Beaumont

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Sweat Chloride Quantitation: Chloride Titration

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This procedure describes the process of analyzing sweat for chloride concentration as an aid in the diagnosis of cystic fibrosis (CF). In this condition, sweat glands secrete fluid with an abnormally high concentration of sodium and chloride. Although both can be measured, the chloride concentration correlates best with the clinical condition and is the preferred diagnostic test. Deoxyribonucleic acid (DNA) can be tested for mutations associated with cystic fibrosis, but not all possible mutations are detected by this approach. Therefore, the sweat chloride remains the definitive diagnostic test.

II. PRINCIPLE:

The theory of operation of the Chloridometer is based on established principles of coulometric titration using electrochemical generation of reagent and amperometric indication of the end-point. A constant direct current is passed between a pair of silver generator electrodes in the generator (coulometric) circuit, causing release of silver ions into the titration solution at a constant rate. The end-point is indicated after all chloride has been precipitated, by detecting the increasing concentration of free silver ions using indicator electrodes and a meter-relay in the indicator (amperometric) circuit. At a preset increment of indicator current the relay is actuated, stopping a timer which runs concurrently with generation of silver ion. Since the rate of generation of silver ion is constant, the amount of chloride precipitated is proportional to the elapsed time, after correction for the blank time.

III. SPECIMEN COLLECTION AND HANDLING:

A minimum of 0.075g of sweat must be collected for accurate results (Clinical & Laboratory Standards Institute (CLSI) recommendation). If less than 0.075g is collected, the collection should be repeated. DO NOT do a sweat test on an infant less than 48 hours old. The closed sample may be stored up to 4 hours at room temperature or 72 hours refrigerated.

IV. REAGENTS AND SUPPLIES:

A. Elution Reagent - Combine 800 mL of DI H_2O, 6.4 mL of concentrated 70% Nitric Acid and 100 mL of Glacial Acetic Acid. Be certain the water is thoroughly stirred during these additions. Dilute to 1000 mL with DI H_2O. Stable one month at room temperature.

B. Gelatin from Porcine Skin Gel strength 175g Bloom, Type A - (Sigma G2625 - 100g)

C. Thymol ≥99% - (Sigma T0501-100g)

D. Thymol Blue ACS Reagent - (Sigma 114545-5g)

E. Gelatin Reagent - Combine 1.5g Gelatin, 0.025g Thymol and 0.025g Thymol Blue, add 250 mL of DI H_2O and heat gently with continuous swirling (use the heating block with magnetic stir bar in place), until the gelatin is dissolved and the solution is clear. Aliquot cooled gelatin into test tubes, cover, and refrigerate. Stable 6 months refrigerated. DO NOT FREEZE.

F. ICT Serum Calibrator Low - (Abbott 01E4603) Obtain fresh aliquot daily and then discard after use.

G. ICT Serum Calibrator High - (Abbott 01E4603) Obtain fresh aliquot daily and then discard after use.

H. Chloride Standard, 0.100 Normal (N/10) 100 mEq/L NaCl - (Ricca 1960-16)

I. Glass Vials - (Labconco Corp. 586-0007) 100 20 x 40 mm vials

J. Simichrome Polish - (Fisher Scientific NC9748910)

K. Kimwipes

V. PRECAUTIONS:

Glacial Acetic Acid and concentrated Nitric Acid are very caustic - handle with care.

VI. EQUIPMENT:

Labconco Digital Chloridometer

VII. CALIBRATION:

Calibration is checked each day of use using Abbott ICT Serum Calibrators:

Standard Low	80 mmol/L
Standard High	120 mmol/L

If the calibration check fails, refer to the Chloridometer manual calibration section and notify a supervisor.

Calibration Verification Procedure

Calibration verification is additionally performed twice per year using standard 100 mEq/L solution.

A. Titrate five 100 mcL samples of the 100 mEq/L solution utilizing the HIGH range.

B. Submit the results to a Medical Technologist II or the Technical Director. The acceptability criteria for the 100 mEq/L standard is +/- 5 mmol/L.

NOTE: If the results are consistently low or high, the instrument readout may need to be compensated. See the LABCONCO instruction manual for the compensation procedure.

VIII. QUALITY CONTROL:

BioRad Liquid Unassayed Multiqual® Level 1 - dispense 100 mcL into 4.0 mL of Elution Reagent

BioRad Liquid Unassayed Multiqual® Level 3 - dispense 100 mcL of control material onto a gauze in a

covered glass vial. Add 8.0 mL of Elution Reagent and let sit at least 10 minutes. Run as a patient sample.

IX. PROCEDURE:

A. 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 Simichrome Polish, rinse with distilled water and buff with Kimwipes. Be certain no residue remains between the indicator electrodes at their common mounting post. Avoid getting skin oils on the electrodes.
3. After cleaning, place a vial filled with 4 mL of Elution Reagent and 4 drops of gelatin reagent in the vial holder. Set the RANGE switch to HIGH 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. Rinse electrode with DI H_2O after every specimen.
6. When not in use, leave electrodes in DI H_2O.

B. Blank Determination:

1. Set the TITRATION switch to AUTO.
2. Select the High Range.
3. Set the Blank Adjust to 00.0.
4. Fill each of two (2) vials with 4.0 mL of Elution Reagent plus four drops of gelatin reagent.
5. Place each vial in turn on the vial holder, raise the holder and the instrument determines the blank adjustment.
6. Calculate the average of the blank determinations (see step 5) and enter that number on the BLANK thumbwheel switch. (If the blank is greater than 10, re-clean the electrodes.)

