



BIOMEDICAL SYSTEMS

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Product Bulletin:

Laboratory Procedure for the ChloroChek® Chlorodometer® Sweat Chloride Analyzer Model 3400

These instructions are provided to help meet CLSI guidelines and CLIA mandated requirements.

These instructions are based on the ChloroChek Chloridometer Sweat Chloride Analyzer Applications manual (RP-497)¹, which should be consulted for more in-depth discussion of the following information.

CLINICAL SIGNIFICANCE

The quantitative measurement of the chloride in sweat (commonly called the “sweat test”) is used to confirm the laboratory diagnosis of cystic fibrosis (CF). With an approximate incidence of 1:3200 in Western Europe and the USA, CF is the most common life-threatening genetic disease within the Caucasian population. It is an autosomal recessive disorder characterized by viscous secretions that affect the exocrine glands, primarily in the lungs and pancreas. Patients with CF have an increased concentration of sodium, chloride, and potassium in their sweat. The criteria for the diagnosis of CF include the presence of one or more characteristic phenotypic features, a history of CF in a sibling, or a positive newborn screening result; and an increased sweat chloride concentration by pilocarpine iontophoresis on two or more occasions, or identification of two CF-causing mutations or demonstration of abnormal nasal epithelial ion transport.

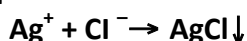
METHOD ²

Based on CLSI recommendations. C34-A3 (2009): Coulometric titration.

PRINCIPLE

The Elitech Group ChloroChek operates according to the principle of coulometric titration. Two silver electrodes—the generator electrodes (anode and cathode) —are dipped into a measuring vessel filled with working solution. The working solution consists of an acid buffer and a colloid stabilizer that keeps the silver chloride, which arises later on, in suspension.

Since the buffer does not contain any silver ions, the silver ion concentration, and thus the indicator current (see below) is brought to a specific end point. By means of a constant current (generator current) between the two silver electrodes, a constant amount of silver ions is released at the anode. The silver ion concentration is maintained by the measurement electrodes (indicator electrodes), which are dipped into the solution. By adding a chloride sample, the free silver ions form a non-soluble silver chloride precipitate together with the free chloride ions of the sample.



The indicator current drops, and by controlling the generator current, silver ions are released until all chloride ions are precipitated as silver chloride. This restores the original silver ion concentration (end point).

The period of flow of the generator current is measured during the titration process and is proportional to the chloride ion concentration.

Depending on the type of sample, no more than 50 measurements should be carried out using one batch of working solution.

INDICATIONS FOR USE

The Elitech Group ChloroChek Chloridometer test system is intended for the quantitative in vitro diagnostic determination of chloride in human sweat using the principle of coulometric titration. Sweat chloride measurements are used in the diagnosis of Cystic Fibrosis. It is for use in Clinical Laboratory settings. The ChloroChek Chloridometer test system consists of the ChloroChek Chloridometer and the ChloroChek Reagent Set.

The ChloroChek Reagent Set (SS-248), is to be used on the ChloroChek. It is used as the titration matrix during the titration process.

The 100 mmol/L NaCl/H₂O Standard Solution (SS-251), is to be used on the ChloroChek. It is used as a calibration verifier, and quality control solution.

The Elitech Group Sweat Controls (SS-150), levels #1, #2, and #3, are to be used as quality control solutions on the ChloroChek.

SAMPLE INFORMATION

Specimen

Human sweat collected with Elitech Group's Macroduct® Collector (REF: SS-032) or by other means (see CLSI C34-A3 guideline for more information).

Storage

Sweat is stable for at least 72 hours across a reasonable temperature range, 25-77 °F, (2-25°C) without significant evaporation when stored in 0.2-mL microcentrifuge tubes with snugly fitting caps (see CLSI C34-A3 guideline for more information).

Interferences

Any salts containing chloride or other halides (halogens) such as fluoride, bromide, or iodide will interfere and cause an elevated reading. CLSI acknowledges this in the C34-A3 guideline. "In addition to chloride, other halides such as bromide and iodide are also detected using a chloridometer. Therefore, if a sweat sample contains other halides in addition to chloride, they will be detected and can falsely elevate the sweat chloride result." ². Halides including chloride may be present in lotions or creams, so it is important that the patient's skin is properly cleaned prior to collecting the sweat. Refer to the CLSI C34-A3 guidelines for cleaning the skin prior to pilocarpine iontophoresis. Improperly cleaned skin prior to sweat collection can lead to higher than normal results, thus leading to false intermediate or false positive results.

