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Breathing Institute

# Improvements to Confirmatory Testing

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NewSteps 360 CF NBS QI & Timeliness Meeting
June 21, 2016

Affiliated with
University of Colorado
Anschutz Medical Campus

# Disclosures

None

# Timeliness of CF Diagnosis is Important

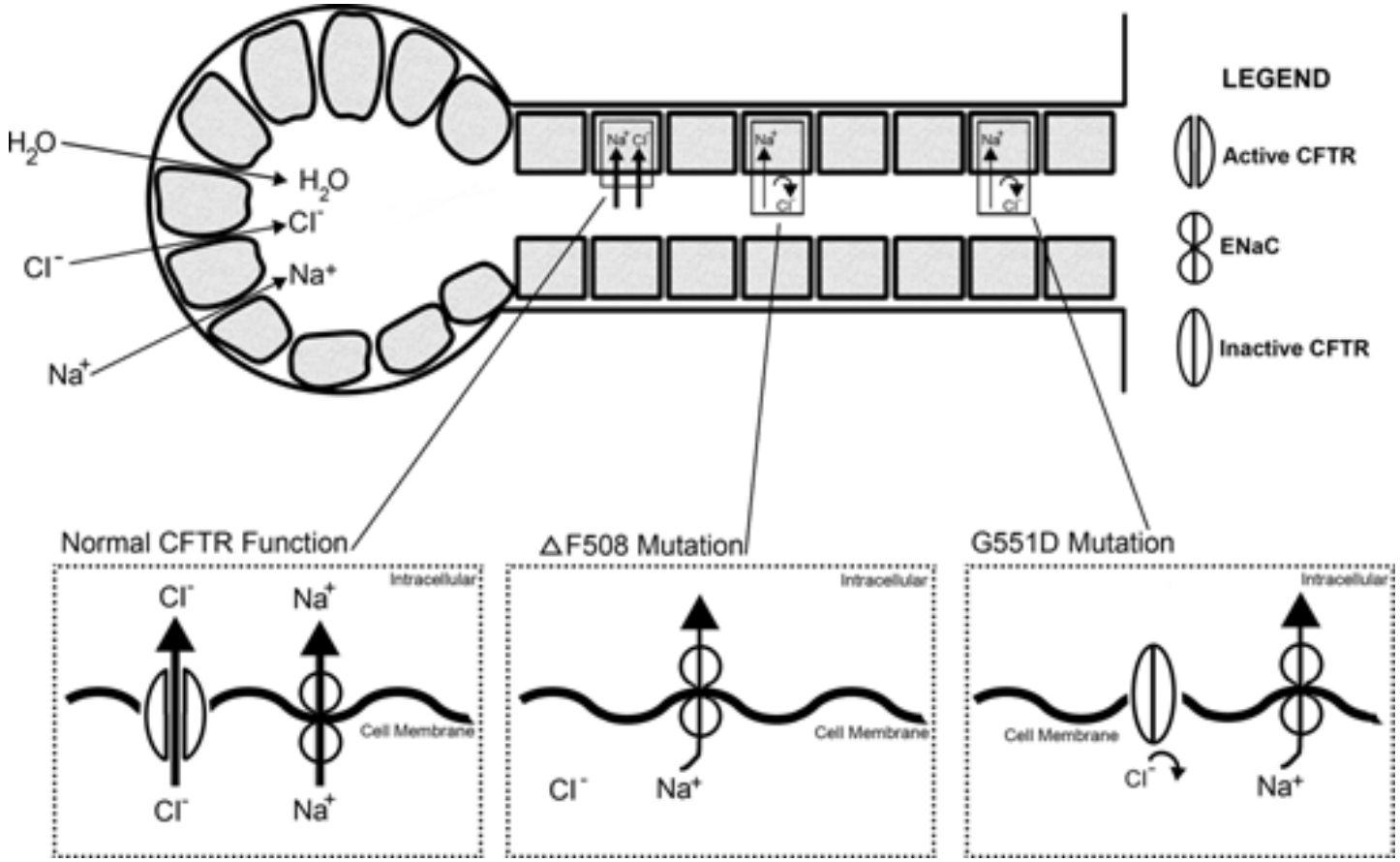
- Timeliness of NBS discussed by Carol Johnson, RN and Dr. Susanna McColley, MD today
  - Goal of diagnosis is < 30 days
- Confirmatory testing must follow newborn screen
  - **Positive sweat test = gold standard**
    - **Normal sweat chloride < 30 mEq/L**
    - **Intermediate 30 - 59 mEq/L**
    - **Abnormal and diagnostic of CF ≥ 60 mEq/L**
  - CF genotype with 2 CF-disease causing mutations
  - Typically the date of confirmed diagnosis recorded is the date of the positive sweat test, though often treatment may start before then due to a presumed diagnosis with positive NBS with 2 CF causing mutations

