SFRI Medical Diagnostics

IONIX

IONIX ANALYTICAL PERFORMANCES

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1 ISE theory

An ion-selective membrane is the key component of all potentiometric ion sensors. It establishes the preference with which the sensor responds to the analyte in the presence of various interfering ions from the sample. If ions

can penetrate the boundary between two phases, then an electrochemical equilibrium will be reached, in which different potentials in the two phases are formed. If only one type of an ion can be exchanged between the two phases, then the potential difference formed between the phases is governed only by the activities of this target ion in these phases. When the membrane separates two solutions of different ionic activities (αC1 and αC2) and provided the membrane is only permeable to this single type of ion, the potential difference (E) across the membrane is described by the Nernst equation:

- E = E° + RTlog (αC)/nF,
- E = potentiel of the electrode in the measured solution
- E° = potentiel of the electrode in reference solution
- RT/nF = constant (depending of absolute temperature T)

With:

- R is Ideal gas constant, equal to 8,3144 J · mol $^{-1}$· K^{-1}
- T is the temperature in kelvin
- F is the Faraday constant, equal to 96485 C·mol^{-1}
- n is the number of electrons transfered in the half reaction
- α ionic activity depends of ionic strength. a is the chemical activity for the relevant species, where aRed is the activity of the reduced form and aOx is the activity of the oxidized form. Similarly to equilibrium constants, activities are always measured with respect to the standard state (1 mol/L for solutes, 1 atm for gases). The activity of species x, aX , can be related to the physical concentrations cX via aX = γXcX, where γX is the activity coefficient of species X. Because activity coefficients tend to unity at low concentrations, activities in the Nernst equation are frequently replaced by simple concentrations.

So the Nernst equation can be simplified as:

E = const + S · log (C).

S, the Slope is equal to 59 mV/decade at 25°C when n = 1 (pH, Na^+, K^+, Li^+), -59 when n = -1 (Cl^-), and 30 when n = 2 (Ca^{++}).

2 Measured ions

Sodium, Potassium, Chloride, ionized Calcium, pH and Lithium with ion specific electrodes. Total CO_2 (bicarbonates and dissolved CO_2) is measured with a barometer.

The pH is measured only for the standardisation of iCa at 7.4

3 Limits of detection

- Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) are terms used to describe the smallest concentration of a measure and that can be reliably measured by an analytical procedure.
- LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested.
- LoB = mean blank + 1.645(SD blank)
- LoD is the lowest analyte concentration likely to be reliably distinguished from the LoB and at which detection is feasible.
- LoD = LoB + 3SD blank
- LoQ is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and impreci-

sion are met. The LoQ may be equivalent to the LoD or it could be at a much higher concentration.

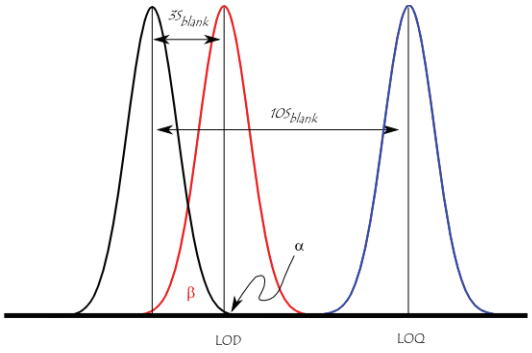


Illustration of the concept of detection limit and quantitation limit by showing the theoretical normal distributions associated with blank, detection limit, and quantification limit level samples.

For a signal at the LOD, the alpha error (probability of false positive) is small (1%). However, the beta error (probability of a false negative) is 50% for a sample that has a concentration at the LOD (red line).

For the test, the sample has been replaced by dionized water, and measured thirty times (ten times for tCO_2). The averages and the standard deviations are calculated, in order to determine the limits of detection and of quantification.

Table 1 : Blank, Limit of Detection and of Quantification

	Na	K	Cl	Ca	pH	tCO_2
	2,3	0,08	3,5	0,08	7,32	-1,83
	2,6	0,08	3,7	0,08	7,3	-1,34
	2,7	0,1	3,5	0,09	7,31	-3,21
	2,6	0,08	3,9	0,08	7,34	-2,92
	2,5	0,1	3,7	0,09	7,33	-2,32
	2,9	0,08	3,7	0,09	7,37	-2,08
	3,1	0,09	3,7	0,08	7,32	-2,06
	2,5	0,09	3,6	0,08	7,31	-1,63
	2,6	0,08	3,5	0,08	7,38	-1,15
	2,6	0,1	3,8	0,08	7,26	-1,09
	2,5	0,1	3,7	0,09	7,29	
	2,3	0,09	3,5	0,08	7,31	
	4	0,08	3,3	0,08	7,36	
	2,4	0,08	3,7	0,07	7,35	
	2,5	0,1	3,6	0,09	7,3	
	2,5	0,08	3,9	0,08	7,29	
	2,6	0,1	3,6	0,09	7,3	
	2,5	0,08	3,7	0,08	7,38	
	2,5	0,09	3,4	0,08	7,34	
	3	0,11	3,7	0,09	7,26	
	2,6	0,1	3,7	0,09	7,31	
	2,4	0,09	3,7	0,08	7,33	
	2,7	0,1	3,9	0,09	7,3	
	2,5	0,09	3,6	0,08	7,32	
	2,6	0,08	3,5	0,08	7,36	
	3,5	0,11	3,8	0,1	7,21	
	3,2	0,1	4,1	0,1	7,19	
	2,7	0,1	3,6	0,08	7,24	
	2,6	0,07	2,9	0,07	7,38	
	3,4	0,08	3,9	0,08	7,09	
	2,3	0,1	3,5	0,08	7,32	
	2,8	0,09	3,8	0,09	7,36	
	2,5	0,1	3,8	0,08	7,33	
	2,4	0,08	2,4	0,07	7,45	
Blank	2,69	0,09	3,61	0,08	NA	-0,03
Standard deviation	0,372	0,010	0,301	0,007	NA	0,64
Limit Of Detection	3,80	0,12	4,52	0,11	NA	1,90
Limit Of Quantification	6,40	0,19	6,62	0,16	NA	6,41

4 Sensitivity and Specificity

The sensitivity is the capacity to measure a positive result (false negative), and the specificity is the capacity to measure a negative result (false positive).

