



ELYTE 3 KIT

(Colorimetric Method)

(For veterinary invitro diagnostic use only)

INTENDED USE

QUADRAPED™ Elyte 3 kit is used for the determination of sodium, potassium & chloride in serum.

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 + K₂Fe(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 → HgCl₂ + 2(SCN)⁻

3(SCN)₂ + Fe³⁺ → Fe(SCN)₃

EXPECTED VALUES

Species	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Dog	142 - 152	3.9 - 5.1	110 - 124
Cat	146 - 156	3.7 - 6.1	115 - 130
Cow	136 - 144	3.6 - 4.9	99 - 107
Horse	128 - 142	2.9 - 4.6	98 - 109
Pig	135 - 150	4.4 - 6.7	94 - 106

Species	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Sheep	139 - 152	3.9 - 5.4	95 - 103
Goat	142 - 155	3.5 - 6.7	99 - 110
Rabbit	138 - 150	3.5 - 6.9	95 - 115
Buffalo	135 - 150	4.0 - 6.0	95 - 110

It is recommended that each laboratory establish its own range as reference ranges may vary between laboratories.

PRESENTATION

REF	1126090015
Pack Size	15 Tests
SL1 Precipitating Reagent	35 ml
SL2 Acid Reagent	45 ml
SL3 Colour Reagent	5 ml
KL1 Potassium Reagent	45 ml
S Na ⁺ /K ⁺ Standard (150 / 5 mmol/l)	5 ml
CL1 Chloride Reagent	45 ml
CS Chloride Standard (100 mmol/l)	5 ml

COMPOSITION

Sodium: Uranyl Acetate >15mmol; Potassium Ferrocyanide >200mmol; Magnesium ions >210mmol.

Potassium: Sodium Tetraphenyl Boron 90mmol.

Chloride: Mercuric Thiocyanate >2mmol; Ferric Nitrate 40mmol; Nitric Acid 29 mmol; Non Reactive Stabilizers and Preservatives.

STORAGE / STABILITY

Contents are stable at 23-29°C till the expiry mentioned on the labels.

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. Sodium & Potassium are reported to be stable in serum for 7 days at 2-8°C.

For Chloride : Serum/ Plasma. Chloride is reported to be stable in sample for 7 days at 2-8°C.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.

Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

PROCEDURE

Wavelength / filter **Sodium** : 530 nm (Hg 546) / Green
 Wavelength / filter **Potassium** : 630 nm (Hg 623) / Red
 Wavelength / filter **Chloride** : 505 nm (Hg 546) / Green
 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 (SL1)	1.0	1.0
Na ⁺ /K ⁺ Standard (S)	0.02	-
Sample	-	0.02

Mix well and let stand at R.T. for 5 mins. 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 (SL2)	1.0	1.0	1.0
Supernatant from Step 1.	-	0.02	0.02
Precipitating Reagent (SL1)	0.02	-	-
Colour Reagent (SL3)	0.1	0.1	0.1

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

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 (KL1)	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 mins. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against Blank, within 15 mins.

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 (CL1)	1.0	1.0	1.0
Deionised Water	0.01	-	-
Chloride Standard (CS)	-	0.01	-
Sample	-	-	0.01

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

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 :

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

QUALITY CONTROL

The following process is recommended for QC during the assay of Elyte 3. Define and establish acceptable range for your laboratory.

- Two levels of control (Normal and Abnormal) are to be run on a daily basis.
- If QC results fall outside acceptance criteria, re-calibration may be necessary.
- Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

Sodium

LOD: 1.0 mmol/l
 LOQ: 5.0 mmol/l
 Lower Limit: 1.0 mmol/l
 Higher Limit: 200 mmol/l

Potassium

LOB: 0.07 mmol/l
 LOD: 0.15 mmol/l
 LOQ: 0.31 mmol/l
 Lower Limit: 0.15 mmol/l
 Higher Limit: 8 mmol/l

Chloride

LOB: 0.29 mmol/l
 LOD: 1.0 mmol/l
 LOQ: 5.0 mmol/l
 Lower Limit: 1.0 mmol/l
 Higher Limit: 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.

