

# *Biological Variation: From Interpretation of Test Results to Clinical Protocols*



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## *Content and expected outcomes*

*After this lecture, participants should :*

- *realise the limitations of conventional population-based reference values ,*
- *be able to calculate reference change values and appreciate their use in reporting, auto-verification and delta checking, and*
- *know how to analyse scientific and clinical guidelines that make use of numerical laboratory test results.*

# *A traditional report*

*The “recommended” laboratory report:*

<u>Matrix</u>	<u>Component</u>	<u>Result</u>	<u>Units</u>
<i>serum</i>	<i>sodium</i>	<i>139</i>	<i>mmol/L</i>

*It is also recommended [by IFCC] that laboratories report reference values or other aids to interpretation [here, 135 - 147 mmol/L].*

*Reference values not always appropriate - fixed limits are "trendy"*



- *glucose/GGT - ADA, WHO*
- *cholesterol/lipids - NCEP and others*
- *many dynamic function tests*
- *many modern guidelines - troponins, hs-CRP, etc*

## *Use of reference values*

*Reference values most often used as aids to interpretation in:*

- *screening*
- *case-finding*
- *diagnosis*

*But – as we have seen in previous presentation individuality makes of less value unless very well stratified by age and gender*

*Monitoring - investigation of serial results in an individual*



*Change in serial results due not only to -  
patient improving or deteriorating  
but also to  
pre-analytical, analytical and biological  
sources of variation.*

## *Monitoring a patient over time before and after statin Rx*

<i>Time</i>	<i>Alb</i>	<i>AP</i>	<i>Bili</i>	<i>ALT</i>	<i>AST</i>
	<i>g/L</i>	<i>U/L</i>	<i>μmol/L</i>	<i>U/L</i>	<i>U/L</i>
<i>Initial results</i>	<i>38</i>	<i>89</i>	<i>10</i>	<i>18</i>	<i>16</i>
<i>Pre-dose</i>	<i>42</i>	<i>81</i>	<i>12</i>	<i>14</i>	<i>18</i>
<i>After 2 months</i>	<i>40</i>	<i>86</i>	<i>9</i>	<i>27</i>	<i>24</i>
<i>After 4 months</i>	<i>41</i>	<i>94</i>	<i>13</i>	<i>35</i>	<i>40</i>
<i>After 6 months</i>	<i>39</i>	<i>85</i>	<i>8</i>	<i>45</i>	<i>54</i>

## *The BNF advice on interpretation*

*“Treatment should be discontinued if serum transaminase concentration rises to, and persists at, 3 times the upper limit of the reference range.”*

*A clinical criterion fixed limit  
based on a  
a population based fixed limit*



## *Reference change values*

*In order to decide whether a change is due to the patient improving or deteriorating, the “critical difference” or “reference change value” must be exceeded -*

*these depend on probability [Z], analytical [CV<sub>A</sub>] and within-subject biological [CV<sub>I</sub>] variation -  $RCV = 2^{1/2} \times Z \times [CV_A^2 + CV_I^2]^{1/2}$*

## *Calculation of RCV [P < 0.05]*

<u>Analyte</u>	<u>CV<sub>A</sub></u>	<u>CV<sub>I</sub></u>	<u>RCV</u>
<i>Albumin</i>	<i>0.8</i>	<i>3.1</i>	<i>8.6</i>
<i>AP</i>	<i>1.4</i>	<i>6.4</i>	<i>18.1</i>
<i>Bilirubins</i>	<i>1.0</i>	<i>25.6</i>	<i>70.9</i>
<i>ALT</i>	<i>0.9</i>	<i>24.3</i>	<i>67.3</i>
<i>AST</i>	<i>1.1</i>	<i>11.9</i>	<i>33.2</i>

## An example laboratory report

### BIOCHEMICAL MEDICINE

DMR 374

Tayside Clinical Laboratory Services

Telephone 660111 Ext 32601

Name:

Sex: M PID:

DoB: 21 Jan 1936

N/W Ward 20

Clinician: Dr S.

