Biological Variation:
From Principles to Practice

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Dundee     Scotland
Content and expected outcomes

After this lecture, participants should:

• be able to list the types of biological variation,
• know how to generate and/or find data on random biological variation, and
• appreciate how to use the data in laboratory medicine.
Content and expected outcomes

Participants should appreciate how to use data on biological variation in:

• setting quality specifications,
• assessing the significance of changes in serial results from an individual,
• deciding the utility of conventional population based reference values, and
• managing aspects of quality.
Biological variation

Variation over the span of life
Predictable rhythmical/cyclical variation
Random variation around setting points
Life-long biological variation

Some analytes change over the span of life -

• neonatal period
• childhood
• puberty
• menopause
• old age

This is taken care of by creation of age stratified reference values when needed.
Predictable rhythmical/cyclical rhythms

- **Daily**
  - cortisol, growth hormone

- **Monthly**
  - LH, FSH, progesterone

- **Seasonal**
  - vitamin D - also cholesterol, HbA1c
Problems associated with rhythms/cycles

• it is impossible to develop good reference values for every time point during the cycle
• knowledge of the expected values throughout the cycle is vital for clinical interpretation
• for some analytes, samples should be taken at relevant times for the clinical purpose
• absence of the expected cycle may provide clinically useful information
**Most analytes - random variation**

A series of four results taken from an individual

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Unit</th>
<th>Results</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>mmol/L</td>
<td>137, 139, 136, 138</td>
<td>135 - 147</td>
</tr>
<tr>
<td>Potassium</td>
<td>mmol/L</td>
<td>4.3, 4.6, 4.5, 4.4</td>
<td>3.5 - 5.0</td>
</tr>
<tr>
<td>Urea</td>
<td>mmol/l</td>
<td>4.0, 4.4, 4.1, 3.9</td>
<td>3.3 - 6.6</td>
</tr>
<tr>
<td>Creatinine</td>
<td>μmol/L</td>
<td>88, 97, 89, 92</td>
<td>64 - 120</td>
</tr>
<tr>
<td>Bilirubins</td>
<td>μmol/L</td>
<td>19, 21, 16, 20</td>
<td>up to 17</td>
</tr>
</tbody>
</table>

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Pre-analytical sources of variation

- Preparation of subject
  - fasting
  - exercise
  - posture
Sources of random variation

• Pre-analytical - preparation of subject
  sample collection and handling

Examples of increase in large molecules
and quantities bound to large molecules
on venous stasis
Pre-analytical sources of variation

- Preparation of subject
  - fasting
  - exercise
  - posture

- Sample collection and handling
  - type of sample
  - anticoagulant
  - tourniquet
  - transport time
  - centrifugation
Sources of random analytical variation

Analytical - imprecision
changes in bias
Sources of random analytical variation

Imprecision - random error - usually expressed as SD or CV \[SD/\text{mean}] \times 100

- intrinsic to all measurements,
- due to factors such as variations in temperature, volume of sample or reagent delivered, AND
- is method dependent.
Sources of random analytical variation

Bias - systematic error [due to calibration and other constant sources of 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 a source of variation in serial results
## A quality control material for sodium - replicate analyses

<table>
<thead>
<tr>
<th>Series 1</th>
<th>Series 2</th>
</tr>
</thead>
</table>
| 140      | 140      | Series 1 done with one calibrator - mean = 140 mmol/L  
| 141      | 142      | SD = 1.15 mmol/L  
| 139      | 141      |  
| 140      | 141      |  
| 142      | 139      | Series 2 - after re-calibration  
| 138      | 143      | mean = 141 mmol/L  
| 139      | 140      | SD = 1.15 mmol/L  
| 141      | 141      |  
| 140      | 142      | Overall SD = 1.24 mmol/L  
| 140      | 141      |  

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Sources of random variation

- **Pre-analytical** - preparation of subject sample collection/handling

- **Analytical** - imprecision changes in bias

- **Biological** - within-subject biological variation
Random test result variation

