Clin Chem Lab Med 2006;44(3):346–352 © 2006 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2006.060

# Recommendation for measuring and reporting chloride by ISEs in undiluted serum, plasma or blood

**International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)[1),2)]**

IFCC Scientific Division, Committee on Point of Care Testing and Working Group on Selective Electrodes[3)]

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## Abstract

The proposed recommendation for measuring and reporting chloride in undiluted plasma† or blood by ion-selective electrodes (ISEs) will provide results that are identical to chloride concentrations measured by coulometry for standardized normal plasma or blood samples. It is applicable to all current ISEs dedicated to chloride measurement in undiluted samples that meet the requirements. However, in samples with reduced water concentration, results by coulometry are lower than by ion-selective electrode due to volume displacement. The quantity measured by this



[2)] IFCC Sections reprinted in J. Clin. Chem. Clin. Biochem. are listed in the Cumulative Index, which appeared in connection with the contents of this journal in Volume 27, 1989 and since 1991 have been printed in (Eur.) J. Clin. Chem. Clin. Biochem.
IFCC 1991/1 Vol. 29, 435–457
IFCC 1991/2 Vol. 29, 531–535
IFCC 1991/3 Vol. 29, 577–586
IFCC 1991/4 Vol. 29, 767–772
IFCC 1992/1 Vol. 30, 901–905
IFCC 1994/1 Vol. 32, 639–655
IFCC 1995/1 Vol. 33, 247–253
IFCC 1995/2 Vol. 33, 399–404
IFCC 1995/3 Vol. 33, 623–625
IFCC 1995/4 Vol. 33, 627–636
IFCC 1995/5 Vol. 33, 637–660
IFCC 1997/1 Vol. 35, 317–344
IFCC 1997/2 Vol. 35, 345–349
IFCC 1997/3 Vol. 35, 805–831
IFCC 1997/4 Vol. 35, 833–843
For IFCC sections printed in Clin. Chem. Lab. Med. since 1998, please visit the link http://degruyter.com/journals/extenza, where they are freely accessible.

[3)] The membership of the Committee on Point of Care Testing in 2005 is as follows: Chairman, W.R. Külpmann (DE); members, M.C. Ben Rayana (TN), N. Fogh-Andersen (DK), C. Ritter (AT), A. St John (AU).
The membership of the Working Group on Selective Electrodes in 2005 is as follows: Chairman: W.R. Külpmann (DE); members, R.W. Burnett (US), A.K. Covington (UK), P. D'Orazio (US), N. Fogh-Andersen (DK), E. Jacobs (US), R. Kataky (UK), K. Kuwa (JP), L. Larsson (SE), A. Lewenstam (FI), A.H.J. Maas (NL), G. Mager (DE), J.W. Naskalski (PL), A.O. Okorodudu (US).

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Received December 13,2005

† In this document, the general term ''plasma'' refers to circulating plasma and the forms in which it is sampled to determine its chloride concentration, namely the plasma phase of anticoagulated blood, plasma separated from blood cells, or serum.

standardized ISE procedure is called the ionized chloride concentration. It may be clinically more relevant than the chloride concentration as determined by coulometry, photometry or by ISE after dilution of the sample.

**Keywords:** blood; chloride (ionized and total); coulometry; ion-selective electrode; plasma; standardization.

**Contents**



## 1. Introduction

### 1.1 Background

Ion-selective electrodes used in blood or undiluted plasma respond to the activity of the appropriate ion. They do not sense substance concentration, which, in fluids such as plasma, is related to activity in a complex way. Therefore, results should be expressed in terms of ion activity. However, this is difficult to attain in practice.

For many years, results of determinations of chloride in physiological fluids have been expressed in terms of substance concentration, i.e., the concentration of total chloride (mmol/L). Hence, the use of substance concentration of chloride and its reference range is firmly established in clinical interpretation and practice. Furthermore, it can be envisaged that analytical systems that measure total substance concentration will continue to be used alongside ion-selective electrode determinations in undiluted plasma. Thus, it is recognized that introducing the reporting of results by ion-selective electrodes in terms of ion activity may lead to incorrect clinical interpretation.

The convention proposed here represents a compromise to facilitate the use of ion-selective electrode determinations of chloride ion concentration in blood or undiluted plasma in routine clinical practice while minimizing the risk of clinical misinterpretation.