Lab No: C01487819

N20 CROS1H

SODIUM	139		mmol/L	(	135-147	)
POTASSIUM	4.3	*	mmol/L	(	3.5-5.0	)
UREA	17.2	**	mmol/L	(	3.3-6.6	)
CREATININE	103		umol/L	(	66-128	)
ALT	53	**	U/L	(	13-43	)
BILIRUBINS	35	>	umol/L	(	0-17	)
ALKALINE PHOSPHATASE	236	>	U/L	(	45-130	)
ALBUMIN	21	<<	g/L	(	36-50	)
CALCIUM	2.03	<	mmol/L	(	2.10-2.55	)
CALCIUM (CORRECTED)	2.48		mmol/L	(	2.10-2.55	)
MAGNESIUM	1.05	*	mmol/L	(	0.70-1.15	)
PHOSPHATE	1.24	*	mmol/L	(	0.80-1.50	)
C-REACTIVE PROTEIN	352	>>	mg/L	(	up to 5	)

Lab. Comments:

Sample Date/Time

10 Oct 2001 07:30

Request Entered: 10 Oct 2001 09:13

Report Printed: 12 Oct 2001

REPORT RECEIVED

DOCTOR'S INITIALS

## *Our approach to using reference values and reference change values*

- *“no flag” means inside RI AND no changes*
- *< means lower than LRL*
- *> means higher than URL*
- *\* means significant change at  $P < 0.05$*
- *<< means significantly lower than LRL*
- *>> means significantly higher than URL*
- *\*\* means significant change at  $P < 0.01$*

## *Auto-verification*

*Population-based “reference values” - best age and sex matched - are often used in AUTO-VERIFICATION -*

*are other data, based upon biological variation, of value in this context?*

## *Disadvantages of reference values in auto-verification*

- *By definition, 5% outside reference values.*
- *Because of inherent variation, some will have values that change from inside to outside reference intervals.*
- *Individuality means some always have values outside reference limits.*
- *Individuality means some always have values inside reference limits in spite of the occurrence of significant change for them.*

## *An example approach*

*do NOT hold for review - results with*

- *“no flag” - means inside RI AND no changes*
- *< - means lower than LRL*
- *> - means higher than URL*
- *\* - means significant change at  $P < 0.05$*

*the “only” results held are those flagged as follows*

- *<< - means significantly lower than LRL*
- *>> - means significantly higher than URL*
- *\*\* - means significant change at  $P < 0.01$*

## *Setting delta check values*

### *A. Scientific*

- collect large numbers of consecutive pairs of patient data*
- $\Delta$  -values are calculated and then plotted in a frequency distribution histogram*
- $\Delta$  - values to be used are set at either 5% or 1%*



## *Setting delta check values*

- B. Empirical - set  $\Delta$ -values on experience  
- adjust with time so as not to  
generate too many failures*
  
- C. Use RCV - since these are objectively set  
values taking analytical  
imprecision and biological  
variation into account*

## *Are published protocols based on laboratory data appropriate?*

- *Is internal QC performed in your laboratory?*
- *Do you have access to the internet?*
- *If “yes” to both questions - you too can become an expert in deciding whether the many published guidelines that are based on numerical clinical laboratory data are appropriate.*

# *Cholesterol - analytical aspects*

*Current status of blood cholesterol measurement in clinical laboratories in the United States: a report from the Laboratory Standardization Panel of the National Cholesterol Education Program*

*Clinical Chemistry 1988 34: 193-201*

*NCEP maximum allowable imprecision - CV 3%*

## *Cholesterol - practical aspects*



*A minimum of two blood lipid measurements, at least one of which should be fasting, should be made before commencing lipid lowering drug therapy.*

**S I G N**  
Scottish Intercollegiate Guidelines Network

Lipids and the Primary Prevention of  
Coronary Heart Disease

## *A single analysis of cholesterol*

- *The test result is usually reported as a single number, but it represents a range of numbers, due to -*
- *analytical random variation - imprecision - CVa, and*
- *inherent biological variation - within-subject biological variation - CVb.*

## *Addition of variations*

- *Addition of variations cannot be done by simple addition of CV - variations MUST be mathematically manipulated as variance -  $CV^2$ .*
- *The total variance is the sum of the variances.*
- *If we have analytical imprecision of  $CVa$  and the biological variation is  $CVb$ , then -*

$$CVt^2 = CVa^2 + CVb^2$$

$$CVt = [CVa^2 + CVb^2]^{1/2}$$

## *Dispersion of a cholesterol test result*

- $CVt = [CVa^2 + CVb^2]^{1/2}$
- $CVa$  - NCEP says 3% - so we have accepted this
- $CVb$  - 6% - this is a constant - easily found from [www.westgard.com/guest26.htm](http://www.westgard.com/guest26.htm)
- thus,  $CVt = [3.0^2 + 6.0^2]^{1/2} = [9 + 36]^{1/2} = 6.7\%$