A series of four results taken from each of a cohort of four individuals - sodium [mmol/L]

| Individual 1 | 137 | 139 | 136 | 138 | [135 -147] |
| Individual 2 | 144 | 146 | 145 | 144 | [135 -147] |
| Individual 3 | 141 | 143 | 142 | 140 | [135 -147] |
| Individual 4 | 136 | 138 | 137 | 135 | [135 -147] |

Homeostatic setting points do vary amongst individuals - between-subject biological variation
## Variation of derived indices

<table>
<thead>
<tr>
<th>Date</th>
<th>Creat</th>
<th>Urea</th>
<th>Alb</th>
<th>Age</th>
<th>eGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 March 1992</td>
<td>92</td>
<td>4.4</td>
<td>45</td>
<td>47</td>
<td>87.4</td>
</tr>
<tr>
<td>21 April 1995</td>
<td>97</td>
<td>4.3</td>
<td>46</td>
<td>50</td>
<td>82.9</td>
</tr>
<tr>
<td>23 January 1996</td>
<td>86</td>
<td>3.7</td>
<td>45</td>
<td>51</td>
<td>94.9</td>
</tr>
<tr>
<td>28 August 1998</td>
<td>94</td>
<td>4.7</td>
<td>44</td>
<td>53</td>
<td>82.3</td>
</tr>
<tr>
<td>12 July 1999</td>
<td>88</td>
<td>4.3</td>
<td>44</td>
<td>54</td>
<td>88.9</td>
</tr>
<tr>
<td>13 January 2003</td>
<td>89</td>
<td>4.4</td>
<td>42</td>
<td>58</td>
<td>85.2</td>
</tr>
</tbody>
</table>

*Derived indices have random variation over time!*

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Generation of numerical estimates of components of biological variation

- select a small number of reference individuals
- apply exclusion criteria [just as for reference values]
- 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 $CV_A$, $CV_P$, $CV_G$
Mean and absolute ranges for serum creatinine in 10 subjects
To generate or not to generate?

It is dogma that all laboratories 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!
### Within-subject biological variation of sodium and urea as CV

<table>
<thead>
<tr>
<th>No</th>
<th>Time</th>
<th>Sex</th>
<th>Na</th>
<th>Urea</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2 weeks</td>
<td>M</td>
<td>0.7</td>
<td>12.3</td>
<td>Denmark</td>
</tr>
<tr>
<td>10</td>
<td>4 weeks</td>
<td>M</td>
<td>0.9</td>
<td>14.3</td>
<td>USA</td>
</tr>
<tr>
<td>10</td>
<td>8 weeks</td>
<td>M</td>
<td>0.6</td>
<td>9.5</td>
<td>Germany</td>
</tr>
<tr>
<td>14</td>
<td>8 weeks</td>
<td>F</td>
<td>0.5</td>
<td>11.3</td>
<td>Germany</td>
</tr>
<tr>
<td>9</td>
<td>12 weeks</td>
<td>M</td>
<td>1.4</td>
<td>13.6</td>
<td>USA</td>
</tr>
<tr>
<td>11</td>
<td>15 weeks</td>
<td>M</td>
<td>0.6</td>
<td>15.7</td>
<td>Denmark</td>
</tr>
<tr>
<td>37</td>
<td>22 weeks</td>
<td>M</td>
<td>0.5</td>
<td>11.1</td>
<td>England</td>
</tr>
<tr>
<td>15</td>
<td>40 weeks</td>
<td>M&amp;F</td>
<td>0.7</td>
<td>13.9</td>
<td>Scotland</td>
</tr>
</tbody>
</table>
## Biological variation in young and elderly as CV

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Young</th>
<th>Elderly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Urea</td>
<td>13.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Albumin</td>
<td>2.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.9</td>
<td>5.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.8</td>
<td>4.7</td>
</tr>
</tbody>
</table>
### Within-subject variation in urine as CV

