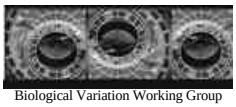


Biological Variation Working Group

Critical appraisal checklist for papers on biological variation

Dr Bill Bartlett

Biological Variation Working Group

EFLM

These fundamental data have many applications that under-pin our practice!

These are reference data!

Do I have confidence in the data and understand their limitations?

Rodin's Thinker
Burrell Collection Glasgow

Reference Data?

"The components of the human organism are subject to variation caused by physiological processes, genetic differences, diseases and environmental factors. A rational interpretation of laboratory results demands knowledge of the variation of these components in the individual under study or in one or more adequately defined sets or reference individuals. An important task for clinical chemists and haematologists is therefore to provide relevant sets of reliable ***reference values."***

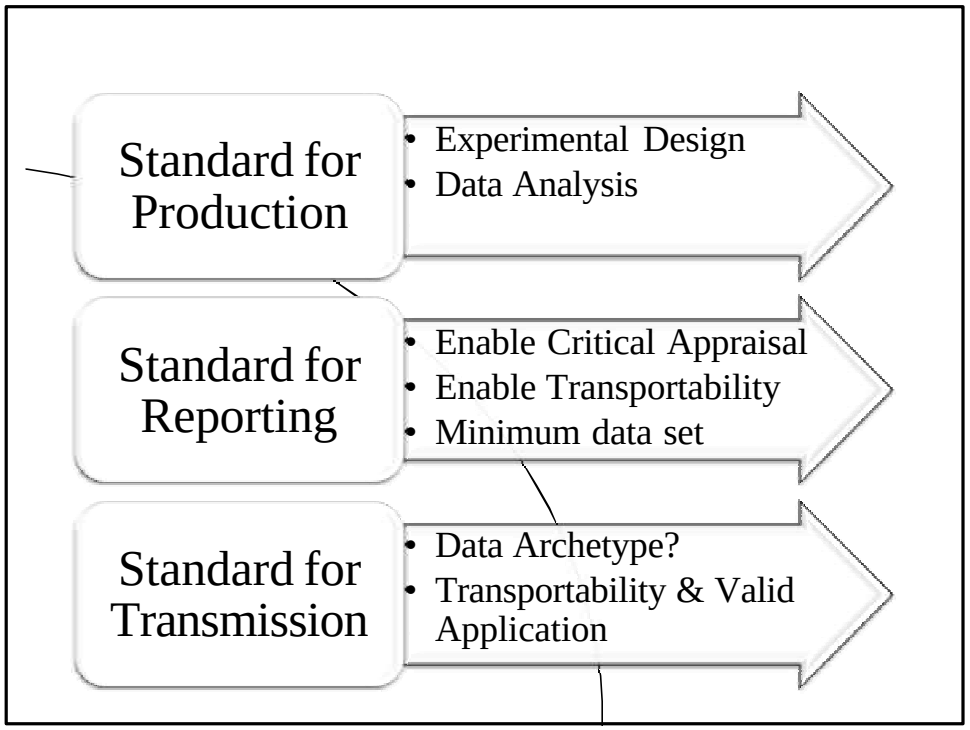
Solberg HE. Approved recommendations on the theory of reference values. Part 1. The concept of reference values. Clin Chim Acta 1987;165:111-18.

IFCC Expert panel on the Theory of Reference Values

1. **The Concept of Reference Values.** ***1987;25:337-342***
2. **The selection of Individuals for the Production of reference values.** ***1987;25:639-644***
3. **Preparation of individuals and collection of specimens for the production of reference intervals.** ***1988;26:593-598***
4. **Control of analytical variability in the production of reference values.** ***1991;29:531-535***
5. **Statistical treatment of collected reference limits.** ***1987;25:645-656***
6. **Presentation of observed values related to reference values.** ***1987;25:657-662***

