|  |
| --- |
| **Sweat Chloride on ChloroCheck Chloridometer** |
| **Purpose** | This procedure provides instructions for performing SWEAT CHLORIDE on the ChloroCheck Chloridometer. |
| **Policy Statements** | This procedure applies to Chemistry personnel responsible for the analysis of sweat chloride samples by coulometric titration on the ChloroCheck Chloridometer |
| **Principle** | The ChloroCheck 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.ChloroCheck 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 a 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 forma a non-soluble silver chloride precipitate together with the free chloride ions of the sample.Ag+ + CI-  AgCI↓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 concentration. |
| **Clinical Significance** | The quantitative measurement of the chloride in sweat (commonly called the “sweat test”) is used to confirm the 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, or identification of two CF-causing mutations or demonstration of abnormal nasal epithelial ion transport. |
| **Instrument** | ChloroCheck Chloridometer, Model 3400 |
|  |  |
|  | **Equipment** |
|  | * Model 3400 ChloroCheck Chloridometer
* Analytical Balance with accuracy to 0.1 mg (Sartorius Scale or Ohaus Explorer)
 |

|  |  |
| --- | --- |
| **Materials** | **Reagents**  |
|  | **Reagent** | **Product Number** | **CHC Number** | **Storage/Stability** | **Description** |
| Reagent Set | SS-248 | 34927 | See individual components below | Contains Buffer Solution x 37, and Gelatin Solution x 1 |
| Buffer Solution | SS-248BS | Included in Reagent Set | Store at 10-30° C until expiration or day of use | 10mL Buffer Sol. of acetic acid <0.5%, nitric acid <0.5%, DI H2O |
| Gelatin Solution | SS-248GS | Included in Reagent Set | Store at 10-30° C until expiration  | 30mL Gelatin Sol. with indicator, pH 5-7 |
| Working Solution | N/A | N/A | Make each day of use. May be used for up to 50 determinations. | The Working Solution is composed of 20 drops of Gelatin Solution in 1 vial of Buffer Solution |
| Standard Solution | SS-251 | 34928 | Store at 5-45° C Stable until expiration or 30 min once opened | Contains 10 vials of 100 mmol/L NaCl/H2O (equivalent 100 mEq/L Cl-) |
| CLRW | N/A | N/A | Use fresh daily, or store up to 7 days in squirt bottle. | Clinical Laboratory Reagent grade Water |
| Sweat Controls | SS-150 | 34926 | Store at 15-25° C until expiration or day of use | 3 levels of controls, 12 vials each, 0.75 mL |
|  |  |  |  |  |  |
|  | **Supplies and Accessories** |
|  | **Supply** | **Product Number** | **CHC Number** | **Description** |
| Silver Electrode, red | RP-481 | 34925 | Anode |
| Silver Electrode, black | RP-482 | 34923 | Cathode |
| Measurement Electrode | RP-483 | 34924 |  |
| Titration Beaker | RP-484 | 34929 |  |
| Magnetic Stir Bar | RP-485 | 34930 |  |
| Stir Bar Retriever | RP-486 | 34934 |  |
| Silver Cleaning Cloth and Microfiber Cloth | RP-487 | 34935 |  |
| Data Cable Set | RP-535 | SO | RS232/USB |
| 10 uL pipette | N/A | SO | MLA fixed D-tipper |
| Pipette tips | 9025 | 14679 | 5-200 uL MLA style |
| Ampule Organizer | AC-071 | SO |  |
| Squirt Bottle | AC-180 | SO | 500 mL volume, for water |
| Small Sealable Container | SS-107 | N/A | Included with MacroDuct supplies.  |
| Kimwipes | KCP34155H | 31128 | Lint free tissue |
|  | Nippers | RP-066 | 20186 |  |
|  | Sweat Dispenser | RP-065 | 19276 |  |
|  |  |  |  |  |
|  | **Documents** |
|  | * SunQuest worksheet SCL (MIN) or SCL2 (STP)
* [CH 6.78.f1 ChloroCheck Chloridometer Maintenance Log](https://starnet.childrenshc.org/References/labsop/chem/forms/ch-6.78.f1-chloridometer-maintenance.pdf)
* [CH 4.06 Sweat Collection](https://starnet.childrenshc.org/References/labsop/chem/collect/ch-4.06-sweat-collection.pdf) by Macroduct Advanced
 |
