Analytical performance of a biomarker: what the clinician should know

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## Biochimie Sanguine

### Protéine C-Réactive (CRP)

<table>
<thead>
<tr>
<th>Valeur</th>
<th>Unites</th>
</tr>
</thead>
<tbody>
<tr>
<td>18,0 mg/l</td>
<td></td>
</tr>
</tbody>
</table>

### Clarté de la Creatinine (Ccr)

<table>
<thead>
<tr>
<th>Valeur</th>
<th>Unites</th>
</tr>
</thead>
<tbody>
<tr>
<td>12,0 mg/l</td>
<td>μmol/l</td>
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### Creatinine

<table>
<thead>
<tr>
<th>Valeur</th>
<th>Unites</th>
</tr>
</thead>
<tbody>
<tr>
<td>155,6 μmol/l</td>
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</tbody>
</table>

**Clairance estimée selon Cockcroft**

63,6 ml/min

### D. P. G. selon MDRD

63,3 ml/min

**Classification de la maladie rénale chronique en 5 stades selon les recommandations internationales**

- **Stade 1** : atteinte rénale avec DPG normal ou augmenté (DPG ≥ 90)
- **Stade 2** : atteinte rénale avec légère diminution du DPG (60 ≤ DPG < 90)
- **Stade 3** : insuffisance rénale chronique modérée (15 ≤ DPG < 60)
- **Stade 4** : insuffisance rénale chronique sévère (15 ≤ DPG < 15)
- **Stade 5** : insuffisance rénale chronique terminale (DPG < 15)

### Transaminase TGO (ASAT)

11 U/l

### Transaminase TGP (ALAT)

11 U/l
Laboratory Workflow

Pre-analytical
- Test Request
- Patient preparation
- Sample Collection
- Sample Transport
- Sample Receipt

Analytical
- Testing Review
- Lab Interpretation

Post-analytical
- Reporting
- Record Management

Testing
- Response

Patient
The hourglass model representing the errors in Laboratory medicine
The pre-analytical variation
Size of the needle

Choice of the sampling tube

Stability of the analyte

Choice of the vein

Tourniquet time

Transport of the samples

Qualification of the phlebotomist

Patient's position

Fasting status

Indications???

Patient ID
Tests routinely ordered by Nephrologists particularly subject to pre-analytical variation (non exhaustive)

- **Parathormone** (type of sample tube and temperature of conservation).
- **Active renin** (temperature of conservation)
- **Potassium and phosphorus** (hemolysis)
- **Coagulation factors** (incomplete filling and prolonged use of a tourniquet)
- **Aldosterone** (posture of the patient)
The analytical variation
The « true » value of an analytical measurement is always unknown (even with a « reference method »)

At best, the result of an analytical process is an estimation of the « true » value

Two types of error impact any analytical result, namely the **systematic** and the **random** error
The random error

Random error is constituted by the addition of different uncontrolled sources of variation.

Theses numerous and independent sources of variation can have opposite effects, leading to a Gaussian dispersion of the results around the expected "true" value.
Evaluation of the random error

The random error is evaluated by repeating multiple measurements on samples presenting different levels.

We calculate the mean, the standard deviation and the coefficient of variation

\[ CV(\%) = \frac{SD}{\text{Mean}} \times 100 \]
The systematic error

The systematic error represents the constant bias observed between the observed value and the "true" value.

The systematic error is evaluated by the bias (in %) between the true value and the value found by the method.
The total error

The total error is the combination of the random and systematic error
If a PTH method has a CV of 5% and a negative bias of 10%. The lab found that the patient's value was 90 pg/mL:

![Graph showing errors and true values with values 81, 90, 99 on the left and 100 on the right.]
Exemple

If a PTH method has a CV of 5% and a negative bias of 10%. The lab found that the patient’s value was 90 pg/mL:

The true value was 100 pg/mL, but the lab will provide results ranging from 81 to 99 pg/mL with a probability of 95%.
5 times on 100, the value will be below 81 or above 99.
The biological variation
Biological variation

Biological variation corresponds to the **natural** and **physiological** fluctuation of body fluid constituents around a homeostatic setting point which is specific for each individual.

