Biological Variation and Analytical Quality Specifications

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Content and expected outcomes

After this presentation, participants should :

- *know the types of biological variation,*
- *know how to generate and/or find data on random biological variation, and*
- *appreciate the many uses of data in laboratory medicine, particularly to set* ***Quality Specifications*** *for analytical reliability performance characteristics.*

Variation over the span of life

Some quantities change over the span of life – particularly when rapid physiological change occurs:

- *neonatal period*
- *childhood*
- *puberty*
- *menopause*
- *old age*

Solution - age stratified reference values.

Predictable rhythmical variation

Daily, monthly and seasonal cycles.

It is impossible to develop good reference values for every time point during a cycle and samples should be taken at relevant times for the clinical purpose at which RV should be available.

Knowledge of the expected values is vital for clinical interpretation and absence of the expected cycle may provide clinically useful information.

Random variation

A series of four results taken from an individual

Haemoglobin	*15.4*	*15.1*	*15.3*	*15.5*	*[13.0-18.0 g/dL]*
RBC	*5.00*	*5.07*	*5.14*	*5.15*	*[4.5 - 6.0 x 10^{12}/L]*
WBC	*7.2*	*4.7*	*4.5*	*6.4*	*[4.0 - 11.0 x 10^{9}/L]*
Platelets	*346*	*287*	*288*	*328*	*[150 - 400 x10^{9}/L]*

Variation is pre-analytical, analytical and biological.

Pre-analytical variation

Preparation of subject

- *fasting*
- *exercise*
- *posture*

Sample collection and handling

- *type of sample*
- *anticoagulant*
- *tourniquet*
- *transport time*
- *centrifugation*

Random analytical variation

Imprecision [inherent random error]

Changes in bias [systematic error]

Constant bias does not affect results over time [they may all be low or all be high - due to bias] but changes in bias - most importantly on recalibration of methods – is often a significant source of variation in serial results.

Biological variation

A series of four results taken from each of a cohort of four individuals - haemoglobin [g/dL]

Individual 1	*13.7*	*13.1*	*13.6*	*14.2*
Individual 2	*14.4*	*14.6*	*14.9*	*15.1*
Individual 3	*16.1*	*16.3*	*16.8*	*17.0*
Individual 4	*15.0*	*15.8*	*15.7*	*15.5*

Homeostatic setting points do vary amongst individuals - between-subject BV

Generation of estimates of components of variation

- *select a small number of reference individuals and apply exclusion criteria*
- *take samples at intervals - minimizing pre-analytical sources of variation [subject preparation and sample collection and handling]*
- *store as to ensure stability*
- *analyze in random duplicate in one batch*
- *look for outliers [complex]*
- *use nested ANOVA to determine CV_A, CV_I, CV_G*

To generate or not to generate?

It is dogma that all laboratories must generate their own reference values - analogously, do all laboratories have to generate their own data on components of biological variation?

The answer is ……..absolutely NOT!

Biological variation in young [20-45] and elderly [70-83] men

Quantity	*Young*	*Elderly*
Haemoglobin	*2.8*	*2.7*
RBC	*3.0*	*2.3*
MCV	*1.1*	*1.1*
WBC	*10.8*	*9.4*
Platelets	*6.7*	*10.0*
MCH	*1.3*	*0.8*