| **Sample** | Sweat is stimulated using Pilogel iontophoresis, and collected using the MacroDuct coils. Refer to the procedure [CH 4.06 Sweat Collection](https://starnet.childrenshc.org/References/labsop/chem/collect/ch-4.06-sweat-collection.pdf) by Macroduct Advanced for instructions on proper collection of sweat samples.**Volume:**The minimum volume for a 30 minute collection is 15 microliters based on an average sweating rate of 1 gm/m2 per minute. Collections are never to be pooled. ALL samples should be measured and weighed. Determination of acceptability will be made by the chemistry tech performing measurement, and weights/volumes must be recorded for each and every sample. Record all volumes on the worksheet regardless of final acceptability.**Stability:**The sweat specimen is stable for same day analysis while sealed in the MacroDuct coils and placed in a sealed plastic bag immediately after collection to minimize evaporation. Sample must be immediately transferred to a Small Sealable Container (SSC) upon receipt by chemistry tech. Every effort should be made to prioritize the testing of sweat chloride and to complete it as soon as possible. Only in the case of an analyzer downtime, the sample may be kept for up to 24 hours when immediately transferred to a SSC and stored at 2-8C.**Sample preparation:*** Do not pool collections.
* Do not send samples by pneumatic tube, walk to lab only.
* Label Small Sealable Containers (SSC) with small foot label and differentiate between the two collections with 1st/2nd, left/right, or collection times.
* While wearing powder-free gloves, weigh the appropriately-labeled, dry, empty Small Sealable Container on an analytical balance capable of measuring to 0.1 mg (0.0001g). Record the weight for each vial on the worksheet.
* Use the dispenser device or a needle attached to an air filled syringe to slowly and carefully dispense the sample into the small cup. (Use care to avoid contaminating the dispensing needle with sample. If external contamination occurs, clean with DI water. Discard contaminated dispensing needles.)
* Follow directions for the scale located at each testing site: Sartorius Scale in Minneapolis and Ohaus Explorer in St. Paul. Measure the Small Sealable Container (SSC) again, making sure the outside of the container is dry and the cap is tightly sealed.
* Record this weight on the worksheet. Subtract the weight of the empty vial from each measurement. Be sure you are subtracting the weight of the correct vial from the weight of the correct collection, because each labeled SSC has a different weight. Record final weight on the worksheet and determine acceptability. (The Ohaus Explorer scale in St. Paul and SunQuest will perform calculations to determine the weight of sweat; check the scale calculation against SunQuest to be sure the values are the same).
* The density of sweat is 1.001 to 1.008, and using the *density = weight/volume* equation we can extrapolate that for the purposes of sweat gravimetric volume measurement, 1 mg = 1 uL and therefore 15 mg = 15 uL.
* Inadequate collections:
	+ If weight is less than 15 mg (15uL) for only one arm, credit using “SWEA1”, this will credit one of the two tests. If weight is less than 15 mg (15uL) for both arms, credit using “SWEA2” for both fields, this will credit both tests. Only use the SWEA1/SWEA2 so that QNS failure rates can be adequately compiled, any other cancel or QNS codes are not acceptable.
	+ Notify technical specialist and ordering provider if either one or both arms fail to produce enough sweat.
	+ SWEA1 will add comment stating “Unable to collect sweat.” and credit one test.
	+ SWEA2 will add comment stating “Unable to collect sweat from either site. Suggest collection at a later date.” and credit both tests.
	+ See additional instructions in [Result Reporting](#ResultReporting) section

