# TITLE: Chloride VITROS Microslide Assay

**PRINCIPLE:**

The VITROS Cl- Slide method is performed using the VITROS Cl- Slides and the VITROS Chemistry Products Calibrator Kit 2 the VITROS 5600 Integrated System. The VITROS Cl- Slide is a multilayered, analytical element coated on a polyester support that uses direct potentiometry 3 for measurement of chloride ions. The slide consists of two ion-selective electrodes, each containing a protective layer, a silver layer and a silver chloride layer coated on a polyester support. The protective layer inhibits interference from normal levels of bromide and uric acid. A drop of patient sample and a drop of VITROS Reference Fluid on separate halves of the slide results in migration of both fluids toward the center of the paper bridge. A stable liquid junction is formed connecting the reference electrode to the sample indicator electrode. Each electrode produces an electrical potential in response to the activity of chloride ions applied to it. The potential difference poised between the two electrodes is proportional to the chloride concentration in the sample.

**Test Type and Conditions:**

| **Test Type** | **VITROS System** | **Approximate Incubation Time** | **Temperature** | **Reaction Sample Volume** | **Reference Fluid Drop Volume** |
| --- | --- | --- | --- | --- | --- |
| Potentiometric | 5600 | 2 minutes | 37> °C  | 10 µL | 10 µL |

**Warnings and Precautions**

For *in vitro* diagnostic use only.

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| --- | --- |
| **WARNING:** | ***Take care when handling materials and samples of human origin. Since no test method can offer complete assurance that infectious agents are absent, consider all clinical specimens, controls, and calibrators potentially infectious. Handle specimens, solid and liquid waste, and test components in accordance with local regulations and CLSI Guideline M29***[***3***](#d6e2458) ***or other published biohazard safety guidelines.*** |

For specific warnings and precautions for calibrators, quality control materials, and other components, refer to the Instructions for Use for the appropriate VITROS product, or to other manufacturer’s product literature.

**CLINICAL SIGNIFICANCE:**

 Chloride is the major anion in the extracellular water space; its physiological significance is in maintaining proper bodywater distribution, osmotic pressure, and normal anion-cation balance in the extracellular fluid compartment. Chloride is increased in dehydration, in renal tubular acidosis (hyperchloremia metabolic acidosis), and in excessive infusion of isotonic saline. Chloride is decreased in overhydration, chronic respiratory acidosis, salt-losing nephritis, metabolic alkalosis, and congestive heart failure. 1 Urine chloride levels aid in determination of type of metabolic alkalosis (chloride responsive, and chloride resistant). 2

VITROS Chemistry Products Cl- Slides quantitatively measure chloride (Cl-) concentration in serum, plasma and urine using the VITROS 5600 Integrated System. Chloride measurements are used in the diagnosis and treatment of electrolyte and metabolic disorders such as cystic fibrosis and diabetic acidosis.

**SPECIMEN COLLECTION:**

**Specimens Recommended**

 • Serum

 • Plasma: Heparin

 • Urine

|  |  |
| --- | --- |
| **IMPORTANT:** | *Certain collection devices have been reported to affect other analytes and tests.*[*4*](#d6e2465) *Owing to the variety of specimen collection devices available, Ortho-Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this test.* |

**Specimens Not Recommended**

 Urine with the following preservatives:

 • Hydrochloric acid (12N HCl)

 • 10% Thymol in isopropanol

**Serum and Plasma**

 **Specimen Collection and Preparation**

 Collect specimens using standard laboratory procedures. 6,7

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| --- | --- |
| **Note:** | For details on minimum fill volume requirements, refer to the operating instructions for your system. |

**Patient Preparation**

 No special patient preparation is necessary.

**Special Precautions**

 • Do not draw specimen from an arm receiving an intravenous transfusion.

 • Fibrin clots may cause incomplete sampling of the specimen. 8

 – Allow specimens to clot completely in order to prevent fibrin clots.

 – Inspect plasma specimens for the presence of fibrin clots.

