Hitch-hiker’s Guide
to
Interpreting Serial Results

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Is the difference between consecutive serial results explained by inherent variability of the results?

OR

Is the magnitude of the difference larger than can be explained by inherent variation?

i.e. a real biological change that may be clinically significant?
<table>
<thead>
<tr>
<th>Collection Date</th>
<th>04/04/12</th>
<th>05/12/13</th>
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<tbody>
<tr>
<td>Request Number</td>
<td>93452782</td>
<td>33127940</td>
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</tbody>
</table>

**Analytical System:** Siemens Centaur

**Total PSA (ug/L)**

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>8.6</td>
</tr>
</tbody>
</table>
Can the assay reliably measure the difference in concentration between the two specimens?
PSA QC results for 3 months

Precision under intermediate reproducibility conditions

Can use data to estimate MU of each result
PSA QC results for 3 months

Mean value: 2.34 \( \mu \text{g/L} \)

SD \( (u_{QC}) \): 0.09 \( \mu \text{g/L} \)

CV: \( (u_{rel}) \): 3.8 %
CV: 3.8 % of 7.3 = SD = 0.28; 2 SD = 0.56 = 0.6 μg/L

8.6 = SD = 0.33; 2 SD = 0.66 = 0.7 μg/L
Total PSA (μg/L) 7.3 ± 0.6  g/L 8.6 ± 0.7  g/L

Interval of possible values (95 % probability):
6.7 - 7.9  g/L 7.9 - 9.3  g/L
Cannot add or subtract SDs – must use **variances**
\[ s_N = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (x_i - \bar{x})^2}, \]

Variance = \[ \frac{1}{N} \sum_{i=1}^{N} (x_i - \bar{x})^2, \]

SD^2 = variance

Combining SD_A and SD_B:

Combined SD = \( \sqrt{(SD_A^2 + SD_B^2)} \)

or using alternative notation for \( \sqrt{ \)

Combined SD = \( (SD_A^2 + SD_B^2)^{1/2} \)
\[ \text{SD} = 0.28 \quad \text{Variance} = 0.28^2 = 0.078 \]
\[ \text{SD} = 0.33 \quad \text{Variance} = 0.33^2 = 0.109 \]

Combined variance = 0.187

Combined SD = \( \sqrt{0.187} = 0.43 \) g/L

PSA g/L
For 95% probability: $0.43 \times 1.96 = 0.84 \text{ g/L}$

\[ \geq 0.8 \]

Bi-directional probability: Allows possibility that the 2 results could have been up/down due to analytical imprecision.

MP can reliably distinguish 7.3 and 8.6 g/L
Information of limited use because wrongly assumed
PSA concentration is constant at all times

Most measurand concentrations show significant
inherent variability over time

Biological variation
Biological variation

Within-individual biological variation

Between-individual biological variation
Within-individual biological variation

Concentration of many measurands in a presumed healthy individual fluctuate with time.

Minutes
Hours
Days
Weeks
Months
Biological variation within the individual

Many causes e.g.

*Endogenous factors:*
- gender, biological age, genes, ethnicity,
- homeostatic mechanisms e.g. serum TSH, calcium
- anabolic/catabolic balance, compartment distribution

*Exogenous factors:*
- lifestyle and environment e.g. nutritional intake, activity,
- alcohol/drugs, season
Quantifying within-individual biological variation

Presumed healthy’ subjects– gender & age range?

Standardise specimen collection:

Specimens: same time & day, same phlebotomist etc

Frequency: each week/fortnight/month

Analysis: Single run to reduce imprecision
Figure 1.10  Mean Values and Absolute Ranges of Serum Creatinine in Four Samples Taken from Each of 10 Apparently Healthy Men.
Total variability of results for each individual: $CV_T$

Repeatability imprecision of assay: $CV_A$

Within-individual biological variation: $CV_I$

$CV_T = (CV_A^2 + CV_I^2)^{1/2}$

$CV_I = (CV_T^2 - CV_A^2)^{1/2}$
For each individual:

Calculate Mean, total SD & total $CV_T\%$

Mean value = personal homeostatic set point
    for analyte concentration/enzyme activity

$CV_T\% = \text{combined analytical imprecision \& within subject BV}$

Express this relationship as relative variances

Calculate $CV_I$ for each individual

$$CV_I\% = (CV_T^2\% - CV_A^2\%)^{1/2}$$
Calculate the mean CV$_1$ % for the total individuals

Mean CV$_1$:

quantifies average variability of results
due to within-individual biological variation

(ANOVA – ANalysis Of VAriance often used for calculations)