**Criteria for Rejection:*** Specimens with a total volume of less than 15 microliters (0.0150g or 15mg)
* CAUTION: inadequate sample volume may cause false negative sweat chloride results.
* Unlabeled specimens
 |
| **Special Safety Precautions** | * Refer to laboratory safety policies and procedures.
* Dispose of reagents according to Safety Guidelines. See Procedure, Step 19
* Do not perform instrument maintenance, including replacing electrodes, while power is on.
* Use protective sleeve (included in control kits) when opening control or standard ampules.
 |
| **Maintenance** | Day of useElectrode inspection and cleaning: With the instrument off, inspect the electrodes for placement or deterioration. Clean or install new electrodes as needed, following the applicable procedures below. Cleaning is only required if there is visible build up or deterioration on the electrodes, or if the conditioning step fails. Install new electrodes when severe deterioration is present.Instrument inspection and cleaning: Inspect the instrument for spills or residue; clean if required using DI water and mild detergent. Do not use bleach or solvents.As NeededCleaning Electrodes:Required materials: CLRW water, catch beaker, silver cleaning cloth and microfiber cloth.Instructions: Place the small microfiber cloth and large silver cleaning cloth near each other on the lab bench. At an approximately 30-degree angle from the benchtop, pull the electrode across the microfiber cloth; dark marks should appear on the cloth. If electrode bends, straighten. Clean all sides of electrode with polishing cloth, dampened with CLRW. Rinse with CLRW and dry with a Kimwipe.* **Installing Electrodes:** For all electrodes, the carriage must be in the up position and the instrument turned off. For the anode (red) or cathode (black) match to the same colored receptacle. Ensure the short side of the electrode goes into the receptacle leaving the long end exposed. For the measurement electrode, place the electrode in the front facing receptacle and align the 3-pin connections. Push the measurement electrode straight into the receptacle, it is not screwed into place.
 |
| **Troubleshooting** |  |
|  | **Error** | **Component Group** | **Effect** | **Possible Cause** | **Solution** |
| Fluctuating measurement values during measurement of standards or controls | Titration electrode; Anode or Cathode | No Plausible measurement values | Contaminated electrodes or mechanically defective electrodes | Clean electrodes with silver cleaning cloth |
| Pipette | No Plausible measurement values | Pipette defective or incorrect pipette | -Inspect pipette, recalibrate or replace-Inspect pipetting technique, retrain |
| Conditioning not successful | Measurement Electrode | Unable to begin the measurement series | -Contaminated electrode-Mechanically defective electrode | -Clean the measurement electrode with the silver cleaning cloth and rinse with CLRW-Replace the measurement electrode |
| Working Solution | Unable to begin the measurement series | Incorrect solution used or old solution | Use new working solution |
|  |  |
| **Calibration** | * The instrument self-calibrates during the conditioning phase. See Procedure section below.
* Calibration verification, linearity, and reportable range

Calibration Verification Dilution Table utilizing the 100 mEq/L standard:

|  |  |  |
| --- | --- | --- |
| Dilution | Formulation | Target Value |
| A (1:10) | 50 uL standard, 450 uL CLRW | 10 mEq/L |
| B | 50 uL Dilution A, 50 uL dilution C | 32.5 mEq/L |
| C | 200 uL standard, 200 uL Dilution A | 55 mEq/L |
| D | 50 uL Dilution C, 50 uL standard | 77.5 mEq/L |
| E (Neat) | 50 uL standard | 100 mEq/L |

* Perform every 6 months and after major service.
* Must use a different lot of 100 mEq/L standard than that used for daily calibration/conditioning
* Recommended to make samples in this order: E → A → C → B and D
* Measure each dilution in triplicate by following the routine operating procedure.
* Enter all values in EP Evaluator, under project ChloroCheck, Linearity and Calibration Verification. Record lot numbers of calibrators, reagents, QC. Export the project as a PDF, have documentation reviewed by technical specialist.
* When standards and controls cannot be brought into accepted ranges following normal troubleshooting procedures, factory calibration of the ChloroCheck Chloridometer may be needed. See user manual for details.
 |
| **Quality Control** | Measurement of three levels of quality control, and the 100 mEq/L standard, are due once each day of use and with each new tech performing analysis. The EliTech Sweat Controls are assayed, liquid, and ready to use. Stored room temperature (15-25°C), and when unopened, are stable until expiration date stated on the label. After opening, controls are stable for immediate use. **Acceptable ranges:*** The standard solution must measure 100 mEq/L +2
* Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* Acceptable ranges are current in Unity Real Time only. Quality Control results must be rejected in SunQuest when the results cross the interface.
* Do not load or release patients until QC is acceptable in Unity Real Time.
 |