J Clin Chem Clin Biochem

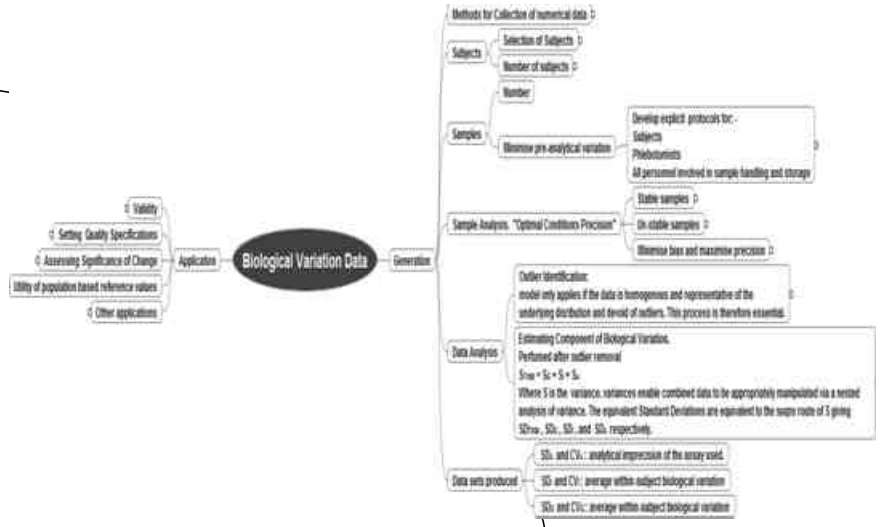
Given their importance, we should have accepted standards for production and characterisation of BV Data!



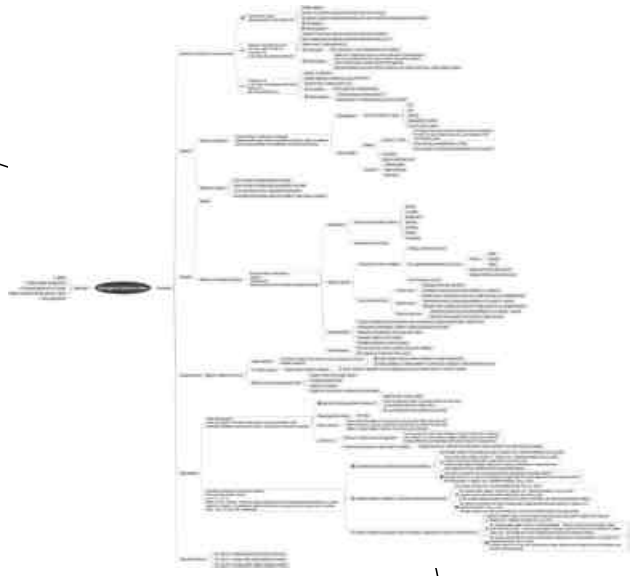
Standard for Production

"Our hope is that the comparability of such data might be provided by use of a common study design and analysis of data"

Fraser & Harris 1989
Crit Rev in Clin Lab Sci. 1989;27(5)409-437

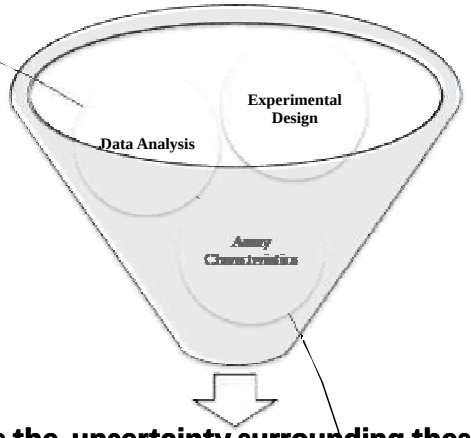


A few factors to consider!



And a few more!

Fundamental Questions?



What is the uncertainty surrounding these data?

What are the quality standards for BV Data?