Limitation:
Calculations assumes distribution of values around
personal set-point is Gaussian – often not true
Total biological variation = \((CV_I^2 + CV_G^2)^{1/2}\)
12 monthly measurements of serum TSH in healthy subjects

Fig. 2. The distribution of 12 monthly measurements of total T₄ in 15 healthy men (□) and in one individual, number 11 (■). The distribution in one individual is about half the width of the distribution in the group.
Index of Individuality (II) = \( \frac{CV_I}{CV_G} \)

II >0.6:
– RI relatively sensitive to abnormal values in the individual

II <0.6:
– RI relatively insensitive to abnormal values in the individual
Westgard Biological Database

Lists the extensive studies by Ricos et al - many measurands

www.westgard.com/biodatabase1

or

Google westgard biological
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Biological Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CVw</td>
</tr>
<tr>
<td>B- pCO2</td>
<td>4.8</td>
</tr>
<tr>
<td>B- pH [H+]</td>
<td>3.5</td>
</tr>
<tr>
<td>B- pH (pH units)</td>
<td>0.2</td>
</tr>
<tr>
<td>S- Paraoxonase 1</td>
<td>13.4</td>
</tr>
<tr>
<td>S- Paraoxonase 1 substrate inhibition (PON 4SI)</td>
<td>3.9</td>
</tr>
<tr>
<td>S- Paraoxonase, activity (salt stimulated)</td>
<td>8.0</td>
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<tr>
<td>S- Parathyroid hormone (PTH)</td>
<td>25.9</td>
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<tr>
<td>S- Phenylacetate</td>
<td>6.6</td>
</tr>
<tr>
<td>P- Phenylalanine</td>
<td>9.5</td>
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<tr>
<td>S- Phosphate</td>
<td>8.5</td>
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<td>U- Phosphate, concentration, 24h</td>
<td>26.4</td>
</tr>
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<td>U- Phosphate, output, 24h</td>
<td>18.0</td>
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<td>Patient- Phosphate tubular reabsorption</td>
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<tr>
<td>S- Phospholipids</td>
<td>6.5</td>
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<tr>
<td>B- Piruvate</td>
<td>15.2</td>
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<tr>
<td>P- Plasminogen</td>
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<tr>
<td>B- Platelets, count</td>
<td>9.1</td>
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<td>B- Platelet distribution wide</td>
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<td>B- Plateletcrit</td>
<td>11.9</td>
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<tr>
<td>U- Porphobilinogen</td>
<td>17.0</td>
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<tr>
<td>U- Porphyrins (total)</td>
<td>40.0</td>
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<tr>
<td>(B)Leuc- Potassium</td>
<td>13.6</td>
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<tr>
<td>S- Potassium</td>
<td>4.8</td>
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<tr>
<td>U- Potassium, concentration, 24h</td>
<td>27.1</td>
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<tr>
<td>U- Potassium, output</td>
<td>24.4</td>
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<tr>
<td>S- Prealbumin</td>
<td>10.9</td>
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<tr>
<td>S- Prolactin</td>
<td>23.0</td>
</tr>
<tr>
<td>P- Proline</td>
<td>17.0</td>
</tr>
<tr>
<td>P- Prolyl endopeptidase</td>
<td>16.8</td>
</tr>
<tr>
<td>S- Properdin factor D</td>
<td>9.5</td>
</tr>
<tr>
<td>S- Prostatic specific antigen (PSA)</td>
<td>18.1</td>
</tr>
</tbody>
</table>

**PSA**

$CV_I: \sim 18\%$

$CV_A: \sim 2-3\%$
Analytical imprecision (CV$_A$): unavoidable cause of result variability

Within-individual biological variation (CV$_I$): unavoidable cause of result variability

Comparing 2 serial results for a patient:
Must consider: Combined effect of CV$_A$ and CV$_I$ on each result

Combining SDs or CVs:
requires conversion to variances, relative variances
In the diagram, the combined effect of two variables is shown with a value of 7.3. The coefficients of variation are given as follows:

- $CV_A = 3.8\%$
- $CV_I = 18\%$

The text mentions "PSA g/L" which typically refers to the prostate-specific antigen concentration in blood.
First result (7.3)       Second result (8.6)

Combine the 2 variances: \( CV_A^2 + CV_I^2 \)    \( CV_A^2 + CV_I^2 \)

Convert each to total CV: \( (CV_A^2 + CV_I^2)^{1/2} \)    \( (CV_A^2 + CV_I^2)^{1/2} \)

\[ 1.96 \times (CV_A^2 + CV_I^2)^{1/2} \quad 1.96 \times (CV_A^2 + CV_I^2)^{1/2} \]

