Chloride – Single Reagent

Diagnostic reagent for the in-vitro quantitative determination of Chloride in human serum, plasma and urine on manual systems.

REF: V/CL02.025	50 test	REF: V/CL02.050	100 test
REF: V/CL04.025	100 test	REF: V/CL02.100	200 test

CLINICAL SIGNIFICANCE

Chloride is the most abundant extracellular anion. Together with sodium, chloride is responsible for the maintenance of osmotic pressure, the anion-cation balance and therefore of the water distribution in the extracellular fluid compartment.
Decreased plasma Cl^- concentrations (hypochloremia) can result from salt-losing nephritis, persistent gastric secretion, prolonged vomiting and metabolic acidosis that are caused by increased production or reduced secretion of organic acids.
Increased plasma Cl^- concentrations (hyperchloremia) occur with dehydration, renal tubular acidosis, acute renal failure, in adrenocortical hyperfunction , salicylate in- toxication and metabolic acidosis associated with prolonged diarrhoea and loss of sodium bicarbonate.

METHOD PRINCIPLE

Chloride ions react with undissociated mercuric thiocyanate to form undissociated mercuric Chloride and free thiocyanate ions.
thiocyanate ions react with ferric ions to form a highly coloured reddish complex of ferric thiocyanate.
The increasing extinction is directly proportional to the concentration of chloride ions.

$$Hg(SCN)2 + 2Cl^- \longrightarrow HgCl2 + 2\,SCN^-$$

$$3(SCN)^- + Fe^{3+} \longrightarrow Fe(SCN)3$$

REAGENT COMPOSITION

R1: Standard	354.6 mg/dl (100 mmol/L)
R2: Reagent Hg II - thiocyanate Fe III - nitrate HNO3	 2 mmol/L 30 mmol/L 40 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the **Lab.Vie**. Chloride reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie.Chloride reagents are supplied ready-to-use. All reagents are stable until expiration date stated on label when stored at 2-8°C.

Deterioration
The **Lab.Vie**. Chloride reagent is normally clear, do not use reagent if it is turbid.

SPECIMEN COLLECTION AND PRESERVATION

Unhemolysed serum or Heparinized plasma or urine.
Chloride is stable for 7 days at 2-8°C.

SYSTEM PARAMETERS

Wavelength	492 nm (460 - 500 nm)
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio e.g.: Reagent volume Sample volume	1:100 1 ml 10 µl
Temperature	15-25°C
Incubation time	5 min at 15-25°C
Zero adjustment	Reagent blank
Sensitivity	1.0 mmol/L
Linearity	130 mmol/L

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Blank	Standard	Specimen
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Specimen			10 µl

Mix, incubate for 5 minutes at room temp. Measure absorbance of specimen and standard against reagent blank. The color is stable for at least 30 minutes.

CALCULATION

$$\text{Chloride concentration (mmol/L)} = \frac{\text{Abs. specimen}}{\text{Abs. standard}} \times 100$$

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie**. technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mmol/L	85.1	136.3	84.6	133.4
SD. mmol/L	0.51	0.94	0.67	1.95
CV. %	0.60	0.69	0.79	1.5

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from **Lab.Vie**. Chloride reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.994.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 1.0 mmol/L.

Linearity
The reaction is linear up to concentration of 130 mmol/L. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

INTERFERING SUBSTANCES

Haemoglobin
It interferes with Over-estimation value.
Icterus
No significant interference.
lipemia
No significant interference.
Bromide and Fluoride
They can cause falsely elevated chloride values.
Drugs
No interference was observed by ascorbic acid up to 10 mg/dl.
Others
Other drugs and substances may interfere.

EXPECTED VALUES

	mmol/l
Serum / Plasma	97 – 108
Urine(24 h)	95 – 240 mmol/24h
Urine(morning)	54 – 158

DYNAMIC RANGE

1.0– 130 mmol/l.

REFERENCES

1. Tietz N.W. Clinical Guide to Laboratory Tests, 3rd Philadelphia:W.B. Saunders Company, 1995:516-519.
2. Batlle DC. et al. The use of the urinary anion gap in the diagnosis of hyperchloremic metabolic acidosis. N Engl J Med 1988, 318:594-599.
3. Krieg M. et al. Comparative quantitative clinico-chemical analysis of the characteristics of 24-hour urine and morning urine (in German). J Clin Chem Clin Biochem 1986, 24:863.
4. YOUNG D.S., Effect of Drugs on Clinical Laboratory Tests,4th Ed. (1995) p. 3-137 a 3-141.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		