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)

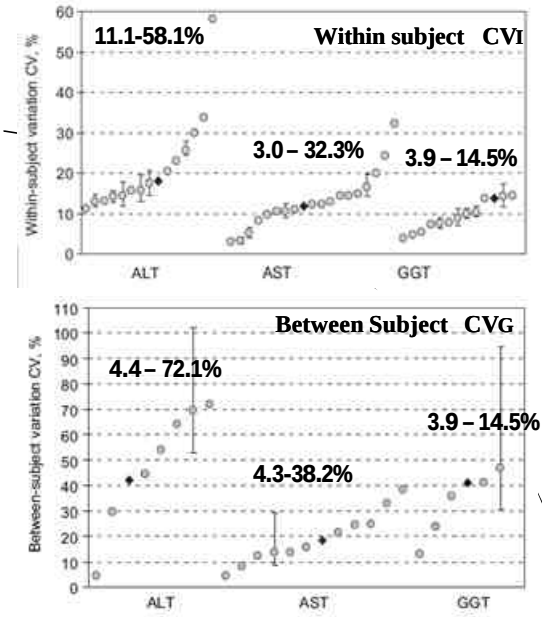
- design of an experiment to estimate biological variation should take into account the analytical imprecision.

Standard ?

- Estimates of biological variation should always be reported with confidence intervals (CIs)

Factors affecting confidence intervals around CV_I and RCV

- Study design: number of subjects, number of samples, number of replicates .
- The effects of variables vary with the **ratio of CV_A to CV_I**
  - Low ratio = narrower CI around estimate CV_I
  - Low ratio = higher power study
- Number of samples more important than number of subjects



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

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 this approach

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

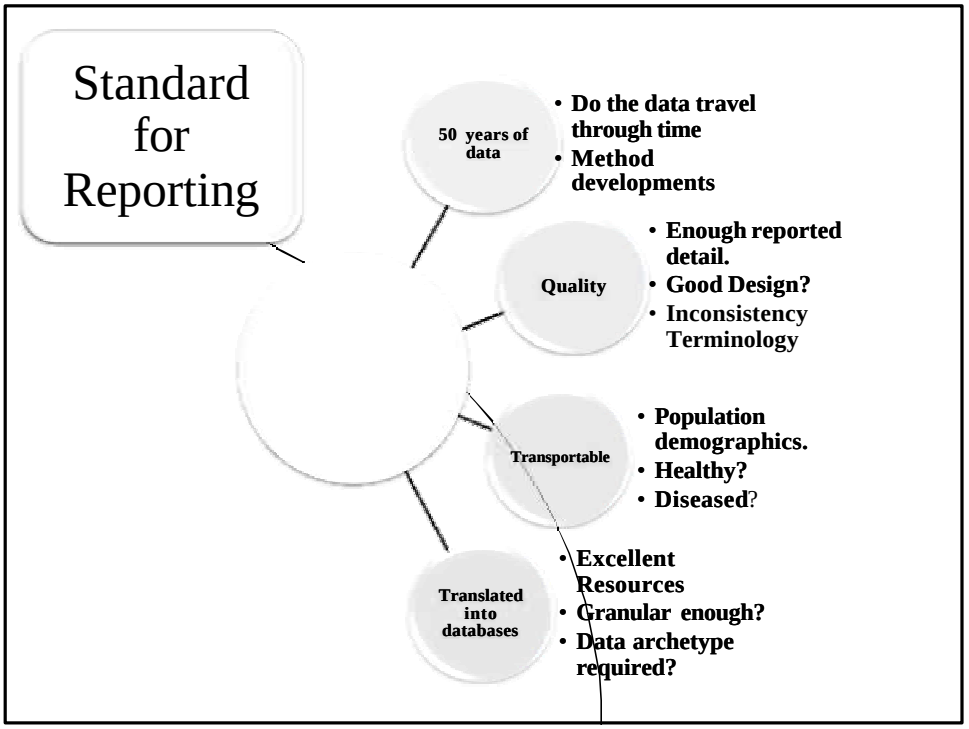
  Issues: -

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

Summary

- BV data are complex reference data
- Need for standards
- Safe application requires prior critical appraisal
- Published data are of varying quality
- Need to identify a minimum set of attributes to enable the data to be effectively transmitted and applied (archetype).
- Confidence intervals critically dependant upon ratio of CV_A to CV_I .