CAUTION!

It is critical that the skin is free of contamination before collecting a sweat sample to be used.

SPECIFICATION OF SAFE USE

Using this instrument in a manner not specified by Elitech Group may impair the safety protection designed into the equipment and may lead to injury.

SAFE USE ENVIRONMENT

This device has been designed for indoor use only, between 41-104 °F, (5-40 °C), maximum relative humidity 80%, at up to 87.8 °F (31 °C). For use at altitudes up to 2000 meters.

For use with a supply voltage of 85 to 264 Volts AC @ 50 to 60 Hz, ±10%. Transient Overvoltage Category II. Pollution Degree 2 in accordance with IEC 664 (non-conductive pollution).

PRECAUTIONS AND WARNINGS

- This equipment is for professional *in vitro* diagnostic use.
- Take standard precautions and adhere to good laboratory practices.
- To avoid contamination, clean the skin thoroughly and use clean or single use laboratory equipment while collecting the sweat sample.
- To avoid contamination, use clean or single use laboratory equipment during the analysis of the sweat sample on the ChloroChek.
- When opening the 100 mmol/L NaCl/H₂O Standard Solution (ref: SS-251) ampule, carefully break ampule at the correct location. The SS-251 ampule has a One Point Cut (OPC). Locate this point and break ampule by pushing the top of the ampule back while holding onto the base. It is recommended to use a plastic sleeve when breaking ampules. Elitech Group provides two reusable protective sleeves with each new box of sweat controls (SS-150).
- The reagent set contains acetic acid and nitric acid. If discharged in the plumbing system, rinse with plenty of water.

Initial Setup and Description

- 1 Place the instrument onto a flat and solid surface, such as a laboratory table. Avoid direct sunlight on the LCD screen for easier reading.
- 2 Use the provided power cord to connect power from an outlet to the power entry module.

NOTE:

Ensure that the line voltage matches the voltage indicated by the arrow on the power entry module of the instrument. If it does not, see Section 4.5 to change the voltage on your instrument.

- 3 Turn the power switch ON (I). After a short self-test the welcome screen appears on the display. This screen features three touch-sensitive buttons. This is the home screen:
- 4 Operating the ChloroChek is very easy using the touch screen display. Users can select between the menu items **INFORMATION (INFO)**, **MEASURE**, or **SETTINGS** by touching the corresponding button.

In the **INFORMATION** you will find brief information about the system. To continue, touch the **CONTINUE** button. At the end, you will return to the start which will return you to the home screen.

In the **SETTINGS** menu, you can perform basic settings; to leave this menu press BACK. Setting options include Language (German or English), Display (adjust contrast of the screen), screen saver (select the amount of time until the screen saver activates, from 30 seconds to 60 minutes). In order to avoid damage to the screen it is recommend that the screen saver be used.

In the Settings menu there are also the Lab and Service menus. The date and time displayed on the ChloroChek is changed inside the Lab menu. The Lab menu can be password protected with a password set by the lab if they desire. The Service Menu is protected by a manufacturer-generated password. Only trained personnel should access this Service menu.

3.2 Installing Electrodes

Hands should be gloved so that the electrodes and beaker are not contaminated.

- 1 The Electrode Carriage must be in the up position.
- 2 Install the anode (red housing) and cathode (black housing) electrodes in the appropriate colored receptacle. The short side of the electrode goes into the receptacle leaving the long end exposed.
- 3 Install Measurement Electrode. The Measurement Electrode must be aligned to match the 3-pin connections without binding. Push the Measurement Electrode straight into the receptacle when the pins have been correctly aligned. The Measurement electrode is not screwed into place.

Preparing the Working Solution (Reagents)

A fresh Working Solution must be prepared each day before using the ChloroChek.

Appropriate good laboratory practices should be followed, including using appropriate personal protective equipment (PPEs). Refer to SDS.

NOTE:

The Reagent Set (SS-248) contains 1 dropper-bottle of SS-248GS and 37 bottles for SS-248ABS. The 1 bottle of SS-248GS contains enough Gelatin Solution to prepare all 37 bottles of SS-248ABS. Each bottle of SS-248ABS contains 10 mL of Acid Buffer Solution.

