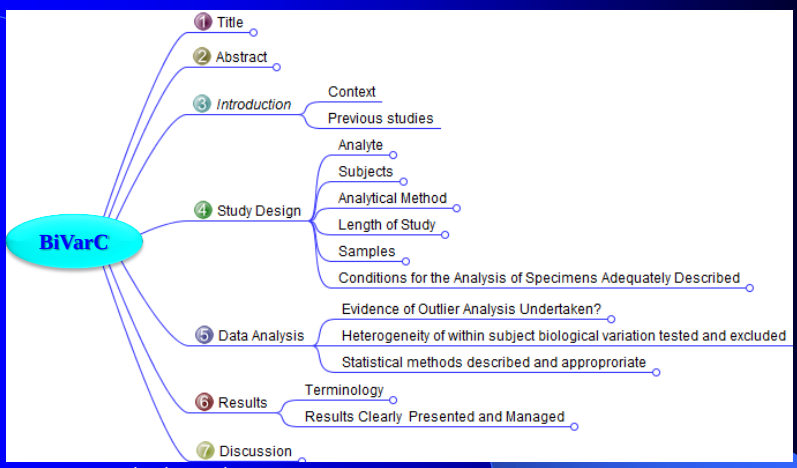
STARD Statement STAndards for the Reporting of Diagnostic accuracy studies

- The objective of the STARD initiative is to improve the accuracy and completeness of reporting of studies of diagnostic accuracy, to allow readers to assess the potential for bias in the study (internal validity) and **to evaluate its generalisability (external validity).**

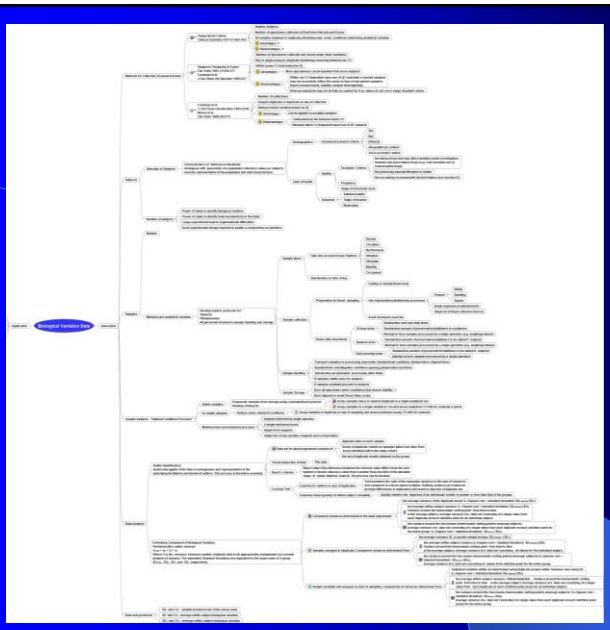


Section and Topic	Item		On page
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy(recommend MeSH heading 'sensitivity and specificity').	
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	
METHODS			
Participants	3	Describe the study population: The inclusion and exclusion criteria, setting and locations where the data were collected.	
	4	Describe participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the (evaluated) index tests or the (golden) reference standard?	
	5	Describe participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in items 3 and 4? If not, specify how participants were further selected.	
	6	Describe data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	
Test methods	7	Describe the reference standard and its rationale.	
	8	Describe technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	
	9	Describe definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	
	10	Describe the number, training and expertise of the persons executing and reading the index tests and the reference standard.	
	11	Describe whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	
Statistical methods	12	Describe methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	
	13	Describe methods for calculating test reproducibility, if done.	
RESULTS			
Participants	14	Report when study was done, including beginning and ending dates of recruitment.	
	15	Report clinical and demographic characteristics of the study population (e.g. age, sex, spectrum of presenting symptoms, co morbidity, current treatments, recruitment centers).	
	16	Report the number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test (a flow diagram is strongly recommended).	
Test results	17	Report time interval from the index tests to the reference standard, and any treatment administered between.	
	18	Report distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	
	19	Report a cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	
	20	Report any adverse events from performing the index tests or the reference standard.	
Estimates	21	Report estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	
	22	Report how indeterminate results, missing responses and outliers of the index tests were handled.	
	23	Report estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	
	24	Report estimates of test reproducibility, if done.	
DISCUSSION	25	Discuss the clinical applicability of the study findings.	

Critical Appraisal Checklist for BV Data Publication



www.biologicalvariation.com
www.biologicalvariation.com/Tools.html



And a few more!

Table 1. Biological Variation Data Reporting Checklist (BiVarC).

Section and Topic	Item # (MDS Domain Mapping: A-F)[a]	
Methods	3	Described in enough detail to facilitate transportability of the derived data across populations and health care economies. The biological variation data produced are effectively reference data and their applicability requires delivery of appropriately described metadata to enable their use as such.
Analyte/ Measurand.	3.1 (A)	The described study should clearly identify the target analyte and measurand/s. Where available internationally agreed terminology and codings should be utilised.
Subjects	3.2 (B)	The description of the subjects and population studied should be detailed enough to enable transportability of the biological variation data set. Minimum data set should be present [21,22,23]
Measurement Procedure.	3.3 (A)	A clear description of the analytical methodology used should form part of the metadata. This may be made available *via* an appropriate reference or be presented within the publication. Deviation from standard operating procedures, use of adaptations of published methods, and deviation from manufacturers recommended methods in the case of commercially available systems should be documented. Standardisation and traceability should be clearly identified.
Length of Study	3.4 (C)	Length of the study periods should be clearly identified.
Samples	3.5 (C)	Sampling protocols that minimise pre-analytical variation should be adequately described to enable transportability of the data and numbers of samples taken sufficient to deliver the required power to the study.[25, 26] Sampling conditions and sample type should be described in detail. Pre-analytical storage conditions of samples should be described. Recorded details should include the beginning and end date of the study and timings of sampling.
Conditions for analysis of samples	3.6 (C)	A description of conditions under which the samples were analysed. Analytical protocols should be designed to minimise sources of analytical variability (Optimal Conditions Precision). [24]