4. 1 : Specificity

For all the measured ions, the probability of false positive is almost zero:

- Sodium: LOD = 3.8 mmol/L, physiological range from 135 to 145 mmol/L
- Potassium: LOD = 0.12 mmol/L, physiological range from 3.5 to 5 mmol/L
- Chloride: LOD = 4.5 mmol/L, physiological range from 90 to 110 mmol/L
- Calcium: LOD = 0.11 mmol/L, physiological range from 1.1 to 1.3 mmol/L
- tCO_2: LOD = 1.9 mmol/L, physiological range from 23 to 27 mmol/L
- For the pH these data are not available, by definition: the minimum measurement range of a pH electrode is from 2 to 12, and the physiological range is from 7.32 to 7.42

4. 2 : Sensitivity

For all the measured ions, the probability of false negative is almost zero:

- Sodium: LOQ = 6.4 mmol/L, physiological range from 135 to 145 mmol/L
- Potassium: LOQ = 0.19 mmol/L, physiological range from 3.5 to 5 mmol/L
- Chloride: LOD = 6.6 mmol/L, physiological range from 90 to 110 mmol/L
- Calcium: LOD = 0.16 mmol/L, physiological range from 1.1 to 1.3 mmol/L
- tCO_2: LOQ = 6.4 mmol/L, physiological range from 23 to 27 mmol/L

5 Precision

5. 1 : Serum mode

1. Tests on water based controls:
   Three controls, low, normal and high level have been tested 10 times in blood mode (serum or plasma), averages, standard deviations and CV calculated. Refer to table 2 page, 5

Table 2 : Precision on water based solutions

Code	Type	Na	K	Cl	Ca	pH	Li	tCO_2
ctl h	Blood	150,1	5,92	118,8	1,38	7,59	0,83	31,21
ctl h	Blood	149,8	5,94	118,8	1,38	7,59	0,83	31,45
ctl h	Blood	150,0	5,94	118,5	1,39	7,59	0,83	29,96
ctl h	Blood	150,3	5,94	118,5	1,38	7,59	0,84	30,77
ctl h	Blood	149,7	5,94	118,9	1,38	7,59	0,83	29,47
ctl h	Blood	150,1	5,91	118,5	1,39	7,59	0,83	29,40
ctl h	Blood	149,9	5,90	118,6	1,38	7,59	0,83	29,71
ctl h	Blood	149,6	5,89	118,8	1,38	7,59	0,83	30,45
ctl h	Blood	149,2	5,91	118,6	1,38	7,59	0,83	29,76
ctl h	Blood	148,6	5,92	118,3	1,38	7,58	0,83	31,99
Average		149,7	5,921	118,7	1,38	7,59	0,831	30,42
SD		0,52	0,02	0,20	0,01	0,003	0,003	0,90
CV		0,35 %	0,31 %	0,17 %	0,36 %	0,04 %	0,38 %	2,97 %
Mini		148,6	5,89	118,3	1,38	7,58	0,83	29,40
Maxi		150,3	5,94	118,9	1,39	7,59	0,84	31,99
ctl l	Blood	126,9	2,54	80,3	1,04	7,07	0,14	
ctl l	Blood	126,7	2,53	80,3	1,04	7,07	0,14	
ctl l	Blood	126,8	2,52	80,0	1,04	7,07	0,14	
ctl l	Blood	126,5	2,52	80,2	1,04	7,07	0,14	
ctl l	Blood	126,5	2,52	80,1	1,04	7,07	0,14	
ctl l	Blood	126,6	2,54	80,0	1,04	7,07	0,15	
ctl l	Blood	126,4	2,54	79,9	1,04	7,07	0,14	
ctl l	Blood	126,1	2,52	80,0	1,04	7,07	0,14	
ctl l	Blood	126,0	2,52	79,9	1,04	7,07	0,14	
ctl l	Blood	126,3	2,52	79,5	1,04	7,06	0,14	
Average		126,5	2,53	80,1	1,04	7,07	0,14	
SD		0,3	0,01	0,2	0,00	0,00	0,003	
CV		0,24 %	0,38 %	0,21 %	0,00 %	0,00 %	2,36 %	
Mini		126,0	2,52	79,9	1,04	7,07	0,14	
Maxi		126,9	2,54	80,3	1,04	7,07	0,15	
ctl n	Blood	137,2	3,81	97,7	1,24	7,35	0,42	
ctl n	Blood	137,1	3,81	98,0	1,24	7,35	0,42	
ctl n	Blood	136,9	3,80	98,1	1,24	7,35	0,42	
ctl n	Blood	137,2	3,81	97,8	1,24	7,35	0,42	
ctl n	Blood	136,7	3,79	98,0	1,24	7,35	0,42	
ctl n	Blood	137,1	3,80	97,8	1,24	7,35	0,42	
ctl n	Blood	137,0	3,80	97,8	1,24	7,35	0,42	
ctl n	Blood	136,7	3,79	97,8	1,24	7,34	0,42	
ctl n	Blood	136,3	3,79	97,8	1,24	7,34	0,42	
ctl n	Blood	135,6	3,78	97,6	1,23	7,34	0,42	
Average		136,78	3,80	97,83	1,24	7,35	0,42	
SD		0,52	0,01	0,16	0,004	0,005	0,000	
CV		0,38 %	0,27 %	0,16 %	0,30 %	0,07 %	0,00 %	
Mini		135,6	3,78	97,6	1,23	7,34	0,42	
Maxi		137,2	3,81	98,1	1,24	7,35	0,42	

