

CHLORIDE(Thiocyanate)

INTENDED USE

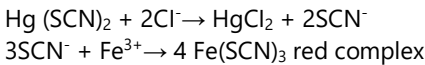
Bioline Chloride is used for the quantitative colorimetric determination of chloride in human serum.

CLINICAL SIGNIFICANCE

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 of proper hydration, osmotic pressure, and acid & base equilibrium. Low serum chloride values are found with extensive burns, excessive vomiting, intestinal obstruction, nephritis, metabolic acidosis, and in Addisonian crisis. Elevated serum chloride values may be seen in dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary obstruction.

METHOD AND PRINCIPLE

Earlier methods for the determination of chloride involved precipitation as an insoluble salt. Chloride can also be determined by titration of the chloride with standard mercuric nitrate solution using diphenylcarbazone as the indicator. These methods require protein precipitation. Our chloride procedure is a direct method based on a modification of the colorimetric method of Skeggs and Hochstrasser.³



Chloride ions form a soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a color complex that absorbs light at 480 nm. The intensity of the color produced is directly proportional to the chloride concentration.

REAGENT COMPOSITION

1. Chloride Reagent (Active Ingredients):

Mercuric Nitrate	0.058 mM
Mercuric Thiocyanate	1.75 mM
Mercuric Chloride	0.74 mM
Ferric Nitrate	22.3 mM

Non-reactive ingredients and stabilizers in dilute acid and methanol.

2. Chloride Calibrator:

Sodium Chloride	100 mEq/L
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WARNING AND PRECAUTIONS

Chloride Reagent: **Poison**. Contains Mercury and Methanol. Maybe harmful or fatal if swallowed. **DO NOT PIPETTE BY MOUTH**. Call physician if taken internally.

REAGENT PREPARATION

Reagents are in ready to use form.

REAGENT STORAGE AND STABILITY

The reagents are stable until the expiration date stated on the label. Store between 15 - 30°C and protected from light.

REAGENT DETERIORATION

Do not use if the reagent is red-brown color and/or cloudy. The reagent should be a clear, pale-yellow solution.

SPECIMEN COLLECTION AND STORAGE

1. Use serum that has been separated from the blood clot soon after drawing.
2. Grossly hemolyzed serum should not be used as it may create falsely decreased values.
3. Avoid contamination of blood with tissue fluid.
4. Store serum in tightly stoppered tubes.
5. Chloride is stable in serum for one (1) day at room temperature, up to one (1) week at refrigerator temperature and for three (3) months frozen when stored tightly capped.

INTERFERENCES

1. Bromide and Fluoride can cause falsely elevated chloride values.
2. Other substances can influence chloride determination. For a comprehensive list, see Young, D.S. et al.

MANUAL PROCEDURE

Wavelength : 505 nm

Temperature : RT Read against reagent blank

	Blank	Standard	Sample
Reagent	1000 µL	1000 µL	1000 µL
Distilled water	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL

Mix and read the optical density (OD) after 5 minutes of incubation at RT

CALCULATIONS

Abs. = Absorbance

$$\frac{\text{Abs. of unknown}}{\text{Abs. of calibrator}} \times \text{Concentration of calibrator} = \text{Concentration of chloride (mEq/L)}$$

Example:

$$\text{Abs. of unknown} = 0.349 \quad \text{Abs. of calibrator} = 0.357$$

$$\text{Concentration of calibrator} = 100 \text{ mEq/L}$$

$$\frac{0.349}{0.357} \times 100 \text{ mEq/L} = 98 \text{ mEq/L}$$

CALIBRATION

The procedures are calibrated with the standard solution which is included with each series of tests. Its absorbance is used to calculate results. It is recommended to establish a linearity curve up to 140 mEq/L with other available commercial standard solutions to verify the performance of the instruments and reagents.

LIMITATIONS

1. Samples with chloride values above 140 mEq/L should be diluted 1:1 with distilled water, re-run and resulting value multiplied by two (2).
2. Care should be exercised not to touch pipette tips with the fingers.
3. Hydrochloric acid fumes may cause high results.

QUALITY CONTROL

Normal and abnormal control sera of known concentrations of

chloride should be analyzed routinely with each group of unknown samples.

EXPECTED VALUES¹

Serum: 98 - 106 mEq/L.

The above values are taken from literature and should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values since differences exist between instruments, laboratories, and local populations.

3. Mosby Co., St. Louis. p. 182 (1970).
3. Skeggs. L.T. and Hochstrasser, H.C., Clin. Chem., 10:918 (1964).
4. Henry, R. J., et al., Clinical Chemistry Principles and Techniques, 2nd Ed., Harper and Row Hagerstown, p. 712 (1974).
5. Young, D.S., et al., Clin. Chem. 21:10 (1975).

PERFORMANCE CHARACTERISTICS

1. Linearity:70-140 mEq/L.
2. Sensitivity:Based on an instrument resolution of A = 0.001 the present chloride procedure has a sensitivity of 0.28 mEq/L.
3. Comparison:Studies done between this procedure and an identical procedure yield a correlation coefficient of 0.97 with a regression equation of $y = 1.00x + 0.45$
4. Precision:
Day-to-Day precision was obtained by assaying two commercial control sera representing normal and elevated results for a period of thirty (30) days.

	<u>Normal</u>	<u>Abnormal</u>
Mean	122.4	91.7
S.D.	4.0	3.8
C.V. (%)	3.3	4.1

Within Run precision was obtained by assaying two control sera twenty (20) times.

	<u>Normal</u>	<u>Abnormal</u>
Mean	95.2	77.7
S.D.	5.7	3.0
C.V. (%)	5.1	3.8

Mode	End Point
Wavelength (Filter)	505 nm(500-520)nm
Reaction Direction	Increasing
Reagent Blank	Yes
Sample Vol.	10 µL
Reagent Vol.	1000 µL
Incubation Time	5 min at RT
Reagent Blank Abs.(Max)	NMT 0.10 Abs
Calibration Method	1- Point
Standard (Conc.)	100 mEq/L
Linearity	140mEq/L
Decimal Places	1
Temp.	RT
Unit	mEq/L
Ref. Low	98 mEq/L
Ref. High	106mEq/L

REFERENCES

1. Tietz, N.W.: Fundamentals of Clinical Chemistry, W.B. Saunders, Philadelphia, PA, p. 897 (1976).
2. White, W.L., et al., Chemistry for Technologist, 3rd Ed., The C.V.