Cystic Fibrosis (CF) is an autosomal recessive disorder characterized by chronic obstructive pulmonary disease, pancreatic insufficiency, and elevated sweat electrolytes.

The quantitative analysis of chloride in sweat, performed in the clinical laboratory, is considered the diagnostic standard for CF.

Cystic Fibrosis is a chronic, genetic disease affecting over 20,000 Americans. Approximately 1 out of 3000 Caucasian has CF and 1 out of 25 carry the mutation. Most patients have a severe, classical form of CF characterized by chronic obstructive pulmonary disease, pancreatic insufficiency, and elevated sweat electrolytes. The laboratory plays a critical role in diagnosing CF phenotypically and genotypically. This article will describe the phenotypic features of CF, the genetic defect involved, the pathophysiology of the disorder, and the criteria for diagnosis. The diagnostic standard, the sweat test, will be discussed to include stimulation, collection, analysis, quality assurance, potential sources of errors, and educational resources for technologists, physicians, and patients.

Phenotypic Features
Most patients with CF have mucous plugging and thickened secretions obstructing small airways. A cycle of inflammation and bacterial infection leads to lung tissue destruction, diminished pulmonary function, and decreased life expectancy. A list of phenotypic features of CF is found in Table 1. The median predicted survival age of CF patients in 1999 was 29 years. The pulmonary damage begins in infancy and is managed with chest physiotherapy, antibiotics, anti-inflammatory agents such as steroids, and medication to decrease sputum viscosity. Approximately 85% of CF patients are pancreatic insufficient because of obstructed pancreatic ducts preventing the secretion of digestive enzymes into the intestines. Patients are unable to absorb fat and protein from their diet and often present with failure to thrive. Malabsorption of fat leads to steatorrhea and foul smelling stools. Pancreatic insufficiency can be treated with oral pancreatic enzyme and vitamin supplements. Patients with CF have increased concentrations of sodium and chloride in their sweat, but do not have increased sweat volume.

Genetic Defect and Pathophysiology
CF, an autosomal recessive disease, is due to mutations in the CF Transmembrane Conductance Regulator (CFTR) gene. This gene codes for the CFTR protein that functions as a chloride channel in epithelial cells. There are more than 900 mutations of the CFTR gene. Approximately 52% of CF patients are homozygous for the most common CFTR mutation, ∆F508, and 36% are heterozygous ∆F508/other CF mutation. Patients homozygous for ∆F508 have the classical, severe form of CF and are pancreatic insufficient. In the lungs, the effect of the CFTR mutation is increased sodium reabsorption and an inability to secrete chloride leading to a desiccation of airway surface liquid and thickened mucus secretions. In CF sweat glands, the defect results in the inability to reabsorb chloride and sodium as sweat moves along the sweat ducts to the skin surface. Certain mutations of CFTR are associated with pancreatic sufficiency, borderline sweat chloride concentration, and overall milder disease.

Criteria for Diagnosis
A diagnosis of CF is based on the presence of 1 or more phenotypic features, or CF in a sibling, or a positive result on CF newborn screening test, along with laboratory evidence of CFTR mutation. The laboratory evidence is provided by increased sweat chloride concentration, or by identification of 2 CF mutations, or abnormal electrophysiological studies of nasal epithelium. Half of all CF patients are diagnosed before 6 months of age and 90% are diagnosed by age 8. Accurate
and timely diagnosis is necessary to provide appropriate treatment and avoid unnecessary testing. Indications for sweat testing are found in T2.

**Sweat Testing**

The sweat test is comprised of 3 components: stimulation, collection, and analysis. Each phase must be carefully performed and monitored to ensure valid test results. Sweat is produced by iontophoresis of the cholinergic drug, pilocarpine nitrate, into an area of the skin on the arm or leg. Iontophoresis uses a small electric current to deliver pilocarpine into the sweat glands. After iontophoresis, sweat is collected onto preweighed gauze pads or filter paper or into Macroduct coils, using techniques to minimize vaporization and contamination. For a detailed procedure for stimulation and collection, refer to NCCLS document C34-A2.5

Sweat tests can be categorized into screening and diagnostic (confirmatory) assays. Patients with positive or borderline screening tests should be followed by a diagnostic sweat test. Examples of qualitative screening sweat tests currently in use are the Wescor Sweat-Chek conductivity analyzer (Wescor M, Logan, UT), the Advanced Instruments conductivity analyzer (Advanced Instruments M, Norwood, MA), the Orion skin electrode for chloride (Orion Research M, Cambridge, MA), the CF Indicator System chloride patch (Poly-Chrome Medical Inc M, Brooklyn, MN), and sweat osmolality measurements. Some of these methods have been associated with false results. For example, the Orion Skin Measuring System and older conductivity analyzers using unheated collection cups are not recommended as diagnostic tests because problems have been reported with sample evaporation, condensation, and the inability to quantify sweat sample volume.6,7 The Cystic Fibrosis Foundation approved the Wescor Macroduct Sweat-Chek for CF screening with the criteria that a patient having a sweat conductivity ≥50 mmmol/L should be referred to an accredited CF care center for a quantitative sweat chloride test.8 When evaluating sweat conductivity results, it should be noted that values from sweat conductivity methods are approximately 15 mmol/L higher than sweat chloride concentration.9 Laboratories performing screening tests need to inform physicians what is being measured in the analysis, that the test offered is for screening, and provide the appropriate reference intervals.

