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CHLORIDE

(Mercuric Thiocyanate Method)
For the determination of Chloride in serum
(For In vitro Diagnostic Use Only)

CLINICAL SIGNIFANCE

Chloride, a major anion, is important in the maintenance of the cation/anion balance between intra and extra-cellular fluids. This electrolyte is therefore essential to the control proper hydration, osmotic pressure and acid/base equilibrium.

INCREASES

Elevated serum chloride values may be seen in dehydration, hyperventilation, congestive heart valve and prostatic or other types of urinary obstruction.

DECREASES

Low serum chloride values are found with extensive burns, excessive vomiting, intestinal obstruction, nephritis, metabolic acidosis, and in Addisonian crisis.

METHODOLOGY : Mercuric Thiocyanate method

PRINCIPLE

The Chloride ions react with mercuric thiocyanate to release thiocyanate ions, which in turn react with ferric ions to form a red coloured complex of ferric thiocyanate. The intensity of the colour is proportional to the chloride concentration.

$2Cl^- + Hg(SCN)_2 \longrightarrow HgCl_2 + 2(SCN)^-$

$3(SCN)^- + Fe^{3+} \longrightarrow Fe(SCN)_3$ (red coloured complex)

REAGENT COMPOSITION

Mercuric (II) thiocyanate	:	2 mmol/L
Iron (III) Nitrate	:	18 mmol/L
Nitric acid	:	40 mmol/L
Mercuric stabilizer and surfactants		

STANDARD CONCENTRATION : 100 mmol/L

STABILITY AND STORAGE

It's stable at room temperature up to the date of expiration as specified, avoid direct sunlight.

REAGENT PREPARATION

Reagnets are ready to use.

SAMPLE MATERIAL

Serum, heparinised plasma, urine and .CSF

ASSAY PARAMETERS

Reaction	End point	Interval	
Wavelength	505 nm	Sample Vol.	0.01 ml
Zero Settings	Reagent blank	Reagent Vol.	1.00 ml
Incub. Temp	37°C	Standard	100 mmol /L
Incub Time	-	Factor	-
Delay Time	-	React. Slope	Increasing
Read Time	-	Linearity	150 mmol/L
No. of read.	-	Units	mmol/L

ASSAY PROCEDURE

Pipette into clean dry test tubes labelled as blank (B), standard (S) and Test (T) :

Addition Sequence	B (ml)	S (ml)	T (ml)
MTC (A_1)	1.0	1.0	1.0
Standard (S)	-	0.01	-
Specimen	-	-	0.01

Mix well read absorbance of standard (S) and Test (T) against Reagent blank (B) at 505nm

CALCULATIONS

$$\text{Chloride (mmol/L)} = \frac{\text{Abs. of Test}}{\text{Abs.of Standard}} \times 100$$

mEq/l. = mmol/L

LINEARITY

This method is linear up to 150 mmol/L

QUALITY CONTROL

To ensure adequate quality control each run should include assayed normal & abnormal controls.

NORMAL REFERENCE VALUES

Serum : 98 – 107 mEq/l (98-107 mmol/l)

CSF : 123-128 mEq/l (123-128 mmol/L)

Urine : 170 – 250 mmol/24h.

REFERENCE

1. Schoenfeld RG, Lerveller CV.Clin Chem 10,533 (1964)
2. Levinson,S.S (1976) Clin Chem,22,273

PRESENTATION

PRODUCT CODE	PACK SIZE	MTC REAGENT (A_1)	STANDARD (S)
ACL 0609	1 x 60ml	1 x 60ml	3.0 ml

PRODUCT FEATURES AT A GLANCE:

1. Mono reagent (Ready to use).

2. Rapid, simple, one step assay procedure.

3. Results Comparable with ISE Titrimetic method.

4.Reagents & standard specially stabilized.

5.Linearity-150mmol/L.

6.Convenient pack size-1x 60ml.

7.Store at RT.

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SODIUM

(COLORIMETRIC METHOD)
FOR THE DETERMINATION OF SODIUM IN SERUM
(For In vitro Diagnostic Use Only)

CLINICAL SIGNIFICANCE

Sodium is the major cation of extra-cellular fluid. It plays a central role in the maintenance of the normal distribution of water and the osmotic pressure in the various fluid compartments. The main source of body sodium is sodium chloride contained in ingested foods. Only about one third of the total body's sodium is contained in the skeleton since most of it is contained in the extra-cellular body fluids.