### 1.2 Purpose

Potentiometric measurement of chloride in undiluted plasma is now widely used, and ionized chloride – the definition is a subject of this recommendation – is measured and reported as total chloride. However, a means for proper and uniform standardization is still lacking. Therefore, this document presents a recommendation for standardization of the measurement and reporting of ionized chloride in undiluted plasma using ion-selective electrodes (1) using an approach similar to that for sodium and potassium (2).

Special emphasis is placed on issues related to calibration, the reference method and liquid junction effects, which may be sources of analytical variability.

## 2. Measurement

### 2.1 Measuring cell

Currently the measurement of ionized chloride ($Cl^-$) is predominantly performed by potentiometry with an ion-selective chloride electrode ($Cl^-$-ISE). A $Cl^-$-ISE will change its electrical potential difference ($E_{Cl\text{-}ISE}$) in response to a change in the chemical potential of chloride ions in solution. Since no perfectly specific $Cl^-$-ISE is available, this response is estimated by a modified Nernst equation, often called the Nikolsky-Eisenman equation [1]:

$$E_{Cl\text{-}ISE} = E^0 - s \log\left( a_{Cl^-} + \sum_j K^{pot}_{Cl^-,j}\, a_j^{z_{Cl^-}/z_j} \right), \quad [1]$$

where $a_{Cl^-}$ is the activity of $Cl^-$ in the calibrator or sample and $z_{Cl^-}$ is the electrical charge of $Cl^-$, $a_j$ is the activity of any interfering ion and $z_j$ is its electrical charge, $E^0$ is the standard potential, $s$ is the slope and $K^{pot}_{Cl^-,j}$ is the selectivity coefficient; $s = 2.303\ RTz_{Cl^-}^{-1}\ F^{-1}$, where the gas constant

$R$=8.3144 J/(mol K), $T$ is absolute temperature (K) and the Faraday constant $F$=96,485 C/mol.

The activity of the chloride ion is measured with a potentiometric cell comprising a $Cl^-$-ISE and a reference electrode. These two electrodes are immersed in the calibrator or sample. Two electrical leads connect the electrodes to a millivoltmeter, which measures the potential difference between the $Cl^-$-ISE and the reference electrode, with the latter including the liquid-junction potential:

External reference electrode || test solution | $Cl^-$ membrane | internal reference electrode [2]

The $E_{Cl\text{-}ISE}$ value of the sample is compared to those of the calibrators. Using the Nikolsky-Eisenman equation [1], the concentration of $Cl^-$ in the sample is estimated from the $E_{Cl\text{-}ISE}$ of the sample and the calibrators.

### 2.2 Calibration

$Cl^-$-ISEs are standardized to give accurate results in terms of substance concentration (mmol/L) in standard normal serum (see Section 5). During routine operation, calibration of the $Cl^-$-ISE is performed with working calibrators obtained from the manufacturer, which may be used for other calibration purposes as well. These calibrators may include a slowly diffusing ion, such as acetate or lactate, replacing chloride and mimicking the slowly diffusing protein anion and bicarbonate ion of plasma.

### 2.3 Liquid junction

**2.3.1 Origin of the liquid junction potential** A diffusion potential ($-E_j$) will always arise when two electrolytes of different composition are in contact. Hence, this potential is unavoidable at the liquid junction between the sample and the salt bridge of the reference electrode. Even with equitransferent salts such as KCl of $>2$ mol/L and at steady state, which is seldom achieved, the diffusion potential is not completely constant with different samples or calibrators. The millivoltmeter measures the total electrical potential difference, so it is necessary to consider $E_j$.

The change that occurs in $E_j$ when plasma is substituted by a calibrator is called the residual liquid junction potential or $\Delta E_j$ [$=E_j$(plasma)$-E_j$(calibrator)]. It is subtracted from the measurement, since it causes a bias unless taken into account.

$\Delta E_j$ should also be taken into account during slope calibration. The bridge solution should consist of an equitransferent salt of not less than 2 mol/L (3, 4).

**2.3.2 Design of the liquid junction** An open liquid junction is recommended to provide an uncontaminated and undiluted bridge solution for each measurement.

However, use of a restricted liquid junction with a porous plug, or membrane, separating the filling solution of the reference electrode from the sample, is permissible, provided that similar results are obtained as those with a hypertonic open liquid junction. A washout effect may arise, due to the diffusion of water from the rinse solution into the porous plug, which may make the bridge solution isotonic. If the bridge solution does become diluted, $\Delta E_j$ may be much larger than with an open junction, increasing the bias.