Data on biological variation

Over the years, many compilations - most recent in literature -

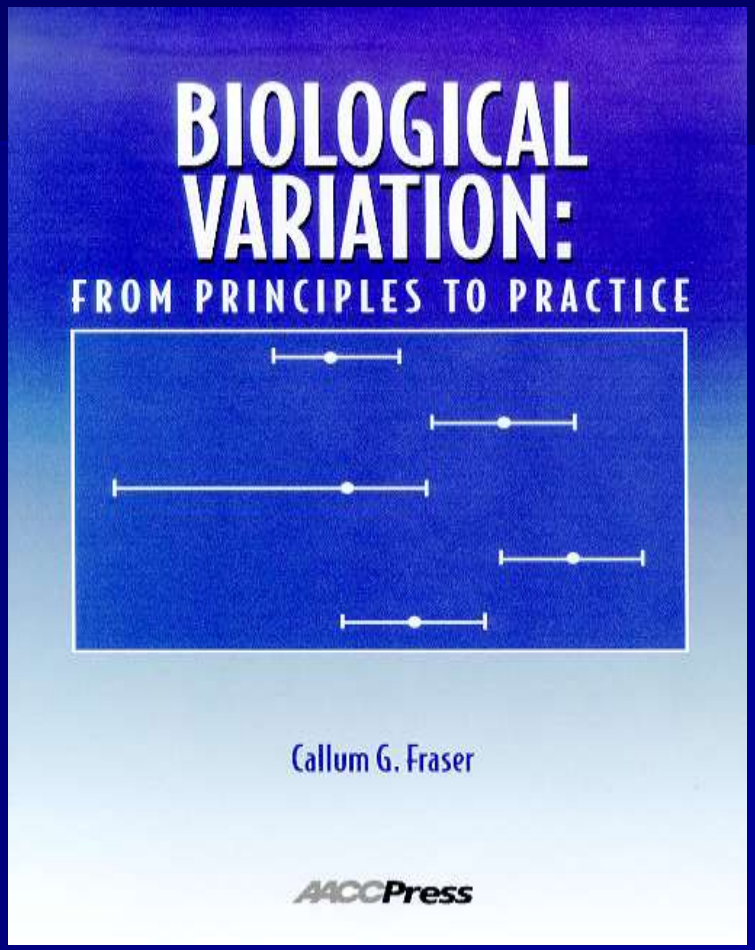
Ricos C, et al. *Current databases on biologic variation: pros, cons and progress.* *Scand J Clin Lab Invest 1999;59:491-500.*

2008 update at:

www.westgard.com/biodatabase1.htm

Data on biological variation and the content [some, at least] of this presentation.

Available at www.aacc.org



Application of data

- *assessing the significance of changes in serial results from an individual*
- *deciding the utility of conventional population based reference values*
- *managing aspects of quality and assessing professional guidelines*
- ***setting quality specifications***

Setting quality specifications - difficulties

- *many published recommendations*
- *further new recommendations published*
- *test results are used in many situations*
- *neither patients [nor clinicians] are harmed by current performance*
- *manufacturers do not use in either development or marketing*

Stockholm – Consensus Conference

See all the papers in: SJCLI 1999;57:475-585

The hierarchy of strategies to set quality specifications

1. *Assessment of the effect of analytical performance on specific clinical decision-making.*
2. *Assessment of the effect of analytical performance on general clinical decision-making.*
3. *Professional recommendations.*
4. *Quality specifications laid down by PT or EQAS organisers.*
5. *Published data on the state of the art.*

Level 2 - Effect of analytical performance in general terms

- *Formulae using biological variation data look complex – BUT*

- *directly related to use of tests clinically,*
- *many different applications,*
- *many data on biological variation, and*
- *widely supported by professionals.*

Quality specifications best based on biological variation

- *Imprecision* $< 0.5 \bullet CV_I$
- *Bias* $< 0.25 \bullet [CV_I^2 + CV_G^2]^{½}$
- *Total error* $< 1.65 \bullet 0.5 \bullet CV_I + 0.25 \bullet [CV_I^2 + CV_G^2]^{½}$
- *Other specifications based on biology include for reference methods and the allowable difference for two analysers in the same laboratory.*

Some quality specifications [as %]

Quantity	CV_I	CV_G	CV_A	|B|	TEa
MCV	1.3	4.8	0.7	1.2	2.3
Haemoglobin	2.8	6.6	1.4	1.8	4.1
RBC	3.2	6.1	1.6	1.7	4.4
WBC	10.9	19.6	5.5	5.6	14.6
Platelets	9.1	21.9	4.6	5.9	13.4
Reticulocytes	11.0	29.0	5.5	7.8	16.8

Potential problems with BV-based quality specifications

- *Some seem to be similar to state of the art and similar to professional recommendations.*
- *Some too difficult to obtain with current technology and methodology - should we stop doing these?*
- *Some quality specifications are very easy to achieve - should we let quality slip?*

Optimum and minimum quality specifications based on BV

Imprecision

- $CV_A < 0.25\ CV_I$
- $CV_A < 0.75\ CV_I$

Bias

- $B_A < 0.125\ [CV_I^2 + CV_G^2]^{1/2}$
- $B_A < 0.375\ [CV_I^2 + CV_G^2]^{1/2}$

Total error allowable

- $TE_a < 1.65 \times 0.25\ CV_I + 0.125\ [CV_I^2 + CV_G^2]^{1/2}$
- $TE_a < 1.65 \times 0.75\ CV_I + 0.375\ [CV_I^2 + CV_G^2]^{1/2}$