2. Tests on serum
   A pool of fresh serum, collected in a blood bank in dry tubes with separation gel, has been tested 31 times (15 times for tCO_2). Refer to table 3 page, 6. For the lithium, the test has been performed on a control serum, see table 5 page, 7

Table 3 : Precision on a serum pool

Code	Na	K	Cl	Ca	pH	Li[a]	tCO_2
Pool 06-12	139,2	4,79	104,0	1,18	7,55		
Pool 06-12	139,2	4,80	103,9	1,18	7,56		
Pool 06-12	139,3	4,79	104,0	1,19	7,56		
Pool 06-12	139,4	4,79	103,9	1,19	7,56		
Pool 06-12	139,3	4,80	104,1	1,18	7,57		
Pool 06-12	139,4	4,79	103,9	1,18	7,55		
Pool 06-12	139,4	4,79	104,2	1,20	7,58		
Pool 06-12	139,6	4,81	104,0	1,19	7,58		
Pool 06-12	139,5	4,80	103,9	1,19	7,57		
Pool 06-12	139,6	4,84	104,4	1,20	7,58		
Pool 06-12	139,5	4,79	104,0	1,19	7,58		
Pool 06-12	139,2	4,82	104,2	1,21	7,54		
Pool 06-12	139,3	4,84	104,3	1,21	7,55		
Pool 06-12	138,5	4,79	105,0	1,19	7,53		
Pool 06-12	139,4	4,80	104,0	1,21	7,55		
Pool 06-12	139,3	4,78	104,9	1,19	7,56		
Pool 06-12	139,4	4,83	104,2	1,21	7,53		24,56
Pool 06-12	139,5	4,80	103,8	1,19	7,54		26,34
Pool 06-12	139,3	4,79	104,8	1,19	7,53		25,59
Pool 06-12	139,3	4,80	103,9	1,20	7,52		25,68
Pool 06-12	139,5	4,80	104,0	1,20	7,53		24,68
Pool 06-12	138,5	4,79	104,6	1,19	7,52	0,78	22,99
Pool 06-12	139,2	4,82	104,2	1,20	7,52	0,77	25,10
Pool 06-12	138,4	4,77	104,6	1,20	7,51	0,79	23,89
Pool 06-12	139,2	4,78	104,5	1,19	7,51	0,79	24,21
Pool 06-12	139,1	4,80	103,8	1,20	7,52	0,78	24,32
Pool 06-12	139,2	4,78	103,8	1,21	7,51	0,79	23,58
Pool 06-12	138,4	4,79	104,4	1,19	7,52	0,78	24,21
Pool 06-12	139,2	4,79	104,4	1,19	7,52	0,78	23,77
Pool 06-12	139,3	4,82	103,6	1,20	7,52	0,78	24,26
Pool 06-12	139,7	4,82	104,1	1,20	7,52	0,76	23,78
Average	139,24	4,80	104,17	1,19	7,54	0,78	24,46
SD	0,34	0,02	0,34	0,01	0,02	0,01	0,89
CV	0,24 %	0,36 %	0,33 %	0,78 %	0,31 %	1,21 %	3,65 %
Mini	138,40	4,77	103,60	1,18	7,51	0,76	22,99
Maxi	139,70	4,84	105,00	1,21	7,58	0,79	26,34

a. Repetability for lithium measure on a control serum

5. 2 : Urine

Tests have been performed on solutions of sodium and potassium choride at different levels. Urine mode: sample volume is increased, no dilution. Results in table 4 page, 6

Table 4 : Precision on water based solution (urine)

Code	Na	K	Cl
repet 25	25,2	25,43	54,3
repet 25	25,9	25,52	53,2
repet 25	25,0	25,56	54,4
repet 25	25,0	25,58	53,0
repet 25	25,7	25,43	53,0
repet 25	24,9	25,52	54,4
Average	25,28	25,51	53,72
SD	0,42	0,06	0,72
CV	1,6 %	0,3 %	1,3 %
Mini	24,9	25,43	53
Maxi	25,9	25,58	54,4
repet 100	102,3	102,64	208,5
repet 100	100,5	99,84	211,7
repet 100	101,9	101,88	210,2
repet 100	98,2	101,80	210,6
repet 100	100,7	101,72	211,5
repet 100	100,3	101,57	211,6
Average	100,65	101,58	210,68

Table 4 : Precision on water based solution (urine)

Code	Na	K	Cl
SD	1,44	0,93	1,23
CV	1,4 %	0,9 %	0,6 %
Mini	98,2	99,84	208,5
Maxi	102,3	102,64	211,7
repet 150	139,4	161,09	306,4
repet 150	149,5	151,21	303,3
repet 150	154,1	149,21	306,9
repet 150	151,3	151,52	303,2
repet 150	153,1	151,25	303,0
repet 150	155,8	152,98	298,7
repet 150	153,0	148,93	304,0
repet 150	154,4	151,28	302,9
Average	153,62	150,86	303,12
SD	1,53	1,53	2,63
CV	1,0 %	1,0 %	0,9 %
Mini	151,3	148,93	298,7
Maxi	155,8	152,98	306,9

6 Accuracy

6. 1 : Lithium, sodium and potassium

The sodium, potassium and lithium electrodes have been tested on Seronorm™ Trace Elements Serum L-1 LOT 1309438 and Seronorm™ Trace Elements Serum L-2 LOT 1309416.

The value assignment procedures has been established in accordance with the ISO 17511 [1]) International standard using reference method procedures traceable to primary international standards.

For the lithium, the sodium and the potassium, the method is ICP-SFMS: Inductively Coupled Plasma-Sector Field Mass Spectrometry.