Combine both terms

\[ [1.96 \times (CV_A^2 + CV_I^2)^{1/2}]^2 + [1.96 \times (CV_A^2 + CV_I^2)^{1/2}]^2 \]

Take square root to give combined CV\(_T\)

\[ \left\{ [1.96 \times (CV_A^2 + CV_I^2)^{1/2}]^2 + [1.96 \times (CV_A^2 + CV_I^2)^{1/2}]^2 \right\}^{1/2} \]
\[
\left\{ 1.96 \times (CV_A^2 + CV_I^2)^{1/2} \right\}^2 + \left\{ 1.96 \times (CV_A^2 + CV_I^2)^{1/2} \right\}^2 \right\}^{1/2}
\]

Above simplifies to:

\[2^{1/2} \times 1.96 \times (CV_A^2 + CV_I^2)^{1/2}\]

\[1.41 \times 1.96 \times (CV_A^2 + CV_I^2)^{1/2}\]

\[2.77 \times (CV_A^2 + CV_I^2)^{1/2} = 2.77 \times CV_T\]

Reference Change Value (RCV)
\[
2.77 \times (CV_A^2 + CV_I^2)^{1/2}
\]

\[
2.77 \times (3.8^2 + 18.0^2)^{1/2}
\]

CV\(_A\) = 3.8 %

CV\(_I\) = 18 %

PSA g/L
2.77 \times (C_{VA}^2 + C_{VI}^2)^{1/2}

2.77 \times (3.8^2 + 18.0^2)^{1/2}

2.77 \times 18.4

Reference Change Value = 51 \%

PSA \ g/L
If Reference Change Value = 51 %

\[ 8.6 - 7.3 = 1.3 = \text{only } \sim 18\% \text{ of } 7.3 \text{ g/L} \]
If Reference Change Value = 51 %

Or: 51 % of 7.3 = 3.7 μg/L (95 % probability)

PSA g/L

\[ 2.77 \times (CV_A^2 + CV_I^2)^{1/2} \]

7.3 + 3.7 ≥11.0
SUMMARY

when calculating a Reference Change Value that is bi-directional

i.e. Inherent variability of both results could be up or down

Use

\[
RCV = 2.77 \times (CV_A^2 + CV_I^2)^{1/2}
\]

From Lab QC data  From Westgard BV website

Assuming Z-score of 1.96 used (95 % probability)
Uni-Directional Changes

Used to answer questions such as:

- Is a result definitely above a clinical decision value?
- Is a result definitely below a clinical decision value?
- Is a serial result biologically higher than the previous result?
- Is a serial result biologically lower than the previous result?
Is the result biologically different from a clinical decision value?

Requires different Z score
Table 3.3  Z-scores and Probability

<table>
<thead>
<tr>
<th>Probability (%)</th>
<th>Unidirectional Z-score</th>
<th>Bidirectional Z-score</th>
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<tbody>
<tr>
<td>99</td>
<td>2.33</td>
<td>2.58</td>
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<tr>
<td>98</td>
<td>2.05</td>
<td>2.33</td>
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<tr>
<td>97</td>
<td>1.88</td>
<td>2.17</td>
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<tr>
<td>96</td>
<td>1.75</td>
<td>2.05</td>
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<tr>
<td><strong>95</strong></td>
<td><strong>1.65</strong></td>
<td><strong>1.96</strong></td>
</tr>
<tr>
<td>90</td>
<td>1.28</td>
<td><strong>1.65</strong></td>
</tr>
<tr>
<td>85</td>
<td>1.04</td>
<td>1.44</td>
</tr>
<tr>
<td>80</td>
<td>0.84</td>
<td>1.28</td>
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<tr>
<td>75</td>
<td>0.68</td>
<td>1.15</td>
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<tr>
<td>70</td>
<td>0.52</td>
<td>1.04</td>
</tr>
<tr>
<td>60</td>
<td>0.25</td>
<td>0.84</td>
</tr>
</tbody>
</table>
Is the result biologically different from a clinical decision value?

\[ 1.65 \times (CV_A^2 + CV_I^2)^{1/2} = 1.65 \times 18.4 = 30.4 \%
\]

\[ RCV = 30\% \text{ of } 4.0 = 1.2 \text{ g/L} \]
For result to be biologically higher from the fixed clinical decision value (95 % confidence)

RCV = 5.2 g/L

Z score: 1.65
Is 8.6 biologically higher than 7.3?

