CONCEPT DIAGNOSTICS
ONTARIO, CALIFORNIA 91761 USA

CRESTLINE SCIENTIFIC CORPORATION
2902 Finlandia cor Copernico Streets, San Isidro Village, Makati City
Telefax No.: 887-5777 / 887-2150

CHLORIDE REAGENT SET

CHLORIDE REAGENT SET
For the quantitative colorimetric determination of chloride in serum.

INTRODUCTION
Chloride, a major anion, is important in the maintenance of the cation/ anion balance between intra- and extracellular 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.[1,2]

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 Hochestrasser.[3]

PRINCIPLE
$Hg(SCN)_2 + 2\ Cl^- \text{------> } HgCl_2 + 2SCN^-$
$3\ SCN^- + Fe^{3+} \text{-------> } 4\ Fe(SCN)_3$ red complex

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.

REAGENTS
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

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

REAGENT PREPARATION
Reagents are in ready to use form.

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.[4]
2. Other substances can influence chloride determination. For a comprehensive list, see Young, D.S. et al.[5]
3. Lipemic and/or icteric sera do not interfere in the reaction.

MATERIALS PROVIDED
1. Chloride Reagent.
2. Chloride Calibrator.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Test tubes/rack.
2. Timer.
3. Accurate pipetting devices.
4. Spectrophotometer able to read at 460-550nm.

PROCEDURE (AUTOMATED)
See appropriate instrument application instructions.

PROCEDURE (MANUAL)
1. Label test tubes "blank," "calibrator," "patient," etc.
2. Pipette 1.5ml chloride reagent to each tube.
3. Add 0.01ml (10 µl) of calibrator or sample to respective tubes, mix.
4. Incubate at room temperature for at least five (5) minutes.
5. Set spectrophotometer to 480nm and zero with reagent blank. Wavelengths of 480-520nm may be used.
6. Read and record the absorbance readings of all tubes.
7. See "CALCULATIONS" section to determine values.
   Note: Final color is stable for thirty (30) minutes at room temperature.

* USE MULTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

CALIBRATION
It is not necessary to determine a standard curve with this procedure since the reaction is essentially linear in a range of 70 - 140 mEq/L.

QUALITY CONTROL:
Normal and abnormal control sera of known concentrations of chloride should be analyzed routinely with each group of unknown samples.

CALCULATIONS
Abs. = Absorbance

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

Example:
Abs. of unknown = 0.349
Abs. of caliabrator 100 mg/dl = 0.357

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

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.

EXPECTED VALUES [1]
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.

PERFORMANCE
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.

	Normal	Abnormal
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.

	Normal	Abnormal
Mean	95.2	77.7
S.D.	5.7	3.0
C.V.(%)	6.1	3.8

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. 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 Hagerstrown, p. 712 (1974).
5. Young, D.S., et *al.*, *Clin. Chem.* 21:10 (1975).

Date revised: 6/95