Table 5 : Tests on Seronorm™ Trace Elements

Time	Code	Na	K	Cl	Ca	pH	Li
11 juil. 2018 15:44	level 1	135,2	3,54	114,7	1,87	6,98	0,78
11 juil. 2018 15:43	level 1	135,0	3,54	114,1	1,89	6,99	0,77
11 juil. 2018 15:42	level 1	135,3	3,58	114,1	1,87	6,99	0,79
11 juil. 2018 15:41	level 1	135,3	3,56	114,2	1,88	6,99	0,79
11 juil. 2018 15:40	level 1	135,2	3,54	114,0	1,87	6,99	0,78
11 juil. 2018 15:39	level 1	135,1	3,55	114,2	1,87	6,99	0,79
11 juil. 2018 15:37	level 1	135,1	3,54	114,5	1,86	6,98	0,78
11 juil. 2018 15:36	level 1	134,6	3,53	114,9	1,86	6,99	0,78
11 juil. 2018 15:35	level 1	134,7	3,54	114,7	1,87	6,99	0,78
11 juil. 2018 15:34	level 1	136,3	3,58	113,3	1,81	6,98	0,76
Average		**135,18**	**3,55**	**114,27**	**1,87**	**6,99**	**0,78**
SD		**0,46**	**0,02**	**0,46**	**0,02**	**0,00**	**0,01**
CV		**0,34 %**	**0,50 %**	**0,40 %**	**1,14 %**	**0,07 %**	**1,21 %**
Mini		**134,6**	**3,53**	**113,3**	**1,81**	**6,98**	**0,76**
Maxi		**136,3**	**3,58**	**114,9**	**1,89**	**6,99**	**0,79**
Expected value (Seronorm Trace Elements Serum L-1 Lot 1309538)		**127**	**3,2**				**0,758**
SD		13	0,35				0,0765
Target - SD		114	2.85				0.6815
Target + SD		140	3.55				0.8345
11 juil. 2018 15:56	level 2	154,6	5,77	115,90	2,09	6,91	1,42

1. *ISO 17511. In Vitro Diagnostic Medical Devices – Measurement of quantities in samples of biological origin – Metrological traceability of values assigned to calibrators and control materials. Geneva, International Organisation for Standardization 2003.*

Table 5 : Tests on Seronorm™ Trace Elements

Time	Code	Na	K	Cl	Ca	pH	Li
11 juil. 2018 15:55	level 2	154,7	5,75	115,9	2,11	6,91	1,44
11 juil. 2018 15:54	level 2	152,7	5,66	117,4	2,04	6,92	1,40
11 juil. 2018 15:53	level 2	155,8	5,70	116,5	2,07	6,92	1,41
11 juil. 2018 15:51	level 2	155,9	5,72	116,5	2,09	6,91	1,42
11 juil. 2018 15:50	level 2	154,8	5,70	114,6	2,07	6,91	1,41
11 juil. 2018 15:49	level 2	154,6	5,77	115,9	2,09	6,91	1,43
11 juil. 2018 15:48	level 2	154,7	5,71	114,9	2,07	6,91	1,42
11 juil. 2018 15:47	level 2	154,3	5,69	115,8	2,09	6,91	1,42
11 juil. 2018 15:46	level 2	155,1	5,72	116,6	2,08	6,91	1,42
Average		**154,72**	**5,72**	**116,00**	**2,08**	**6,91**	**1,42**
SD		**0,88**	**0,04**	**0,82**	**0,02**	**0,00**	**0,01**
CV		**0,57 %**	**0,62 %**	**0,71 %**	**0,91 %**	**0,06 %**	**0,78 %**
Mini		**152,7**	**5,66**	**114,6**	**2,04**	**6,91**	**1,4**
Maxi		**155,9**	**5,77**	**117,4**	**2,11**	**6,92**	**1,44**
Expected value (Seronorm Trace Elements Serum L-2 Lot 1309416)		**154**	**5,7**				**1,396**
SD		15,5	0,55				0,1405
Target - SD		138.5	5.15				1.26
Target + SD		169.5	6.25				1.54

These tests show a good correspondance between the results obtained by the Ionix and a reference method for the sodium, the potassium and the lithium.

6. 2 : Calcium and pH

6. 2. 1 pH

The pH electrodes are very well known since a long time, and theoritically the measure of pH is easy. But, if the measure is simple, the pH of the internal reference and calibration solutions are not stable and vary with the temperature. For the calibration, there is no problem, as the two solutions use the same buffer at different pH, with the same variation. The measured slope is not affected by the temperature.

1. For the measure, as the sample is compared to the internal reference, the pH of this solution must be corrected according to the room temperature.
2. The pH of serum or plasma changes also with the temperature. For standardization, in «Blood» mode, the pH of the sample is corrected for 37°C [1]:
   $pH = pH_m - 0.0146(t-37)$,
   with pH_m = pH measured, and t the room temperature

6. 2. 2 Calcium

The precision and the linearity of the calcium measures are very good. The major difficulty comes from the samples handling: the exchanges with the atmosphere and the glycolysis cause a change the sample pH, and so the balance between the ionized calcium and the calcium bounded to proteins is disturbed. [2]

The raw measure of calcium is standardized to a pH at 7.4:

$$Ca_{7.4} = Ca_{mes} \times (1-0.53(7.4-pH_{37}))$$

1.Temperature Correction of Blood-Gas and pH Measurement, Clinical Chemistry 29/11, 1877-1885(1983)
2.IFCC recommendation on sampling, transport and storage for the determination of the concentration of ionized calcium in whole blood, plasma and serum

7 Measuring range

7. 1 : Serum

1. Sodium, potassium, choride, calcium and pH

A first series of tests has been performed on normal patient samples (serums and heparine lithium plasma) in order to determine some plausible coefficients of correction for sodium, potassium, cloride and calcium.