 • Centrifuge specimens and remove the serum or plasma from the cellular material within 4 hours of collection. 9

**Specimen Handling and Storage**

 • Handle and store specimens in stoppered containers to avoid contamination and evaporation.

 • Mix samples by gentle inversion and bring to room temperature, 18–28 °C, prior to analysis.

**Specimen Storage and Stability 9**

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**Urine**

**Specimen Collection and Preparation**

 Collect specimens using standard laboratory procedures.

|  |  |
| --- | --- |
| **Note:** | For details on minimum fill volume requirements, refer to the operating instructions for your system. |

 **Patient Preparation**

 No special patient preparation is necessary.

**Special Precautions**

 None. Urine specimens will not be pre-treated.

**Specimen Handling and Storage**

 • Handle and store specimens in stoppered containers to avoid contamination and evaporation.

 • Mix samples by gentle inversion and bring to room temperature, 18–28 °C , prior to analysis.

**Specimen Storage and Stability for Urine** 9



**REAGENTS:**



**Reagent Handling**

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| --- | --- |
| **Caution:** | **Do not use slide cartridges with damaged or incompletely sealed packaging.** |

* Inspect the packaging for signs of damage.
* Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.

**Reagent Preparation**

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| **IMPORTANT:** | *The slide cartridge must reach room temperature, 18–28 °C (64–82 °F), before it is unwrapped and loaded into the slide supply.* |

1. Remove the slide cartridges from storage.
2. Warm the wrapped cartridge at room temperature for 30 minutes when taken from the refrigerator or 60 minutes from the freezer.
3. Unwrap and load the cartridge into the slide supply.

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| **Note:** | Load the cartridges within 24 hours after they reach room temperature, 18–28 °C (64–82 °F). |

**Reagent Storage and Stability**

VITROS Cl- Slides are stable until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date.

Verify performance with quality control materials:

• If the system is turned off for more than 2 hours.

• After reloading cartridges that have been removed from the slide supply and stored for later use.

**CALIBRATION:**

 **Required Calibrators**

 VITROS Chemistry Products Calibrator Kit 2

 **Calibrator Preparation, Handling, and Storage**

 Refer to the Instructions for Use for VITROS Calibrator Kit 2.

 **Calibration Procedure**

 Refer to the operating instructions for your system.

 **When to Calibrate**

 Calibrate:

* + When the slide lot number changes.
	+ When critical system parts are replaced due to service or maintenance.
	+ When government regulations require.

 For example, in the USA, CLIA regulations require calibration or calibration verification at least once every six months.

 When the VITROS Reference Fluid lot number changes.

 The VITROS Cl- test may also need to be calibrated:

* + If quality control results are consistently outside acceptable range.
	+ After certain service procedures have been performed.

For additional information, refer to the operating instructions for your system.

 **Calculations**

The VITROS Analyzer measures the potential difference in millivolts between the two electrodes of a potentiometric slide—one in contact with the sample to be analyzed and the other in contact with the electrolyte reference fluid. A linear relationship exists between the measured potential difference observed on the slide and the logarithm of chloride concentration, i.e., the Nernst equation for ion-selective electrodes. Once the calibration has been established for each slide lot, unknown chloride concentrations for a given sample can be determined using the software-resident math model and the measured potential difference.

 **Validity of a Calibration**

Calibration parameters are automatically assessed by the system against a set of quality parameters detailed in the on Review Assay Data screen the VITROS 5600.

Ch-168 Failure to meet any of the pre-defined quality parameters results in a failed calibration. The calibration report should be used in conjunction with quality control results to determine the validity of a calibration.

 **Measuring (Reportable or Dynamic) Range**



 For out-of-range samples, refer to “Sample Dilution.”

 **Traceability of Calibration**

Values assigned the VITROS Chemistry Products Calibrator Kit 2 for chloride are traceable to the Certified NIST (National Institute of Standards and Technology) Reference Material, SRM® (Standard Reference Material) 919. The Ortho-Clinical Diagnostics calibration laboratory uses SRM® 919 to calibrate the coulometric-amperometric titration method 10,11 to support chloride value assignment for VITROS Calibrator Kit 2.

