POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

Chloride Reagent Set

Intended Use
For the quantitative determination of Chloride in serum.

Method History
In 1878 chloride was determined by precipitation of the anion as silver chloride and the measurement of the excess silver nitrite with standardized thiocyanate solution.[1,2] Since then, many methods were introduced including mercurimetric titration using diphenylcarbazone as indicator.[3,4] An electrometric method was introduced in the 1950's which was based on the exposure of a chloride solution to electrodes that emit silver ions in an acid medium.[5] The time required for chloride precipitation as silver chloride is recorded and is directly proportional to chloride concentration.

The present method was developed in the late 1950's and later adapted to the Technicon Auto Analyzer.[6] This is a direct method and can be adapted to a variety of automated instruments.

Principle

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

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

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

Reagents
Chloride Reagent (Active Ingredients):
Mercuric Nitrate 0.105mM.
Mercuric Thiocyanate 1.01mM
Ferric Nitrate 37.63mM
In dilute acid and methanol.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. This reagent is a TOXIC POISON as well as CAUSTIC. Avoid all contact with skin or clothing. Flush with water if contact occurs.
3. DO NOT PIPETTE BY MOUTH. Call physician if taken internally.

Reagent Preparation
Reagent comes in a ready to use form.

Reagent Storage
Store at room temperature protected form light.

Reagent Deterioration
Do not use if:
The reagent is a 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 day at room temperature and for three months frozen when stored tightly capped.

Interferences
1. Bromide and Fluoride can cause falsely elevated chloride values.[8]
2. Other substances can influence chloride determination. For a comprehensive list see Young, D.S. et al.[9]
3. Lipemic and/or icteric serums do not interfere in the reaction.

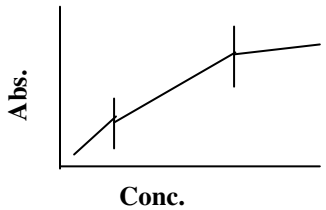
Materials Provided
Chloride reagent.

Materials Required but not Provided
1. Accurate pipetting devices.
2. Timer.
3. Test tubes/rack
4. Spectrophotometer able to read at 460-550nm.

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Label test tubes "Blank", "Calibrator", "Sample", etc.
2. Add 1.0ml chloride reagent to each tube.
3. Add 0.01ml (10ul) of calibrator or sample to respective tubes, mix.
4. Incubate at room temperature for at least five minutes.
5. Set spectrophotometer at 480nm and zero with reagent blank. Wavelengths of 460-550nm may be used.
6. Read and record the absorbance reading of all vials.
7. See "CALCULATIONS" section to determine values. NOTE: Final color is stable for thirty minutes at room temperature.

Calibration
1. This reaction does not follow Beer's Law but has a linear range of 80-120 mEq/L on most instruments.
2. The linear range of the instrument to be used must be verified before actual testing. To verify a linear range; prepare a calibration curve by plotting the concentration of standards (ranging from 80-120mEq/L) versus their respective absorbance readings. Commercial standard/calibrator kits are recommended for this procedure.



3. Once the linear range has been established a single standard (included in the kit) can be used to standardize the reaction. Results are then calculated ratiometrically. (see "CALCULATIONS").

Quality Control
Control serums with known concentrations of chloride should be used to monitor the integrity of the reaction.

Calculation

Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Calibrator}} \times \text{Concentration of Calibrator} = \text{Chloride (mEq/L)}$$

Example: If a 100mEq/L calibrator has an absorbance reading of 0.550 and unknown has 0.450 then:

$$\frac{0.450}{0.550} \times 100 = 82\text{mEq/L}$$

Limitations

1. Samples with chloride above 120 mEq/L should be diluted 1:1 with distilled water, re-run, and resulting value multiplied by two.
2. Do not touch pipette tips with fingers as chloride in skin perspiration may influence results.
3. Hydrochloric acid fumes may cause falsely elevated results.

Expected Values[8]

Serum 98-106 mEq/L

The above values are taken from literature and are intended as a guideline only. Each laboratory should establish its own range of expected values. Chloride levels in clinically healthy individuals vary by diet, sex, age, diurnal variation, physical activity, menstrual cycle, pregnancy and environmental factors.

Performance

1. Linearity: 80-120 mEq/L
2. Comparison: Studies done between this procedure and a similar procedure yielded a correlation coefficient of 0.991 with a regression equation of: y=1.01x + 0.11.
3. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
97	0.9	0.9	97	1.4	1.4
111	1.2	1.1	109	1.6	1.5

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Manufactured by Pointe Scientific, Inc.
5449 Research Drive Canton MI 48188

"European Authorized Representative"
(O.E.A.R.C.) Av. De Tervueren 34 bte
44 B-1040 Brussels, Belgium

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