- 1 Add 20 drops of Gelatin Solution (SS-248GS) to one bottle of Acid Buffer Solution (SS-248ABS).
- 2 Put the cap back on the bottle and carefully swirl or invert it to mix it thoroughly. This forms the "Working Solution."
- 3 Place the Magnetic Stir Bar (RP-485) in the Titration Beaker (RP-484). Pour the freshly prepared Working Solution into the Titration Beaker.

Conditioning

NOTE:

Conditioning must take place whenever a new Working Solution is prepared and used. A new Working Solution should be prepared and used each day. The stability of a used working solution is 24 hours.

- 1 With the instrument on, press MEASURE on the display.
- 2 Place the filled titration beaker in the beaker receptacle.
- 3 Lower the handle to bring the electrode set down into the Working Solution.
- 4 Press the READY button on the display.

NOTE:

Pressing CANCEL ends the conditioning cycle and returns you to the Main screen. If this is done, the Working Solution must be replaced.

- 5 In the following display you will be asked to wait until the system has reached its Working Point. A runtime display under the message visualizes this process. Proceed immediately to the next step.
After the working point is reached, you will be instructed to pipette 10 μL of the 100 mmol/L NaCl/H₂O Standard (SS-251) into the Working Solution.
To do so, hold the One Point Cut (OPC) ampule of SS-251 steady in one hand. With the other hand, carefully break off the head of the ampule with only slight pressure across the printed point (blue dot) on the neck of the ampule. Use a protective plastic sleeve when opening the ampule.
- 6 Place a correctly-sized pipette tip onto a 10 μL fixed-volume piston pipette. Elitech Group has an appropriate pipette (AC-178) and pipette tips (SS-253) available. Make sure the tip are securely fitted to the pipette.
- 7 Place the pipette tip well into the 100 NaCl/H₂O mmol/L Standard Solution, holding the tip in the solution during the entire filling phase. Avoid drawing air into the pipette during the filling phase. Allow the piston to smoothly and slowly retract until it has returned to its initial position. Wait for about one second and then remove the pipette tip from the solution. If you see drops on the outside of the pipette tip, carefully remove them with a lint free tissue—do not draw out any solution from the pipette tip (through capillary forces of the paper tissue)—or if necessary, repeat the process with a new pipette tip.
- 8 Using one of the pipette guides found on the sensor carriage, place the loaded pipette tip as near as possible in the center of the stirring Working Solution. Dispense the sample above the working solution, allowing the sample to drop into the working solution. Avoid dispensing the sample near the electrodes or the sides of the beaker. Press the pipette piston down to the second pressure point to completely eject the solution. Remove the pipette from pipette guide and discard the tip.
- 9 When the ChloroChek recognizes the added standard solution, the display will read: PROCEEDING WITH CONDITIONING. When the conditioning is complete, the ChloroChek is ready to use.

NOTE:

In cases where conditioning is not successful, you must clean the electrodes and prepare a new Working Solution (see Section 3 of the manual).

Quality Control

To ensure adequate quality control, Sweat Control Solution (SS-150) should be run at all three levels and the 100 mmol/L NaCl/H₂O Standard Solution (SS-251) should be assayed before running any patient samples. This must be done at least once a day and after conditioning with a freshly prepared Working Solution.

The control frequency should be adapted to Quality Control procedures of each laboratory and any regulatory requirements. Results should be within the defined ranges.

If values fall outside of the defined ranges, then clean the measurement electrodes (see Section 4.2) or check the accuracy of the pipette (see Section 4.1). Quality control materials should be used in accordance with local, state, and/or federal guidelines.

NOTE:

Over time and use the anode (red housing) silver electrode will be used up as it precipitates with the chloride ions. This electrode will have to be replaced periodically as it gets smaller. As long as the ChloroChek passes quality control the anode is still usable for sample measurement.

Sample Measurement

PROCEDURE

NOTE:

Before each sample series, perform quality control procedures (see Section 3.5). Elitech Group normally recommends testing the 100 mmol/L NaCl/H₂O Standard Solution until it consistently reads 100 ± 2 mmol/L. Also, the three levels of sweat control should be tested; results should be within the specified values for the sweat controls. These controls are tested according to the instructions below.

