Beaumont

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Sweat Chloride Collection: Gibson Cooke Method

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This procedure describes the process of collecting sweat for analysis of 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 can be linked to disease phenotype. Therefore, the sweat chloride remains the definitive diagnostic test.

The sweat chloride test measures chloride ions in sweat by coulometric titration. In order to stimulate production of sweat, pilocarpine is delivered into the skin on the arm or leg by means of iontophoresis (small electrical current). After 5 minutes of iontophoresis, sweat is collected for thirty minutes onto a clean, dry, pre-weighed gauze pad, then analyzed for chloride ions.

II. SPECIMEN COLLECTION AND HANDLING:

A minimum of 0.075g of sweat must be collected for accurate results (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.

III. REAGENTS AND SUPPLIES:

A. Stock K_2SO_4 (0.2 M) - Weigh out 3.49 g of K_2SO_4 dissolve in DI H_2O; dilute to 100 mL with DI H_2O. Stable for 2 years when stored at 4 – 8^oC. Label as stock with the following precaution on the label. **Caution: Do Not Use Undiluted!**

B. Working K_2SO_4: (0.02 M) - Dilute 10mL stock K_2SO_4 with 90 mL with DI H_2O, for a total volume of 100 mL of solution. Stable for 1 year when stored at 4 – 8^oC.

C. Stock Pilocarpine Nitrate (4.0%) - Weigh out 4.00 g of Pilocarpine Nitrate (U.S.P. grade or equivalent), dissolve in DI H_2O dilute to 100 mL. Store in a brown glass bottle at 4 – 8 ^oC. Stable for 2 years. Label as stock with the following precaution on the label. **Caution: Do Not Use Undiluted!**

D. Working Pilocarpine Nitrate (0.4%) – Dilute 10 mL of stock 4.0% Pilocarpine Nitrate with 90 mL with DI

H_2O, for a total volume of 100 mL of solution. Stable for 1 year when stored at 4 – 8^{o}C.

E. Kendall Lisco Sponges 2 in x 2 in (ref. # 3041) – Sodium-free gauze pads
F. Avant Deluxe Gauze Sponges 2 in x 2 in (ref. # NON26223)
G. 70% Isopropanol
H. Glass Vials
I. Disposable Pipettes
J. Volumetric Pipettes
K. Rubber Pipette Bulb
L. DI H_2O
M. Emesis Pan
N. Timer
O. Plastic Forceps
P. Paper Towels
Q. Chloride-free Bar Soap (e.g. Dial or Ivory) and Soap Dish
R. Parafilm
S. Cellophane Tape
T. Simichrome Polish

IV. PRECAUTIONS:

DO NOT use the pilocarpine nitrate or potassium sulfate undiluted.

DO NOT do a sweat test on a patient in an oxygen tent. Check with a nurse to see if the patient can be removed from the tent.

V. EQUIPMENT:

Gibson-Cooke Sweat Test Apparatus (C & S Electronics, Inc.) – The battery must be tested each day of use. The battery is replaced twice per year, as indicated on the maintenance log. The instrument must be checked annually for current leakage and current control. Disinfection procedure must be reviewed biennially.

VI. PROCEDURE:

A. Preparation of Equipment:
    1. Weigh a clean sodium-free gauze pad in a labeled weighing vial and record both the weight and the label ID on the sweat testing form as the pre-collection weight. Weigh a separate pad and vial for each patient, plus an extra one to be used in the event of breakage or a need to repeat the test.
    2. Check to be sure that all the following items are available on the sweat testing cart before proceeding to the patient:
        a. Pre-weighed vials with sodium-free gauze collection pads
        b. Iontophoresis unit with clean and disinfected electrodes and straps

c. 0.4% Pilocarpine Nitrate solution
d. 0.02 M Potassium Sulfate (K_2SO_4) solution
e. Package of sodium-free gauze pads (Kendall Lisco Sponges)
f. Package of gauze pads (Avant Deluxe Gauze Sponges)
g. Disposable pipets and rubber bulb
h. Squeeze bottle of DI H_2O
i. Emesis pan
j. Timer
k. Plastic forceps
l. Paper towels
m. Dial soap and soap dish
n. Parafilm
o. Cellophane tape
p. 70% Isopropanol
q. Simichrome Polish

3. For an inpatient, call the floor to see if the patient is in the room

B. Preparation of the Patient:

Confirm that the patient or parent received the Information Relative to Sweat Chloride Testing form (see attachment). If not, provide them with one to read before proceeding.

NOTE: Sweat chloride collection may be performed on patients in isolation. Beaumont infection control policies provide instructions for minimizing the transmission of infection and decontamination of patient care equipment.

1. Wash the area of skin to be used with soap and DI H_2O, wipe twice with DI H_2O gauze pads, and rinse with DI H_2O. Use either the flexor surface of the forearm in an older child or adult, or the back of the thigh in an infant. Select areas which are free from inflammation, rash, moles, etc.
   NOTE: Do not use the small electrodes. If necessary, it is permissible to bend the large electrodes to match the curve of the limb.
2. Be certain to position both electrodes on the same limb so they DO NOT allow current to pass from one side to the patient's trunk to the other or serious risk of shock to the heart could occur.
3. Place 3 gauze pads over the area and saturate with 0.4% pilocarpine nitrate (should be wet to the touch).
4. Adjust the electrode shape to fit the curvature of the area to be tested. To ensure the surface of the electrodes are free of oxidation, clean the face of the electrodes with an abrasive pad until they are shiny copper. Wipe the electrode clean with sodium-free gauze moistened with DI H_2O and adjust the holding strap for a snug fit. BE SURE THE ELECTRODE DOES NOT TOUCH BARE SKIN AT THE EDGES.
5. Place 3 gauze pads over a nearby area on the same limb and saturate with 0.02 M K_2SO_4.