Test of linearity and precision performed with a commercial kit provided by Phœnix Diagnostics [1]. These solutions contain a bovine serum matrix, allow-

Table 6 : Phœnix Diagnostics electrolyte linearity test, lot 60101. Ampules have been equilibrated to room temperature during one day, and tests performed immediately after opening ampules, according to Phœnix instructions. Each level has been assayed five times.

Electrolyte	Na		K		Cl		Ca		pH		
	Phœnix	Ionix	Phœnix	Ionix	Phœnix	Ionix	Phœnix	Ionix	Phœnix	Ionix (37)	Ionix (RT)
		103		1,73		69		4,21		6,94	7,16
		104		1,55		70		4,29		6,95	7,17
		105		1,55		73		4,26		6,96	7,18
		105		1,54		73		4,29		6,95	7,17
		106		1,51		72		4,25		6,96	7,17
Level 1	110		1,30		65		3,00		6,96		
Mean		104,7		1,58		71,4		4,26		6,95	7,17
SD		1,0		0,09		1,8		0,03		0,01	0,01
		123		2,46		94		2,68		7,26	7,48
		124		2,43		94		2,70		7,25	7,47
		124		2,49		94		2,70		7,27	7,49
		125		2,47		93		2,65		7,24	7,46
		126		2,46		94		2,71		7,26	7,48
Level 2	130		2,30		85		2,10		7,25		
Mean		124,3		2,46		93,8		2,69		7,26	7,476
SD		1,1		0,02		0,3		0,02		0,01	0,01
		146		4,31		119		1,68		7,43	7,65
		148		4,28		119		1,70		7,44	7,66
		149		4,32		118		1,68		7,42	7,63
		149		4,30		119		1,69		7,45	7,66
		150		4,37		116		1,75		7,42	7,64
Level 3	145		4,25		105		1,40		7,43		
Mean		148,4		4,32		117,9		1,70		7,43	7,648
SD		1,5		0,03		1,4		0,03		0,01	0,01
				6,24		135		1,08		7,59	7,81
		168		6,22		137		1,09		7,59	7,81
		168		6,18		134		1,05		7,60	7,82
		170		6,12		135		1,09		7,59	7,81
		171		6,33		131		1,13		7,59	7,81
Level 4	170		6,45		120		0,95		7,58		
Mean		169,2		6,22		134,4		1,09		7,59	7,812

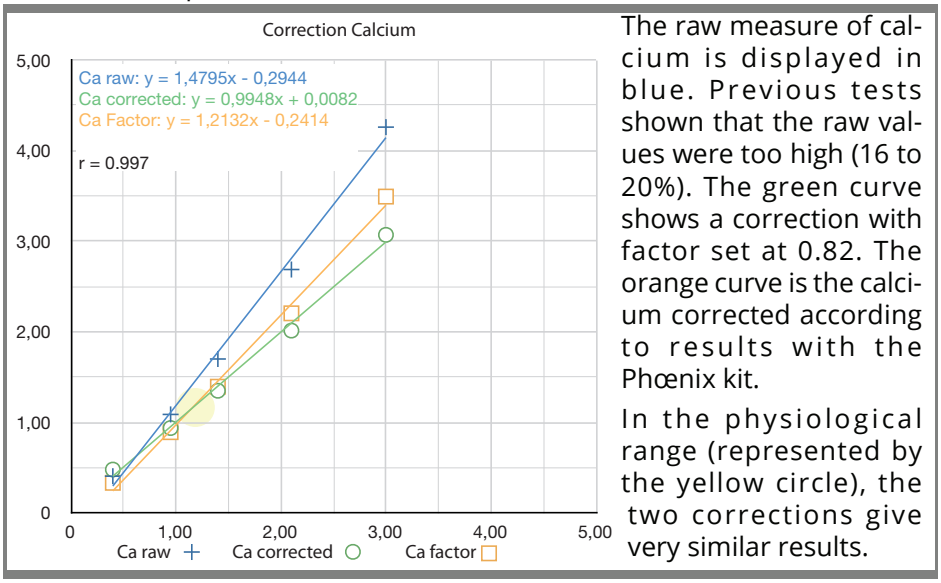
1.http://www.phoenixdiagnostics.com

Table 6 : Phœnix Diagnostics electrolyte linearity test, lot 60101. Ampules have been equilibrated to room temperature during one day, and tests performed immediately after opening ampules, according to Phœnix instructions. Each level has been assayed five times.

Electrolyte	Na		K		Cl		Ca		pH		
	Phœnix	Ionix	Phœnix	Ionix	Phœnix	Ionix	Phœnix	Ionix	Phœnix	Ionix (37)	Ionix (RT)
SD		1,4		0,08		2,4		0,03		0,00	0,00
		194		8,58		165		0,41		7,76	7,98
		194		8,57		162		0,41		7,76	7,97
		194		8,57		157		0,39		7,76	7,98
		195		8,64		158		0,38		7,77	7,98
				8,75		154		0,44		7,74	7,96
Level 5	185		9,00		140		0,40		7,73		
Mean		194,1		8,62		159,2		0,41		7,76	7,974
SD		0,5		0,08		4,3		0,02		0,01	0,01
Slope		1,17		0,91		1,17		1,48		1,05	
Intercept		-24,85		0,38		-5,05		-0,29		-0,35	
r		0,995		1,000		1,000		0,997		1,000	
Correction											
Slope		0,85		1,09		0,86		0,67		0,95	
Offset		22,58		-0,42		4,35		0,21		0,34	

ing a «true» measure of ionized calcium. Each level has been measured six times, without correction.