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Australia</th>
<th>Scotland</th>
<th>Spain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>28.0</td>
<td>26.5</td>
<td>28.7</td>
</tr>
<tr>
<td>Calcium</td>
<td>25.1</td>
<td>26.2</td>
<td>27.5</td>
</tr>
<tr>
<td>Creatinine</td>
<td>11.2</td>
<td>11.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Phosphate</td>
<td>16.6</td>
<td>16.9</td>
<td>20.6</td>
</tr>
</tbody>
</table>
Over the years, many compilations, most recent -


2006 update at www.westgard.com/guest32.htm

Available at www.aacc.org
Estimation and Application of Biological Variation of Urinary Delta-Aminolevulinic Acid and Porphobilinogen in Healthy Individuals and in Patients with Acute Intermittent Porphyria

Aasne K. Aarsand¹,a, Per Hyltoft Petersen² and Sverre Sandberg¹,²
Application of data on biological variation

- setting quality specifications
- assessing the significance of changes in serial results from an individual
- deciding the utility of conventional population based reference values
- other uses in quality management
**Setting quality specifications**

Imprecision $< x \cdot CV_1$

*For most situations, $x$ should be 0.5 [1/2].*
Setting quality specifications

\[ \text{Bias} < y \cdot [CV_I^2 + CV_G^2]^{1/2} \]

For most situations, \( y \) should be 0.25 \([1/4]\).
Setting quality specifications

- Total error < $Z \cdot x \cdot CV_I + y \cdot [CV_I^2 + CV_G^2]^{1/2}$

- Other specifications based on biology include:
  - reference methods
  - fixed limits for EQAS
  - allowable difference between 2 methods.
Application of data

- setting quality goals
- **assessing the significance of changes in serial results from an individual**
- deciding the utility of conventional population based reference values
- other uses in quality management
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” that would be expected due to inherent sources of variation must be exceeded

RCV depend on probability \([Z]\), analytical \([CV_A]\) and within-subject biological \([CV_I]\) variation, if pre-analytical variation is minimized -

\[
\text{Change} > RCV = 2^{1/2} \times Z \times [CV_A^2 + CV_I^2]^{1/2}
\]
Reference change values

Each result has a dispersion dependent on - probability \([Z]\), analytical \([CV_A]\) and biological \([CV_I]\) variation -

First result - \(Z \times [CV_A^2 + CV_I^2]^{1/2}\)
Second result - \(Z \times [CV_A^2 + CV_I^2]^{1/2}\)

Total variation = [sum of squares]^{1/2}

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

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

- use $2^{1/2}$ because we have 2 samples
- use 1.96 and 2.58 as the Z-scores for significant and highly significant respectively
- use $CV_A$ from your own IQC program - at “clinically significant levels”
- use $CV_I$ from the most recent data base available [as per Carmen Ricos and colleagues]
# An example laboratory report

## Biochemical Medicine

**Tayside Clinical Laboratory Services**

- **Name:** [Blank]
- **Sex:** M
- **PID:** [Blank]
- **DoB:** 21 Jan 1936
- **Lab No:** C091687819
- **Ward:** 20
- **Clinician:** Dr S.

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>139 mmol/L</td>
<td>135-147 mmol/L</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.3 mmol/L</td>
<td>3.5-5.0 mmol/L</td>
</tr>
<tr>
<td>Urea</td>
<td>17.2 mmol/L</td>
<td>3.3-6.6 mmol/L</td>
</tr>
<tr>
<td>Creatinine</td>
<td>103 umol/L</td>
<td>66-128 umol/L</td>
</tr>
<tr>
<td>ALT</td>
<td>53 U/L</td>
<td>13-43 U/L</td>
</tr>
<tr>
<td>Bilirubins</td>
<td>35&gt; umol/L</td>
<td>0-17 umol/L</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>236&gt; U/L</td>
<td>45-130 U/L</td>
</tr>
<tr>
<td>Albumin</td>
<td>21&lt; g/L</td>
<td>36-50 g/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.03 mmol/L</td>
<td>2.10-2.55 mmol/L</td>
</tr>
<tr>
<td>Calcium (Corrected)</td>
<td>2.48 mmol/L</td>
<td>2.10-2.55 mmol/L</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.05 mmol/L</td>
<td>0.70-1.15 mmol/L</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.24 mmol/L</td>
<td>0.80-1.50 mmol/L</td>
</tr>
<tr>
<td>C-Reactive Protein</td>
<td>352&gt; mg/L</td>
<td>up to 5 mg/L</td>
</tr>
</tbody>
</table>

