

CHLORIDE

(THIOCYANATE METHOD)

Reagent kit for quantitative estimation of chloride in serum or plasma.

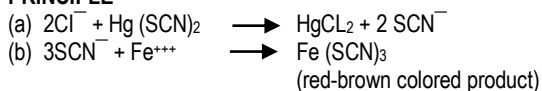
BACKGROUND & SYNOPSIS:

Chloride is a major extra cellular anion. It plays a significant role in maintaining water and electrolyte balance in the extra cellular compartment.

Earlier titrimetric methods of chloride estimation were based on the reactions of chloride with silver and mercuric salts. Schales and Schales (1) described a titration method using mercuric thiocyanate and diphenyl carbazone. The method was modified by Schoenfeld and Lewellen (2) in which diphenyl carbazone was replaced by ferric nitrate. Thus mercuric thiocyanate reacts with 'Cl' ions to form mercuric chloride. The liberated thiocyanate ions form a red-brown colored ferric thiocyanate. The colour thus generated by ferric thiocyanate is proportional to the chloride content in the sample and can be measured photometrically.

CHEMPAK Chloride estimation method is based on the modification of Schoenfeld and Lewellen technique.

PRINCIPLE



Chloride ions in the sample react with mercuric thiocyanate to form mercuric chloride, an undissociated salt and free thiocyanate ions. These free thiocyanate ions react with ferric ions in the reagent to form red-brown colored ferric thiocyanate. The absorbance of ferric thiocyanate at 480 nm is proportional to the concentration of chloride in the sample.

DIAGNOSTIC SIGNIFICANCE:

Chloride may be lost through sweating and urination. Chloride concentration in body is very well controlled. The losses are made up partly by dietary intake and partly by reabsorption of chloride ions. In conditions like, chronic pyelonephritis, diabetic acidosis, renal failure and prolonged vomiting due to any cause there is a loss of chloride ions and hence serum chloride level may decrease.

High serum chloride values may be observed in dehydration, congestive heart failure, renal tubular pathology etc.

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
• Mercuric Thiocyanate	0.2 mmol/L
• Ferric Nitrate	30 mmol/L
• Mercuric Chloride	0.2 mmol/L

Chloride Standard (100 mmol/L)

Also contains non-reactive fillers and Stabilizers.

PRESENTATION:

	No. of Bottle
• CHLORIDE Ready for use. Store at room temperature.	2x50ml 2
• CHLORIDE STANDARD, 100 mmol/L Store at 2-8° C. SEPARATELY PROVIDED	1

PRECAUTIONS:

- CHEMPAK Chloride is for *IN-VITRO* diagnostic use only.
- The use of disposable plastic ware is recommended.
- All the glasswares should be thoroughly washed and made chloride free. Finally, these should be thoroughly rinsed with glass distilled water and dried completely. (Refer note.)

PREPARATION OF WORKING REAGENT:

Chloride reagent is ready for use.

REAGENT STORAGE AND STABILITY:

Chloride reagent is stable at room temperature until the expiry date stated on the label.

CHLORIDE STANDARD (100 mmol/L) is stable at 2-8° C until the expiry date stated on the label.

SPECIMEN COLLECTION:

Fresh, clear, unhemolysed serum is the specimen of choice. Heparinized plasma may be used.

REACTION PARAMETERS :

Type of reaction	: End Point
Wavelength	: 480 nm (470-510 nm)
Flow cell Temperature	: 30°C
Sample volume	: 0.01 ml (10 µl)
Working Reagent Volume	: 1.0 ml
Incubation	: 5 min at R.T
Std. Concentration	: 100 mmol/L
Light path	: 1 cm
Zero setting with	: Distilled water

PROCEDURE:

For instruments having 1.0 ml. cuvette capacity.

PIPETTE INTO TEST TUBE	BLANK	STANDARD	TEST
• WORKING REAGENT (ml)	1.0	1.0	1.0
• SAMPLE (ml)	-	-	0.01
• STANDARD (ml)	-	0.01	-

Mix well and incubate at room temperature (25-30° C) for 5 minutes and read absorbance at 480 nm. (470-510 nm) or with blue green filter against reagent blank.

For instruments having 2.5 ml. cuvette capacity.

PIPETTE INTO TEST TUBE	BLANK	STANDARD	TEST
• WORKING REAGENT (ml)	2.5	2.5	2.5
• SAMPLE (ml)	-	-	0.02
• STANDARD (ml)	-	0.02	-

Mix well and incubate at room temperature (25-30° C) for 5 minutes and read absorbance at 480 nm (470-510 nm) or with blue green filter against reagent blank.

STABILITY OF FINAL REACTION MIXTURE:

The colour of the final reaction mixture is stable for 30 minutes.

TEST RESULTS:

$$\text{Chloride concentration (mmols/L)} = \frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times 100$$

Chloride is expressed as mmol/L or mEq/L.
mmol/L = mEq/L.

NORMAL VALUES:

Serum Chloride: 98-109 mmols/L (mEq/L).

These values are suggested guidelines. It is recommended that each laboratory establishes its own normal range.

LINEARITY:

This method is suitable and linear between 75 mmols/L to 150 mmol/L concentration of chloride. For values higher than 150 mmol/L, dilute the sample with distilled or deionized water and incorporate a suitable dilution factor in the final test results. For values below 75 mmols/L, increase the sample volume suitably and apply the necessary correction.

NOTE:

For washing or decontaminating glasswares from chloride ions, acid chromate solution may be used. The acid chromate solution for washing glasswares can be prepared by dissolving 10 gms. of potassium dichromate in 100 ml. of 1N sulfuric acid.

REFERENCE:

Schales o., Schales S.S., A simple and accurate method for the determination of chloride in biological fluids, J. Biol.Chem, 140, 879, (1941).

1. Schoenfeld R.C., Lewellen C.J., A colorimetric method