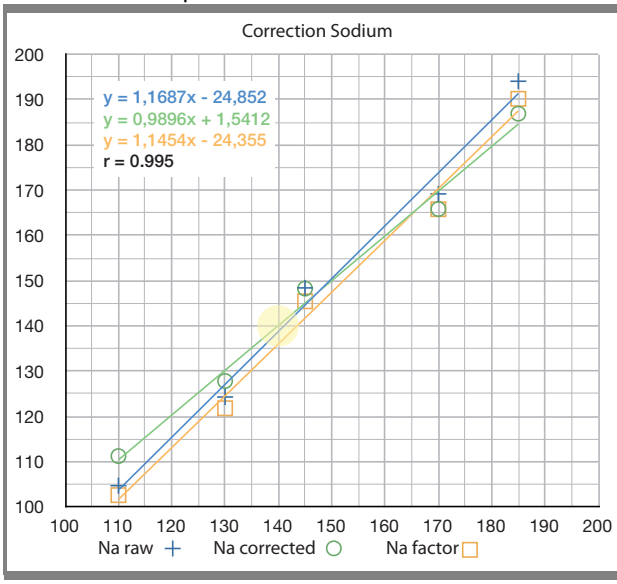
- pH response: for the pH, no correction is necessary for the pH corrected at 37°C
- Calcium response



The raw measure of calcium is displayed in blue. Previous tests shown that the raw values were too high (16 to 20%). The green curve shows a correction with factor set at 0.82. The orange curve is the calcium corrected according to results with the Phœnix kit.

In the physiological range (represented by the yellow circle), the two corrections give very similar results.

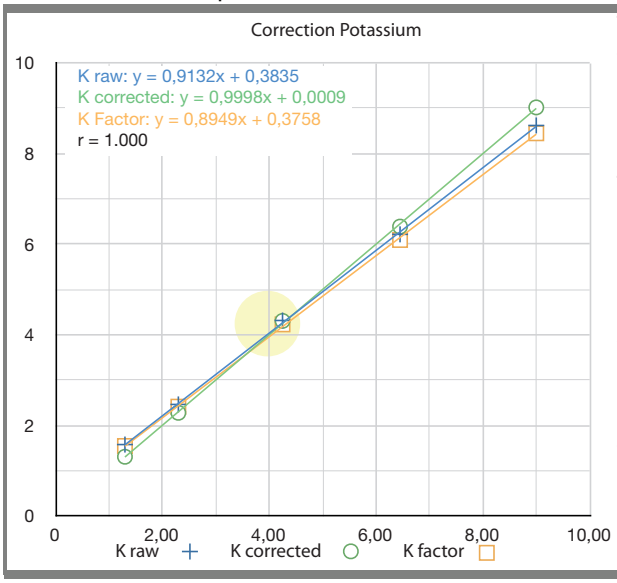
- Sodium response



The raw measure of sodium is displayed in blue. Previous tests shown that the raw values were too high (2%). The green curve shows a correction with factor set at 0.98. The orange curve is the sodium corrected according to results with the Phœnix kit.

In the physiological range (represented by the yellow circle), the two corrections give very similar results.

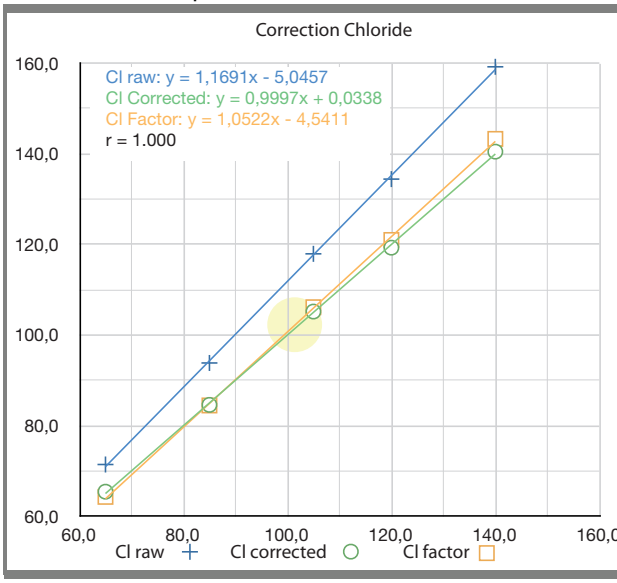
- Potassium response



The raw measure of potassium is displayed in blue. Previous tests shown that the raw values were almost correct. The green curve shows a correction with factor set at 0.98. The orange curve is the potassium corrected according to results with the Phœnix kit.

In the physiological range (represented by the yellow circle), the two corrections give very similar results. Even the raw measure can be used.

- Chloride response



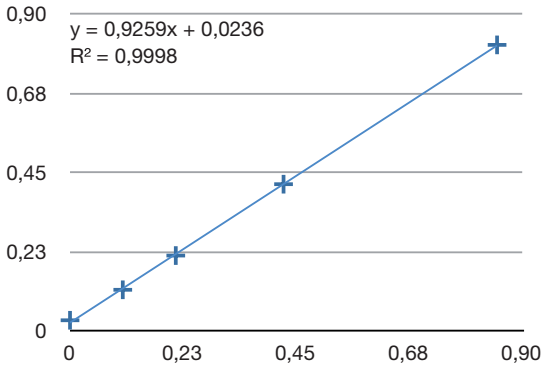
The raw measure of chloride is displayed in blue. Previous tests shown that the raw values were too high. The green curve shows a correction with factor set at 0.88. The orange curve is the chloride corrected according to results with the Phœnix kit.

In the physiological range (represented by the yellow circle), the two corrections give very similar results.

