Analytical performance of a biomarker: what the clinician should know

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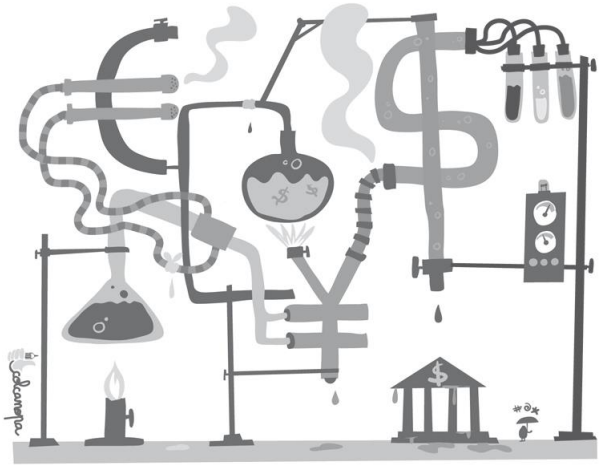
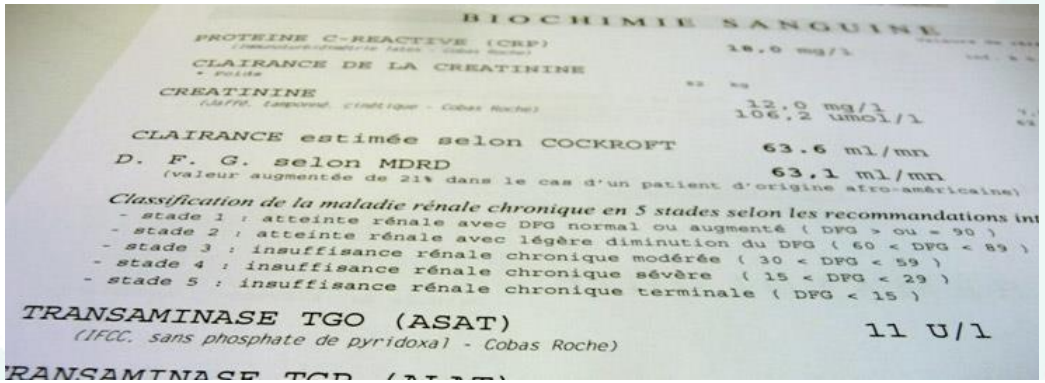
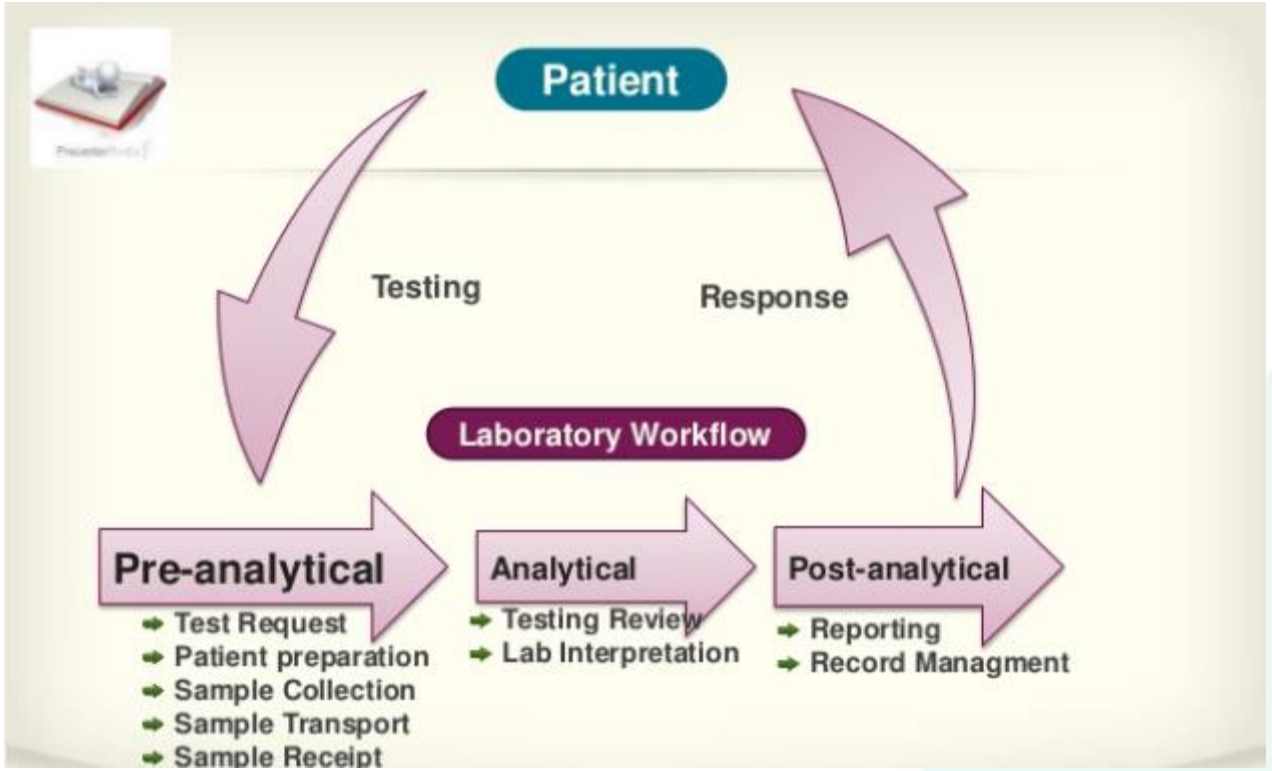
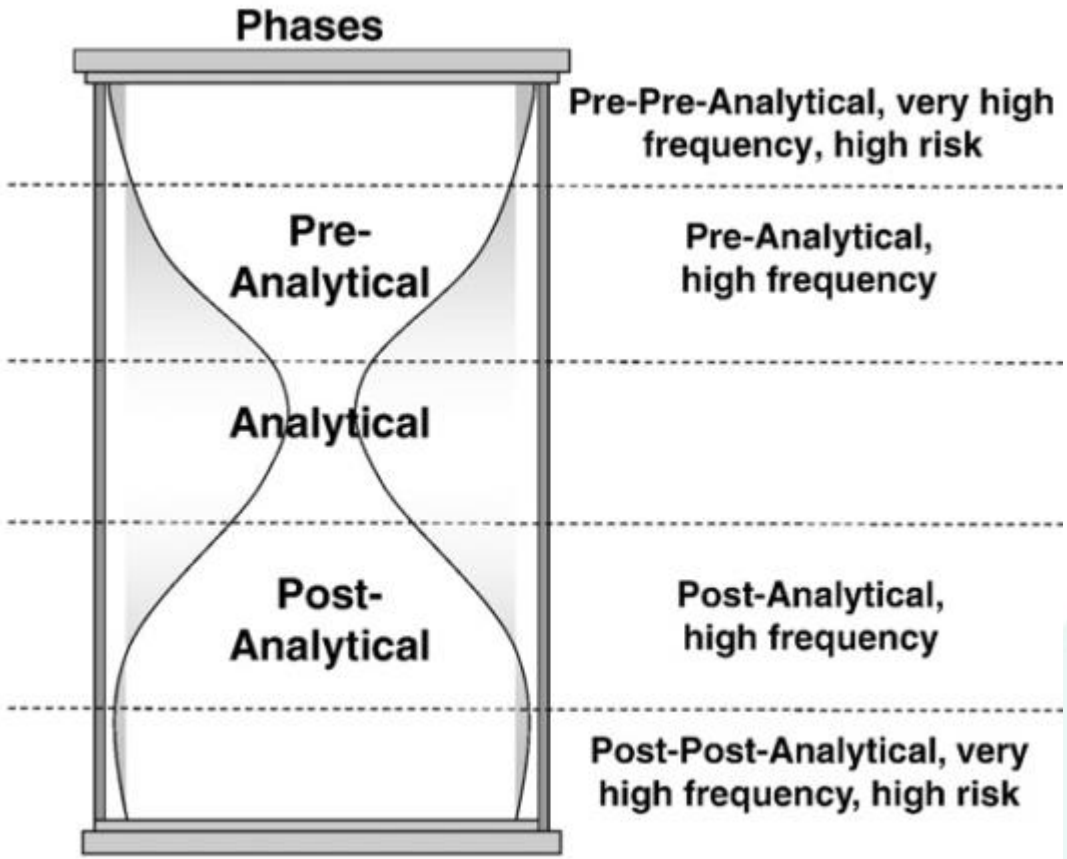