## *Actual dispersion of the result*

*to get the actual range in which the test result lies - say 5.00 mmol/L - the CV must be multiplied by the Z-score appropriate for the selected probability*

*1.96 - for 95% probability [ $P < 0.05$ ]*

*2.58 - for 99% probability [ $P < 0.01$ ]*

*95% dispersion for cholesterol =  $\pm 1.96 [CVa^2 + CVb^2]^{1/2}$*

*5.00 mmol/L lies, with 95% probability, between*

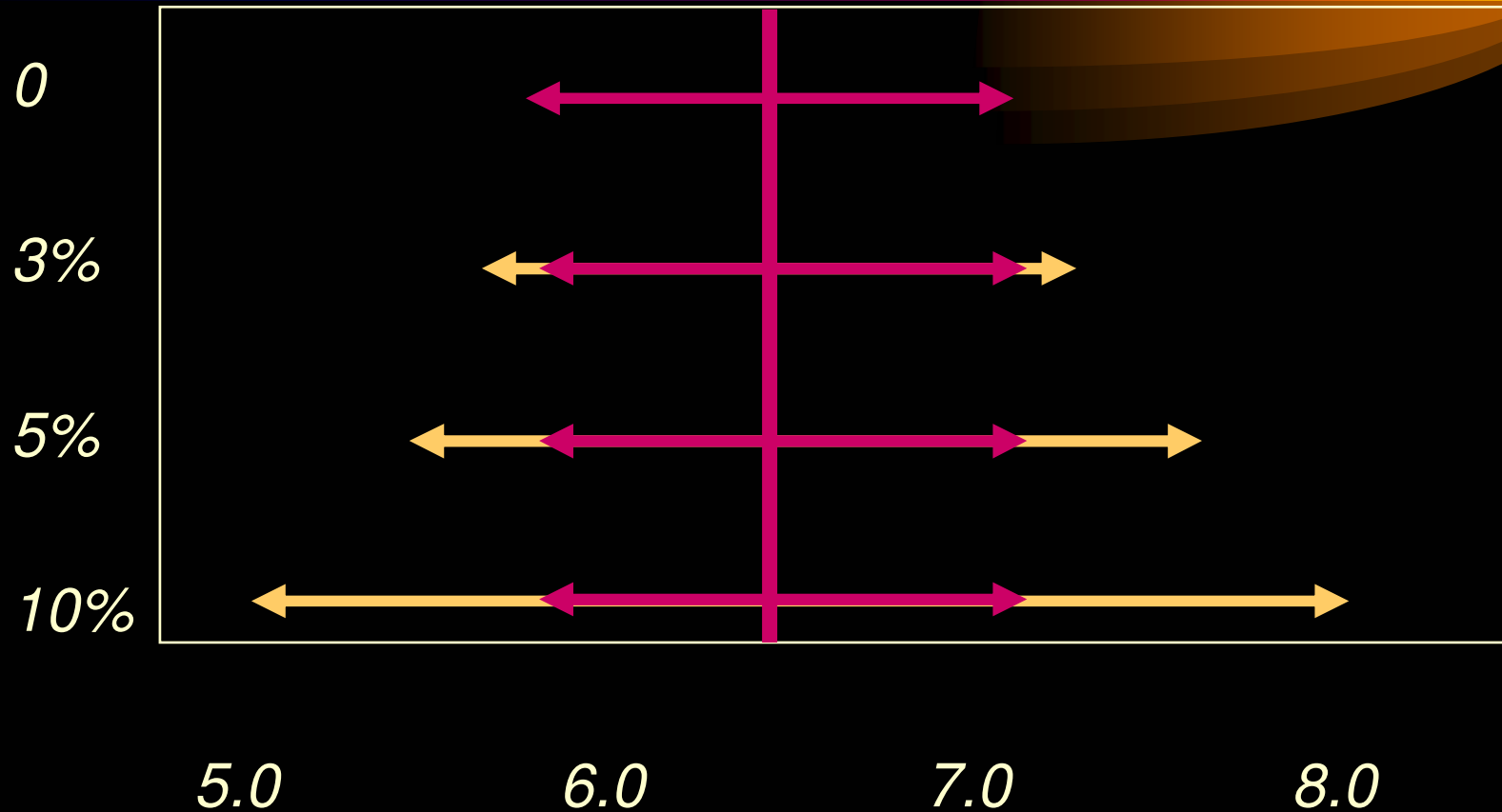
*$5.00 \pm 1.96 [3.0^2 + 6.0^2]^{1/2} = 5.00 \pm 0.66 = 4.34 - 5.66$  mmol/L*