6. Fit the second electrode similarly and adjust its holding strap for a snug fit, again being careful to avoid contact with the skin.

C. Iontophoresis:

1. Be sure the iontophoresis unit is turned off and the current adjust knob is set at its minimum position before connecting the electrodes.
2. Attach the positive (red) wire to the pilocarpine electrode and the negative (black) wire to the K_2SO_4 electrode.
   NOTE: Be sure these are not reversed since pilocarpine will not be delivered from a negative electrode.
3. Set the current limit to the lowest possible current, turn on the power. Slowly bring the current up to 3 mA over a period of 5 - 10 seconds. Never exceed 4 mA because burns can occur.
4. Iontophorese for 5 minutes ± 15 seconds.
5. If the patient complains of discomfort, pressing down slightly on the electrode may provide relief. If this does not help, stop the iontophoresis by decreasing the current slowly to zero, and check to be sure that the electrode is not in contact with the skin. If needed, add more of the appropriate solution to the gauze of the electrode involved and restart. If the patient continues to complain of excessive discomfort, discontinue the procedure.
6. **Observe the electrodes during the entire iontophoresis period** to be sure that they have not moved and made contact with the skin.
7. At the end of the iontophoresis period, gradually decrease the current level to zero BEFORE turning off the iontophoresis unit.
8. Disconnect the electrode wires and remove the electrodes and gauze pads being careful not to touch the area to be measured with your fingers.
9. Wipe the pilocarpine treated area first with sodium-free gauze moistened with DI H_2O and then dry the area with dry sodium-free gauze. Keep covered until ready for collection.
10. If either electrode area shows evidence of excessive redness, hives or a burn, refer the patient to the emergency center for evaluation by a physician and notify a supervisor.
11. Clean electrodes and straps with 70% Isopropanol after patient use. See Disinfection Procedures below. Simichrome Polish may be used to clean the electrodes. Rinse with copious amounts of water after using the polish.
    NOTE: Disinfectant wipes may contain chloride. Deviation from Infection Control policy is necessary when cleaning the electrodes to avoid chloride contamination that may affect the patient results.

D. Sweat Collection:

1. Remove the preweighed sodium-free gauze pad from the vial with plastic forceps and place on the iontophoresed area of skin. The area of iontophoresis (2 x 2 in.) is equivalent to the area of collection (2 x 2 in.).
2. Wrap the limb tightly with Parafilm taking care to ensure that the Parafilm seals the gauze airtight on all sides. Secure the Parafilm with a piece of paper tape along all edges.
3. Allow the sweat to collect for 30 minutes. The sweat collection time may not exceed 30 minutes.
4. After the 30-minute collection period is over, place the weighing vial near the limb with the top removed and unwrap the Parafilm. Quickly transfer the gauze with forceps to the weighing vial and

replace the top so as to minimize evaporation.

5. Reweigh the vial with the gauze as soon as possible, record as the post-collection weight and determine the weight of sweat collected by difference. For reliable assay, a minimum of 0.075 g should be collected. Samples less than the required weight must be rejected and not pooled to achieve the weight requirement.

E. Disinfection Procedures: Equipment and facilities used for sweat collection are to be disinfected after each collection.

1. Equipment: Clean electrodes and straps of the Gibson-Cooke Sweat Test Apparatus and other equipment that comes in contact with the patient or the bed thoroughly with 70% Isopropanol. Document cleaning on maintenance checklist.
2. Facility: Cover patient contact areas with disposable blue pads. After each patient, dispose of pads and clean contact surfaces with disinfectant wipes (containing dimethyl ethylbenzyl ammonium chloride). Linens on the patient bed should be changed as needed. Remove trash each day of patient testing (weekly), and call housekeeping to clean the floor (weekly).

F. Assay of Sweat Chloride:

1. After the sweat is weighed, add 8.0 mL nitric-acetic acid reagent (see Sweat Chloride Quantitation procedure) to the vial and elute the sweat from the gauze pad for at least 10 minutes with the lid in place. The closed sample may stored up to 4 hours at room temperature or 72 hours refrigerated (Elution Mixture).
2. See Sweat Chloride Quantitation Procedure to continue.

VII. LIMITATIONS AND INTERFERING SUBSTANCES:

A. Patient must be at least 48 hours old.

B. Patient must be adequately hydrated for proper sweat collection.

C. DO NOT perform sweat collection in an Oxygen tent. The test may be performed on a patient with a nasal cannula who is not in a tent.

D. DO NOT perform a sweat collection unless the patient or parent has first read the “Information Relative to Sweat Chloride Testing” form (see attachment).

VIII. REPORTING:

See Sweat Chloride Quantitation Procedure.

IX. 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-A2--Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guideline—Third Edition, CLSI Vol. 29, No. 27, October 2009.

Attachments

Information Relative to Sweat Chloride Testing

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

Applicability

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