BIOCHIMIE SANGUINE
PROTEINE C-REACTIVE (CRP)
18.0 mg/l
CLAIRANCE DE LA CREATININE
CREATININE
12.0 mg/l
106.2 umol/l
CLAIRANCE estimée selon COCKROFT
63.6 ml/mn
D. F. G. selon MDRD
63,1 ml/mn
(valeur augmentée de 21% dans le cas d'un patient d'origine afro-américaine)
Classification de la maladie rénale chronique en 5 stades selon les recommandations int
- stade 1 : atteinte rénale avec DFG normal ou augmenté (DFG > ou = 90)
- stade 2 : atteinte rénale avec légère diminution du DFG (60 < DFG < 89)
- stade 3 : insuffisance rénale chronique modérée (30 < DFG < 59)
- stade 4 : insuffisance rénale chronique sévère (15 < DFG < 29)
- stade 5 : insuffisance rénale chronique terminale (DFG < 15)
TRANSAMINASE TGO (ASAT)
(IFCC, sans phosphate de pyridoxal - Cobas Roche)
11 U/l


Patient
Testing
Response
Laboratory Workflow
Pre-analytical
Test Request
Patient preparation
Sample Collection
Sample Transport
Sample Receipt
Analytical
Testing Review
Lab Interpretation
Post-analytical
Reporting
Record Managment