Standard for Reporting



Ricos et al Database

Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation

This most recent and extensive listing of biologic goals has been provided by Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minchinela J, Perich C, Simon M. "Current databases on biologic variation: pros, cons and progress." Scand J Clin Lab Invest 1999;59:491-500. *This database was most recently updated in 2012.*

Annex I, Part I: Within-subject and between-subject CV values of analytes and *Desirable Analytical Quality Specifications for imprecision, bias and total error*

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

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

Utility of Reference Values

1. **These are only meaningful and transferable/transportable if defined for the population or individual in terms of: -**
   - **Inclusion and exclusion criteria**
   - **Intake of food & drugs**
   - **Physiological and environmental conditions**
   - **State of well being**
   - **Specimen collection criteria**
   - **Performance characteristics of the analytical method**
   - **The statistical methods used for estimation of the limits**

Critical Appraisal Checklist

STARD Statement STAndards for the Reporting of Diagnostic accuracy studies

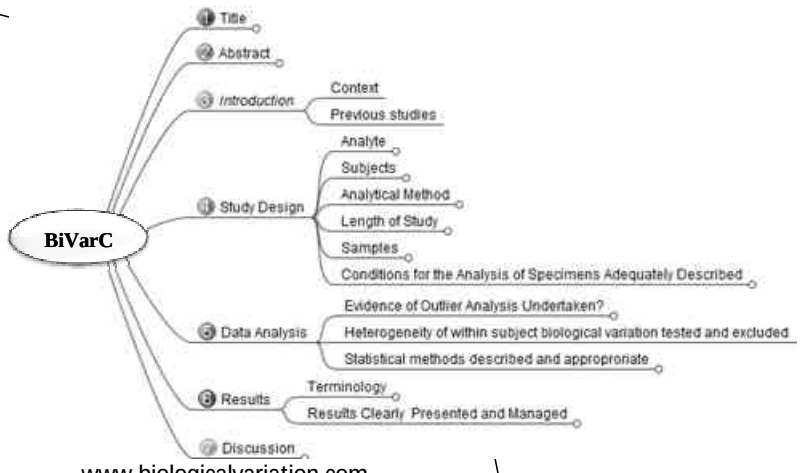
- The objective of the STARD initiative is to improve the accuracy and completeness of reporting of studies of diagnostic accuracy, to allow readers to assess the potential for bias in the study (internal validity) and **to evaluate its generalisability (external validity).**

STARD Statement

STAndards for the Reporting of Diagnostic accuracy studies

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 (gold) 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 centres).	
	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



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

Standards for Transmission

Utility of Reference Values

1. **These are only meaningful and transferable/transportable if defined for the population or individual in terms of: -**
   - **Inclusion and exclusion criteria**
   - **Intake of food & drugs**
   - **Physiological and environmental conditions**
   - **State of well being**
   - **Specimen collection criteria**
   - **Performance characteristics of the analytical method**
   - **The statistical methods used for estimation of the limits**

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.

Minimum Data Set: BiVarC MDS

- Granularity
  - Drill down into detail (needs to be reported)
  - Detail needs to be available and understood
  - Use of standardised terminology and coding.
    - Terminology Simundic et al Clinical Chemistry November 2014
    - C-NPU, LOINC, SNOMED-CT

Standard ?

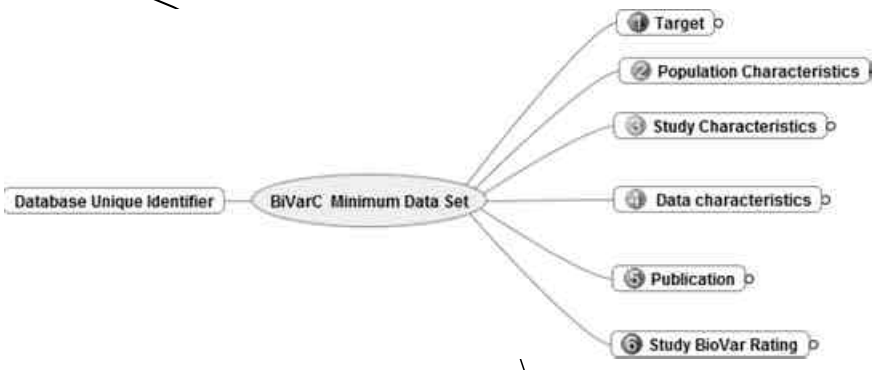
- Definition of a Data Archetype required.

