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| **Sweat Chloride on Labconco Chloridometer** |
| **Purpose** | This procedure provides instructions for performing SWEAT CHLORIDE ON LABCONCO CHLORIDOMETER. |
| **Policy Statements** | This procedure applies to Chemistry personnel responsible for the analysis of sweat chloride samples by titration on the Labconco Chloridometer |
| **Principle** | The Labconco Digital Chloridometer is a dedicated coulometric titrator designed to determine the chloride ion concentration of a solution. It displays this concentration directly in millequivalents chloride per liter when 10 microliter samples are used. Some typical samples are serum, urine, biological extracts, food product extracts, industrial effluents and other aqueous solutions. Our application involves analyzing the Chloride concentration in sweat. The combination of silver ions and chloride ions is a quantitative reaction that results in an insoluble precipitate of silver chloride (AgCI). Ag+ + CI- → AgCI (ppt.)This reaction is carried out at a constant rate by passing a fixed direct current between a pair of silver electrodes immersed in an acid solution. The anode, which is consumed in this reaction, is a continuous spool of silver wire. As the immersed portion of the wire is consumed, fresh wire is drawn from the spool. As the equivalence point of the reaction is reached, an increase in current between a pair of separate indicator electrodes is detected. At a preset indicator current, the instrument automatically stops the incremental counter and the generation of silver ions. Since the generator current is constant, the total titration time is directly proportional to the number of chloride ions that are introduced into the sample vial. The instrument displays this relative time in units of millequivalents chloride per liter. |
| **Clinical Significance** | To provide appropriate care for Cystic Fibrosis (CF) patients and genetic counseling to their families, it is important that the diagnosis of CF be made accurately and promptly. CF is one of the most common autosomal recessive diseases in people of Northern European ancestry with an estimated incidence in the United States of about 1 in 3200 and a carrier frequency of 1 in 29. It is a multisystem disorder affecting the pulmonary, gastrointestinal, and reproductive organs. CF is recognized as a syndrome with a wide spectrum of clinical presentations ranging from meconium ileus and severe respiratory disease in infants to mild pulmonary symptoms and no evidence of gastrointestinal problems even in adulthood. Morbidity and mortality of the disease are most related to mucus accumulation, recurrent infections with unusual pathogens, and excessive inflammation in the lung. CF is associated with a defect in the cystic fibrosis transmembrane conductance regulator protein (CFTR), a protein that normally regulates electrolyte transport across epithelial membranes. Several hundred mutations of CFTR have been identified. Genetic analysis and sweat tests are using in conjunction to diagnose CF.  |
| **Instrument** | Labconco Chloridometer |
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|  | **Equipment** |
|  | * Labconco Digital Chloridometer SN143574 Model 442-5000 [MPLS]
* Labconco Digital Chloridometer SN144122 Model 442-5000 [St Paul]
* Analytical Balance with accuracy to 0.1mg
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| **Materials** | **Reagents**  |
|  | * Chloridometer Acid Reagent, 475 mL Labconco Cat. No. 442-5068 Supplied ready for use. Store at room temperature. Stable until the expiration date printed on the bottle.
* Chloridometer Standard, 100mEq/L, 120rnL Labconco Cat. No. 442-5069. Supplied ready for use. Contains 100mEq/L chloride for use in assuring the proper operating performance of Digital Chloridometers.
* Chloridometer Standard, 20mEq/L, Prepare a working standard using 1.0 mL of 100 meq/L standard and 4.0 mLs of reagent grade water. Stable for 3 months at room temperature.
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|  | **Supplies and Accessories** |
|  | * Glass Sample vials, 20 x 40 mm, Box of 100 Cat. No. 586-0007
* Vial Rack Cat. No. 442-5080
* Silver Wire Cat. No. 442-5120
* Silver Polish Cat No. 442-5094
* Replacement Fuse ½ Amp, 220V AC, Slo Blo Cat.No. 531-0095
* Power Cord, 10 A, 120V Cat. No. 515-1001
* 10.0 microliter pipette and pipette tips
* Oxford Repetitive Pipettor calibrated to deliver 4.0 mLs
* Rinse bottle with reagent grade water
* Kimwipes
* Small Sealable Containers (SSC) (supplied in Wescor Macroduct Supply Kit)
* Wescor Macroduct Supply Kit (PN SS-032)
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|  | **Documents** |
|  | Generate the worksheet SCL (MIN) or SCL2 (STP) from Sunquest for use in recording results.Labconco Chloridometer Maintenance Log, found on-line [Chloridometer Maintenance Log](http://khan.childrensmn.org/Manuals/Lab/SOP/Chem/Forms/204091.pdf) . |
| **Sample** | Sweat is stimulated using Wescor Pilogel iontophoresis, and collected using the Wescor Macroduct coils. Refer to the Sweat Chloride Collection procedure 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.
* Label one Small Sealable Container (SSC) for each collection device with patient name and specific collection information: “1” vs. “2”, or times collections ended).
* 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 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.
* If weight is less than 15mg/15uL, credit using “SWEA” for any inadequate collection. Only use the SWEA cancel comment for the collection(s) that fail so that QNS failure rates can be adequately compiled. Always notify the provider whenever volumes are inadequate for any/either collection.