|  |  |  |
| --- | --- | --- |
| **Procedure** | **Step** | **Action** |
|  |  | Print the worksheet SCL (MIN) or SCL2 (STP) from SunQuest to record standard, QC and patient values. Sample weight should be recorded on this worksheet according to procedure listed in Sample Preparation procedure (above). |
|  |  | Inspect the instrument and electrodes per daily maintenance procedure (above). Ensure all electrodes are in good condition, then place them in the correct locations. |
|  |  | Gently mix Gelatin Solution (SS-248GS) then add 20 drops to a new vial of Buffer Solution (SS-248BS). Replace the caps. Gently mix the Buffer Solution vial. This is now the Working Solution. |
|  |  | Place a clean magnetic stir bar into a clean titration beaker. Pour the freshly prepared Working Solution into the titration beaker. Save the Buffer Solution vial for disposal, step 19. |
| **Conditioning** |  | Conditioning must be done each day of use or with each new Working Solution. |
|  |  | Power on the instrument. Select “Measure” option. |
|  |  | Place the filled titration beaker into the beaker receptacle (case). Lower the carriage to bring the electrode set down into the Working Solution and the “Ready” button will appear. |
|  |  | Click the “Ready” button. The instrument will ask the user to wait until it has reached the working point. Do not progress or cancel until the “Inject” screen appears. |
|  |  | Once the “Inject” screen appears, add 10 uL of the 100 mEq/L standard to the Working Solution. Dispense sample just above the Working Solution, and avoid dispensing near the sides of the beaker or near electrodes.***Note:*** *to open the standard, use a protective sleeve and apply pressure across the side of the ampule with the blue dot.****Note:*** *if drops adhere to the exterior of the pipette tip, they may be removed with a kimwipe but be sure to avoid drawing liquid from the inside of the pipette.* |
|  |  | The instrument will recognize the standard solution and now read “Conditioning”. Once conditioning is complete, the instrument will read “Ready”. |
|  |  | Perform steps 12-14 for standard solution and all three control levels before patient testing. |
|  |  | Add 10 uL of standard, quality control, or patient sample to the Working Solution. Dispense sample just above the Working Solution, and avoid dispensing near the sides of the beaker or near electrodes. |
|  |  | The ChloroCheck will automatically detect the added sample and will begin counting up from 0 until it reaches the final value. The final value will remain on the screen until another sample is pipetted into the Working Solution, or until the “standby” button is pressed. |
|  |  | Record the result on the worksheet under the correct sample.NOTE: Patient samples should be tested in duplicate if volume is sufficient, and result averaged. |
|  |  | Each collection is attempted in duplicate (typically one collection on each arm). Perform steps 12-14 on both samples, if adequate volume was collected. |
|  |  | If additional patient samples are expected same day, press the “standby” button. Press the “continue” button to resume measurement when samples arrive. Otherwise go to step 17.**Note:** *Use standby for any break of 5 or more minutes between measurements. Standby of more than 1 hour may have a reading when “continue” is pressed due to excess chloride becoming unbound from electrodes or silver chloride precipitate.* |
| **Shutdown** |  | When ready to shut down for the day, press “standby” and then “Exit”. This will end the measurement sequence. Power down the instrument. |
|  |  | Raise the electrode carriage into the upward position. |
|  |  | Remove the titration beaker. Remove the stir bar with the magnetic stir bar retriever. Decant the working solution back into the Buffer Solution vial. Cap and dispose of in Acid waste. Remove the electrodes from the electrode carriage. |
|  |  | Rinse the titration beaker, stir bar, and electrodes with CLRW water. |
|  |  | Dry the titration beaker, stir bar, and electrodes with lint free tissue. To prevent oxidation, store electrodes in their individual plastic containers. |
|  |  |
| **Calculations** | Sweat volume is calculated by the following formula, where VT is Total Volume including both sample and vial, VV is Vial Volume, and VS is Sample Volume.$$V\_{S}=V\_{T}-V\_{V}$$ |
| **Interpretation/****Results/Alert Values** | **Reportable Range**: 10-110 mEq/L* Sweat chloride results >160 mEq/L are physiologically impossible, and must be repeated after investigation of possible contamination.
* Sweat Chloride results less than 10 will be entered as their numerical result in SunQuest. SunQuest LIS will change the result to <10.
* Patient sweat chloride results 90 mEq/L and above are very rare; if one collection yields a result of 90 or above, follow the shutdown procedure. Restart the procedure with new reagents, repeating the standard and QC. Run the second sample. If volume allows repeat the first sample as well.

