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Minimum Guidelines for the Follow-Up of Newborns with Positive Cystic Fibrosis Newborn Screening Results

In California, newborn screening for cystic fibrosis involves testing for mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The blood spot specimens of all hypertrypsinogenemic infants are tested using a panel of known deleterious mutations. Those with one mutation are further tested by focused DNA sequencing, an open-ended method that can detect previously known as well as novel mutations/variants. Infants with two mutations/variants are defined by the California Newborn Screening Program to have a positive cystic fibrosis newborn screening test result, and are referred for diagnostic testing and evaluation at cystic fibrosis specialty care centers. Not all individuals with two mutations/variants will have a sweat chloride value ≥ 60 mEq/L, the gold standard diagnostic criterion for cystic fibrosis. There are several reasons why this can happen. These include: (i) the infant is a carrier (heterozygote) because the two mutations/variants identified are on the same chromosome (*cis*), (ii) the infant is functionally a carrier because the CFTR variant in one allele is a non-pathogenic polymorphism that does not alter CFTR function (iii) the infant has a CFTR-related disorder because one or both of the two mutations/variants enable partial CFTR function, (iv) the sweat chloride was falsely low because of the infant's physiological status (e.g., edema, hyponatremia, elevated serum aldosterone level) or because of technical problems at the time of the test. The minimum guidelines set forth below are provided to enable clinicians the ability to differentiate between these reasons and to determine the correct clinical follow up for the infant and family.

1. Preparation for Sweat Chloride Test

- a. The Cystic Fibrosis (CF) Center is responsible for advising parents of the following necessary preparations prior to the scheduled test date.
- b. On the two days before and on the day of the sweat test, infants should be given 1/8 teaspoon salt supplement per day.¹
- c. On the day of the test, infants should be well fed and well hydrated (i.e., infants should be fed three or less hours before testing).

2. Sweat Chloride Test

- a. A sweat test should be scheduled for and conducted at the first available appointment after the infant has been referred to the CF Center. All sweat tests must be conducted at Cystic Fibrosis Foundation (CFF) Accredited Sweat Test Laboratories.
- b. Laboratories should follow the CFF *Sweat Testing Standards for CFF Accredited Care Centers*² and the CFF Diagnostic Sweat Testing Guidelines³.

- i. To increase the likelihood of collecting a sufficient sample size, the infant should be ≥ 37 weeks gestation and ≥ 2 kg body weight before a sweat test is conducted.
 - ii. Sweat collection should be conducted bilaterally from the lower arms or upper legs.
 - iii. Laboratories should not use hybridized methods for sweat stimulation and collection:
 - 1. “If gauze or filter paper collection is used the stimulated area **MUST** be 2 x 2 inches (total area 4 square inches). A slightly smaller electrode (e.g. 1 ½ x 1 ½ inches) is used for iontophoresis. Other electrode sizes are permissible if they cover greater than 50% of the 2 x 2 inch area (i.e. an area of greater than 2 square inches). The iontophoresis should be carried out using USP grade pilocarpine for 5 minutes. After stimulation the sample **MUST** be collected from a single site using 2 x 2 inch gauze or filter paper. The minimal sample weight using this method is 75 mg in 30 minutes.”³
 - 2. “If Macroduct® coil is used for collection then sweat must be stimulated with a disposable Pilogel® electrode using the Webster Sweat Inducer (Wescor, Logan, Utah) for 5 minutes. After a 30-minute collection, the minimum acceptable sample is 15 µL.”³
 - 3. Other methods may be used in the future if they are certified by the CFF.
 - c. Hospital Laboratory Directors should share all sweat test results with the associated CF Center Director, as well as with the child’s primary care provider.
 - d. Appropriately trained staff at CF Centers (generally medical doctors, nurses and/or genetic counselors) should have a face-to-face visit with the child and parents shortly after the sweat test to discuss the sweat test results.
 - e. A repeat sweat test will almost always be needed regardless of the value of the first test. Rarely can a diagnosis be confirmed without further testing (described below).
3. Interpretation of Sweat Chloride Test Results
- a. Interpretation of initial sweat chloride test results in infants **<6 months of age** with two mutations/variants (see Table 1). How to use Table 1: Locate the infant’s initial sweat chloride test value in the left hand column. Based on the presence of (i) a family history of CF, (ii) CF-like signs or symptoms, and (iii) family genetic testing results, choose the appropriate row to determine the timing of the infant’s next sweat test and the suggested Case Status. If at any time the infant develops CF-like signs or symptoms, please move to the appropriate row to determine the appropriate timing of the infant’s next sweat test and the suggested Case Status.