The hourglass model representing the errors in Laboratory medicine



Plebani M CCA 2009

The pre-analytical variation

Transport of the samples

Tourniquet time

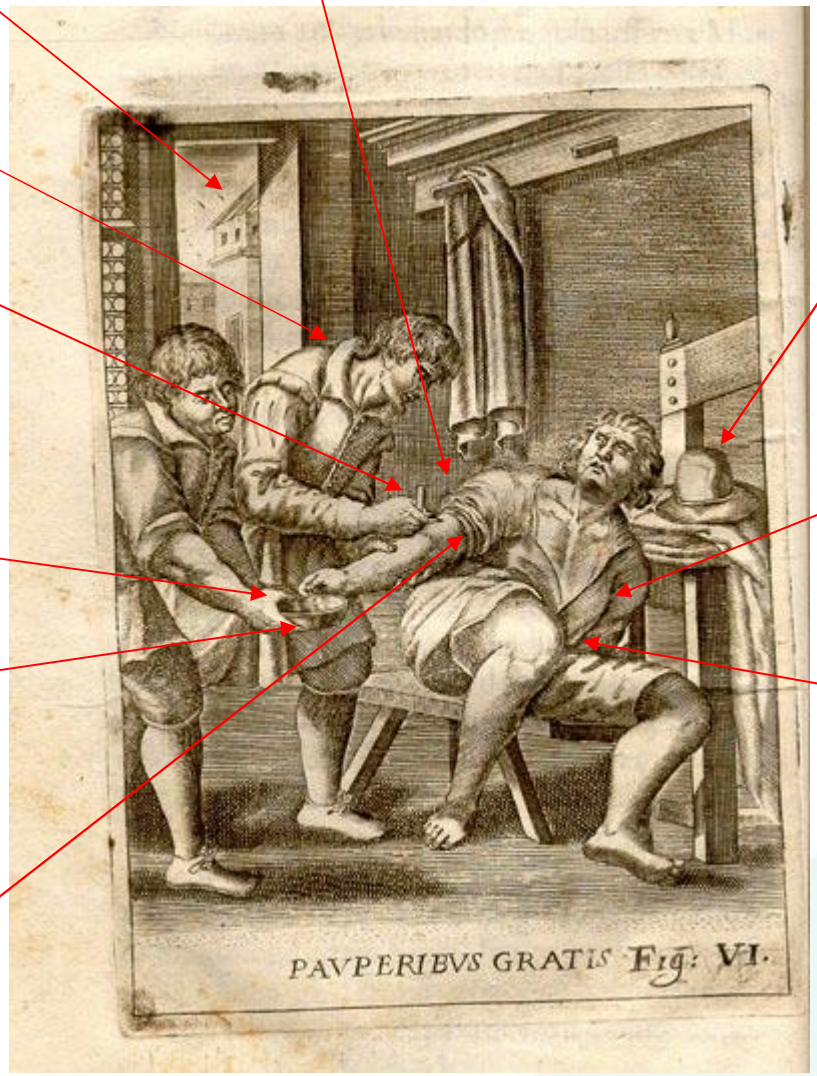
Qualification of the phlebotomist

Size of the needle

Stability of the analyte

Choice of the sampling tube

Choice of the vein



Patient ID

Patient's position

Fasting status

Indications???

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

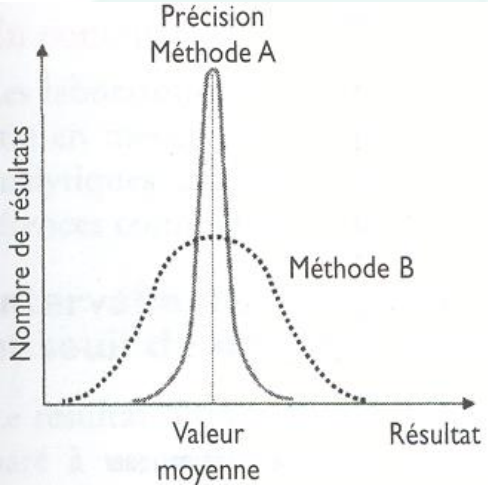
- Random error is constituted by the addition of different uncontrolled sources of variation
- Theses numerous and independent sources of variation can have opposite effects, leading to a Gaussian dispersion of the results around the expected ñtrueò value.

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**

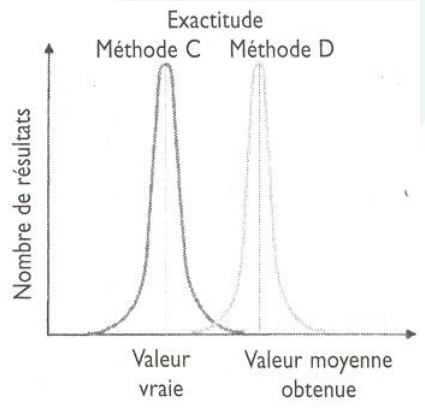
- **CV(%) = SD / Mean x 100**



The systematic error

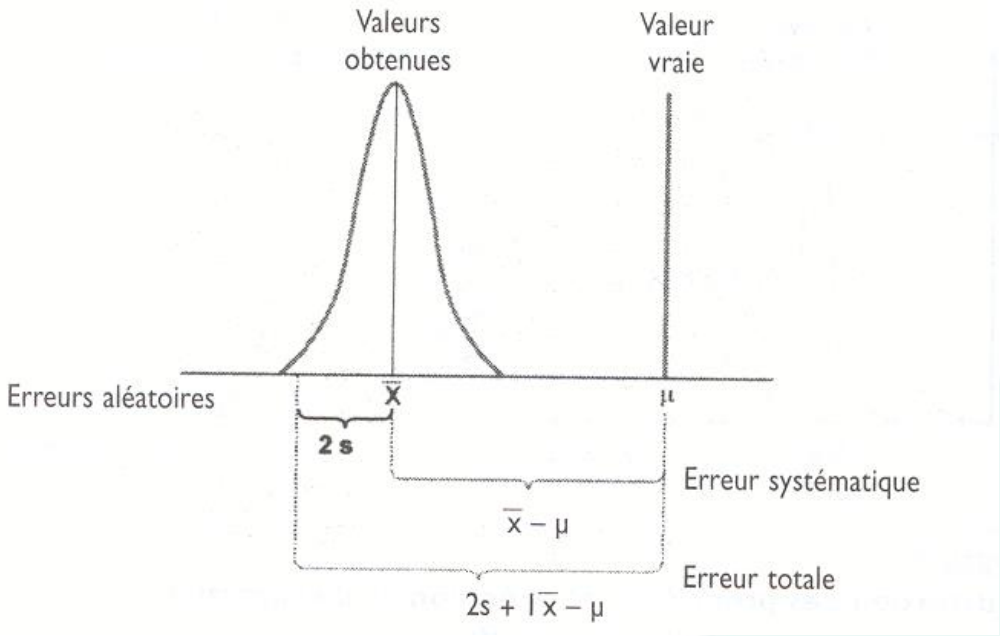
The systematic error represents the constant bias observed between the observed value and the ñtrueòvalue.