**2.3.3 Erythrocyte effect on the liquid junction potential** Erythrocytes lower $E_j$ by approximately 1 mV (depending on hematocrit), with corresponding bias of approximately $-4$% for $Cl^-$ as measured with a saturated KCl bridge. Water, extracted from the erythrocytes near the hypertonic salt bridge solution, dilutes the plasma and restores the osmotic equilibrium across the erythrocyte membrane (5). Diffusion potentials between plasma, diluted plasma and the bridge solution are the major contributors to $E_j$ observed with blood.

### 2.4 Interferences

**2.4.1 Anion interference** Membranes of most currently used $Cl^-$-ISEs contain an ion-exchanger, a quaternary ammonium chloride. The selectivity of such membranes is in principle governed by the ion hydration energy (6). Hence, all ions that have hydration energy higher than or equivalent to chloride are considered as potentially interfering ions, e.g., bromide, iodide, thiocyanate, salicylate, bicarbonate and heparin. However, only bicarbonate is normally a significant contributor to the last term in Eq. [1].

**2.4.2 Influence of lipophilic cations** Lipophilic cations may enter the membrane by co-extraction, resulting in Donnan exclusion (membrane permselectivity) failure (7–9). Drugs in the form of quaternary ammonium compounds and their salts are representatives of this group.

**2.4.3 Influence of proteins** Due to the rather high polarity of $Cl^-$-ISE membranes, proteins tend to precipitate at the membrane surface. This may impair ionic partition and slow down ion exchange, or even impede the signal generation process. Typically, changes in sensitivity and response time are observed, resulting in possible deterioration of the precision and trueness.

## 3. Reporting results of ISE chloride determination

The $Cl^-$ activity determined should be adjusted by a constant factor to yield the total concentration in normal plasma. In this way, the usual reference intervals and units of concentration are maintained, which allows direct comparison of ionized chloride by potentiometry to the total chloride concentration.

## 4. Reference measurement procedure for the determination of chloride

It is proposed that coulometric titration with amperometric end-point determination be the reference measurement procedure for the potentiometric method of determination of chloride (10). All potentiometric methods should be traceable to this reference method.

## 5. Standardization

### 5.1 Objective

The objective of standardization is to obtain results from $Cl^-$-ISE analyzers that are verified as accurate in terms of substance concentration (mmol/L) in standard normal serum (constituted according to Table 1). There is a need to enable laboratories to attain numerically identical results for both total and ionized chloride in normal plasma. The deviation of the ISE measurement from total chloride concentration after standardization should not exceed $\pm$2%, irrespective of the chloride concentration of the specimen, within the limits 80–120 mmol/L chloride in plasma. A method is suggested whereby this standardization may be achieved in setting up the $Cl^-$-ISE instrument. This method may be employed both by manufacturers and by users in verifying, or adjusting, the results of their instruments.

### 5.2 Use of certified serum-based reference materials

A series of three human serum reference materials should be obtained that is traceable to certified human serum (CRM). These should provide a range of chloride concentrations from approximately 80 to 120 mmol/L with a midpoint sample at 100 mmol/L ($\pm$2 mmol/L). All three materials should have values for water mass concentration, total $CO_2$, albumin, total protein, triacylglycerols, cholesterol concentrations and pH in serum within the ranges given in Table 1. They should contain bicarbonate in the normal range and should not contain any other interferent ion causing a bias in concentration higher than 0.1 mmol/L.

Ultrafiltration, dilution and spiking may be used to achieve an acceptable range of concentrations.

Samples of each material must be analyzed in random order, in duplicate, on both the ISE instrument (calibrated according to Section 2.2) and the coulometric titrator (calibrated according to Section 4). The coefficient of variation (CV) of both procedures must be $\leq$1.0%. For coulometric titration, the bias from the target value of the certified reference material must be less than $\pm$1%. Results are analyzed by linear regression analysis ($y = Ax + B$, where y is the original ISE result and x is the result of the coulometric titration). The coefficient of correlation r should be greater than 0.990. The output of the ISE analyzer is adjusted to provide an intercept of 0 and a slope of 1.000 using a slope correction factor of 1/A and an intercept correction of –B/A. The equation for adjusting the ISE results is thus: $y' = y/A - B/A$, where y′ is the adjusted, and y the original ISE result. In this way, the results of the ISE analyzer are standardized to those of the coulometric titrator (Figure 1).