Minimum Data Set: BiVarC MDS

Domain	Area for Application	Attributes
(A) 1	Checklist & database	Target - analyte and measurand, sample matrix, method characteristics.
(B) 2	Checklist & database	Population characteristics- demographics, state of well being, physical/physiological characteristics, medication.
(C) 3	Checklist & database	Study Characteristics- study duration and design, power of study to detect BV indices, model assumptions, statistical approach.
(D) 4	Checklist & database	Data Characteristics- indices of biological variability, confidence intervals, tests for model assumptions
(E) 5	For database	Publication Details- links to the original publication.
(F) 6	For database	Data rating- new concept to be developed to indicate the quality of the BV data against a set of key criteria.

Challenge: -

- Identification of key questions to enable practical application of a checklist to assess:
  - Existing data- *Identifies usable historical data fo inclusion in a new database.*
  - New publications – *Drives up quality of BV Data*

New Database Development

- EFLM Task and Finish Group set up post the Milan EFLM Strategic Conference. Sverre Sandberg Chair.
- Includes
  - Biological variation working group members
  - Spanish Quality Commission (SEQC)
- Barcelona /Paris 2015

TFG 5 - TFG-BVD "Biological variation database"
Chair: Sverre Sandberg (NO)
Aasne Karine Aarsand (NO)
Maria-Carmen Alsina Perich (SP)
Bill Bartlett (UK)
Federica Braga (IT)
Anna Carobene (IT)
Abdurrahman Coskun (TR)
Pilar Fernández Calle (SP)
Callum Fraser (UK)
Niels Jonker (NL)
Jorge D Marco (SP)
Mauro Panteghini (IT)
Per Hyltoft Petersen (DK)
Carmen Ricos (SP)
Karen Robijns (NL)
Thomas Helge Røraas (NO)

Building for the Future

- Pragmatic approach
  - Use data already published and help users recognise limitations
  - Identify the key areas for future work and inform the structure of the publication checklist.
  - 14 questions identified and rating of A to D Classified on lowest rated with subscript identifying area of concern ($C_{8,10}$)

4) Are the measurand and the measurement procedure documented?

i	**Category A requires either**
	(a) method described in detail.
	(b) reference to article where method is described in detail.
	(c) an identifiable method has been applied and is described with sufficient detail e.g. samples run on hexokinase method at Cobas 6000, Roche Diagnostics.
ii	**If no/little information is given, category B, C or D depending on the amount of detail given and the measurand in question.**
iii	**If the method is considered no longer valid i.e. that current methods in practise estimate another measurand, category C or D depending on the consequences.**

Fit for Publication ?
Fit to Recycle?

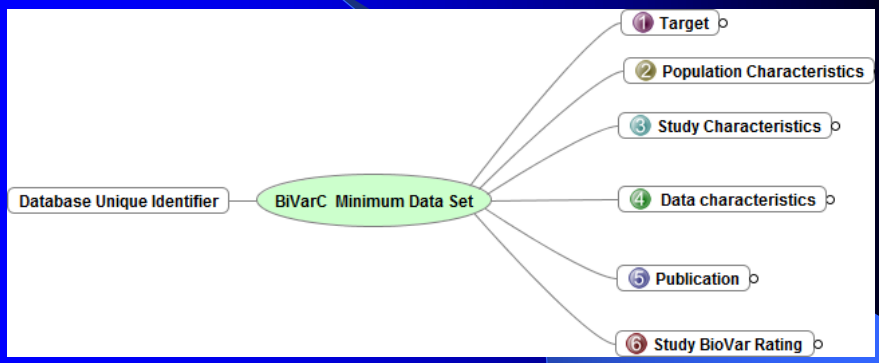
A B C D

Checklist Compliance

Transportability

Archetype: definition?

A computable expression of a domain content model.
Structured content to enable communication of key information



Minimum Data Set: BiVarC MDS

Definition of a Data Archetype required.

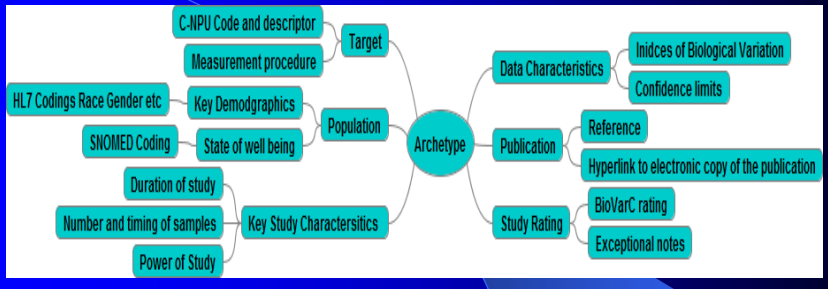
Provides granularity

- Enable drill down into detail

Use of standardised terminology and coding.

- Terminology Simundic et al Clinical Chemistry November 2014
- C-NPU, LOINC, SNOMED-CT

Simple?



- Derived as far as possible from historical studies
- Mandated for future studies

Summary

- Biological variation data are reference data.
- Existing databases are a valuable resource but need to be used with care
- A critical appraisal checklist has been developed to:
  - enable assessment of historical data
  - drive up quality of future publications
  - EFLM TFG hard at work to deliver a new database

Biological Variation Database, time for an update?

Yes!

Slides available next week:

www.biologicalvariation.com