Coding systems

Are coding systems granular enough? :
Serum creatinine in Diabetes Stage 3 CKD:

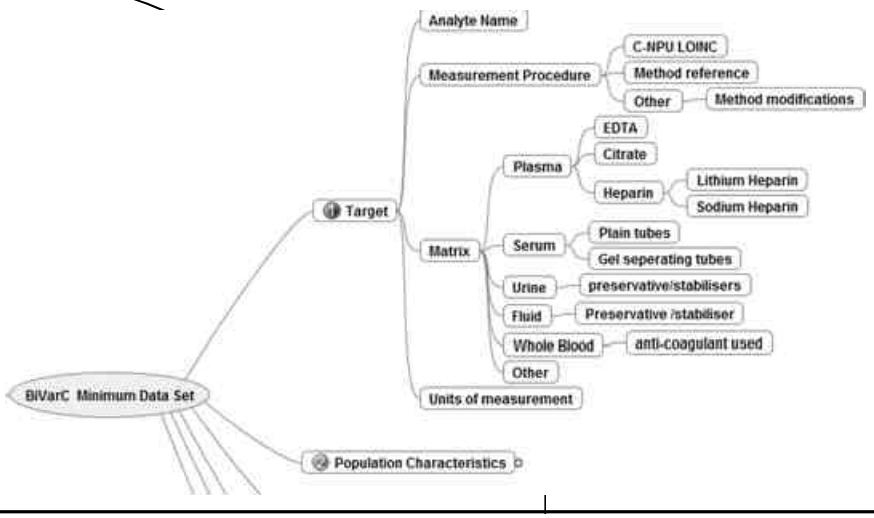
- C-NPU: NPU04998 P Creatininium; subst.c.(enz.) = ? µmol/L
- LOINC: 14682-9 Serum / Plasma Creat SerPl-sCnc umol/L
- SNOMED CT: **Concept ID:** 731000119105
  Chronic kidney disease stage 3 associated with type 2 diabetes mellitus (disorder)

Databases & BiVarC Minimum Data Set

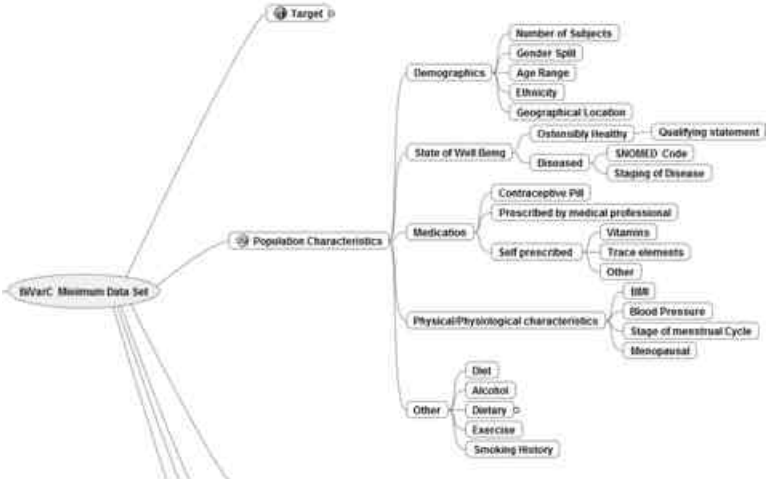


Use of recognised coding systems as metadata?