**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
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| **Special Safety Precautions** | Refer to laboratory safety policies and procedures. Dispose of reagents according to Safety Guidelines. See Procedure, Chloride Determination, # 8. |
| **Maintenance** | Day of use:Electrode Cleaning and Conditioning 1. With the instrument off, check that the generator electrode (silver wire spool) is the same length as the other electrodes and is thicker than the shaft of an ordinary pin. If it is not, snip off the thinning segment and draw off enough wire from the spool to even its length with the other electrodes. Tighten the binding post (located to the left of the wire spool) so that it makes good contact with the silver wire. Do not put the wire through the hole in the binding post since this will deform the soft metal.
2. Thoroughly clean all four electrodes with silver polish, rinse with distilled water and buff with lint free tissue. Be certain no residue remains between the indicator electrodes at their common mounting post. Avoid getting skin oils on electrodes.
3. After cleaning, place a vial filled with 4 mL of Chloridometer Acid Reagent in the vial holder. Set the Blank to 0.0. Set the RANGE switch to LOW, the TITRATION switch to AUTO and raise the holder so that the electrodes are immersed and the stirrer begins.
4. If a reading does not appear after 30 seconds, re-rinse the electrodes and re-titrate using a fresh vial. Do this until a reading is obtained.
5. Immerse electrodes in distilled water each day at the end of the run, or if patient sample analysis will be delayed.
6. It is acceptable to prepare the analyzer in anticipation of patient testing, up to 6 hours ahead of time. Immediately prior to testing the patient sample, however, Quantimetrix controls levels 1 and 3 must be run to ensure analyzer function has remained stable. Record all results and time testing was completed next to each result.

As Needed * Clean outside of instrument with mild detergent or soap and distilled water. DO NOT USE BLEACH.
* Replace entire electrode assembly if it becomes damaged or deteriorated.
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| **Calibration** | * Calibration is performed each day of use by titration of 4 DI water blanks (0), the 20 mEq/L standard, and the 100 mEq/L standard.
* Once every 6 months verify reportable range:
	+ Prepare a 1:2 of the 20 standard
	+ Run dilution, daily DI water blank, 20 mEq/L and 100mEq/L standards in triplicate. Results must match within 10% of the assigned target value.
	+ Enter replicates in EP Evaluator, Project CAL VER, Linearity and Calibration Verification. Save previous results to History
* When standards and controls cannot be brought into accepted ranges following normal troubleshooting procedures, factory calibration of the Labconco Digital Chloridometer may be needed.
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| **Quality Control** | At least once each day of use, and immediately prior to patient runs, analyze two levels of quality control material with known chloride concentrations. Use Quantimetrix Levels 1 and 3.Quantimetrix Sweat Controls for Cystic Fibrosis testing are assayed, liquid, and ready to use. Stored refrigerated (2-8°C), and when unopened, are stable until expiration date stated on the label. After opening, controls are stable for three months when stored at 2-8°C in between use. Discard if turbid or any evidence of microbial contamination is present.**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the [Westgard Rules in Chemistry procedure](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.18-westgard-rules-in-chemistry.pdf) for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.