INCREASES

Hypernatremia (increased serum sodium level) is found in the following conditions: hyperadrenalism, severe dehydration, diabetic comma after therapy with insulin, excess treatment with sodium salts.

DECREASES

Hyponatremia (low serum sodium level) is found in a variety of conditions including the following: severe polyuria, metabolic acidosis, Addison's disease, diarrhea, and renal tubular disease.

METHODOLOGY :- Colorimetric Method.

PRINCIPLE

The present method is based on modifications of those first described by Maruna and Trinder in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with Ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Uranyl ions + Mg ions + Na^+ ⟶ Uranyl MgNa Precipitate

Free Uranyl ions + $K_4Fe(CN)_6$ ⟶ Brown colored complex.

REAGENT COMPOSITION

Sodium Reagent (A_1)	Uranyl acetate…….....2,1 mM Magnesium acetate…….20 mM
Reagent 2 Color reagent (A_2)	Potassium ferrocyanide
Standard (S)	NaCl sol……150 mEq/l

For in vitro diagnostic use only. Do not pipette the solution by mouth.

STANDARD CONCENTRATION – 150 m mol/L

STORAGE AND STABILITY

Store all reagent set at 2-8°C. The reagents are stables until the expiration date indicated on the label. Turbidity may be a sign of contamination.

REAGENT PREPERATION

Reagents are ready to use

SAMPLE

Freshly drawn serum is the specimen of choice and (500 µl-5 ml) amount is required. Plasma from non-sodium containing anticoagulants (e.g. lithium, calcium, magnesium or heparin) is an acceptable alternative. Sodium is stable for atleast 24 hours at room temperature and 2 weeks when refrigerated.

ASSAY PARAMETERS

Reaction	End point	Interval	-
Wavelength	530 nm	Sample Vol.	0.02ml
Zero Settings	Reagent Blank	Reagent Vol.	1.2ml
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

ASSAY PROCEDURE

STEP-I :-Precipitation of Sodium.

Pipette into two clean, dry centrifuge tubes labelled standard (S) & Test(T).

Addition Sequence	S	T
Sodium Reagent (A_1)	1.0ml	1.0ml
Standard (S)	10 µl	-
Serum	-	10µl

Shake vigorously & incubate at R.T for 5 min. then centrifuge at 2000-3000 rpm for 2 min. to obtain a clean supernatant.

STEP-II:- COLOUR DEVELOPMENT

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

Addition Sequence	B (ml)	S (ml)	T (ml)
Colour reagent (A_2)	1.0 ml	1.0 ml	1.0 ml
Supernatant from Step I	-	0.02 ml	0.02 ml
Sodium reagent (A_1)	0.02 ml	-	-

Mix well allow it to stand at R.T for 5 min. Then measure the absorbance of Blank (B), Standard (S) & Test (T) against distilled water on a photocolorimeter with green filter or on a spectrophotometer at 530 nm within 10 min.

CALCULATION

$$\text{Sodium (mmol/L)} = \frac{\text{Abs of Blank - Abs of Test}}{\text{Abs of Blank-Abs of Stand.}} \times 150 \text{ mmol/L}$$

LINEARITY

This method is linear up to 200 mmol/L.

NOTES

1. All glassware & cuvettes should be washed with quality distilled water before use.
2. Sodium Assay is an inverse reaction, hence blank is higher than standard and test.
3. Pipetting of Sodium reagent (in Step I) & transfer of supernatant (In step II) should be done quickly to avoid error due to low density of liquid.

QUALITY CONTROL

To ensure adequate quality control each run should include assayed normal & Abnormal controls.

NORMAL REFERENCE VALUE

Sodium 135-155mmol/L

REFERENCE

1. Maruna RFL (1958) clin. Chem, Acta, 2.1.581
2. Tietz NW Fundamentals of Clinical Chemistry , W.B. Saunder Co., Phila, PA, p. 874.
3. Henry RF et al. clinical Chemistry Principles and Technics, 2nd ED., Harper and Row Hagerstein, M.D., (1974).
4. Trindre p. Analyst, 76:596 (1951).