OR

Is 7.3 biologically lower than 8.6?
\[ 2^{1/2} \times 1.65 \times (CV_A^2 + CV_I^2)^{1/2} \]

\[ 1.41 \times 1.65 \times (3.8^2 + 18.0^2)^{1/2} = \]

\[ 2.33 \times 18.4 = \sim 42.9 \% \]

RCV = 7.3 \times 43/100 = 3.1 \text{ g/L}
RCV = 3.1 g/L

Second result needs to be ≥10.4 g/L to have ≥95% confidence it has biologically increased from 7.3 g/L.
\[ 2^{1/2} \times 1.65 \times (CV_A^2 + CV_I^2)^{1/2} \]
\[ 1.41 \times 1.65 \times (3.8^2 + 18.0^2)^{1/2} = \]
\[ 2.33 \times 18.4 = \sim 42.9 \% \]
\[ RCV = 8.6 \times 43/100 = 3.7 \ \text{g/L} \]
PSA = $3.7 \mu g/L$

RCV = 3.7 g/L

Second result needs to be $\leq 4.9 \mu g/L$ to have $\geq 95\%$ confidence it has biologically decreased from 8.6 g/L
<table>
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<th>18/10/12</th>
<th>19/10/12</th>
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<td>Specimen</td>
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<td>PLASMA</td>
<td>PLASMA</td>
<td>PLASMA</td>
<td>PLASMA</td>
<td>PLASMA</td>
<td>PLASMA</td>
</tr>
</tbody>
</table>

**Sodium** 197-140 mmol/L 141 140 146 141 140 142 144
**Potassium** 3.2-4.3 mmol/L 4.4 4.0 3.9 3.2 3.2 3.3 3.0
**Chloride** 100-109 mmol/L 104 101 109 105 104 105 103
**Bicarb.** 22.32 mmol/L 31 28 29 28 27 26 30
**Anion Gap** 7-17 mmol/L 10 15 12 11 12 14 14
**Glucose** 3.2-5.6 mmol/L 4.7 9.6 4.8 5.4 5.1 5.5 6.6
**Urea** 2.7-8.0 mmol/L 7.6 11.0 11.2 7.7 4.2 2.6 4.3
**Creatinine** 50-100 umol/L 81 97 91 79 68 62 76
**eGFR** ml/min/1.73m² 59 48 51 > 60 > 60 > 60 > 60
**calc. Osmo** 285-295 mmol/L 0.45 0.54 0.53 0.52 0.49 0.44 0.47
**Phosphate** 0.45-1.45 mmol/L 1.30 1.12 1.22 0.69 0.83 0.70 1.14
**Tot. Ca** 2.10-2.50 mmol/L 2.22 2.34 2.26 2.17 2.12 2.23 2.25
**calc. Ca** 1.10-1.30 mmol/L 1.17 1.19 1.22 1.19 1.20 1.22 1.20
**Albumin** 34-48 g/L 33 35 28 29 25 28 29
**Glob.** 21-41 g/L 34 40 38 33 32 34 36
**Protein** 65-80 g/L 67 75 66 62 57 62 65
**Tot. Bilii** 2-24 umol/L 7 11 4 7 8 6 6
**Con. Bilii** umol/L
**GGT** < 60 U/L 10 11 13 17 11 12 14
**ALP** 20-110 U/L 95 89 105 114 83 69 72
**ALT** < 35 U/L 9 8 7 4 10 7 12
**AST** < 41 U/L 19 23 14 16 14 17 21
**LD** 110-230 U/L 256 235 221 209 218 226 209
**CK** U/L 218 119 160
**Magnesium** 0.75-1.05 mmol/L 0.85 0.93 0.80
**Amylase** U/L 29 31 29

Reference Ranges refer to the most recent result only.
SUMMARY

For RCV with 95 % probability

Bi-directional changes: $2.77 \times CV_T$

Uni-directional changes: $2.33 \times CV_T$

$CV_I$ often dominant cause of result variability
Aspects not discussed

Variable quality of CV₁ data – often only one study

Validity of specimen timing

Validity of Gaussian distributions

Need to standardise study protocols; data means vs. medians

Applicability of CV₁ data to disease states

Use of more than two serial results

Reporting biological changes in results
REFERENCES

Essential reading:

Biological Variation: From Principles to Practice. Fraser CG.
AACC Press, 2001

Biological variation: a still evolving facet of laboratory medicine
Simundic A-M, Bartlett WA, Fraser CG.
Ann Clin Biochem 52(2);19-190, 2015

www.westgard.com/biodatabase

Rationale for using data on biological variation. C Ricós.
REFERENCES

Calculation of limits for significant bidirectional changes in two or more serial results of a biomarker based on a computer simulation model  
Ann Clin Biochem 52(4);434-440, 2015

Calculation of limits for significant unidirectional changes in two or more serial results of a biomarker based on a computer simulation model  
Ann Clin Biochem 52(2);237-244, 2015

Lund F, Petersen PH, Fraser CG, Sölétormos G.