The biological variation has two components: the **within** and the **between-subject** variation.
Evaluation of the biological variation

Recruit group of apparently healthy volunteers

Take a series of samples from each individual at different time-points

Run the analysis in duplicate in one batch and estimate the biological variations by performing a ANOVA.

http://www.westgard.com/biodatabase1.htm
What can we do with biological variation data?

🔹 Determine the Reference Change Value

🔹 Define the number of specimens required to estimate the homeostatic set point of a parameter.
Reference Change Value (or Least Significant Change)

Â In clinical practice, it is of importance to know if a change between two results in the same patient has significantly occurred.

Â The percentage above which one can consider a change as biologically significant with 95% confidence is called the RCV (or LSC).

Â The resumed formula to calculate the RCV is:

$$ \text{RCV} = 1.96 \times \sqrt{2} \times \sqrt{\left(CV_a^2 + CV_i^2\right)} $$

$$ \approx 3 \times CV_i $$
Note: N = 17 stable patients treated with maintenance hemodialysis. Concentrations given as mean ± SD or median [IQR].
Abbreviations and definitions: ALP, alkaline phosphatise; CVa, analytical coefficient of variation; CVi, intraindividual coefficient of variation; FGF-23, fibroblast growth factor 23; gen, generation; LSC, least significant change (at 95% probability); P1NP, N-terminal propeptide of type 1 procollagen; PTH, parathyroid hormone; TRAP-5B, tartrate-resistant acid phosphatase type 5B.
Exemple

A HD patient presents a PTH at 180 pg/mL (6xUL) and a bAP at 19 µg/mL
8 weeks later, PTH raised to 240 pg/mL (8xUL) and bAP at 24 µg/L
Are these changes significant?

PTH: 180 + 40% = 252 pg/mL
bAP: 19 + 23% = 23.4 µg/L

These changes are not significant.
Nb of samples to estimate the true value in an Individual

To calculate the number of samples to ensure that the homeostatic setting point is within a certain percentage of the true value with a certain probability, we can use this formula

\[ n = \left[ Z \times \sqrt{CV_A^2 + CV_I^2} / D \right]^2 \]

where \( n \) is the number of samples needed, \( Z \) the probability-score and \( D \) is the desired percentage of closeness to the homeostatic set-point.
Exemple

If the intra individual CV of creatinine is 5.3% and the analytical CV is 3% and if we want that a creatinine result to be within 10% of the true homeostatic set-point with a 95% probability, we need:

\[ n = \left[ 1.96 \times \sqrt{\frac{3^2 + 5.3^2}{10}} \right]^2 = 2 \text{ samples.} \]
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Value</th>
<th>CV&lt;sub&gt;a&lt;/sub&gt; (%)</th>
<th>CV&lt;sub&gt;i&lt;/sub&gt; (%)</th>
<th>LSC (%)</th>
<th>No. of Specimens to Estimate True Value in an Individual</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Within 20%</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>39.8 ± 3.1</td>
<td>1.3</td>
<td>2.8</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Total calcium</td>
<td>2.19 ± 0.17</td>
<td>1.4</td>
<td>2.14</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.36 ± 0.35</td>
<td>2.6</td>
<td>11.35</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>Total ALP (UI/L)</td>
<td>89 ± 38</td>
<td>0.9</td>
<td>5.5</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Bone ALP (μg/L)</td>
<td>19.0 ± 11.8</td>
<td>4.9</td>
<td>6.8</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>2nd-gen PTH (pg/mL)</td>
<td>357 [184-461]</td>
<td>2.3</td>
<td>13.8</td>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td>3rd-gen PTH (pg/mL)</td>
<td>157 [81-234]</td>
<td>4.5</td>
<td>14.9</td>
<td>43</td>
<td>3</td>
</tr>
<tr>
<td>Intact P1NP (ng/mL)</td>
<td>124 ± 83</td>
<td>4.7</td>
<td>10.5</td>
<td>32</td>
<td>2</td>
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<tr>
<td>TRAP-5B (U/L)</td>
<td>5.34 ± 1.93</td>
<td>2.6</td>
<td>8.3</td>
<td>24</td>
<td>1</td>
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<tr>
<td>FGF-23 (U/L)</td>
<td>1.678 [770-2.896]</td>
<td>3.5</td>
<td>17.2</td>
<td>48</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: N = 17 stable patients treated with maintenance hemodialysis. Concentrations given as mean ± SD or median [IQR].
Abbreviations and definitions: ALP, alkaline phosphatase; CV<sub>a</sub>, analytical coefficient of variation; CV<sub>i</sub>, intraindividual coefficient of variation; FGF-23, fibroblast growth factor 23; gen, generation; LSC, least significant change (at 95% probability); P1NP, N-terminal propeptide of type 1 procollagen; PTH, parathyroid hormone; TRAP-5B, tartrate-resistant acid phosphatase type 5B.
The post-analytical phase
The reference range established on a « apparently healthy » population