Sources of variation are additive

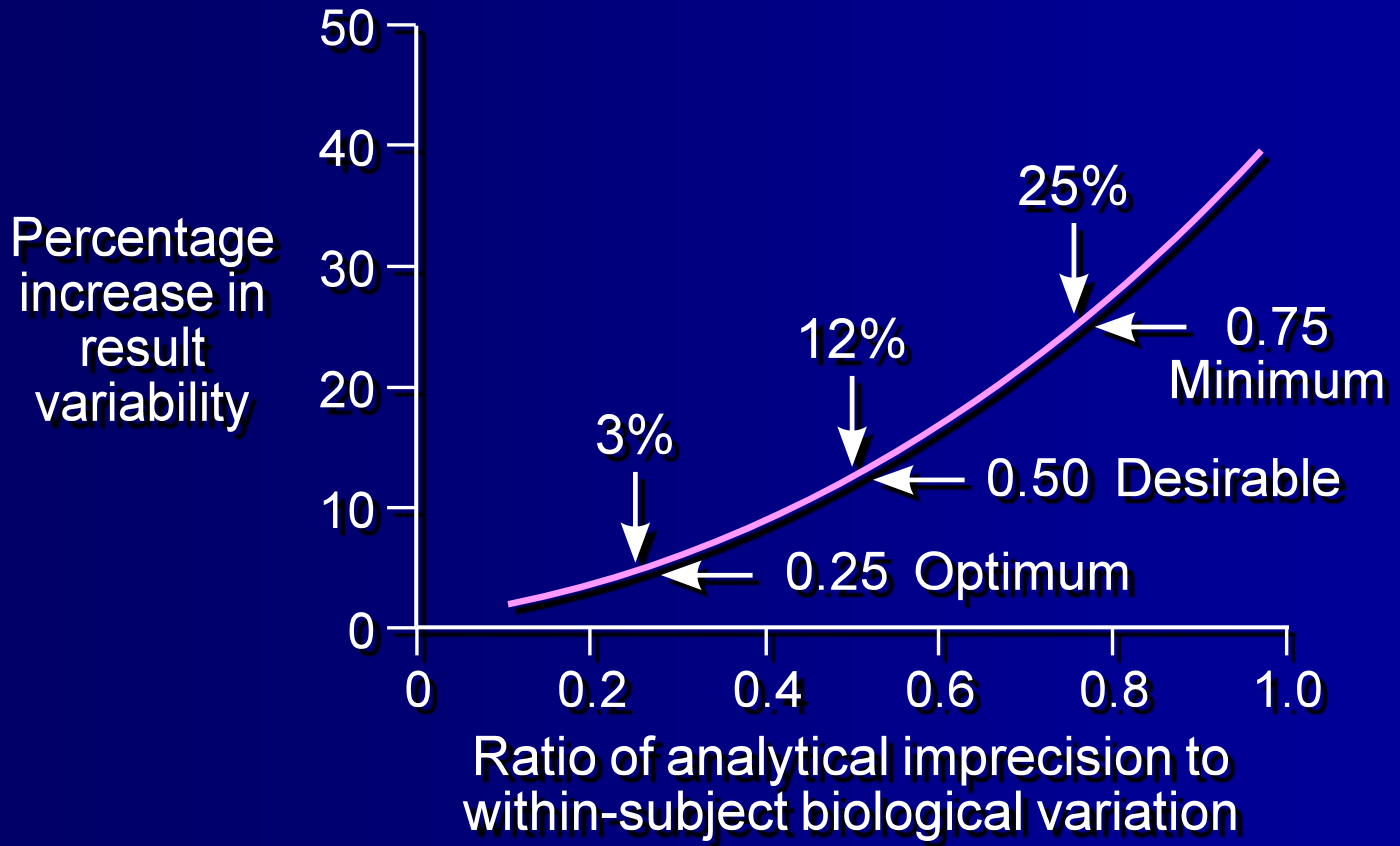
Haemoglobin = 16.60 g/dL

single figure represents a distribution

