CHLORIDE KIT

(Thiocyanate Method)

ELYTE 2 KIT

(Na^+ & K^+ Colorimetric)

ELYTE 3 KIT

(Na^+ / K^+ & Cl^- Colorimetric)

For the determination of Na^+ / K^+ & Cl^- in serum.
(For Invitro Diagnostic Use Only)

Summary

Sodium and Potassium are the major cations of extracellular and intra cellular fluids respectively. Sodium maintains the normal distribution of water and the osmotic pressure in the various fluid compartments. Potassium influences the acid base balance and osmotic pressure including water retention. Increased sodium levels are found in severe dehydration and excessive treatment with sodium salts. Decreased levels are found in severe polyurea, metabolic acidosis, diarrhoea and renal insufficiency. Increased potassium levels are found in renal failure, dehydration, shock and adrenal insufficiency. Decreased levels are found in malnutrition, gastro-intestinal fluid loss, and hyperactivity of the adrenal cortex.Chloride is a major extracellular anion and maintains the cation/ anion balance between intra and extra cellular fluids, mostly as a salt with sodium. Increased levels are usually found in dehydration, kidney dysfunction, and anaemia. Decreased levels are found in extensive burns, vomiting, diarrhoea, intestinal obstructions, & salt losing nephritis

Principle

1. Sodium is precipitated as a triple salt with magnesium and Uranyl acetate. The excess of uranyl ions are reacted with ferrocyanide in an acidic medium to develop a brownish colour. The intensity of the colour produced is inversely proportional to the concentration of sodium in the sample.

Uranyl ions + Mg ions + Na^+ ⟶ UranylMgNa precipitate
Free Uranyl ions + $K_4Fe(CN)_6$ ⟶ Brown coloured complex

2. Potassium reacts with sodium tetraphenyl boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample.

Tetraphenyl Boron + K^+ ⟶ White turbidity

3. Chloride ions combine with free mercuric ions and release thiocyanate from mercuric thiocyanate. The thiocyanate released combines with the ferric ions to form a red brown ferric thiocyanate complex. Intensity of the colour formed is directly proportional to the amount of chloride present in the sample.

$2\,Cl^- + Hg(SCN)_2$ ⟶ $HgCl_2 + 2(SCN)$
$3(SCN) + Fe^{3+}$ ⟶ $Fe(SCN)_3$

Normal reference values

Sodium	:	135 - 155 mmol/l
Potassium	:	3.5 - 5.5 mmol/l
Serum / Plasma Chloride	:	98 - 106 mmol/l
Urine Chloride	:	170 - 250 mmol/24hours
CSF Chloride	:	120 - 135 mmol/l

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	CHLORIDE KIT 75 ml	CHLORIDE KIT 3 x 75 ml	ELYTE 2 15 tests	ELYTE 3 15 tests
Sodium Kit				
L1 : Precipitating Reagent			35 ml	35 ml
L2 : Acid reagent			45 ml	45 ml
L3 : Colour Reagent			5ml	5ml
Potassium Kit				
L1 : Potassium Reagent			45 ml	45 ml
S : Na^+ / K^+ Standard (150/5 mmol/l)			5 ml	5 ml
Chloride Kit				
L1 : Chloride Reagent	75 ml	3 x 75 ml	-	45 ml
S : Chloride Standard (100 mmol/l)	5ml	5 ml	-	5 ml

Storage / stability

All reagents are stable at R.T. till the expiry mentioned.

Reagent Preparation

Reagents are ready to use.

Sample material

For Sodium & Potassium : Serum. Free from hemolysis. Serum should be separated from the clot immediately / as soon as possible.

For Chloride : Serum, plasma, urine, & CSF. Dilute urine samples 1+1 with distilled water before the assay. Chloride is reported to be stable in serum for 7 days at 2-8°C.

Procedure

Wavelength / filter **Sodium**	:	530 nm (Hg 546) / Green
Wavelength / filter **Potassium** / Red	:	630 nm (Hg 623)
Wavelength / filter **Chloride** / Green	:	505 nm (Hg 546)
Temperature	:	R.T.
Light path	:	1 cm.

Sodium Assay :

1 . Precipitation :

Pipette into a clean dry test tubes labelled as Standard (S) and Test (T)

Addition Sequence	S (ml)	T (ml)
Precipitating Reagent (L1)	1.0	1.0
Na^+/K^+ Standard (S)	0.02	-
Sample	-	0.02

Mix well and let stand at R.T. for 5 min. with shaking well intermittently. Centrifuge at 2500 to 3000 RPM to obtain a clear supernatant.