It is acceptable to prepare the analyzer ahead of test time, up to 6 hours prior to patient testing. Prior to testing the patient sample(s), however, two Quantimetrix levels 1 and 3 must be run to ensure analyzer function has remained stable. Record these subsequent results on the worksheet along with the time analysis took place. |

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| **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. |
| **Blank Determination** |  | Place TITRATION switch in STANDBY. |
|  |  | Place the RANGE switch in LOW. |
|  |  | Fill each of four 20 x 40mm-glass sample vials with 4.0 mL of Acid Reagent using the repetitive pipettor. |
|  |  | Place each vial in turn on the vial holder, raise the holder until the electrodes are immersed, and depress the TITRATION switch to the START position. |
|  |  | The final value on the LED display is the blank value. Determine the average of the four blank determinations and enter that number on the BLANK thumbwheel switch. If the blank is greater than 100, enter the blank value minus 100 on the thumbwheel and subtract 100 manually for all determinations. (Note: repeated blank determinations greater than 100 may be lot-related. A new lot of acid reagent should be ordered if blanks are repeatedly above 100.) |
| **Chloride Determinations** |  | For each determination, carefully add 4.0 mL of Acid Reagent using the repetitive pipettor into a 20 x 40mm-glass sample vial. |
|  |  | Aspirate 10.0 uL of standard, control, or patient sample using an appropriately calibrated pipette, making sure the pipette tip does not go deeper than 1-2mm below the surface of the liquid during aspiration or dispensation. Do not wipe the tip between aspiration and dispensation. |
|  |  | Dispense the 10.0 uL of sample into the 4.0 mL of Acid Reagent. “Rinse” the pipette tip 1-2 times in the acid reagent.  |
|  |  | Place each vial in turn on the vial holder, raise the holder until the electrodes are immersed and depress the TITRATION switch to the START position. |
|  |  | Record your results on the worksheet. |
|  |  | Run a 20 mEq/L and a 100 mEq/L standard to determine linearity. Results must be within ±5% of stated concentration. |
|  |  | Run Quantimetrix Level 1 and Level 3 controls. Results must fall within acceptable ranges in Unity Real Time. |
|  |  | Run each of the patient samples and record chloride results and collection volumes on the worksheet. For values less than 60mEq/L, results from each site must agree within 10 mEq/L. For values greater than 60mEq/L, they must agree within 15 mEq/L.  |
|  |  | Do NOT pour vial contents into drains. Dispose of contents into properly labeled Hazardous Waste Satellite Accumulation Containers. Keep containers closed. When full, label with date and place in Hazardous Waste Storage Room (STP Room L122, Mpls Lower Level.) |
|  |  | When finished with run, immerse electrodes in a vial of purified water. Turn instrument off by placing the RANGE switch to POWEROFF position. Reset thumbwheel to 0. |
|  |  | Clean acid reagent vials by rinsing a minimum of 2 times in tap water followed by 3 times in distilled water. Do not add soap. Let drain dry by placing vials upside down in rack lined with paper towel. |
|  |  | Discard etched or cloudy vials. Order new vials as necessary to maintain quality patient results. |
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| **Calculations** | If the average blank reading is greater than 100, subtract 100 from all results, after setting the thumbwheel to the blank reading minus 100. Note on the worksheet and show your calculations, having a second tech review the results and initial. |
| **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, repeat the blanks, standards and QC prior to repeating that sample and the 2nd collection sample.

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

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| 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 Borderline result [40 to 60 mEq/L], recommend repeat testing in 30 to 60 days |
| **Greater than or equal to 60 meq/L**: Consistent with the diagnosis of cystic fibrosis |

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| Critical Values: | * All results greater than or equal to 30 mEq/L must be called to the ordering physician and to Dr. John McNamara’s office, (612-813-3300).
* 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.
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| 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.
* Repeat testing should follow negative sweat chloride results if clinical symptoms of CF persist.
* Patient sweat chloride results 90 mEq/L and above are very rare; if one collection yields a result of 90 or above, repeat the blanks, standards and QC prior to repeating that sample and the 2nd collection sample.