## *Effect of imprecision on dispersion*

<u><i>CVa</i></u>	<u><i>[CVa<sup>2</sup> + CVb<sup>2</sup>]<sup>1/2</sup></i></u>	<u><i>95% Dispersion</i></u>
<i>1.0 %</i>	<i>6.1 %</i>	<i>4.39 - 5.61</i>
<i>3.0 %</i>	<i>6.7 %</i>	<i>4.34 - 5.66</i>
<i>6.0 %</i>	<i>8.5 %</i>	<i>4.17 - 5.83</i>
<i>10.0 %</i>	<i>11.7 %</i>	<i>3.85 - 6.15</i>

# *Influence of imprecision on dispersion of a single result - cholesterol*



## *The effect of replicates*

*Replicates reduce the variation  
by the square root of the number*

$$CV_t = [CV_a^2/n + CV_b^2/n]^{1/2}$$

*Number of  
assays*

*Number of  
samples*

## *Effect of replicate assays on dispersion of one sample*

<u><i>n</i></u>	<u><i>CV<sub>a</sub></i></u>	<u><math>[CV_a^2/n + CV_b^2]^{1/2}</math></u>	<u><i>Dispersion</i></u>
<i>1</i>	<i>3.0 %</i>	<i>6.7 %</i>	<i>4.34 - 5.66</i>
<i>2</i>	<i>2.1 %</i>	<i>6.4 %</i>	<i>4.37 - 5.63</i>
<i>3</i>	<i>1.7 %</i>	<i>6.2 %</i>	<i>4.39 - 5.61</i>
<i>4</i>	<i>1.5 %</i>	<i>6.1 %</i>	<i>4.40 - 5.60</i>

# *Effect of replicate samples on 95% dispersion analysed once*

<u><i>n</i></u>	<u><i>CVa</i></u>	<u><math>[CVa^2 + CVb^2/n]^{1/2}</math></u>	<u><i>Dispersion</i></u>
<i>1</i>	<i>3.0 %</i>	<i>6.7 %</i>	<i>4.34 - 5.66</i>
<i>2</i>	<i>3.0 %</i>	<i>5.2 %</i>	<i>4.49 - 5.51</i>
<i>3</i>	<i>3.0 %</i>	<i>4.6 %</i>	<i>4.55 - 5.55</i>
<i>4</i>	<i>3.0 %</i>	<i>4.2 %</i>	<i>4.58 - 5.42</i>

## *Ramifications for guideline developers*

- *Make imprecision low to cut down the analytical “noise” so that the biological “signal” is not confounded.*
- *Inspect the comparative magnitudes of imprecision and biological variation.*
- *If imprecision > biological variation, then reduce the analytical noise through method improvement OR do the analyses in replicate.*
- *If biological variation is high, then take more than one sample and use the mean of the two results.*

## *A further simple calculation*

*Number of samples required*

$$n = [Z * [CVa^2 + CVb^2]^{1/2}/D]^2$$

*where n is number of samples needed to get D % closeness to the homeostatic setting point with a probability dependent on Z [the number of SD].*

## *A further simple calculation*

*For cholesterol - if we want to get within  
 $\pm 10\%$  with 95% probability, then*

$$n = [Z * [CVa^2 + CVb^2]^{1/2}/D]^2$$

$$= [1.96 * [3.0^2 + 6.0^2]^{1/2}/10]^2 = 2$$



## *The pertinent question*



*Many hundreds, if not thousands of guidelines since 1988 - usually from professional groups - do these stand up to the same rigorous scrutiny?*

## *Example statements in guidelines*

- *Anti-epileptic drugs - long term CV of no more than 10% and preferably < 5%.*
- *Theophylline - precision and accuracy over several days should be such that CV is < 5%.*
- *Cardiac troponin should have CV < 10% at the AMI decision limit.*
- *Assays for ALT activity should have total error of < 10%.*
- *Assays for GGT activity should have total error of < 20%.*

*hs-CRP*



***AHA/CDC Scientific Statement: Markers of  
Inflammation and Cardiovascular Disease  
-- Application to Clinical and Public Health  
Practice***