PRESENTATION

PRODUCT CODE	PACK SIZE	SODIUM REAGENT (A_1)	COLOUR REAGENT (A_2)	STANDARD (S)
ASM 0622	20 T	1 x 50 ml	1 x 65 ml	5 ml

PRODUCT FEATURES AT A GLANCE :

1. Simple End Point Method.
2. Highly stabilized reagents and standard.
3. Can be used in colorimeter and Analyzers.
4. Highest Linearity 200 mmol/L.
5. Convenient pack size – 20 T .
6. Shelf life – 15 months.
7. Store at 2-8 ^{0}C.

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POTASSIUM

(COLORIMETRIC METHOD) (Turbidometric)
For the determination of potassium in serum
(For In vitro Diagnostic Use Only)

CLINICAL SIGNIFICANCE

Potassium is the principle cation of the intra-cellular fluid. It is also an important constituent of the extra-cellular fluid due to its influence on muscle activity. Its intra-cellular function parallels that of its extra-cellular function, namely influencing acid-base balance and osmotic pressure, including water retention.

INCREASES

Elevated potassium levels (hyperkaliemia) are often associated with renal failure, dehydration shock or adrenal insufficiency.

DECREASES

Decreased potassium levels (hypokaliemia) are associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex.

METHODOLOGY : Colorimetric (Turbidometric) method

PRINCIPLE

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2-7 mEq/l.

Tetraphenyl Boron + K^+ ⟶ White Turbidity

REAGENT COMPOSITION

Sodium tetraphenylboron ≥ 50 mmol/L

Sodium hydroxide ≥ 30mmol/L

POTASSIUM STANDARD : 5mmol/L

STORAGE AND STABILITY

Store all reagents at 2-8^{0}C. The reagents are stable until the expiration date indicated on the package label.

REAGENT PREPARATION

Reagents are ready to use.

SAMPLE

Serum is recommended. Potassium in serum is stable for at least 2 weeks 2-8^{0}C. Specimen should be free from hemolysis and blood specimens should also be separated from red cells shortly after collection. Plasma from anticoagulants not containing potassium is also suitable.

ASSAY PARAMETERS

Reaction	End point	**Interval**	-
Wavelength	620 nm	**Sample Vol.**	0.05 ml
Zero Settings	Reagent blank	**Reagent Vol.**	1.00 ml
Incub. Temp	R.T	**Standard**	5 mmol / L
Incub Time	5 min	**Factor**	-
Delay Time	-	**React. Slope**	Increasing
Read Time	-	**Linearity**	8 mmol / L
No. of read.	-	**Units**	mmol / L

ASSAY PROCEDURE

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

Addition Sequence	Standard (S)	Test (T)
Potassium reagent (A_1)	1.0 ml	1.0 ml
Potassium standard (S)	0.05 ml	-
Sample	-	0.05 ml

Mix and allow it to stand at room temperature for 5 min.

read & record the absorbance at 620 nm of all tubes.

CALCULATION

$$\text{Potassium (mmol/L)} = \frac{\text{Abs.of Test}}{\text{Abs. of standard}} \times 5$$

LINEARITY : 8 mmol/L

NOTES

Turbid or icteric samples produced falsely elevated results. Bilirubin above 40 mg/dl, Urea nitrogen above 80 mg/dl and hemolyzed sera will produce elevated results. Sera containing high levels of ammonia should be avoided.

QUALITY CONTROL

To ensure adequate quality control each run should include assayed normal and abnormal controls.

NORMAL VALUES

3.4 – 5.3 mmol/L

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

REFERENCE

1. Henry RF et al. Clinical Chemistry Principles and Technics, 2nd Ed., Harper and Rox, Hagerstown, M.D. (1974).
2. Tietz NW. Fundamentals of Clinical Chemistry WB, Saunders Co., Phila, PA, P. 874.
3. Terri AE and Sesin PG, Am. J. Clin. Path., 29:86 (1958).

PRESENTATION

PRODUCT	PACK	POTASSIUM REAGENT (A_1)	POTASSIUM STANDARD (S)
APM 0621	20 T	1 x 60 ml	5 ml

PRODUCT FEATURES AT A GLANCE :

1. **Liquid stable Mono Reagent (Ready to use).**
2. **Simple End Point Assay.**
3. **Highest Linearity 8 mmol/L.**
4. **Can be tested in Colorimeters and Analyzers.**
5. **Highly standardized and stabilized reagent.**
6. **Convenient pack size – 20 T.**
7. **Shelf life 15 months.**
8. **Store at 2-8^{0}C.**

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