C. Checking Calibration:

1. Dispense 4 mL of Elution Reagent and 4 drops of gelatin into vial. Add 100 mcL of low calibration standard into the vial. (Range switch should be on HIGH). Place the TITRATION switch on AUTO. Raise the vial into position and read result when endpoint is reached. Result should be 80 ± 4 mmol/L.
2. Perform calibration check on the high calibration standard also. Result should be 120 ± 5 mmol/L.

D. Quality Control (QC) I:

1. After the first blank determination and calibration checks, run Multiqual Level 1 by adding 100 mcL of QC material and 4 drops of gelatin reagent to 4.0 mL of acid reagent in a titration vial.
2. Record the QC result when endpoint is reached. This control should be run prior to specimen collection.

E. Specimen Collection:

1. Follow approved sweat collection procedure to collect patient specimens.

F. Gauze Blank Determination:

1. Collection gauze may have a small amount of chloride present, which is accounted for by eluting from blank gauze samples and *re-setting* the chloridometer BLANK thumbwheel.
2. Set the TITRATION switch to AUTO.
3. Select the High Range.
4. Set the Blank Adjust to 00.0.
5. To each of three (3) elution vials, add a blank gauze and 8.0 mL Elution Reagent. Elute from the blank gauze pad for at least 10 minutes with the lid in place.
6. Remove 4.0 mL of elution reagent from each blank and place in a titration vial.
7. Add 4 drops of gelatin reagent to each titration vial.
8. Place each vial in turn on the vial holder, raise the holder and the instrument determines the blank adjustment.
9. Calculate the average of the three (3) blank determinations (see step 7) and enter that number on the BLANK thumbwheel switch for all samples eluted from gauze.
10. If the blank is greater than 12, repeat the blank determination with fresh pieces of gauze. The gauze used for blank determination must be from the same package of gauze used for patient collections.

G. Quality Control II:

1. After specimen collection and gauze blank determination, run a 1:2 dilution of Multiqual Level 3 by dispensing 100 mcL of control material onto a gauze pad in a covered glass vial. Add 8.0 mL of elution reagent and let sit at least 10 minutes.
2. Mix the gauze pad around thoroughly in the solution after 5 minutes and again just before assay with a clean glass volumetric pipette to ensure complete elution and homogeneity. Remove 4.0 mL of elution reagent and place in a titration vial.
3. Add 4 drops of gelatin reagent. Record result of eluate when endpoint is reached.

H. Assay of Sweat Chloride in Patient Samples:

1. Withdraw 4.0 mL of the well-mixed elution mixture from the covered patient sample vial and place into a titration vial.
2. Add 4 drops of gelatin reagent. Record result of eluate when endpoint is reached.

Sample Preparation Summary:

Blank	4 mL Elution Reagent + 4 drops Gelatin
Standard	4 mL Elution Reagent + 0.1 mL Standard + 4 drops Gelatin
Control 1	4 mL Elution Reagent + 0.1 mL Control + 4 drops Gelatin
Gauze Blank	4 mL Eluate + 4 drops Gelatin
Control 2	4 mL Eluate + 4 drops Gelatin
Patients	4 mL Eluate + 4 drops Gelatin

X. CALCULATIONS:

Calculate the sweat chloride concentration using the following formula and record:

$$\frac{\text{Titrator result (mmol/L)} \times 0.2}{\text{g of sweat collected}} = \text{Sweat Chloride in mmol/L}$$

XI. REFERENCE RANGE:

Interpretation	Result (mmol/L)	Comment
Normal	<30	CF unlikely
Intermediate	30 - 59	Intermediate range – need further study to establish or rule out a CF diagnosis.
Possible CF	≥60	Indicative of CF for individuals presenting with a positive newborn screen, clinical features consistent with CF, or a positive family history.

Values greater than or equal to 60 mmol/L are consistent with Cystic Fibrosis in an individual with clinical symptoms of the disease. Elevated values in an adult may not indicate presence of Cystic Fibrosis, but have been linked in carriers of the CF gene.

XII. CLINICALLY REPORTABLE RANGE:

1 – 160 mEq/L.
Do not report >160 mEq/L. If result is >160, contamination is suspected. Consult a Supervisor or Technical Director.

XIII. ANALYTICAL MEASURING RANGE:

The linearity of the chloridometer is 1 - 160 mEql/L.

XIV. LIMITATIONS AND INTERFERING SUBSTANCES:

Erroneously increased results can be caused by the presence of bromide, iodide, sulfa groups, and high protein levels in the sample.

XV. REPORTING:

Results are manually entered into the LIS. Results must undergo two clerical checks according to department procedure to verify accuracy of calculations and manual result entry.

XVI. REFERENCES:

A. Gibson, L.E. and Cooke, R.E. Pediat. 1959, 23:545-9.

B. Gibson, L.E., diSant'Agnese, P.A., Shwachman, H. Procedure for the quantitative iontophoretic sweat test for cystic fibrosis. Cystic Fibrosis Foundation, 1985.

C. CLSI document C34-A3--Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Third Edition, CLSI Vol. 29, No. 27, October 2009.

Attachments

Sweat Chloride Assay Record

Approval Signatures

Step Description	Approver	Date
	Peter Millward: Chief, Clinical Pathology	6/10/2020
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	6/10/2020
Policy and Forms Steering Committee Approval (if needed)	Jillian Trueman: Medical Technologist Lead	6/5/2020
Policy and Forms Steering Committee Approval (if needed)	Jillian Trueman: Medical Technologist Lead	6/5/2020
	Timothy Kennedy: Pathologist	6/5/2020
	Steven Truscott: Clinical Chemist	6/4/2020
	Leah Fontana: Mgr Laboratory	6/4/2020
	Jillian Trueman: Medical Technologist Lead	6/4/2020

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