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. A weekly Sweat Chloride Patient Report is generated in Sunquest and emailed to Dr McNamara (612-813-3300). He is the CF Center Coordinator in Minneapolis. |
| **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 = 1uL.
2. Record results from the LED readout under column for Collection 1 and Collection 2 respectively. The time standards, QC, and patients are run must be recorded on the worksheet using 24-hour clock time. For example, 3 pm would be 15:00.
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
* 2 CL = 2nd collection Chloride value
1. It is acceptable to test one sample if the other collection fails. Follow procedure below to credit the test using the SWEA comment. This will charge for collection but credit the analysis.
2. If unable to collect either sweat sample, receive the sample in CVIS, and credit the tests on the worksheet using the comment SWEA (unable to collect sweat, suggest repeat at a later date) at the 1CL and/or 2CL prompt. Answer 1VOL and 2VOL with “0” only if collection failed to produce any sweat. If collection yielded some sweat but not an adequate volume (weight), enter the actual amount collected, not “0.”
3. 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 SWEA because it will still charge for a sweat collection attempt.
4. Phlebotomists/sweat collectors should only cancel the test if collection was NOT attempted. 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.
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| **References** | 1. Digital Chloridometer Instruction Manual 1997
2. Direction Circular Digital Chloridometer Chloride Standard insert for 442- *5066,* 4/96
3. LeGrys, Vicky A. *Sweat Testing for* the *Diagnosis of Cystic Fibrosis: Practical Considerations J* Pediatr 1996; 129:892- 7.
4. Clinical and Laboratory Standards Institute C34-A3 – Sweat Testing: Sample Collection and Quantitative Chloride Analysis; Approved Guideline – Fourth Edition (ISBN 978-1-68440-035-5), 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, February 2019
5. Guidelines for Diagnosis of Cystic Fibrosis in Newborns through Older Adults, Cystic Fibrosis Foundation Consensus Report, August 2008
6. *Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation*, Journal of Pediatrics 2017; 181S:S4-15
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Deane Reidel  | May 2000 | Initial Version |
|  | Deane Reidel | November 2000 |  |
|  | Linda Lichty | May 2003 | Sweat Chloride by Titration on Labconco Chloridometer |
|  | L.Lichty | February 2004 |  |
|  | L.Lichty | July 2005 |  |
|  | L. Lichty | August 7, 2009 | Revised 20 meq/L standard, reference ranges for newborns |
|  | D. Helfinstine/L. Lichty | April 1, 2011 | New format. Renumbered from CH 6.19. Added clinical significance. Revised 20 meq/L std stability, contact phone number |
|  | L. Lichty | August 22, 2011 | Added missing procedural steps |
|  |  | Erin Bartos | May 1, 2017 | Sample must be transferred from Macroduct coils as soon as possible into SSC. Collector must clearly label samples with start and end times or collection number. Updated ranges to meet 2017 CF guidelines, updated QNS definition, changed measurement of sweat to gravimetric, and procedure for pipetting the sample. Changed who should cancel tests. Added transporting in sealed plastic transport bag. All samples must be weighed to determine acceptability, and ALL volumes must be recorded, regardless of final acceptability. Added acceptability to prepare analyzer in advance. Added verbage regarding new scale in St. Paul and Sunquest worksheet performing calculations of sweat volume. |
|  |  | E Bartos | 5/1/2017 | Changed references to sweat chloride worksheet; changes effective 5/1/17. |
|  |  | Erin Bartos | 9/14/2017 | Changed Lower AMR to 10. Changed Biannual Cal Ver to remove point below 10. Added statement to order new glassware as needed. |
|  |  | Stephen Gripentrog | 5/22/19 | Removed Sunquest QC information. Added Unity Real Time QC Information. |
|  |  | Erin Bartos | 10/2/2019 | Changed information: QC must be run before patient(s) if the instrument is prepared ahead of patient run. Time of testing for standards, QC, and patients must be recorded on the worksheet. All patient results 90 and above must have blanks, standards and QC repeated immediately after the first patient result is returned at or over 90. Added ordering a new lot of acid reagent if blanks are repeatedly over 100.  |
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