**Table 1** Requirements for certified serum-based reference materials (2).

| Component | Range | Unit |
|---|---|---|
| Water | 0.925–0.935 | kg/L |
| $CO_2$, total | 24–26 | mmol/L |
| Protein, total | 68–72 | g/L |
| Albumin | 42–46 | g/L |
| Cholesterol | 3.1–5.2 | mmol/L |
| Triacylglycerols | 0.45–2.26 | mmol/L |
| pH (37°C) | 7.30–7.50 | |

## 6. Supplementary methods necessary for standardization

### 6.1 Calibration of the coulometric-amperometric instrumentation

The accuracy of calibration values for the $Cl^-$-ISE analyzer depends on the accuracy of calibration of the coulometric-amperometric instrumentation. When the coulometer is calibrated according to the manufacturer's specification, calibration values must be traceable to the NIST (National Institute of Standards and Technology) reference method for chloride (10). Accuracy should be verified frequently during use by analysis of human plasma or serum specimens with values that are known with a degree of accuracy, such as the Certified Reference Materials (CRMs) NIST CRM956b and JC (Japanese Clinical Laboratory Use) CRM111.

### 6.2 Determination of serum water mass concentration

A gravimetric method is recommended to determine serum water concentration by mass because it has the highest precision and is simple to perform (11). Pipet approximately 1 mL of serum into a tared weighing bottle and record the starting weight. Dry the sample at 110°C overnight and cool in a desiccator containing silica gel or an equivalent dehydrant. Weigh the residue and the weight loss corresponds to the mass of water present in the aliquot of serum. Results should be expressed in kg of water per liter of serum.

### 6.3 Determination of other relevant quantities

Determination of the serum total $CO_2$ concentration, pH and concentrations of albumin, total protein, cholesterol and triacylglycerols should be performed by

methods that are routinely used in clinical chemistry. These measurements are performed to ensure ''normal'' serum water mass concentration and ''normal'' binding, i.e., normal concentrations of albumin, total protein, cholesterol, triacylglycerols, total $CO_2$ and normal pH.

## 7. Calibration verification

### 7.1 Human sera

Human sera may be used to verify the above procedure immediately, or later, to determine if the standardization is valid or if the procedure should be repeated. The specimens should ideally be no more than 24 h old, maintained in an anaerobic state, and the concentration of their analytes known to be within the ranges given in Table 1. Calibration of the ISE instrument should be in accordance with Section 2.2, and of the coulometric titrator with Section 4. To prove the standardization function, a minimum of 30 patient samples, chosen to span the analytical range of chloride in human plasma (80–120 mmol/L) as closely as possible, should be run in duplicate on each instrument. The data should be normalized to 100 mmol/L chloride by subtraction of 100 from all mean values. Linear regression analysis of the normalized data should be performed by pairing the first ISE duplicate with the first coulometric titrator duplicate, and so on. The ISE instrument should be used as the ''y'' or dependent variable. The slope should be within the interval 0.95–1.05. The intercept should be within the range $-5$ to $+5$ mmol/L for chloride. The correlation coefficient should be greater than 0.990.

### 7.2 Certified serum reference material

It may be more convenient and reliable to apply the proposed calibration verification using target values for certified reference material (see Figure 1 for a summary of the procedures). This alternative procedure will avoid the delays associated with collecting samples, evaluating their suitability by additional (expensive) determinations, and setting up a method for accurate measurements of total chloride.

## Appendix: Specifications for a human serum Certified Reference Material as a calibrant for the ISE measurement of ionized chloride in plasma

### A. Starting material and preparation of reference material

#### A.1 Starting material, biohazards, and base pool specifications

**A.1.1** Only native human serum (not converted ACD plasma) that has been tested and found negative for $HB_S$ antigen, HCV and HIV should be used for reference material. For human serum the concentration of sodium, potassium, and other analytes (see Table A1) in the pooled sera should be within the usual reference intervals for a fasting healthy population. On equilibration with 5% $CO_2$/95% $N_2$ (inert) (v/v) and $pCO_2$ of 4.67 kPa, the pH of this base pool should return to $7.4 \pm 0.1$ and the bicarbonate concentration to $25 \pm 2$ mmol/L. The serum pool should be frozen ($-20$°C) and thawed and a visible cloudiness (presumably lipoproteins) should be observed. If cloudiness is not observed, the freeze-thaw cycle should be repeated.

**A.1.2** Filter the base pool through an Avicel© cellulose slurry under nitrocellulose vacuum (12), or an equivalent clearing process, to reduce turbidity. The absorbance at 700 nm in a cuvet of 1-cm path length should be less than 0.1 against a water blank. This treatment also helps to reduce the rate of clogging in subsequent finer nitrocellulose filtration steps that help to ensure the homogeneity and to reduce the bacterial count.