# How the Sweat Test Began

- NYC, Summer 1948
- CF patients with "heat prostration"
  - Abnormally high electrolyte concentrations in the sweat

From Collins JTB, et al. Ped Pulm 49:106-117 (2014); Photo from S. McColley

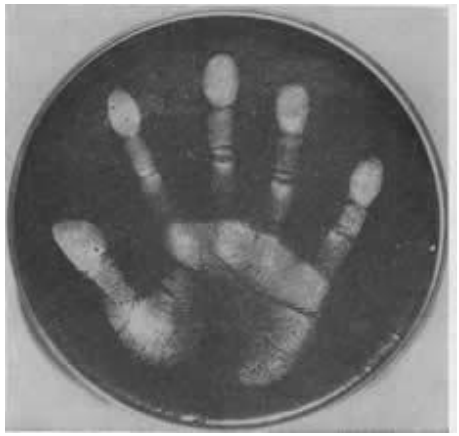
# Sweat chloride is elevated in CF due to absence of functional CFTR




From Collins JTB, et al. Ped Pulm 49:106-117 (2014)

# Sweat test procedure

- Early techniques involved “thermal stress” (1955)
- Finger prints on plates impregnated with silver nitrate and potassium (1956)





From Collins JTB, et al. Ped Pulm 49:106-117 (2014) and cfmedicine.com;
- Schwachman H & Gahm N. NEJM 255:999-1001 (1956)

# Gibson & Cooke 1959

- "A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis"
  - Quantitative Pilocarpine Iontophoretic Test (QPIT) method
    - Topical pilocarpine propelled transdermally by electromotive forces using a small electric current (2.5 – 4 mA for 5 min)
    - Pilocarpine stimulates muscarinic receptors on the eccrine sweat glands inducing sweat secretion
    - Sweat collected on gauze paper (max 30 mins)
- 1983 Macroduct™ system of sweat collection, Wescor (Logan, Utah)

Gibson LE & Cooke RE. Pediatrics. 23:5 (1959); Collins JTB, et al. Ped Pulm 49:106-117 (2014)