2. Lithium

A pool of serum collected on patient without lithium treatment has been prepared. A range of samples with different levels of lithium has been prepared from an heparine lithium plasma sample. The lithium concentration of this sample has been determined with a flame photometer. Dilutions by 2, 4, and 8 have been prepared, then measured three times, with the data determined above:

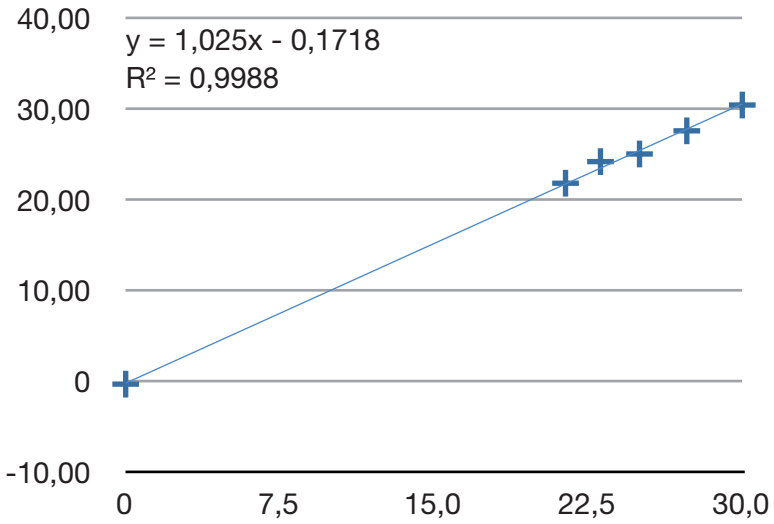
	Theorical value	Measured value
Pool	0,00	0,03
Plasma/8	0,11	0,12
Plasma/4	0,21	0.21
Plasma/2	0,43	0,42
Plasma	0,85	0,81



3. tCO_2

For the total CO_2, a main solution (30 mmol/L) has been diluted by 10, 20 30 and 40%, in order to have concentrations at 30, 27.3, 25, 23.1 and 21.4 mmol/L. Some deionized water has been used for the zero. The different levels have been measured thre times.

	Theorical value	Measured value
Zero	0,00	-0.28
10/14	21.4	21.85
10/13	23.1	24.23
10/12	25	25.07
10/11	27.3	27.62
10/10	30	30.46



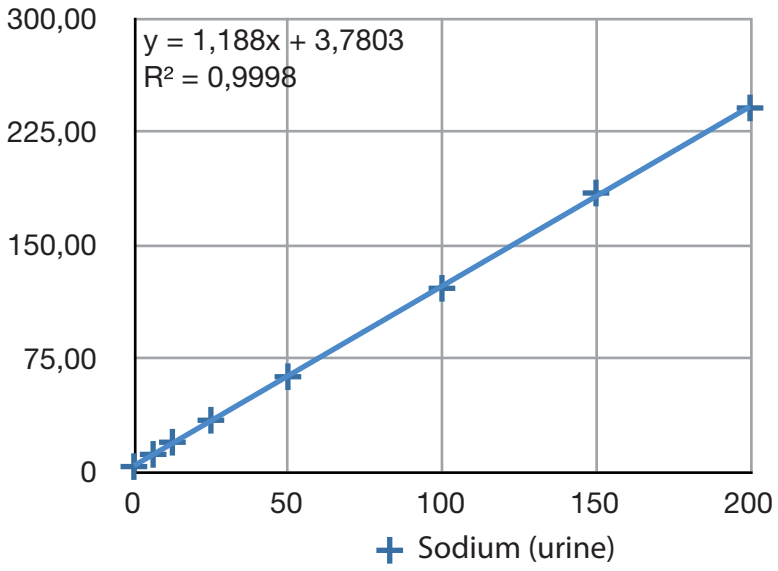
7. 2 : Urine

The linearity has been measured with a mother solution of sodium chloride (200 mmol/L) and potassium chloride (200 mmol/L) in deionized water. This mother solution has been diluted with deionized water.

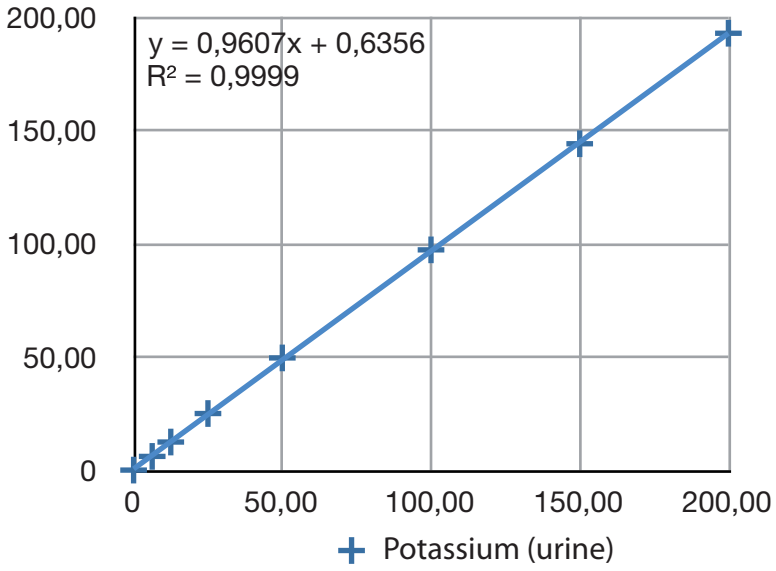
Na	Na measured	K	K measured	Cl	Cl measured
0	3,23	0,00	0,11	0,00	4,97
6,25	11,30	6,25	6,15	12,50	17,10
12,5	19,33	12,50	12,49	25,00	28,83
25	33,87	25,00	25,06	50,00	51,03
50	62,67	50,00	49,58	100,00	93,20
100	121,17	100,00	97,24	200,00	167,63
150	184,20	150,00	144,04	300,00	234,80
200	240,47	200,00	192,77	400,00	299,20

For these three ions, the linearity is very good ($R^2 > 0.99$). Only a simple correction has to be applied.