**A.1.3** After the above clearing step, add and mix gentamicin sulfate into the base pool to give a final concentration of 50 mg/L. The pool is then filtered through a coarse prefilter and then progressively down through nitrocellulose filters of the following pore sizes: 3.00, 1.20, 0.80, 0.65, 0.45, 0.30, and finally 0.22 μm.

**A.1.4** Remove a small aliquot of this filtered base pool and measure its potassium concentration by FAES and total protein by the biuret reaction. Store another 20-mL aliquot at 4°C for future simultaneous comparison of analyte levels against the new low, medium, and high CRMs.

**Table A1** Requirements for serum used in base pool (A1).

| Quantity | Method | Reference interval | Unit |
|---|---|---|---|
| Sodium(S) | FAES | 136–146 | mmol/L |
| Potassium(S) | FAES | 3.5–5.1 | mmol/L |
| Calcium(S) | FAAS | 2.10–2.55 | mmol/L |
| Magnesium(S) | FAAS | 0.65–1.05 | mmol/L |
| Lithium(S) | FAAS | $<0.1$ | mmol/L |
| Chloride(S) | Coulometry | 98–106 | mmol/L |
| Urea(S) | Enzymatic | 2.5–6.4 | mmol/L |
| Creatinine(S) | Enzymatic | 44–106 | μmol/L |

S, serum; FAES, flame atomic emission spectrometry; FAAS flame atomic absorption spectrometry.