- 1 Place a correctly-sized pipette tip onto a 10 μ L fixed-volume piston pipette.
Place the pipette tip well into the sample, holding the tip in the sample during the entire filling phase. Avoid drawing air into the pipette during the filling phase. Allow the piston to smoothly and slowly retract until it has returned to its initial position. Wait for about one second and then remove the pipette from the solution. If you see drops on the outside of the pipette tip, carefully remove them with a lint free tissue—do not draw out any solution from the pipette tip (through capillary forces of the paper tissue)—or repeat the process with a new pipette tip.
- 2 Using one of the pipette guides found on the sensor carriage, place the loaded pipette tip as near as possible in the center of the stirring Working Solution. Avoid expressing solution near the electrodes or the sides of the beaker. Press the piston down to the second pressure point to completely eject the solution, and then remove the pipette tip with the piston still pressed.
- 3 The ChloroChek recognizes the added sample; the display will start counting up from 0 until it reaches the final value. The final value remains on the screen until another sample is pipetted into the Working Solution, or until you press STANDBY on the screen.

NOTE:

Test results must be interpreted by a qualified physician, according to the pertinent guideline. See Appendix E, Reference Intervals for Sweat Chloride.

- 4 After a final value is reached, you can pipette another sample into the Working Solution following Steps 1-3 above. The ChloroChek automatically recognizes each sample when injected and the value for the last sample injection is displayed on the screen.

NOTE:

If the ChloroChek fails to automatically recognize the sample in Step 4, the chloride concentration in the sample is less than 10 mmol/L. Verify this by adding 10 μ L of the 100 mmol/L NaCl/H₂O Standard Solution (SS-251). The reading will likely be above 100 mmol/L. Then subtract 100 mmol/L from this final reading for the approximate final result of the sample. Keep in mind that this result is below the specified linearity range of the ChloroChek and should be recorded only as <10 mmol/L.

NOTE:

Pressing STANDBY on the screen stops stirring the Working Solution, which conserves its functionality. Press STANDBY if there will be a break of at least 5 minutes between measuring samples. The ChloroChek will automatically go into STANDBY mode if no sample has been measured for at least 5 minutes. Press CONTINUE to restart stirring and continue with more measurements. Users should perform quality control with control samples before specimen samples are tested, to be sure that the ChloroChek is functioning correctly. In the STANDBY Menu, if CANCEL is pressed this will cancel the measurement sequence and the Working Solution should be discarded.

NOTE:

Users can run up to 50 samples in each series before needing a fresh Working Solution. A freshly prepared Working Solution should be a transparent red color. As a Working Solution is used it will appear cloudy. If a freshly prepared Working Solution appears cloudy it should be discarded. The titration beaker should then be carefully cleaned and a new Working Solution prepared.

- 5 Once all samples are measured, press STANDBY and then CANCEL. This will end the measurement sequence.
- 6 Raise the handle to lift the electrode set out of the Working Solution.
- 7 Remove the stir bar from the Working Solution by using the Magnetic Stir Bar Retriever (RP-486).
- 8 Discard the Working Solution.
- 9 Rinse the titration beaker, stir bar, and electrodes with deionized water. Remove the electrodes from the electrode carriage for easy rinsing and drying.
- 10 Dry the titration beaker, stir bar, and electrodes with lint-free tissue.
- 11 Store electrodes in plastic tubes to prevent oxidation on electrodes. Store in this manner until next use of the ChloroChek

The ChloroChek is now ready for use again with a freshly prepared Working Solution.

NOTE:

If larger samples are used (>10 µL), take into account that the concentration is reported on the basis of the titration for a 10 µL sample. Adjust the reading according to the volume of pure sweat in the sample using a procedure validated by the laboratory. Equipment and Reagents

EXPECTED RESULTS

Reference Intervals for Sweat Chloride²

Age	Normal Range	Intermediate Range	Indicative of CF Range
Infants (0-6 months)	Cl ⁻ ≤ 29 mmol/L	Cl ⁻ 30-59 mmol/L	Cl ⁻ ≥ 60 mmol/L
Beyond Infancy (6 months-18 years)	Cl ⁻ ≤ 39 mmol/L	Cl ⁻ 40-59 mmol/L	Cl ⁻ ≥ 60 mmol/L
Adults (>18 years)*	Cl ⁻ ≤ 39 mmol/L	Cl ⁻ 40-59 mmol/L	Cl ⁻ ≥ 60 mmol/L

*Exact ranges for adults are not fully defined. Refer to CLSI C34-A3 for more information.

REFERENCES:

- 1 ChloroChek Chloridometer Sweat Chloride Analyzer Applications Manual (RP-497).
- 2 CLSI. Sweat Testing: Sample Collection and Quantitative Chloride Analysis; Approved Guideline - Third Edition. CLSI document C34-A3 [ISBN 156238-000-0]. Clinical Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2009.