Chloride Reagent
Thiocyanate Method

PRODUCT SUMMARY

Stability	:	Until Expiry at 2 - 25°C
Linear Range	:	80 - 120 mmol/L
Specimen Type	:	Serum, Heparinized Plasma
Method	:	Endpoint
Reagent Preparation	:	Reagent supplied ready to use.

INTENDED USE
This reagent is intended for the in vitro quantitative determination of chloride in serum or plasma on both manual and automated systems.

CLINICAL SIGNIFICANCE[1,2]
Chloride is the main extracellular anion. With sodium it accounts for most of the osmotic pressure of plasma and contributes to maintenance of electroneutrality. Chloride ions are ingested with food and absorbed in the intestinal tract. Chloride is removed from the blood by glomerular filtration. It is reabsorbed passively in the proximal convoluted tubule and actively in Henles loop and distal convoluted tubule under the influence of aldosterone. Chloride is seldom performed by itself but usually in conjunction with other electrolytes.
Hypochloraemia is caused in the main by those conditions which result in hyponatraemia. However, in one clinical condition, metabolic alkalosis, there may be a low serum chloride in the presence of a normal sodium. Metabolic alkalosis is associated with bicarbonate excess, which in the presence of normal sodium requires the loss of chloride to maintain electroneutrality. A primary metabolic alkalosis may occur in three situations: Administration of bicarbonate, generation of bicarbonate by the kidney in potassium depletion and generation of bicarbonate by the gastric mucosa when hydrogen and chloride ions are lost in pyloric stenosis or gastric aspiration.
Hyperchloraemia is caused in the main by those conditions which result in hypernatraemia. However, there is one clinical condition, metabolic acidosis, in which chloride may be raised in the presence of a normal sodium. In metabolic acidosis bicarbonate is depleted. In order to maintain electroneutrality extracellular chloride concentration may increase. Conditions which may result in a hyperchloraemic acidosis include renal tubular acidosis, acetazolamide therapy (carbonic anhydrase inhibitor), after administration of ammonium chloride and after transplantation of the ureters.

METHODOLOGY
This Chloride reagent is based on the method of Zall, Fisher and Garner.[3] When chloride is mixed with a solution of undissociated mercuric thiocyanate, the chloride preferentially combines with the mercury to form mercuric chloride. The thiocyanate that is released combines with ferric ions present in the reagent to form ferric thiocyanate which can be measured spectrophotometrically. The procedure is very sensitive and needs to be reduced for routine clinical applications by the addition of mercuric nitrate.
The mercuric nitrate binds a fixed amount of chloride ions and therefore makes them unavailable for reaction with mercuric thiocyanate. Only the chloride present in excess of that bound by the mercury from mercuric nitrate will be able to react with mercuric thiocyanate to produce the red ferric thiocyanate.

REAGENT COMPOSITION

Active Ingredients	Concentration
Mercuric Thiocyanate in methanol	1.3 mmol/L
Ferric Nitrate	59 mmol/L
Mercuric Nitrate	0.26 mmol/L
Chloride standard	100 mmol/L

Warning: Do not ingest. The reagent contains mercuric salts and methanol which may be harmful or fatal if swallowed. Avoid contact with skin and eyes. Dispose of according to local, state and federal regulations. If spilt thoroughly wash affected areas with water. For further information consult the Chloride Material Safety Data Sheet.

REAGENT PREPARATION
Reagent is supplied ready to use.

STABILITY AND STORAGE
The reagent is stable until the expiry date shown on the label when stored at room temperature (2 - 25°C) and protected from exposure to excessive heat and direct sunlight.

Indications of Reagent Deterioration:
- Turbidity; and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING
Serum or heparinised plasma are suitable samples for use with this reagent. For electrolyte determinations serum or plasma should be separated from the red blood cells as soon as possible. In the absence of bacterial contamination, electrolytes are stable at room temperature or refrigerated for at least one week.[4]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 500nm - 520nm.
- Analyzer specific consumables, e.g.: samples cups.
- Normal and Abnormal assayed controls.