Interferences:

For Sodium & Potassium: Sample when spiked with interferent such as upto 20 mg/dl Bilirubin and 1000 mg/dl intralipid does not affect the ability of the kit to determine Sodium and Potassium concentration.

For Chloride: Sample when spiked with interferent such as upto 20 mg/dl Bilirubin and 1000 mg/dl intralipid and 250 mg/dl Haemoglobin does not affect the ability of the kit to determine Chloride concentration.

Precision:

Sodium:

Within run

Within run	n	Mean	SD	% CV
Sample 1	10	138.0	1.73	1.26
Sample 2	10	157.5	1.73	1.10
Sample 3	10	186.8	1.73	0.93

Between run

Between run	n	Mean	SD	% CV
Sample 1	10	137.9	1.88	1.36
Sample 2	10	157.1	1.62	1.03
Sample 3	10	186.8	1.74	0.93

Potassium:
Within run

Within run	n	Mean	SD	% CV
Sample 1	10	3.93	0.07	1.81
Sample 2	10	6.26	0.07	1.17
Sample 3	10	6.38	0.13	2.09

Between run

Between run	n	Mean	SD	% CV
Sample 1	10	3.93	0.07	1.72
Sample 2	10	6.25	0.08	1.25
Sample 3	10	6.41	0.13	2.06

Chloride:

Within run

Within run	n	Mean	SD	% CV
Sample 1	10	98.8	1.86	1.88
Sample 2	10	116.0	1.59	1.37
Sample 3	10	123.8	1.34	1.09

Between run

Between run	n	Mean	SD	% CV
Sample 1	10	99.1	1.65	1.66
Sample 2	10	115.4	1.50	1.30
Sample 3	10	124.5	1.33	1.07

Method comparison:

Comparative studies were done to compare our reagent with another commercial Sodium / Potassium / Chloride Assay. No significant differences were observed. Details of the comparative studies are available on request.

NOTE

In vitro diagnostic reagent for laboratory and professional use only
Not for medicinal use. Avoid contact with skin and mucosa. On disposal flush with large quantities of water. The presence of ammonia does not interfere in this test.

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. Do not use deteriorated or leaking reagents.

REFERENCES

1. Maruna, R.F.L., (1958) Clin. Chem. Acta. 2 : 581
2. Trinder, P., (1951) Analyst 76 : 596
3. Terri, A.E., et. al. (1958) J. Clin. Path. 29 : 86
4. Sunderman, F.W., et. al. (1959) Am. J. Clin. Path. 29 : 95
5. Schales, O., Schales, S.S., (1941) J. Biol. Chem. 140 : 879
6. Schoenfeld, R.G., Lewellen, C.J., (1964) Clin. Chem. 10 : 553.
7. Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, Kenneth S. Latimer, ISBN Jane Wardrop , 6th Edition – 2010.
8. Clinical Biochemistry of Domestic Animals, Sixth Edition, 2008 by Kaneko J.J., Harvey J.W. & Bruss M.L.
9. Data on file : Coral Clinical Systems.

System Parameters Na⁺

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

System Parameters K⁺

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

System Parameters Cl⁻

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

SYMBOL KEYS

 23-29°C Store at 23-29°C	 Manufacturer	IVD <i>In vitro</i> Diagnostic Medical Device	SL1 Precipitating Reagent	Colorimetric Colorimetric Method
 Use by (Last day of stated month)	 Consult Instructions for use	LOT Batch Number	SL2 Acid Reagent	S Na ⁺ / K ⁺ Standard (150/5 mmol/l)
 Date of Manufacture	REF Catalogue Number	 Contains sufficient for <n> tests	SL3 Colour Reagent	KL1 Potassium Reagent
 This way up	CL1 Chloride Reagent	CS Chloride Standard (100 mmol/l)	 H225	



Manufactured by:

Coral Clinical Systems

A Division of Tulip Diagnostics (P) Ltd.

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