**QUALITY CONTROL:**

Refer to Chemistry Quality Control Procedure for Specifics

**Quality Control Material Selection**

 Serum and Plasma

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| --- | --- |
| **IMPORTANT:** | *VITROS Performance Verifiers are recommended for use with the VITROS Chemistry and Integrated Systems. Evaluate the performance of other commercial control fluids for compatibility with this test before using for quality control.* |

 Control materials other than VITROS Performance Verifiers may show a difference when compared with other chloride methods if they:

* + Depart from a true human matrix.
	+ Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives.
	+ Are high or low in total protein concentration.

 Do not use control materials stabilized with ethylene glycol.

 **Urine**

 For urine specimens, use commercially available urine control materials.

**Quality Control Procedure Recommendations**

 • Choose control levels that check the clinically relevant range.

 • Analyze quality control materials in the same manner as patient samples, before or during patient sample processing.

 • To verify system performance, analyze control materials:

 – After calibration.

 – According to local regulations or at least once each day that the test is being performed.

 – After specified service procedures are performed. Refer to the operating instructions for your system.

 • If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.

 • For general quality control recommendations, refer to Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Third Edition 12 or other published guidelines.

 • For additional information, refer to the operating instructions for your system.

**Quality Control Material Preparation and Storage**

 Refer to the Instructions for Use for VITROS Performance Verifier I and II or to other manufacturer's product literature.

**STEPWISE PROCEDURE:**

 **Materials Provided**

 VITROS Chemistry Products Cl- Slides

 **Materials Required but Not Provided**

 **•** VITROS Chemistry Products Calibrator Kit 2

 • Quality control materials, such as VITROS Chemistry Products Performance Verifier I and II and a commercially available urine control

 • Electrolyte Reference Fluid appropriate for your system

**Operating Instructions**

 • Check reagent inventories at least daily to ensure that quantities are sufficient for the planned workload.

 • For additional information, refer to the operating instructions for your system.

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| **IMPORTANT:** | *Bring all fluids and samples to room temperature, 18–28 °C (64–82 °F), prior to analysis.* |

**Sample Dilution**

Chloride concentrations outside the system's measuring (reportable or dynamic) range are not expected. Diluted samples should not be analyzed with VITROS Cl- Slides because dilution changes both the concentration of solids in plasma water and the ionic strength of the sample.

**REPORTING RESULTS:**

 **Reporting Units – mmol/L**

 **Reference Interval**

 These reference intervals were determined per CLSI EP28. 16

 

**Per the Neonatologist Serum Chloride for 0-3 Months is 90-120 mmol/L.**

 + This reference interval is the central 95% of serum results from an internal study of 60 apparently healthy individuals from a working population.

 ++ This reference interval is the central 95% of urine results from an internal study of 135 apparently healthy individuals from a working population.

 \* Chloride concentration (mmol/L) x 24-hour volume (L) = mmol/day

**PROCEDURAL NOTES:**

 **Known Interferences – Serum and Plasma**

 The VITROS Chemistry Products Cl- slides method was screened for interfering substances following CLSI Protocol EP7. 13

 The substances listed in the table, when tested at the concentrations indicated, caused the bias shown

 For substances that were tested and did not interfere, refer to “Specificity.”



 \* It is possible that other interfering substances may be encountered. These results are representative; however, your results may differ somewhat due to test-to-test variation. The degree of interference at concentrations other than those listed might not be predictable.

 \*\*The bias is an estimate of the maximum difference observed.

 • Bromide and iodide from therapeutic drugs and ointments may cause a positive bias of approximately 5 mmol/L and

6 mmol/L, respectively, for each mmol of halide. Normal physiological levels of bromide and iodide do not interfere.