BiVarc MDS - Domain 1

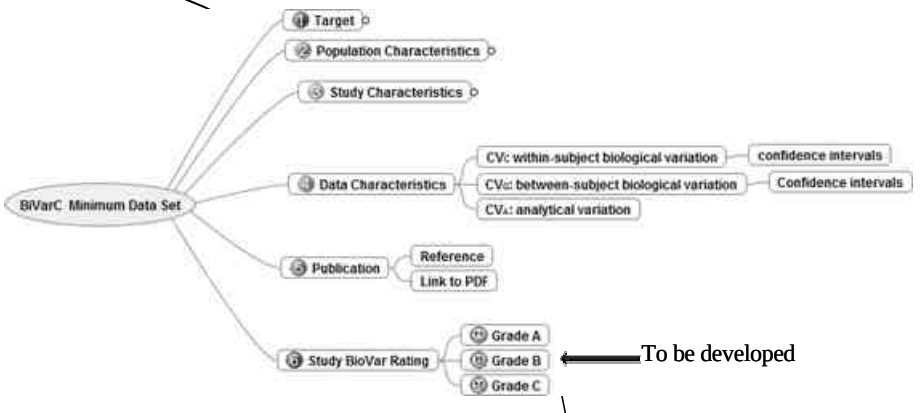


BiVarC MDS - Domain 2



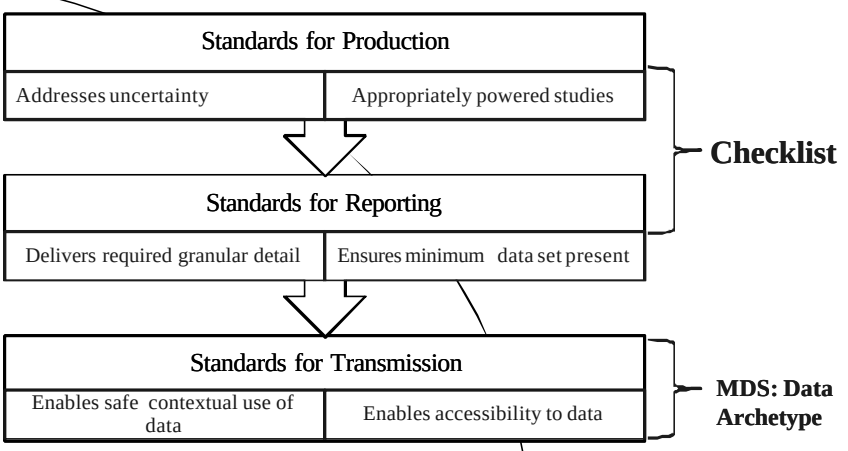
BiVarC MDS - Domain 3

BiVarC MDS - Domain 4,5 & 6

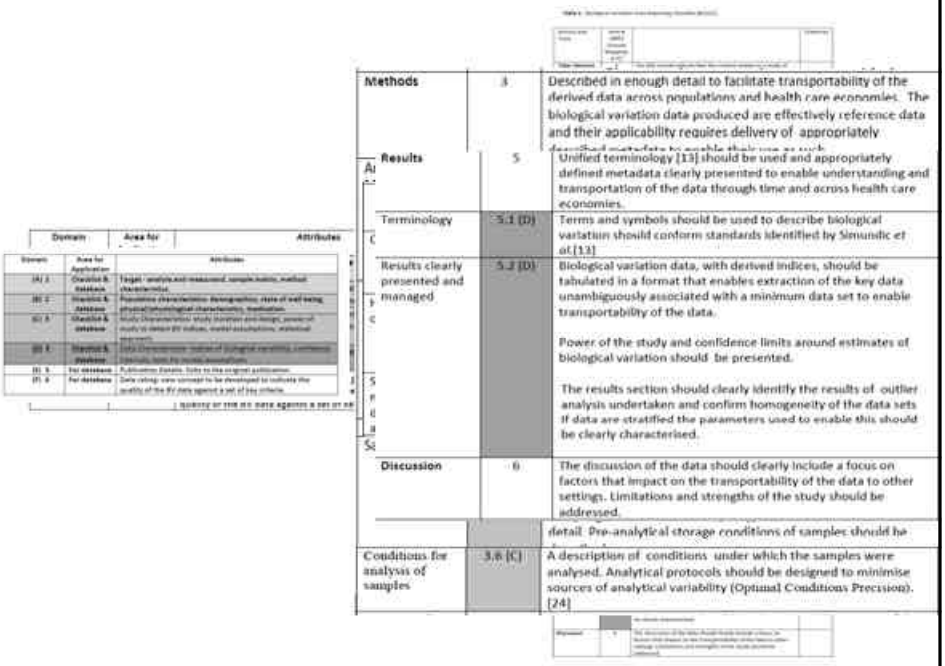


MDS meets Checklist

The Process



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

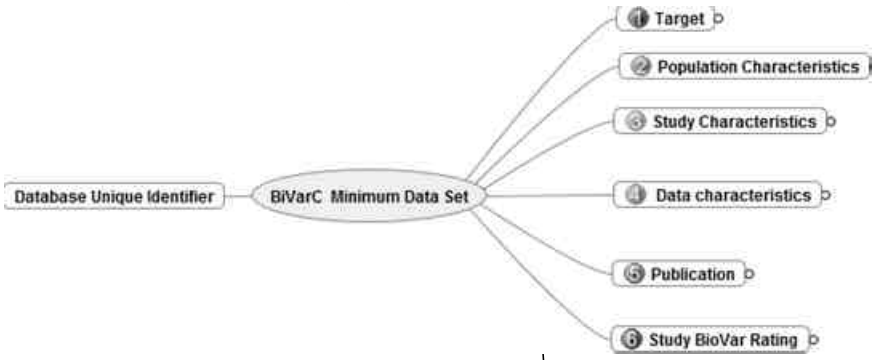


Methods	3	Described in enough detail to facilitate transportability of the derived data across populations and health care economies. The biological variation data produced are effectively reference data and their applicability requires delivery of appropriately
Results	5	Unified terminology [13] should be used and appropriately defined metadata clearly presented to enable understanding and transportation of the data through time and across health care economies.
Terminology	5.1 (D)	Terms and symbols should be used to describe biological variation should conform standards identified by Simundic et al.[13]
Results clearly presented and managed	5.2 (D)	Biological variation 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 transportability of the data. Power of the study and confidence limits around estimates of biological variation 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 parameters used to enable this 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.