Å Reflect only the population on which they have been obtained (ethnicity, habits, food intakes, sports, etc.)

Å Statistically: 5% of healthy subjects will be out of them

Å How should we define the « reference population »: blood donors? Young people?

Å Most of labs use the RR provided by manufacturers but they are poorly defined
X2 ÷ X9

« the upper reference range of the Laboratory »
However…

When establishing reference values for serum PTH, it seems logical to exclude from the reference population any person with a condition potentially leading to an increased PTH concentration. Vitamin D insufficiency is one condition that may increase PTH, but to know whether an apparently healthy individual is vitamin D-insufficient, serum 25OHD must be measured. However, vitamin D status has not been taken into account in most published studies on PTH reference values.

Souberbielle JC, Clinical Chemistry, 2005
<table>
<thead>
<tr>
<th>Methods</th>
<th>Reference range (Manufacturer) (pg/ml)</th>
<th>Lower and Upper Reference limits (95% Confidence – Interval) obtained in our reference population (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2nd generation assays</td>
</tr>
<tr>
<td>Abbott Architect</td>
<td>15.0 - 68.3</td>
<td>16.3 - 64.7</td>
</tr>
<tr>
<td>Beckman Access</td>
<td>12 - 88</td>
<td>10.1 - 47.4</td>
</tr>
<tr>
<td>DiaSorin N-tact IRMA</td>
<td>13 – 54</td>
<td>7.2 - 35.7</td>
</tr>
<tr>
<td>DiaSorin Liaison N-tact</td>
<td>17.3 - 72.9</td>
<td>21.3 - 68.2</td>
</tr>
<tr>
<td>Ortho Vitros</td>
<td>7.5 - 53.5</td>
<td>10.8 - 47.5</td>
</tr>
<tr>
<td>Roche Elecsys</td>
<td>15 - 65</td>
<td>13.7 - 50.2</td>
</tr>
<tr>
<td>Scantibodies Total intact PTH</td>
<td>14 - 66</td>
<td>7.8 - 49.7</td>
</tr>
<tr>
<td>Siemens Immulite</td>
<td>12 - 65</td>
<td>5.4 - 57.1</td>
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<td>3rd generation assays</td>
</tr>
<tr>
<td>DiaSorin Liaison 1-84</td>
<td>5.5 - 38.4</td>
<td>4.6 - 25.8</td>
</tr>
<tr>
<td>Scantibodies Ca-PTH IRMA</td>
<td>5 - 39</td>
<td>6.8 - 30.8</td>
</tr>
</tbody>
</table>
Take-home messages

Å What’s behind a result? Many variations possible!
Å To understand these variations is mandatory to correctly interpret laboratory results
Å In any case: contact the lab!!! (1A in the KDIGO)