A diagnostic (confirmatory) sweat test includes the collection of sweat onto gauze, filter paper, or Macroduct coils, and the quantitative analysis of chloride concentration.4,5 Chloride concentration can be determined by either coulometric titration using a chloridometer or manual titration using mercuric nitrate. If a laboratory chooses to quantify sweat chloride using an automated analyzer that employs an ISE, these methods must be systematically validated for accuracy, precision, and, particularly, lower limit of detection. In the context of clinically significant findings, a sweat chloride >60 mmol/L is consistent with CF; concentrations between 40 and 60 mmol/L are considered borderline, and values <40 mmol/L are generally considered normal.5

**Phenotypic Features Consistent with a Diagnosis of CF**

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<tr>
<td>1. Chronic sinopulmonary disease manifested by:</td>
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<td>a. Persistent colonization/infection with typical CF pathogens including Staphylococcus aureus, nontypeable Haemophilus influenzae, mucoid and nonmucoid Pseudomonas aeruginosa, and Burkholderia cepacia.</td>
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<td>b. Chronic cough and sputum production.</td>
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<td>c. Persistent chest radiograph abnormalities (eg, bronchiectasis, atelectasis, infiltrates, hyperinflation).</td>
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<td>d. Airway obstruction manifested by wheezing and air trapping.</td>
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<td>e. Nasal polyps; radiographic or computed tomographic abnormalities of the paranasal sinuses.</td>
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<td>f. Digital clubbing.</td>
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<td>2. Gastrointestinal and nutritional abnormalities including:</td>
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<td>a. Intestinal: meconium ileus, distal intestinal obstruction syndrome, rectal prolapse.</td>
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<td>b. Pancreatic: pancreatic insufficiency, recurrent pancreatitis.</td>
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<td>c. Hepatic: chronic hepatic disease manifested by clinical or histologic evidence of focal biliary cirrhosis or multilobular cirrhosis.</td>
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<td>d. Nutritional: failure to thrive (protein-calorie malnutrition), hypoproteinemia and edema, complications secondary to fat-soluble vitamin deficiency.</td>
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<td>3. Salt loss syndromes: acute salt depletion, chronic metabolic alkalosis.</td>
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<td>4. Male urogenital abnormalities resulting in obstructive azoospermia (CBAVD).</td>
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**Evaluating the Equivocal Patient**

Fortunately, most CF patients have sweat chloride results and clinical features clearly consistent with the diagnosis. However, the small number of equivocal patients present diagnostic dilemmas for clinicians. These atypical patients present with chronic sinopulmonary disease, pancreatic sufficiency, and borderline or normal sweat chloride.4 Of the more than 20000 patients diagnosed with CF, 0.4% had sweat chlorides of less than 40 mmol/L, 0.5% had sweat chlorides between 40-50 mmol/L, and 1.2% had sweat chloride concentrations
between 51%-60%. Overall, 2.0% of CF patients were diagnosed with sweat chloride concentrations of less than or equal to 60 mmol/L. Most of these equivocal patients had CF confirmed based on DNA analysis and clinical presentation. In addition to mutational analysis, nasal potential difference studies that measure the active transport of ions across nasal epithelial may be useful; however, the technique is rigorous and only performed at a small number of CF centers nationwide. Laboratory tests for pancreatic function such as stool elastase or fecal fat can be helpful in assessing exocrine pancreatic function. Microbiological cultures of sputum or bronchoalveolar lavage fluid for the presence of persistent mucoid *Pseudomonas aeruginosa* is associated with CF and might be supportive evidence in difficult to diagnose cases.

### Quality Assurance

Laboratories should perform and evaluate controls with every sweat analysis using two levels of controls in accordance with CLIA ’88. Sweat testing should be performed by well-trained personnel who pass periodic documented competency testing which includes direct observation of sweat collection. The College of American Pathologists (CAP) offers external proficiency testing (PT) for sweat analysis consisting of 3 unknown samples distributed twice a year. The goal of the sweat analysis proficiency testing program is to provide feedback to institutions on their performance of sweat analysis, and provide educational materials on the total testing process in an effort to improve the quality of sweat testing. Changes in practice have occurred in many institutions performing sweat tests, and a greater awareness of analyte identification has resulted because of the PT program.

### Sources of Errors in Sweat Testing

Mistakes in sweat testing can be significant, resulting in delay in diagnosis and inappropriate treatment. Sources of errors include use of inappropriate methodology, technical mistakes, and misinterpretation of results. Unreliable methods allow for evaporation and contamination of sweat sample. Technical errors include analyzing an inadequate sample, combining inadequate samples for analysis, evaporation, and contamination of the sweat sample, inappropriate instrument calibration, and lack of analytical sensitivity. Errors can occur in result reporting by misrepresenting the analysis and providing inappropriate reference intervals. Accurate sweat testing requires knowledgeable clinical interpretation. A physician ordering a sweat test should be aware of the laboratory’s methodology and should interpret the results in the context of the patient’s clinical presentation, family history, and age. The physician should repeat sweat testing or perform genotyping on patients with: initial borderline or positive results, negative results that are inconsistent with the clinical picture, or positive results in patients that do not follow the expected clinical course.

### Sources of Information about Sweat Testing

Technologists and physicians can obtain specific information about sweat testing procedures and quality assurance from the NCCLS sweat testing document. The CF Foundation has produced videotape for lab-
In addition, the Laboratory Accreditation program of the CAP has developed a checklist for sweat collection and analysis in the Special Chemistry section that can be accessed on the CAP website: www.cap.org.

Laboratories interested in providing informational materials for patients and parents should contact the CF Foundation at 1-800-FIGHT CF. The Foundation has developed print and videotape patient educational materials in English and Spanish.