		detail Pre-analytical storage conditions of samples should be
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]

Archetype: definition?

A computable expression of a domain content model.

Structured content to enable communication of key information



Simple?

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

Summary

- Standards required for production, reporting and transmission of BV Data.
- A critical appraisal checklist has been developed to:
  - enable assessment of historical data
  - drive up quality of future publication
- MDS/Archetype will enable transmission and safe contextual use of BV data across health care systems.

www.biological variation.com

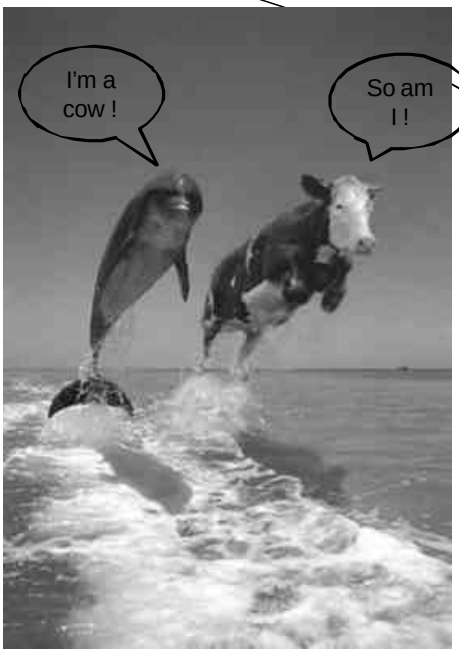
Next Steps?

- Promotion of the checklist
- Definition of MDS/Archetype for application to future database developments.
- Development of supporting information sources/publications to enable understanding and compliance with the approach
- Standards?

Biological Variation Working Group & Collaborators

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

Biological Variation Working Group



Biological Variation ? CV_G?