*Circulation 2003;107:499-511*

## *Scientific Statement Summary*

*“it is most reasonable to limit current assays to hs-CRP, measured twice with the average expressed in mg/L, in metabolically stable patients. Relative risk categories [low, average, high] correspond to approximate tertiles of values [ $<1.0$ , 1.0 to 3.0, and  $>3.0$  mg/dL respectively] based on an aggregation of population studies”.*

## *Scientific Statement*



*“Most of the acute-phase reactant assays have acceptable precision.”*

## *Imprecision of hs-CRP assays*



*Scientific Statement says -*

*“It needs to be emphasized that the assays considered [in Table 2] were for hs-CRP with acceptable precisions down to or below 3.0 mg/L”*

*The fact-*

*in Table 2 - CV < 10%*

# *Scientific Statement*



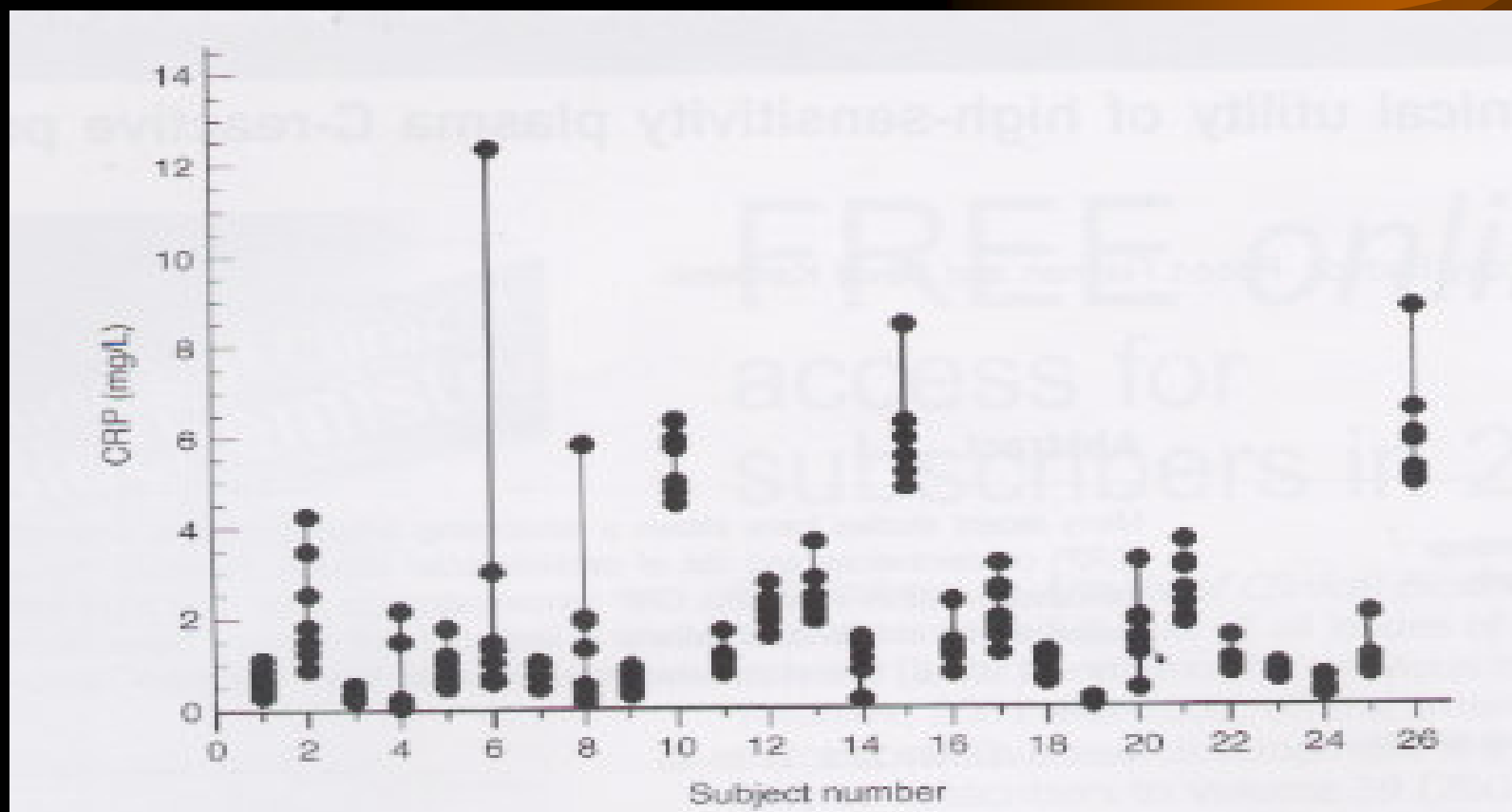
*Scientific Statement says*

*“Considerable within-individual variability exists,  
however for hs-CRP....*

*In fact.....*

*see superb review in Clinical Chemistry 2003;49:1528-1271*

## Biological variation of CRP



*Macy EM et al Clin Chem 1997;43:52-8*

Biological Variation 2 - Prague - 16 May 2006



## *Scientific Statement Summary*



*“The final result is that, in a manner similar to cholesterol, two separate measurements of hs-CRP are adequate to classify a person’s risk level and to account for the increased within-subject variability”.*

## *Number needed*



$$n = [Z * [CVa^2 + CVb^2]^{1/2}/D]^2$$

*if, like cholesterol, we want to get within 10%  
with 95% probability*

$$n = [1.96 * [10^2 + 30^2]^{1/2}/10]^2 = 38$$

## *Total CV for hs-CRP*

$$CV_t = [CV_a^2/n + CV_b^2/n]^{1/2}$$


$$CV_t = [10^2 + 30^2/2]^{1/2} = 23.5\%$$

*Dispersion [95%] of a result of 0.9 mg/L is  
0.5 - 1.3 mg/L*

*Dispersion [95%] of a result of 2.2 mg/L is  
1.2 - 3.2 mg/L*

*Dispersion [95%] of a result of 4.0 mg/L is  
2.2 - 5.8 mg/L*

# *Effect of reducing imprecision on CVt with two samples*



<u>CVa</u>	<u>CVt</u>
10.0 %	23.5 %
5.0 %	21.8 %
2.0 %	21.3 %
1.0 %	21.2 %

# *Effect on CVt of multiple samples analysed once*



<u>Number</u>	<u>CVt</u>
2	23.5 %