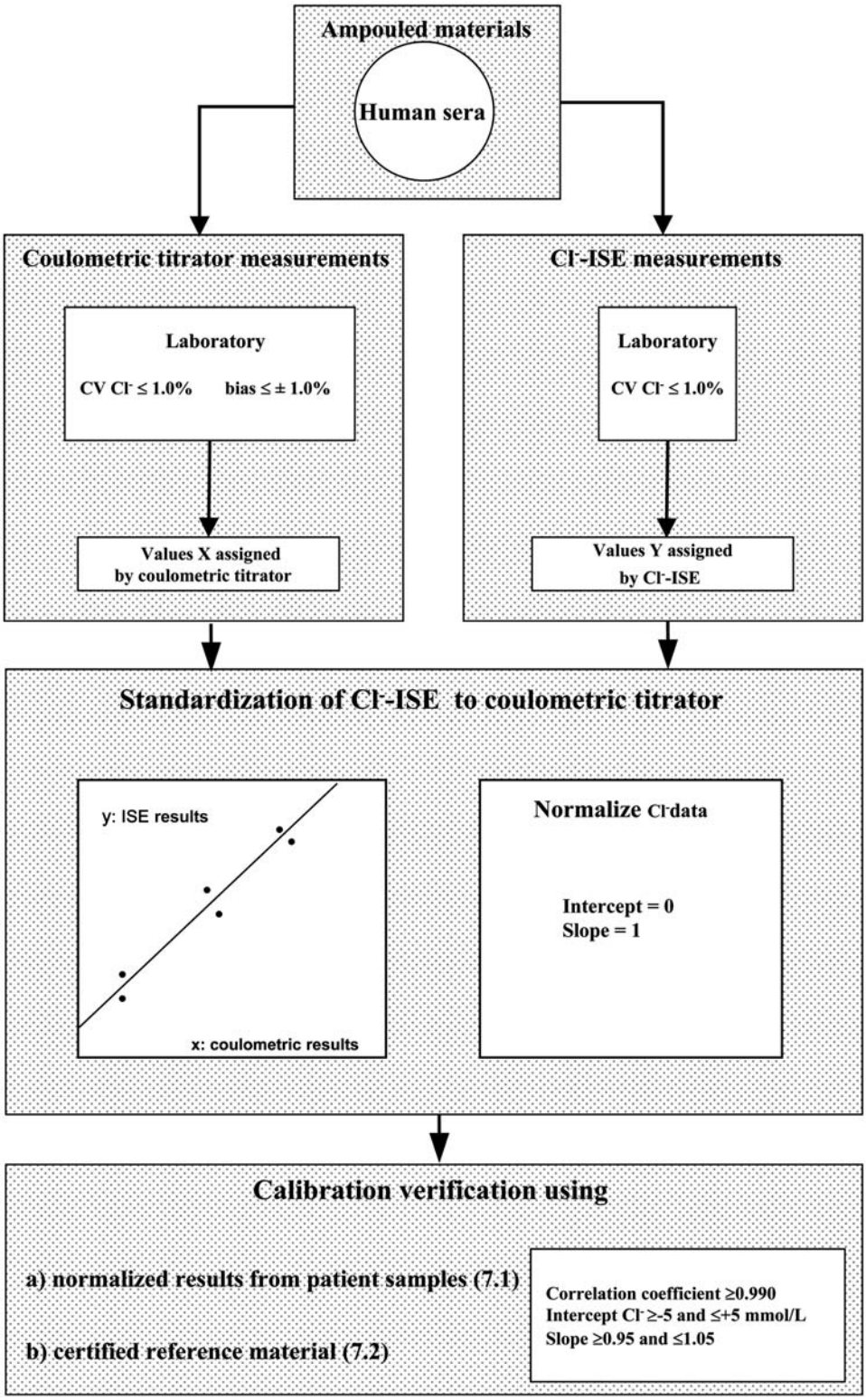


**Figure 1** Standardization of $Cl^-$ ISE measurements and calibration verification.

### A.2 Dilution and ultrafiltration of the base pool

**A.2.1** Dilute the filtered base pool with enough (approx. an equal volume) sterile 25 mmol/L $NaHCO_3$ so that the potassium ion concentration is $2.0 \pm 0.1$ mmol/L by FAES and the total $CO_2$ concentration is $25 \pm 1$ mmol/L by a volumetric technique.

**A.2.2** Ultrafilter this diluted base pool at $6 \pm 3$°C until the total protein of the retentate reaches $70 \pm 2$ g/L using an appropriately sized apparatus and a filter with a relative molar mass cutoff of 20,000.

### A.3 Adjustments to the low and high pools

**A.3.1** Divide the ultrafiltered (0.22 μm) and diluted base pool (A2) into two equal pools labeled low and high. Add the appropriate amounts of sodium, potassium, calcium and magnesium salts of chloride, and sodium lactate to each pool to give the following final

| | Cl, mmol/L | Na, mmol/L | K, mmol/L | Ca, mmol/L | Mg, mmol/L | Lactate, mmol/L | Ionic strength, mol/kg |
|---|---|---|---|---|---|---|---|
| Low pool | 80±3 | 131±3 | 3.0±0.2 | 2.0±0.2 | 1.0±0.1 | 20±0.3 | 0.150 |
| High pool | 120±3 | 148±3 | 6.0±0.2 | 3.0±0.2 | 2.0±0.1 | 4.0±0.1 | 0.175 |

| | Cl, mmol/L | Na, mmol/L | K, mmol/L | Ca, mmol/L | Mg, mmol/L | Lactate, mmol/L | Ionic strength, mol/kg |
|---|---|---|---|---|---|---|---|
| Medium pool | 100±3 | 140±3 | 4.5±0.2 | 2.5±0.2 | 1.5±0.1 | 12±0.2 | 0.163 |

Note: The ionic strength of each of these three pools is different and only the medium pool is approximately 0.160 mol/kg water. The bicarbonate concentration of all pools is approximately 25 mmol/L. A pH range of 7.30–7.50 is realized by adjusting the **p**$CO_2$ accordingly. The composition of the reference material has been chosen such that it may also be used as a calibrant for sodium, potassium, calcium, magnesium and lactate electrodes.

(mmol/L) substance concentrations (Na/K by FAES, Ca/Mg by FAAS, and lactate by an enzymatic method (see above)). The medium pool is made from equal amounts of low and high pools and in theory should give values close to the middle, as above.

### A.4 Final filtration, dispensing, packaging and storage

**A.4.1** Refilter each of these three subpools through a pore size of 0.22 μm to increase the homogeneity and reduce the bacterial count.

**A.4.2** Hold filtered pools at 6±3°C and under a 5% $CO_2$/95% $N_2$ (inert) (v/v) gas mixture at 1 atm during storage and filling operations.

**A.4.3** Dispense 3.5 mL of each subpool into 5-mL glass ampoules that have been flushed with and sealed under a 5% $CO_2$/95% $N_2$ (inert) (v/v) gas mixture. Immediately store all processed CRMs at −50°C and make subsequent transfers under dry ice.

### A.5 Value assignment, unit size and distribution

**A.5.1** The responsibility for the final value assignment and certification of this CRM rests with an accredited reference laboratory.

**A.5.2** A packaged unit of this CRM is, for example, six frozen ampoules (two low, two medium, and two high) shipped in dry ice.

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