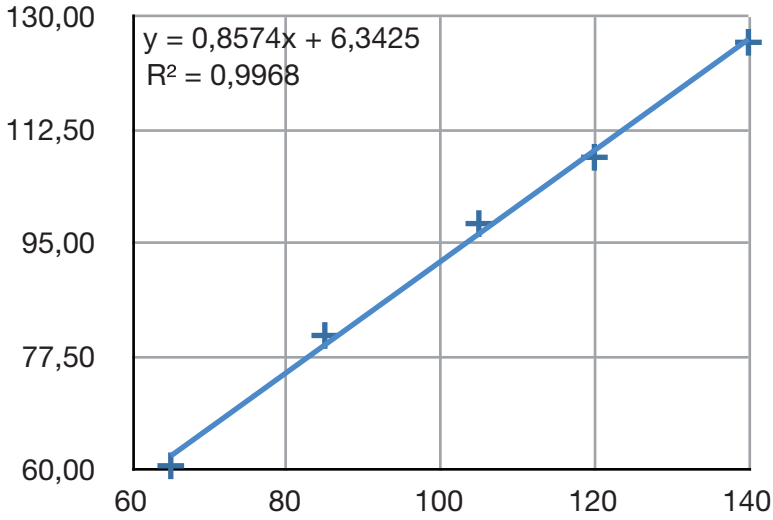
- Sodium response



- Potassium response



- Chloride response



7. 3 : True measuring range

As blood and urine are measured without any dilution, the same measurement ranges can be defined for all kind of samples:

Table 7 : Measuring range

	Low limit	High limit
Sodium	3.8	200
Potassium	0.12	200
Chloride	4.5	400
Ca	0.11	8
pH	4	9
Lithium	0.1	3

8 Interfering substances

The major interferences [1, 2] come from a bad preparation of the samples:

1.Dimeski G, et al, Ion Selective Electrodes (ISEs) and interferences—A review, Clin Chim Acta (2009), doi:10.1016/j. cca.2009.12.005
2.https://acutecaretesting.org/en/articles/useful-tips-to-avoid-preanalytical-errors-in-blood-gas-testing-electrolytes

- Anticoagulants (binding effects)
- Hemolysis (sampling, storage temperature...)
- Metabolism (changes of pH)
- Evaporation

A complete chapter of the user manual describes the risks and gives some tips to avoid the major errors.

9 Stability of samples

Blood samples can be serum of plasma heparin lithium. For serum, a gel separator is highly recommended.

They must be centrifugated and measured as soon as possible after collection (for plasma) or after cloting (for serum). Pre-analytical precaution [1] are very important the accuracy of results.

- Sodium: stable 36 hours at room temperature and 48 hours at 2~4 °C
- Potassium and Chloride: if the serum (or the plasma) has been separated from the red blood cells, there is no change after 12 hours at room temperature or 2~4 °C.
  Without separation, there is a fast hemolysis of the red blood cells, even at 2~4 °C. This hemolysis is not predictable.
- Calcium (and pH): according to IFCC [2], samples must be kept in close tubes and measured with a maximum delay of 15 minutes after the blood collection.

10 Method comparison

- Sodium: flame photometer. Model PHF 104 (Hycel Diagnostics), calibrator Jenway 025006, Lot FCNK517C1.
- Potassium: flame photometer. Model PHF 104 (Hycel Diagnostics), calibrator Jenway 025006, Lot FCNK517C1.
- Lithium: flame photometer. Model PHF 104 (Hycel Diagnostics), controled with Seronorm™ Trace Elements Serum L-1 LOT 1309438 and Seronorm™ Trace Elements Serum L-2 LOT 1309416
- pH: pHmeter Thermoscientific, model Orion 2, sn B44156

1.Dimeski G, et al, Ion Selective Electrodes (ISEs) and interferences—A review, Clin Chim Acta (2009), doi:10.1016/j. cca.2009.12.005
2.Journal of Automatic Chemistry, Vol. 13, No. 5 (September-October 1991), pp. 235-239: IFCC recommendation on sampling, transport and storage for the determination of the concentration of ionized calcium in whole blood, plasma and serum

11 Reference values

Blood	Na	K	Cl	Ca	pH[a]	Li[b]	CO^2
Low	136	3.5	98	1.07	7.32	0	23
High	145	5.1	107	1.15	7.42	1	27

a. Veinous pH at 37°C
b. The lithium is a medicament , not present in normal blood. These values represent a therapeutic range for patients under treatment.

Urine[a]	Na	K	Cl	Ca	pH
Low	40	25	95	2.5	4.6
High	220	125	250	6.2	8

a. Values only for indication. The concentrations have to be reported to the patient diuresis

12 Conclusions

Different organizations publish the desirable specifications for assays performances. From country to country the specifications can vary.

Below, the table shows the desirable biological variation specifications established by Fr. Carmen Ricos and colleagues (https://www.westgard.com/biodatabase1.htm).

Table 8 : Within-subject and between-subject CV values of analytes and Desirable Analytical Quality Specifications for imprecision, bias and total error

Sample	Analyte	Biological variation		Desirable specification		
		CV_i[a]	CVg[b]	I(%)[c]	B(%)[d]	TE(%)[e]
Blood	Sodium	0.6	0.7	0.3	0.23	0.73
Urine	Sodium	28.7	16.7	14.4	8.3	32
Blood	Potassium	4.6	5.6	2.3	1.81	5.61
Urine	Potassium	24.4	22.2	12.2	8.2	28.4
Blood	Chloride	1.2	1.5	0.6	0.5	1.5
Blood	iCalcium	1.7	1.9	0.9	0.6	2.0
Blood	pH(pH units)	0.2	...	0.1	...	...

a. CV_I = within-subject biologic variation
b. CVG = between-subject biologic variation
c. I = desirable specification for imprecision
d. B = desirable specification for inaccuracy
e. TE = desirable specification for allowable total error

The tests performed on serum on Ionix show a imprecision better than requested by Ricos.

The bias and the total error can be easily corrected by the operators if necessary.

Recommandations from other organizations are more or less strict. For more informations, refer to Westgard website (https://www.westgard.com). The performances of the Ionix are better than any of these recommandations.

Tests performed by	Christian Barboux	
Tests validated by	Émilie Soubielle