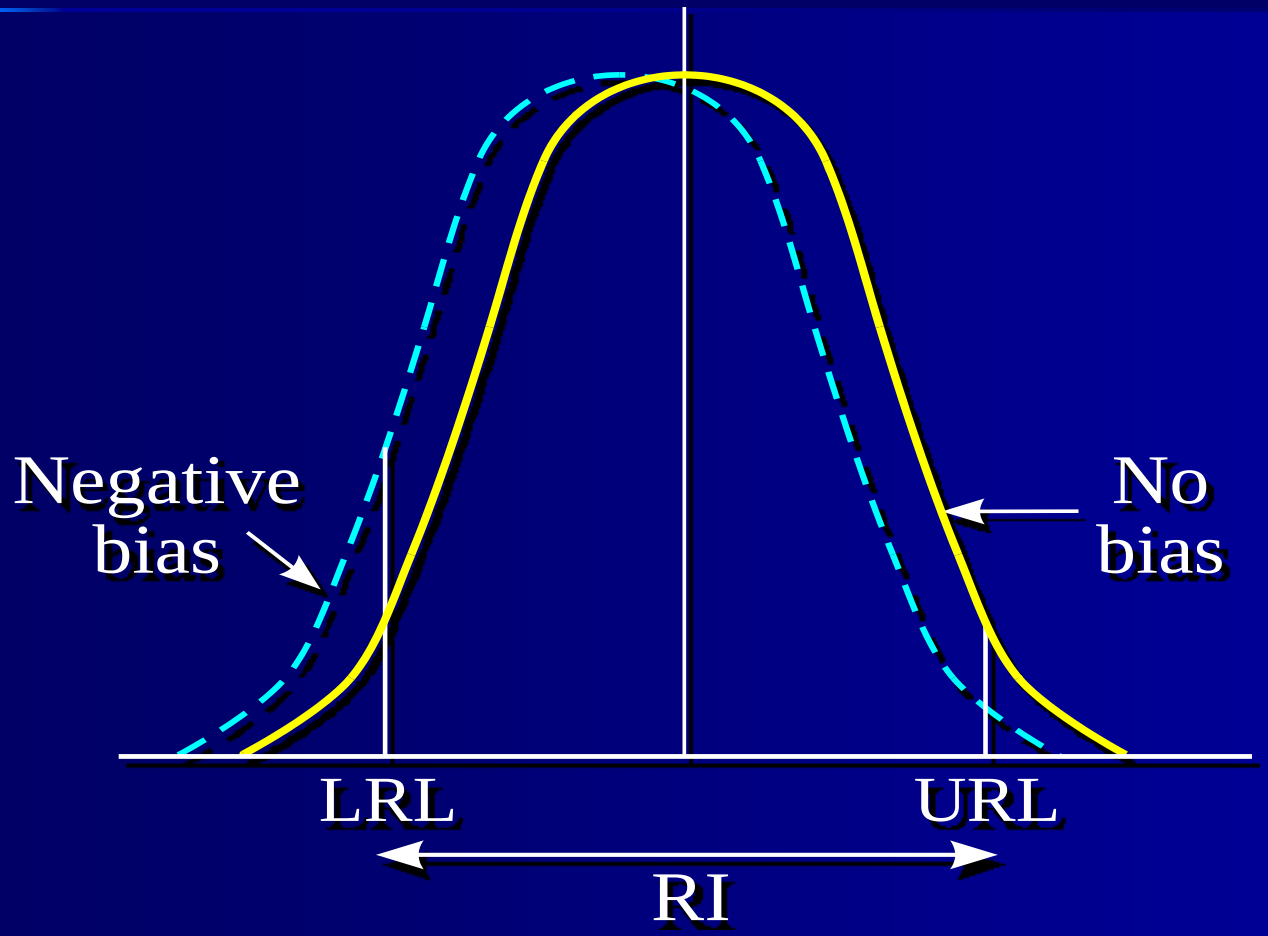
total variation is due to analytical and within-subject biological components