Table 1: Initial Sweat Chloride Test Interpretation Grid for Infants <6 Months of Age

Row	Sweat Cl Result	Family hx of CF	CF-like signs or symptms ^a	Family Studies find <u>All</u> Mutations/ Variants are	Timing of Repeat Sweat Test (following first valid test)	Suggested Case Status
1.a.	≤ 29 mEq/L	No	No	<i>in cis</i>	N/A	'No Disorder/ Carrier'
1.b.	≤ 29 mEq/L	No	No	<i>in trans</i>	6 months	'Pending' follow-up testing
1.c.	≤ 29 mEq/L	Yes	No	N/A	3 months	If repeat sweat test ≤29 mEq/L, 'No Disorder/Carrier' ^b <i>If repeat sweat test ≥ 30 mEq/L, move to row 2a, 2b, or 3 to determine suggested Case Status.</i>
1.d.	≤ 29 mEq/L	No	Yes	N/A	1 month	If repeat sweat test ≤29 mEq/L, 'Cystic Fibrosis' ^c or 'Suspect Cystic Fibrosis' ^d <i>If repeat sweat test ≥ 30 mEq/L, move to row 2a, 2b, or 3 to determine suggested Case Status.</i>
1.e.	≤ 29 mEq/L	Yes	Yes	N/A	1 month	If repeat sweat test ≤29 mEq/L, 'Cystic Fibrosis' ^c or 'Suspect Cystic Fibrosis' ^d <i>If repeat sweat test ≥ 30 mEq/L, move to row 2a, 2b, or 3 to determine suggested Case Status.</i>
2.a.	30-39 mEq/L	N/A	No	N/A	1 month	If repeat sweat test is in 30-39 mEq/L range, 'Suspect Cystic Fibrosis' ^d <i>If repeat sweat test is not in 30-39 mEq/L range, move to the appropriate row to determine the suggested Case Status.</i>
2.b.	30-39 mEq/L	N/A	Yes	N/A	1 month	If repeat sweat test is in 30-39 mEq/L range, 'Cystic Fibrosis' ^c or 'Suspect Cystic Fibrosis' ^d <i>If repeat sweat test is not in 30-39 mEq/L range, move to the appropriate row to determine the suggested Case Status.</i>
3.	≥ 40 mEq/L	N/A	N/A	N/A	N/A	'Cystic Fibrosis' ^c

N/A = Not Applicable

^a CF-like signs/symptoms include but are not limited to: Chronic sinopulmonary disease manifested by persistent colonization/infection with typical CF pathogens, chronic cough and sputum production, persistent chest radiograph abnormalities, airway obstruction manifested by wheezing and air trapping, nasal polyps; Gastrointestinal and nutritional abnormalities including meconium ileus, distal intestinal obstruction, rectal prolapse, pancreatic insufficiency, failure to thrive, hypoproteinemia and edema; Salt loss syndromes including acute salt depletion.⁴

^b In the future, some combinations of mutations and variants may result in other disorders such as congenital bilateral absence of the vas deferens (CBAVD), idiopathic pancreatitis, bronchiectasis, allergic bronchopulmonary aspergillosis, and chronic rhinosinusitis.

^c Cystic Fibrosis: CF Center should follow-up with patient as outlined in the *Clinical Practice Guidelines for Cystic Fibrosis*, Cystic Fibrosis Foundation.

^d Suspect Cystic Fibrosis: CF Center should follow-up with patient every 3-12 months (depending on symptoms) for as long as the CF Center deems appropriate.

- b. Interpretation of **repeat** sweat chloride test results in infants ≥ 6 months of age with two mutations/variants (see Table 2). How to use Table 2: Locate the infant's **repeat** sweat chloride test value in the left hand column. Based on the presence of (i) a family history of CF and (ii) consistently occurring CF-like signs or symptoms, choose the appropriate row to determine the suggested Case Status.

