

Chloride

1.0 INTENDED USE

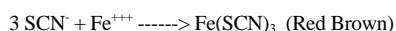
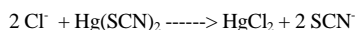
This reagent is intended for the quantitative determination of chloride in serum.

2.0 BACKGROUND

2.1 METHOD AND HISTORY

Chloride has been determined by a range of analytical methods. Schales and Schales (10.1) described a titration method in which chloride is reacted with mercury. The endpoint of the titration is recognized by the formation of a blue color when excess mercury ions react with diphenyl carbazone. The method was modified by Schoenfeld and Lewellen (10.2) who used mercuric thiocyanate to produce mercuric chloride and free thiocyanate ions. The thiocyanate ions react with ferric ions to produce a red-brown color which is proportional to the chloride concentration. This procedure for chloride is a modification of the Schoenfeld and Lewellen technique.

2.2 TEST PRINCIPLE



The absorbance of the ferric thiocyanate at 500 nm is proportional to the concentration of chloride in the sample.

2.3 CLINICAL SIGNIFICANCE (10.5)

Chloride and bicarbonate are the principal anions in blood; sodium and potassium the principal cations. The balance between these electrolytes is frequently affected in disease states, thus meaningful interpretation of chloride levels requires knowledge of other electrolyte concentrations.

Increased chloride levels can occur in nephritis, prostatic obstruction, eclampsia and dehydration. Decreased levels are often associated with impaired gastro-intestinal or renal function.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION.

Fresh, clear, unhemolyzed serum is the preferred specimen.

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25 μl . Call Biotron's technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Serum should be separated from the cells as soon as possible and may be stored at 2-8°C (refrigerated) for up to one week.

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

4.0 MATERIALS (2 X 125 ml) (1 X 500 ml)

Reagents necessary for the determination of chloride are included in the kit.

4.1 REAGENT

Chloride reagent contains:

mercuric thiocyanate	0.031%
ferric nitrate	1.52%
nitric acid	0.24%
methanol	7.5%

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

The reagent contains mercury and hence is toxic. Avoid contact with skin and eyes. Flush contacted area with water. Contact physician immediately if reagent is taken internally.

4.3 REAGENT PREPARATION

The reagent is ready to use as is.

4.4 REAGENT STORAGE AND STABILITY

All reagents included in the kit are stable until the expiration date stated on the label when stored at 18-26° C (room temperature) and away from light.

4.5 ADDITIONAL MATERIALS REQUIRED

- 4.5.1 Spectrophotometer or colorimeter capable of reading absorbance accurately at 500 nm.
- 4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 500 nm.
- 4.5.3 Test tubes capable of holding 3 ml.
- 4.5.4 Pipettes capable of delivering the required volumes.
- 4.5.5 Distilled or deionized water for preparing the reagent blank.
- 4.5.6 A timer for a five minute incubation.
- 4.5.7 Normal and abnormal control for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	500 nm
Temperature	18-26° C
Mode	Endpoint
Reaction time	5 minutes
Sample volume	10 μl
Reagent volume	2.0 ml
Total volume	2.01 ml
Sample to reagent ratio	1:200

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 500 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

The chloride assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator.

5.4 PROCEDURE

- 5.4.1 Into separate test tubes pipette 10 μl of distilled water, calibrator, or serum to be assayed.
- 5.4.2 Add 2.0 ml of chloride reagent and mix well.
- 5.4.3 Incubate at 18-26°C (room temperature) for 5 minutes.
- 5.4.4 Read and record the absorbance of each tube using the distilled water sample as the reagent blank.

5.5 PROCEDURE NOTE

The final reaction mixture is stable for 30 minutes.

Chloride is a common laboratory contaminant and care should be taken to make sure that all glassware or plasticware used in the analysis is free from contamination.

5.6 CALCULATION AND RESULTS

$$\text{Chloride (mEq/L)} = \frac{A}{A_s} \times \text{X concentration of calibrator}$$

A = absorbance of sample, A_s = absorbance of calibrator

Example:

Chloride concentration = $\frac{0.450}{0.550} \times 100 \text{ mEq/L} = 82 \text{ mEq/L}$
with A = 0.450 and As = 0.550, concentration of calibrator = 100 mEq/L

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.4)

The range of expected values is: 98 - 103 mEq/L

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 MEDICAL ALERT VALUES (10.6)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.3 LIMITATIONS OF PROCEDURE

- 6.3.1 Severely icteric, hemolytic, or lipemic samples require the use of a sample blank which may be prepared by using 10 µl of sample and 2.0 ml of distilled or deionized water.
- 6.3.2 This procedure measures total halide concentration including chloride, bromide, and iodide. The latter two do not interfere to a significant degree as their concentrations in serum are normally less than 1 mEq/L.
- 6.3.3 A summary of the influence of drugs on clinical laboratory tests may be found by consulting Young, D.S. (3) Some examples of drugs that affect the serum chloride test are: Acetazolamide, Bromides, M., Intra-Amniotic Saline, Phenylbutazone.
- 6.3.4 This procedure is not recommended for use in determining sweat chloride for cystic fibrosis.

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

The chloride assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the calibrator. Refer to your instrument manual for more details.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

In this study, replicates of 2 control sera were run.

Number of Samples	Mean (mEq/L)	SD (mEq/L)	CV (%)
89	107	0.89	0.8
20	90	0.86	0.9

Between-Run

5 assays were run each day for 10 days on a pool serum specimen.

Number of Samples	Mean (mEq/L)	SD (mEq/L)	CV (%)
50	105	0.88	0.8

9.2 CORRELATION

A correlation study was done comparing this method (y) and a similar comparative chloride procedure (x). The samples range between 61 to 140 mEq/L.

Number of Samples	Regression Equation	Correlation Coefficient
45	$y = .988x + 1.20$	0.987

9.3 RECOVERY STUDY

Chloride of concentrations 25, 37.5 and 50 mEq/L was added to a pool of human serum. The recovery of added chloride averaged 102%.

9.4 LINEARITY

This procedure is linear from 70 to 140 mEq/L. Sample with chloride values above 140 mEq/L should be diluted 1 to 1 with distilled water. Re-run sample and multiply the final answer by 2.

9.5 SENSITIVITY

Absorbance values obtained by the above described procedure show that an absorbance value of 0.005 optical density corresponds to chloride concentrations of 1 mEq/L. This may vary slightly from lot to lot.

10.0 REFERENCES

- 10.1 Schales, O., Schales, S.S., J. Biol. Chem. 140, 879 (1941).
- 10.2 Schoenfeld, R.G., Lewellen, C.J., Clin. Chem. 10, 533 (1964).
- 10.3 Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd ed., Washington DC, AACC Press (1990).
- 10.4 Henry, R.J., (ed.), Clinical Chemistry Principles and Techniques, Harper and Row, New York, (1974), p. 720.
- 10.5 Clinical Diagnosis, 2nd ed., R.J. Henry, D.C. Cannon and J.W. Wilkinson, Eds., Harper & Row, New York, NY 1974.
- 10.6 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5, p.704

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