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



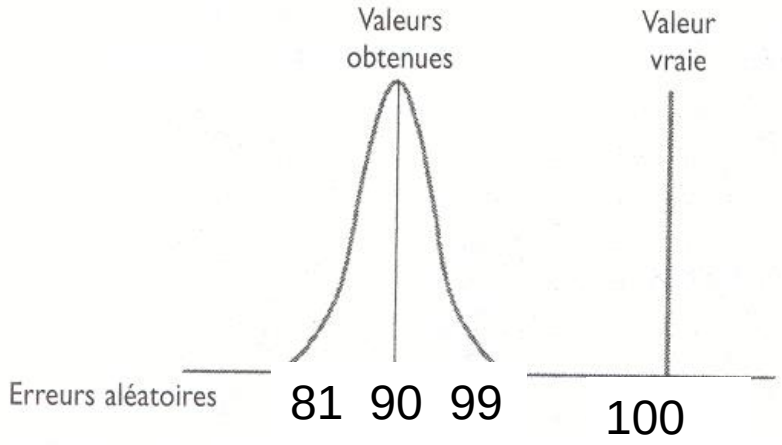
The total error

The total error is the combination of the random and systematic error



Exemple

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



Exemple

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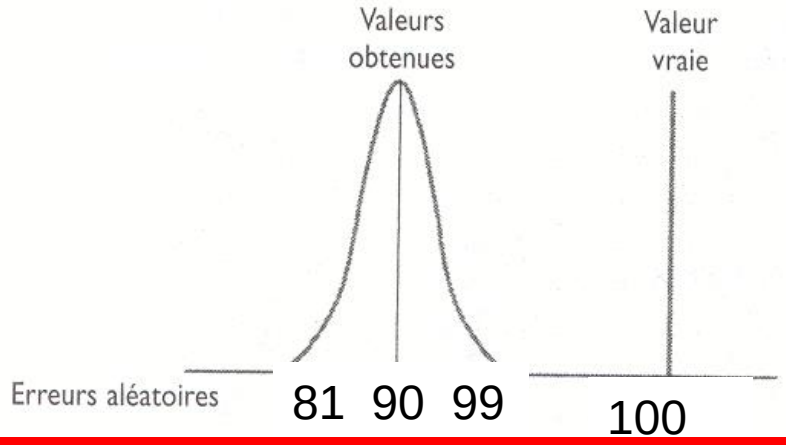

The true value was 100 pg/mL, but the lab will provide results ranging from 81 to 99 pg/mL with a probability of 95%.
5 times on 100, the value will be below 81 or above 99.

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?

☞Detemine 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)

- In clinical practice, it is of importance to know if a change between two results in the same patient has significantly occurred
- The percentage above which one can consider a change as biologically significant with 95% confidence is called the RCV (or LSC)
- The resumed formula to calculate the RCV is **RCV = 1.96 X ã2 X ã(CV_a^2+CV_i^2)**

å 3 x CVi

	No. of Specimens to Estimate True Value in an Individual		
	Within 10%	Within 20%	Within 30%
	1	1	1
	1	1	1
	5	2	1
	2	1	1
	3	1	1
	8	2	1
	10	3	1
	5	2	1
	3	1	1
	11	3	1

Note: N = 17 stable patients treated with maintenance hemodialysis. Concentrations given as mean ± SD or median [IQR].