$$CV_T = [CV_A^{\,2} + CV_I^{\,2}]^{½}$$

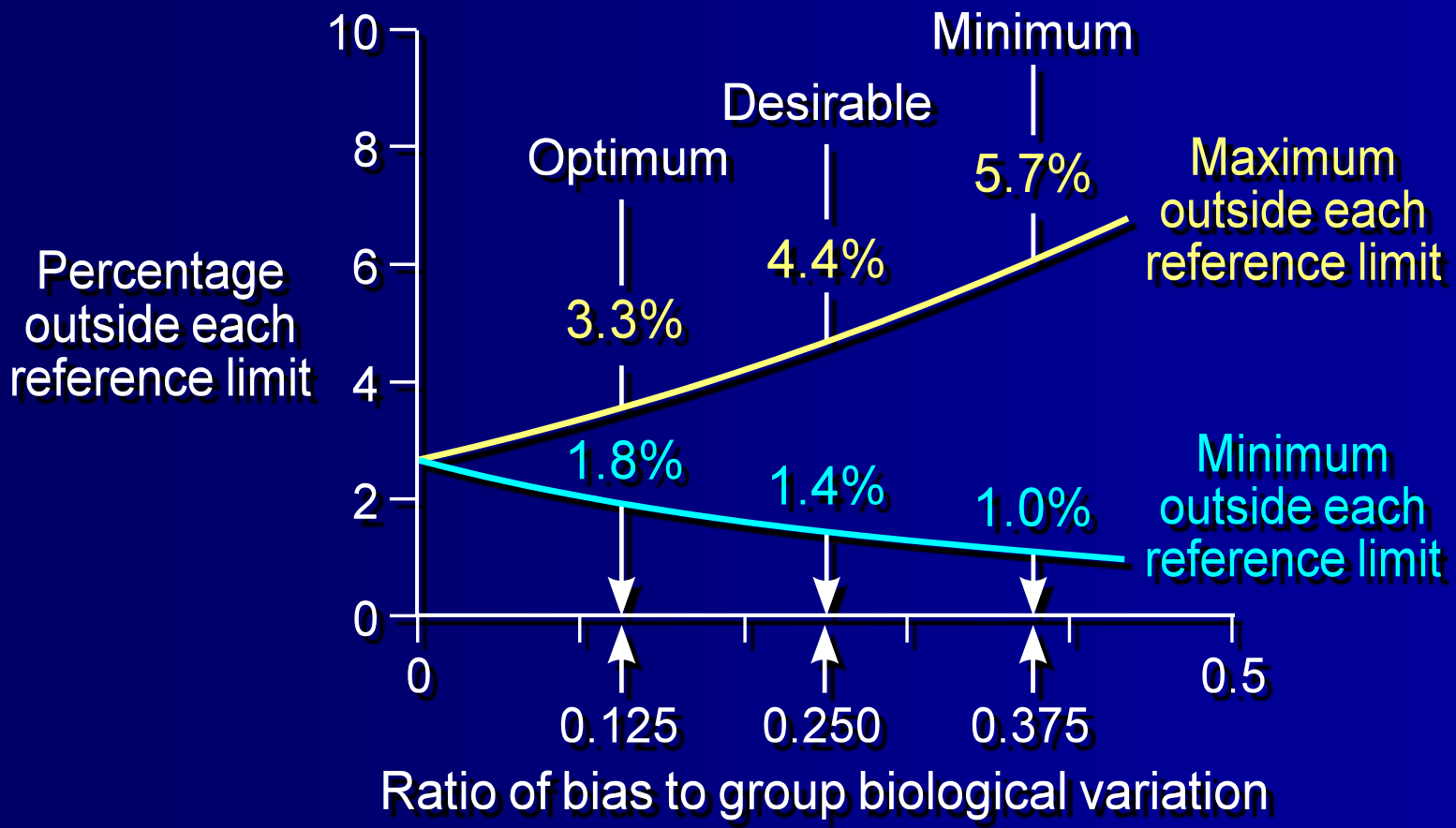
The effect of imprecision on test result variability



Effect of negative bias



Effect of bias on reference values



Quality specifications for EQAS using BV data

$$PT/EQAS < 1.65\,[0.5\,CV_I] + 0.25\,[CV_I^2 + CV_G^2]^{1/2}$$

OR

$$PT/EQAS < 1.65\,[x\,CV_I] + y\,[CV_I^2 + CV_G^2]^{1/2}$$

Interest in this application

Acceptability limits based on biological variation in haematology EQAS.

Milan Skitek

Accred Qual Assur 2005;10:112–115

Final Conclusions

- *Many data on random biological variation - usually not necessary to generate in-house - use existing database for a variety of purposes.*
- *Strategies are available to set quality specifications for imprecision and bias, and models higher in the agreed hierarchy are preferred.*
- *There is a wide consensus that "biology is best" - quality specifications based on BV are easy to obtain, are directly related to clinical outcomes, and are applicable everywhere – including EQAS.*