**Clinical Reportable Range:** If results obtained are greater than 110 mEq/L, report as > 110 mEq/L. |
| **Reference Intervals** | Reference Ranges:

|  |
| --- |
| All Ages: |
| **0-29 mEq/L**: A normal sweat chloride cannot be used as the sole criterion for exclusion of a diagnosis of cystic fibrosis |
| **30-59 mEq/L**: Borderline: repeat testing, further evaluation or CFTR gene mutation analysis recommended |
| **Greater than or equal to 60 mEq/L**: Consistent with the diagnosis of cystic fibrosis |
|  |

 |
| Critical Values: | * All results greater than or equal to 30 mEq/L must be called to the ordering physician.
* Document notification by appending the comment code “Results Phoned and Read back by” using the SunQuest “Phoned Result” button at the top of the result screen.
 |
| Limitations | **Interference:** Bromide and iodide will combine with silver in the same manner as chloride and cannot be differentiated by the Chloridometer. The Chloridometer actually reads the total concentration of CI, Br and I.Sweat chloride results should always be evaluated with regard to the patient’s clinical course.* + Positive sweat chloride results should be confirmed with mutation analysis: the diagnosis of CF should not be based on a single positive determination.
* Borderline (30-59 mEq/L) sweat chloride results should be repeated at a later date.
* Repeat testing should follow negative sweat chloride results if clinical symptoms of CF persist.

Low volumes of sweat contribute to false negative chloride results. Sweat chloride should be collected and reported in duplicate to reduce the potential for false negative reporting, but is not required.  |
| **Result Reporting** | 1. Record sample volume for each collection on SunQuest Worksheet. Volume is determined by measuring gravimetrically on an analytical scale with 0.1 mg accuracy. For purposes of sweat chloride determination, 1 mg = 1 uL.
2. Record results from the readout under column for Collection 1 and Collection 2 respectively.
3. Enter results into SunQuest:
	1. Use function MEM
	2. Method Code= LC1 (MPLS), LC2 (STP)
	3. Worksheet =SCL (MPLS) or SCL2 (STP)
	4. Enter Patient Accession Number: For each of the prompts enter the following information:
* 1VIAL = weight of labeled Small Sample Container (SSC) for 1st Collection
* 1VSW = weight of labeled SSC and sweat for 1st Collection
* 2VIAL= weight of labeled SSC for 2nd Collection
* 2VSW = weight of labeled SSC and sweat for 2nd Collection
* 1 VOL = volume in uL of first collection (SunQuest calculates)
* 2 VOL = volume in uL second collection. (SunQuest calculates)
* 1 CL = 1st collection Chloride value or SWEA1/SWEA2 code for QNS
	+ - Use SWEA1 if only 1 collection is QNS, use SWEA2 if both collections are QNS
* 2 CL = 2nd collection Chloride value or SWEA1/SWEA2 code for QNS
	+ - Use SWEA1 if only 1 collection is QNS, use SWEA2 if both collections are QNS
	1. Always weigh vials, even if no visible sweat is produced. Never enter “0” for any of the VIAL, VSW, or VOL prompts.
1. Always test one sample even if the other collection fails.
2. If the test is being credited for a reason other than unable to collect, credit the test in OER using an appropriate comment. Do not credit in OER if collection was attempted so that we may charge for the collection. If no collection was attempted, use the appropriate test comment; do not use SWEA1/SWEA2 because it will still charge for a sweat collection attempt.
3. If any collection was attempted, collection devices should be sent to the lab in a sealed plastic transport bag for chemistry lab tech to evaluate, document, and cancel inadequate specimens. The testing tech will call the relevant provider for any failed collections and notify the technical specialist.
 |
| **References** | 1. ChloroCheck User’s Manual 57-2006-03C, last updated 06/22/2021
2. *Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation*, Journal of Pediatrics 2017; 181S:S4-15
3. College of American Pathologists, Chemistry and Toxicology Checklist, revised October 24, 2022.
4. Cystic Fibrosis Foundation Sweat Testing guidelines.
5. CLSI. *Sweat Testing: Specimen Collection and Quantitative Chloride Analysis.* 4th ed. CLSI Guideline C34. Wayne, PA: Clinical Laboratory Standards Institute; 2019
6. ELITech Group. MacroDuct Advanced User’s Manual Model 3710 SYS. ELITechGoup Inc. 370 West 1700 South, Logan, Utah, 84321
 |
|  |  |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Matt Johnson  | April 7, 2023 | Initial Version |
|  | Matt Johnson | April 17, 2023 | Added new CHC numbers |
|  |  | Matt Johnson | Jan 15, 2024 | Test each sample in duplicate, average results. Step 14. Per CLSI Doc. |
|  |  |