Abbreviations and definitions: ALP, alkaline phosphatise; CV_a, analytical coefficient of variation; CV_i, 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.

Cavalier et al, AJKD 2013

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 + å40% = 252 pg/mL
- bAP: 19 + 23% = 23.4 µg/L

These changes are not significant.

Nb of samples to esimate 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 ñtrueò value with a certain probability, we can use this formula
- $n=[Z*\tilde{a}(CV_A^2+CV_I^2)/D]^2$ 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:

- $n=[1.96*\sqrt{(3^2+5.3^2)}/10]^2 = 2$ samples.

Analyte	Value	CV_a (%)	CV_i (%)	LSC (%)	No. of Specimens to Estimate True Value in an Individual	
					Within 20%	Within 30%
Albumin (g/L)	39.8 ± 3.1	1.3	2.8	9	1	1
Total calcium	2.19 ± 0.17	1.4	2.14	7	1	1
Phosphate (mmol/L)	1.36 ± 0.35	2.6	11.35	32	2	1
Total ALP (UI/L)	89 ± 38	0.9	5.5	16	1	1
Bone ALP (µg/L)	19.0 ± 11.8	4.9	6.8	23	1	1
2nd-gen PTH (pg/mL)	357 [184-461]	2.3	13.8	39	2	1
3rd-gen PTH (pg/mL)	157 [81-234]	4.5	14.9	43	3	1
Intact P1NP (ng/mL)	124 ± 83	4.7	10.5	32	2	1
TRAP-5B (U/L)	5.34 ± 1.93	2.6	8.3	24	1	1
FGF-23 (U/L)	1.678 [770-2.896]	3.5	17.2	48	3	1

Note: N = 17 stable patients treated with maintenance hemodialysis. Concentrations given as mean ± SD or median [IQR].

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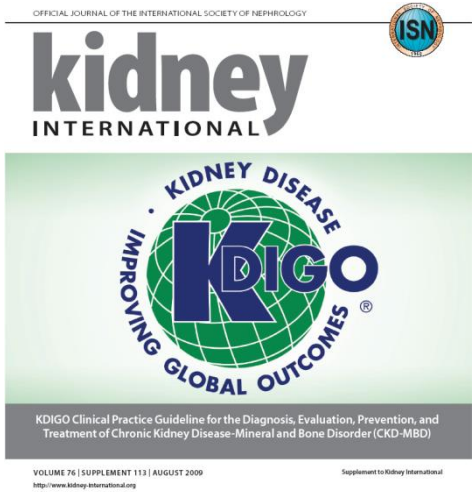
Cavalier et al, AJKD 2013

The post-analytical phase

The reference range established on a « apparently healthy » population

- Reflect only the population on which they have been obtained (ehnicity, habits, food intakes, sports,é)
- Statistically: 5% of healthy subjects will be out of them
- How should we define the « reference population »: blood donors? Young people?
- Most of labs use the RR provided by manufacturers but they are poorly defined



X2 ï X9

« the upper reference range of the Laboratory »