**Lab Comments:**

- **Sample Date/Time:**
  - **Sample Date:** 10 Oct 2001
  - **Sample Time:** 07:30

**Request Entered:**
- **10 Oct 2001 09:13**

**Report Printed:**
- **12 Oct 2001**

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**Biological Variation 1 - Prague - 16 May 2006**
Application of RCV

- in reporting of results to aids clinical interpretation
- in auto-verification
- in delta checking

This will be explored in Biological Variation 2 in more detail.
Application of data

• setting quality goals
• assessing the significance of changes in serial results from an individual
• deciding the utility of conventional population based reference values
• other uses in quality management
**Individuality and reference values: creatinine**

![Graph showing individual creatinine values for different subjects.](image)
Ranges for 4 subjects for creatinine and iron

Creatinine

There is LITTLE variation within each individual but much BETWEEN individuals

Iron

MUCH variation within each individual but LITTLE between individuals

There is LITTLE variation within each individual but much BETWEEN individuals

MUCH variation within each individual but LITTLE between individuals

Biological Variation 1 - Prague - 16 May 2006
Index of individuality

- this is easily calculated as \(-CV_I/CV_G\)

- it is important to note that a low index of individuality means that the analyte has marked individuality whereas a high index of individuality means that the analyte has little individuality

- creatinine has II of 0.33, iron has II of 1.4
Interpretation of index of individuality

- When II is low [0.6], values for any individual span only a small part of the reference interval. Reference values will be of little utility, particularly when deciding whether changes in an individual have occurred. RCV preferred!

- In contrast, when II is high [1.4], values from an individual will cover much of distribution of the reference interval de. Thus, conventional reference values will be of significant value in many clinical settings.
Ranges for 8 women and 7 men for urinary creatinine output
## Individuality of urine creatinine

<table>
<thead>
<tr>
<th>Group</th>
<th>Within</th>
<th>Between</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>13.0</td>
<td>28.2</td>
<td>0.46</td>
</tr>
<tr>
<td>Women</td>
<td>15.7</td>
<td>11.0</td>
<td>1.42</td>
</tr>
<tr>
<td>Men</td>
<td>11.0</td>
<td>6.0</td>
<td>1.83</td>
</tr>
</tbody>
</table>

This provides a scientific basis why stratification of reference values is often desirable.
Is electrophoresis of serum to look for abnormal immunoglobulins an obsolete test? Proposal - measure IgG, IgA, IgM, kappa-chains [κ] and lambda-chains [λ] and calculate the heavy [IgG + IgA + IgM] to light chain [κ + λ] ratio and the κ/λ ratio to see if there was abnormal immunoglobulin and what type of protein was present.

Can biological variation data shed light on the clinical utility of this approach to diagnosis?
### Individuality of Immunoglobulins and Derived Ratios