Assay Procedure
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	500nm (500-520nm)
Assay Type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1:100
e.g.: Sample Vol	3 µL
Reagent Vol	300µL
Incubation Time	60 seconds
Reagent Blank Limits	Low 0.0 AU
(500nm, 1cm lightpath)	High 2.0 AU
Linearity	0 to 120 mmol/L
Sensitivity	0.01 ΔA per mmol/L
(500nm,1cm lightpath)	

Calculations
Calculate the results as follows:

$$\text{Chloride} = \frac{\Delta\text{Absorbance of Unknown}}{\Delta\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:

Absorbance of standard	=	1.00
Absorbance of unknown	=	1.05
Value of standard	=	100 mmol/L

$$\text{Chloride} = \frac{1.05}{1.00} \times 100 = 105 \text{ mmol/L}$$

NOTES
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. The temperature of the reaction is not critical, but the reaction is temperature sensitive and a constant temperature must be maintained to obtain accurate results.

Thermo SCIENTIFIC

Calibration
Calibration is required. This Chloride reagent is supplied with a standard for calibration purposes. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications.
However, if during this period any one of the following events occurs, recalibration is recommended:-

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

Quality Control
To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the upper or lower limits of the established ranges indicate that the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are sill out of control, perform a calibration with freshly prepared reagent, then repeat the test.
- If results are still out of control, contact Technical Services or the local distributor.

LIMITATIONS
1. The assay is linear between 80 - 120 mmol/L.
2. Halides will cause a positive interference with this method, the only clinically significant halide though is Bromide which is administered with some drug preparations[4].
3. High levels or protein such as those found in multiple myeloma may interfere due to the development of turbidity.
4. Young DS5 has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES

101 - 111 mmol/L[4]

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derives a reference interval for the population that it serves.[6]

PERFORMANCE DATA
The following data was obtained using this Chloride reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION
Imprecision was evaluated using two levels of commercial control.

Within Run

	LEVEL I	LEVEL II
Number of Samples	20	20
Mean mmol/L	99	110
SD	2.0	1.3
CV(%)	2.0	1.2

Total

	LEVEL I	LEVEL II
Number of samples	80	80
Mean mmol/L	99	104
SD	3.7	3.1
CV(%)	3.7	3.0

ACCURACY
Comparison studies were carried out using another commercially available Chloride reagent as a reference. Normal and abnormal human serum and plasma samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Number of samples	96
Range of results	87 to 115 mmol/L
Reference method mean	104 mmol/L
Chloride method mean	100 mmol/L
Slope	0.93
Intercept	0.07
Correlation coefficient	0.99

LINEARITY
When run as recommended the assay is linear between 80 - 120 mmol/L.

SENSITIVITY
When run as recommended the sensitivity of this assay is 0.01 ΔAbs per mmol/L (1cm light path, 500nm).

REFERENCES

1. Kleinman LI and Lorenz JM. Physiology and pathophysiology of body water and electrolytes in Clinical Chemistry theory, analysis and correlation Kaplan LA and Pesce AJ Ed. CV Mosby Co. 1984; Chapter 21: 385-6.
2. Zilva JF and Pannall PR: Hydrogen Ion. Homeostasis in Clinical Chemistry in Diagnosis and Treatment. Lloyd-Luke 1979; Chapter IV: 92-8.
3. Zall D, Fisher D and Garner M. Anal Chem 1956; 28:1665.
4. Miller G. Chloride in Clinical Chemistry theory, analysis and correlation. LA Kaplan and AJ Pesce (Ed) CV Mosby 1984; 55: 1059-62.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests Third Edition 1990; 3:90-3.
6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

Fisher Diagnostics
a division of Fisher Scientific Company, LLC
a part of Thermo Fisher Scientific Inc.
Middletown, VA 22645-1905 USA
Phone: 800-528-0494
540-869-3200
Fax: 540-869-8132

840384 (R1)

REF

Reorder Information

Catalogue No.	Configuration
TR38021	2 x 125 mL
TR38026	2 x 250 mL
TR38098	1 x 1000 mL

Each kit comes with 1 x 10 mL Standard