2 . Colour Development :

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T)

Addition Sequence	B (ml)	S (ml)	T (ml)
Acid Reagent (L2)	1.0	1.0	1.0
Supernatant from Step 1.	-	0.02	0.02
Precipitating Reagent (L1)	0.02	-	-
Colour Reagent (L3)	0.1	0.1	0.1

Mix well and incubate at R.T. for 5 min. Measure the absorbance of the Blank (Abs.B), Standard (Abs.S), and Test Sample (Abs.T) against distilled water within 15 min.

Potassium Assay :

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T)

Addition Sequence	B (ml)	S (ml)	T (ml)
Potassium Reagent (L1)	1.0	1.0	1.0
Deionised water	0.02	-	-
Na^+/K^+ Standard (S)	-	0.02	-
Sample	-	-	0.02

Mix well and incubate at R.T. for 5 min. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against Blank, within 15 min.

Chloride Assay :

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T)

Addition Sequence	B (ml)	S (ml)	T (ml)
Chloride Reagent (L1)	1.0	1.0	1.0
Deionised water	0.01	-	-
Chloride Standard (S)	-	0.01	-
Sample	-	-	0.01

Mix well and incubate at R.T. for 2 min. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against Blank, within 60 min.

Calculations

Sodium Assay :

$$\text{Sodium in mmol/l} = \frac{\text{Abs.B} - \text{Abs.T}}{\text{Abs.B} - \text{Abs.S}} \times 150$$

Potassium Assay :

$$\text{Potassium in mmol/l} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 5$$

Chloride Assay :

$$\text{Chloride in mmol/l} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 100$$

Linearity

The Sodium assay is linear upto 200 mmol/l. The Potassium assay is linear upto 8 mmol/l. The Chloride assay is linear between 70-140 mmol/l. If values exceed this limit, dilute the sample with deionised water (free from Na^+ / K^+ / Cl^- ions) and repeat the assay. Calculate the value using the proper dilution factor.

Notes

Bring all reagents to R.T. before use.

The sodium reaction is an inverse reaction and hence the blank has higher absorbance than the standard or test.

During precipitation, inadequate shaking or centrifugation will result in lower values.

Separate serum from the clot as soon as possible as potassium may leach from the RBC's which have a very high potassium level.

Turbid or icteric samples may produce falsely elevated results.

The procedure for chloride measures total halides such as bromides, iodides, & fluorides in addition to chlorides hence their contamination should be avoided. Since the test is temperature sensitive so a constant temperature should be maintained during incubation & reading.

This common package insert is supplied with the Chloride Kit, Elyte 2 Kit (Na^+/K^+) and the Elyte 3 Kit ($Na^+/K^+/Cl^-$)

References

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System Parameters, Na^+

Reaction	:	End Point
Wavelength	:	530 nm
Zero Setting	:	Reagent Blank
Incub. Temp.	:	R.T.
Incub. Time	:	5 min.
Delay Time	:	---
Read Time	:	---
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.02 ml
Reagent Vol.	:	1.10 ml
Standard	:	150 mmol/l
Factor	:	---
React. Slope	:	Decreasing
Linearity	:	200 mmol/l
Units	:	mmol/l

System Parameters, K^+

Reaction	:	End Point
Wavelength	:	630 nm
Zero Setting	:	Reagent Blank
Incub. Temp.	:	R.T.
Incub. Time	:	5 min.
Delay Time	:	---
Read Time	:	---
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.02 ml
Reagent Vol.	:	1.00 ml
Standard	:	5.0 mmol/l
Factor	:	---
React. Slope	:	Increasing
Linearity	:	8.0 mmol/l
Units	:	mmol/l

System Parameters, Cl^-

Reaction	:	End Point
Wavelength	:	505 nm
Zero Setting	:	Reagent Blank
Incub. Temp.	:	R.T.
Incub. Time	:	2 min.
Delay Time	:	---
Read Time	:	---
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.01 ml
Reagent Vol.	:	1.00 ml
Standard	:	100 mmol/l
Factor	:	---
React. Slope	:	Increasing
Linearity	:	70 - 140 mmol/l
Units	:	mmol/l

ELY(Col):01(P)

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