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Within</th>
<th>Between</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>4.4</td>
<td>13.0</td>
<td>0.34</td>
</tr>
<tr>
<td>IgA</td>
<td>5.0</td>
<td>35.0</td>
<td>0.14</td>
</tr>
<tr>
<td>IgM</td>
<td>5.9</td>
<td>48.5</td>
<td>0.12</td>
</tr>
<tr>
<td>κ</td>
<td>4.8</td>
<td>15.3</td>
<td>0.31</td>
</tr>
<tr>
<td>λ</td>
<td>4.8</td>
<td>17.3</td>
<td>0.28</td>
</tr>
<tr>
<td>κ/λ ratio</td>
<td>0.7</td>
<td>12.1</td>
<td>0.06</td>
</tr>
<tr>
<td>H/L ratio</td>
<td>4.2</td>
<td>4.8</td>
<td>0.87</td>
</tr>
</tbody>
</table>
Ranges for the $\kappa/\lambda$ ratio

1.3  1.5  1.7  1.9  2.1
## Individuality of hematological analytes

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Within</th>
<th>Between</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>hemoglobin</td>
<td>2.8</td>
<td>6.6</td>
<td>0.42</td>
</tr>
<tr>
<td>hematocrit</td>
<td>2.8</td>
<td>6.6</td>
<td>0.42</td>
</tr>
<tr>
<td>MCV</td>
<td>1.3</td>
<td>4.8</td>
<td>0.27</td>
</tr>
<tr>
<td>erythrocytes</td>
<td>3.2</td>
<td>6.1</td>
<td>0.52</td>
</tr>
<tr>
<td>leukocytes</td>
<td>10.4</td>
<td>27.8</td>
<td>0.37</td>
</tr>
<tr>
<td>platelets</td>
<td>9.1</td>
<td>21.9</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Individuality

• few analytes have II greater than 1.4
• most analytes have II less than 0.6
• reference values usually not of great utility - especially for monitoring
• hardly surprising that laboratory tests not good for case finding or screening
• stratification of reference values increases II
Number of samples needed to ensure estimate is within a certain percentage of the homeostatic setting point with a predetermined probability –

\[ n = (Z \times [CV_A^2 + CV_I^2]^{1/2}/D)^2 \]

where \( Z \) is the Z-score appropriate for the probability eg 1.96 for \( P < 0.05 \) and

\( D \) is the desired percentage closeness.
An example - cholesterol

- within-subject variation $CV_I = 6\%$ maximum imprecision - NCEP $CV_A = 3\%$
- estimate to be within 5\% of the true homeostatic setting point with 95\% probability

$$n = (Z \times [CV_A^2 + CV_I^2]^{1/2}/D)^2 = (1.96 \times [3^2 + 6^2]^{1/2}/5)^2 = 7$$

decreasing the number of samples required done by -
- lowering probability
- increasing window of acceptability
- decreasing imprecision
### Selection of best sample

Selecting the best sample to collect: microalbumin

<table>
<thead>
<tr>
<th>Sample type</th>
<th>$CV_I$</th>
<th>$CV_G$</th>
</tr>
</thead>
<tbody>
<tr>
<td>First morning</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>Random spot</td>
<td>86</td>
<td>61</td>
</tr>
<tr>
<td>24 hour as concentration</td>
<td>61</td>
<td>53</td>
</tr>
<tr>
<td>24 hour as output</td>
<td>70</td>
<td>55</td>
</tr>
</tbody>
</table>

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### Comparison of tests

<table>
<thead>
<tr>
<th>Analyte</th>
<th>$CV_I$</th>
<th>$CV_G$</th>
</tr>
</thead>
<tbody>
<tr>
<td>cystatin-C</td>
<td>13.3</td>
<td>8.1</td>
</tr>
<tr>
<td>creatinine</td>
<td>4.9</td>
<td>18.2</td>
</tr>
</tbody>
</table>

consider individuality and RCV - cystatin-C will be better in diagnosis [less individual] but creatinine better in monitoring [smaller RCV].
Comparison of creatinine and cystatin-C

There is MUCH variation between each individual - high individuality

There is LITTLE variation between each individual - low individuality

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Generation and application of data on biological variation

Usually not necessary to generate your own data - use existing database - then apply in:

• setting quality specifications,
• assessing the significance of changes in serial results from an individual,
• deciding the utility of conventional population based reference values, and
• other uses in quality management.