3	20.0 %
4	18.0%
5	16.7%
6	15.8%

## *hs-CRP*



- *Is hs-CRP like cholesterol?*
- *Does hs-CRP have "a degree of measurement stability that is similar to that of total cholesterol"?*
- *Is the Scientific Statement flawed - what do you think - objectivity, please?*

*BNP*



*Apple FS, Panteghini M, Ravkilde J, Mair J,  
Wu AHB, Tate J, Pagani F, et al.*

*Quality specifications for B-type natriuretic  
peptide assays.*

*Clin Chem 2005;51:486-93.*

## *Recommendations*

*“because of high biological variation for BNP ( $CV_I$  - 30–50%), very low  $CV_A$  may be unnecessary:*

*however, for monitoring of therapy with serial BNP measurements in clinical cases, it may be desirable to minimize  $CV_A$ .”*



## *Recommendations*

*“Furthermore ... desirable  $CV_A$  of  $<15\%$  at BNP concentrations within the reference interval is recommended.*

*If an eventual goal is to rely on monitoring of marker trends over time, then an optimal imprecision of  $<10\%$  would be advocated.”*

## *Effect of imprecision on RCV*

*A change in serial results only occurs if RCV exceeded.*

*RCV are easily calculated as*

$$RCV = 2^{1/2} \times Z \times [CV_A^2 + CV_I^2]^{1/2}.$$

*If  $CV_A$  was 15%, then the RCV for*

*$P < 0.05$  would be 118% and,*

*if  $CV_A$  was 10%, the RCV would be 114%.*

*The recommendation is NOT cogent!*

*The statement made that an optimal  $CV_A$  of 10% would be advocated for monitoring individuals is NOT evidence-based.*

*If  $CV_I$  is much larger than  $CV_A$  then it is simply not worthwhile reducing  $CV_A$  to less than one-half of  $CV_A$  even in this clinical use of results.*

*To reduce RCV, must take multiple samples [not addressed in the paper].*

## *Conclusions*

- *Easy to calculate RCV and use in reporting, auto-verification and delta checking.*
- *Easy to calculate number of samples, dispersions and RCV and other useful indices from readily available data -  
CVa [IQC/PT] and CVb [database].*
- *Authors of scientific statements, guidelines, recommendations, etc, should do such calculations - BEFORE promulgation of their publications!*