Table 2: Repeat Sweat Chloride Test Interpretation Grid for Infants ≥ 6 Months of Age

Row	Sweat Cl Result	Family history of CF	Consistently occurring CF-like signs or symptoms ^a	Suggested Case Status
1.a.	≤ 39 mEq/L	No	No	'No Disorder/ Carrier' ^b
1.b.	≤ 39 mEq/L	Yes	No	'No Disorder/Carrier' ^b
2.	≤ 59 mEq/L	Yes or No	Yes	'Cystic Fibrosis' ^c or 'Suspect Cystic Fibrosis' ^d
3.	40-59 mEq/L	Yes or No	No	'Suspect Cystic Fibrosis' ^d
4.	≥ 60 mEq/L	N/A	N/A	'Cystic Fibrosis' ^c

N/A = Not Applicable

^a CF-like signs/symptoms include: Chronic sinopulmonary disease manifested by persistent colonization/infection with typical CF pathogens, chronic cough and sputum production, persistent chest radiograph abnormalities, airway obstruction manifested by wheezing and air trapping, nasal polyps; Gastrointestinal and nutritional abnormalities including meconium ileus, distal intestinal obstruction, rectal prolapse, pancreatic insufficiency, failure to thrive, hypoproteinemia and edema; Salt loss syndromes including acute salt depletion.⁴

^b In the future, some combinations of mutations and variants may result in other disorders such as congenital bilateral absence of the vas deferens (CBAVD).

^c Cystic Fibrosis: CF Center should follow-up with patient as outlined in the *Clinical Practice Guidelines for Cystic Fibrosis*, Cystic Fibrosis Foundation.

^d Suspect Cystic Fibrosis: CF Center should follow-up with patient every 3-12 months (depending on symptoms) for as long as the CF Center deems appropriate.

4. Additional early patient evaluation

- a. The CF Center should order the following tests as early as possible:

- i. Fecal elastase
- ii. Serum electrolytes
- iii. Serum albumin
- iv. Blood urea nitrogen
- v. CF Throat cultures

- b. All infants with a pending diagnosis should be seen monthly, by the CF Center or the infant's pediatrician through the first year of life or until a diagnosis is made. After infancy, the CF Center should see the patient with a pending diagnosis 3-4 times per year through age five years or until a diagnosis is made. This may be necessary in persons in which one or more of the CFTR mutations or variants found are not clearly disease-causing because it may take an extended time in such persons to exhibit CF-like signs or symptoms.

- c. Many patients with novel or rare mutations who do not exhibit symptoms or elevated sweat chloride may not receive a definitive diagnosis in the first year of life. These patients should be seen every 3-4 months through age 5 or until a diagnosis is made.

5. Family Studies

- a. The CF Center should conduct the following tests, preferably between the time of the first and second sweat tests of the infant:
 - i. Genetic studies of birth parents when a patient (<6 months of age) has a normal sweat test (≤ 29 mEq/L) and two CFTR mutations/variants.
 1. This will determine whether identified mutations are from one or both parents.
 - a. If mutations are from one parent = on the same chromosome of the newborn (*cis*)= carrier
 - b. If mutations are from both parents = on different chromosomes of the newborn (*trans*)= possible case
 - ii. When an infant's mutations are not part of the ACMG recommended CF carrier mutation panel,⁵ it is critical that genetic studies be conducted at a laboratory capable of detecting the infant's mutations.
 - iii. Determine if either parent has another CFTR mutation to assist with genetic counseling.
 - iv. All siblings of screen positive newborns should be sweat tested and genotyped by the CF Center to provide prognostic information that may be important to making decisions about the infant's care.
6. These guidelines are not meant to delay or limit treatment prior to diagnosis, if treatment is indicated.

References

1. Rowe SM, Accurso F, Clancy JP. Detection of cystic fibrosis transmembrane conductance regulator activity in early-phase clinical trials. *Proc Am Thorac Soc* 2007;4(4):387-98.
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4. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. *J Pediatr* 1998;132(4):589-95.
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