 • Intralipid at 800 mg/dL may cause a positive bias of approximately 1.5 mmol/L.

 • Purines, such as adenine or hypoxanthine, at significantly elevated levels may cause a negative bias of approximately 4

mmol/L.

**Known Interferences – Urine**

 Bromide and iodide from therapeutic drugs and ointments may cause a positive bias of approximately 5 mmol/L and 6 mmol/L, respectively, for each mmol/L of halide. Normal physiological levels of bromide and iodide do not interfere.

 Interference claims were cited when the observed bias exceeded the acceptance criteria for

 Substances exhibiting bias less than the stated acceptance criteria have been cited as substances that do not interfere. It is possible that other interfering substances may be encountered. These results are representative; however results may differ somewhat due to test-to-test variation. The degree of interference at concentrations other than those listed may not be predictable.

**Other Limitations**

Certain drugs and clinical conditions are known to alter chloride concentration *in vivo*. For additional information, refer to one of the published summaries.14, 16

**Performance Characteristics**

 **Limit of Detection – Urine**

The Limit of Detection (LoD) for VITROS Chemistry Products Cl- Slides for urine is 2.2 mmol/L, determined consistent with CLSI EP17 18 and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 180 determinations with 4 blank and 6 low level samples. The Limit of Blank (LoB) is 1.1 mmol/L. The Limit of Quantitation (LoQ) is 5 mmol/L as determined by the lowest concentration at which precision design requirements are met within the linear range of the assay.

 

 \* Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term "analytical sensitivity."

 \*\* Proportions of false positives (α) and false negatives (β) were less than 5%; based on 180 determinations, with 4 blank and 6 low-level samples.

 \*\*\* The level of imprecision used to accept the LoQ was within 5% CV.

**Method Comparison**

 **Serum and Plasma**

The plot and table show the results of a comparison of serum samples analyzed on the VITROS 950 System with those analyzed using the coulometric comparative method. 10,11

The table also shows the results of comparisons with serum and plasma samples on the VITROS 750, 250 and 5,1 FS Systems with the VITROS 950 System.

In addition, the table shows the results of comparisons with serum and plasma samples on the VITROS 5600 Integrated System with the VITROS 5,1 FS Chemistry System. The testing followed NCCLS Protocol EP9. 19

 



**Urine**

The plot and data below show the results of a method comparison study with human urine samples analyzed on the VITROS 5600 Integrated System and the coulometric-amperometric titration reference method 10, 11, based on CLSI Protocol EP09. 20

The table also shows the results of comparisons with human urine samples on the VITROS 350 and VITROS 5,1 FS Chemistry Systems. The testing followed CLSI Protocol EP09.



**Precision**

 Serum and Plasma

Precision was evaluated with quality control materials on VITROS 250, 750 and 5,1 FS Chemistry Systems and the VITROS 5600 Integrated System following NCCLS Protocol EP5. 21

The data presented are a representation of test performance and are provided as a guideline. Variables such as sample handling and storage, reagent handling and storage, laboratory environment, and system maintenance can affect reproducibility of test results.



**Urine**

Precision was evaluated using four sample pools on VITROS 350 Chemistry System, VITROS 5,1 FS Chemistry System and VITROS 5600 Integrated System following CLSI Protocol EP05. 22

The data presented are a representation of test performance and are provided as a guideline. Variables such as sample handling and storage, reagent handling and storage, laboratory environment, and system maintenance can affect reproducibility of test results.



**Specificity**

Substances that Do Not Interfere – Serum/Plasma

The substances listed in the table were tested with VITROS Cl- Slides following CLSI Document EP7 13 and found not to interfere, bias <1.1 mmol/L, at the concentration shown.



**Substances that Do Not Interfere – Urine**

The substances listed in the table below were tested with the VITROS Cl- Slides for urine according to CLSI ProtocolEP07 13, and found not to interfere at the test concentrations shown. The substances were tested at chloride concentrations of approximately 20 and 180 mmol/L and found not to interfere, bias <5%.



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