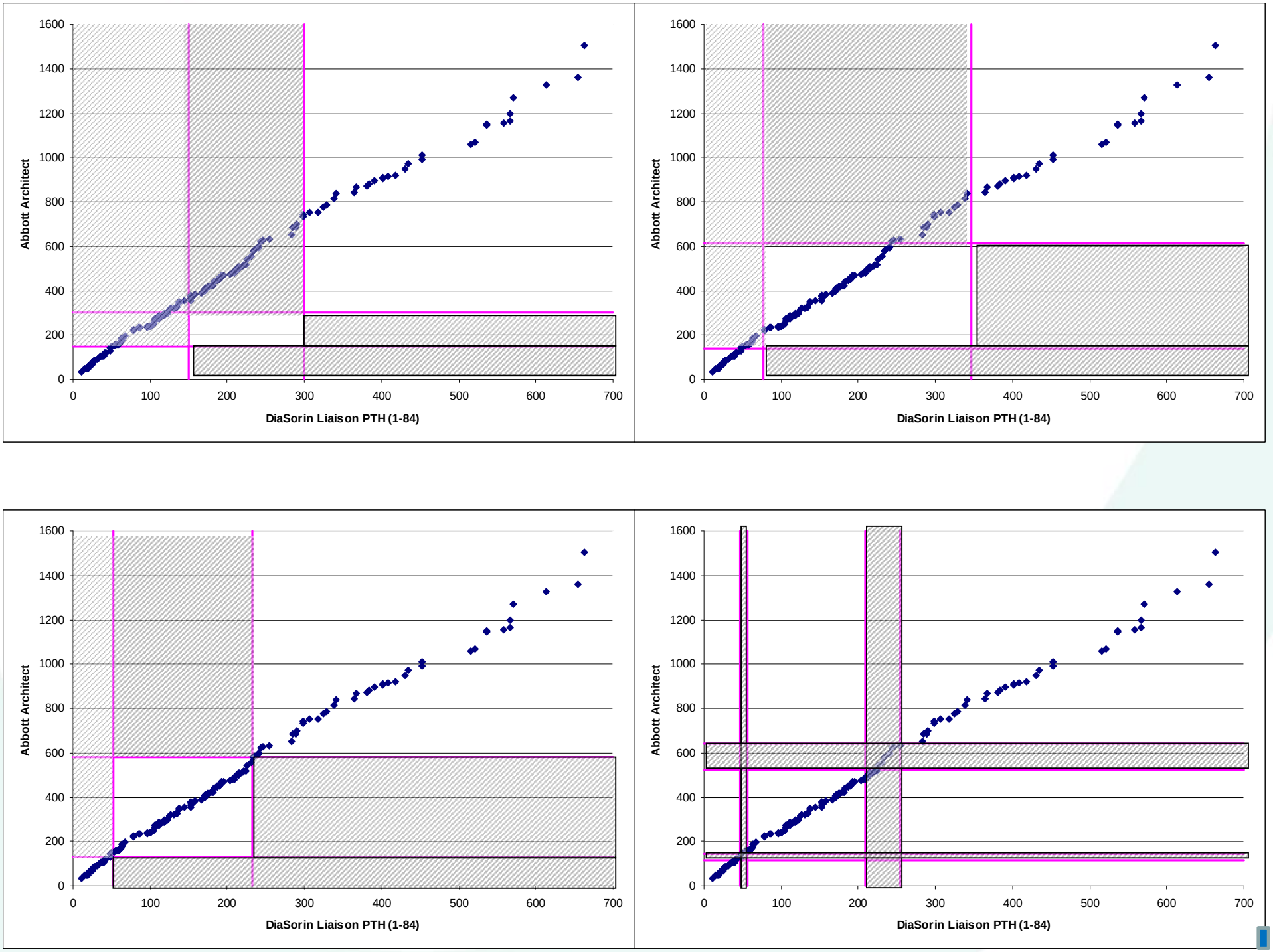
Howeveré

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

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

Cavalier. NDT 2011


Abbott Architect
DiaSorin Liaison PTH (1-84)
0
100
200
300
400
500
600
700
200
400
600
800
1000
1200
1400
1600

Take-home messages

- What's behind a result? Many variations possible!
- To understand these variations is mandatory to correctly interpret laboratory results
- In any case: contact the lab!!! (1A in the KDIGO)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MARIA LATOUR
BERNARD BLIER
MICHEL SERRAULT
PIERRE BRASSEUR
UN FILM REALISE PAR JACQUES BESNARD
le Fou du LABO 4
D'APRES LE ROMAN DE RENE CAMOIN
MARIO DAVID - ROBERT DALBAN - MARGO LION -
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