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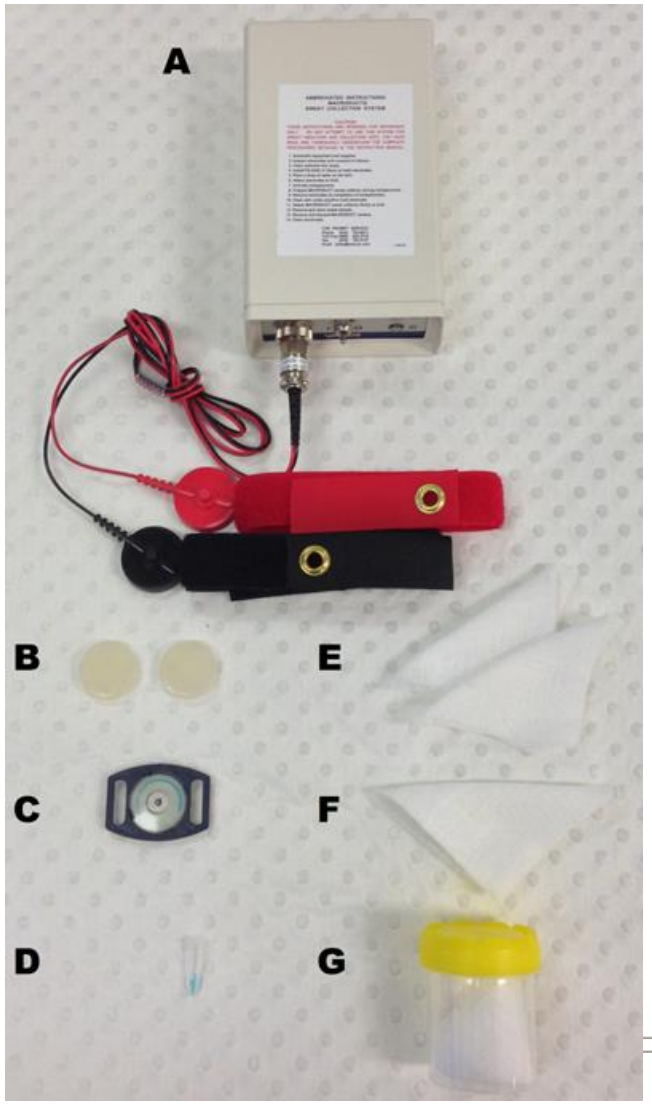
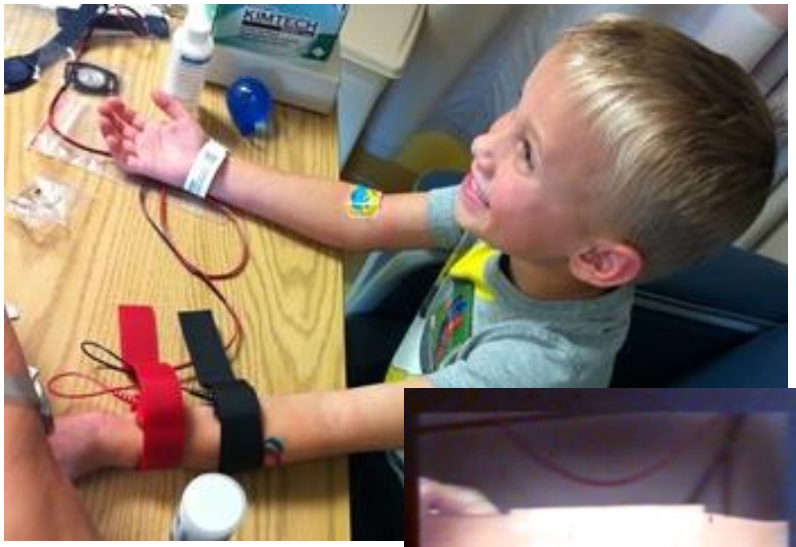
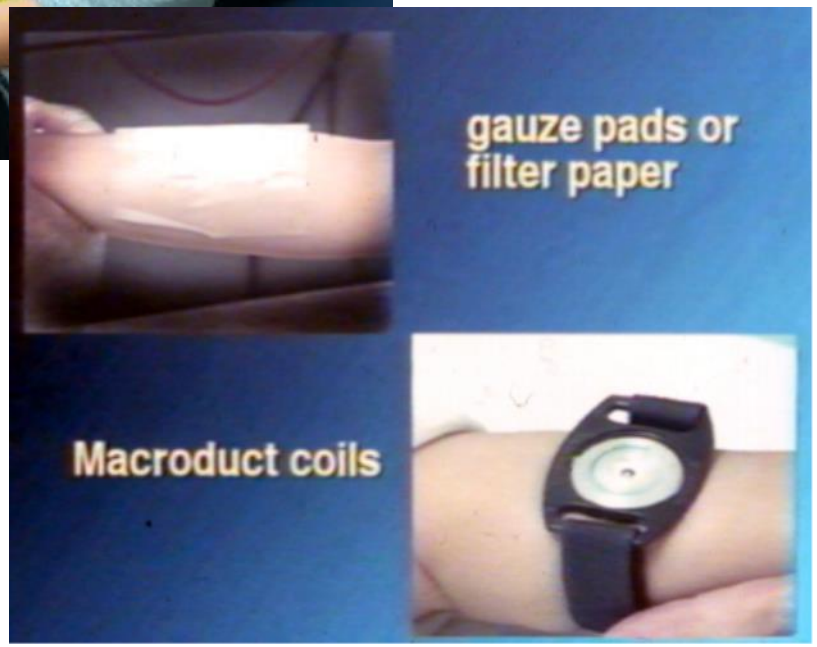
# Gibson-Cooke and Macroduct™ sweat collection






From Collins JTB, et al. Ped Pulm 49:106-117 (2014); Courtesy of McColley and LeGrys, 2015 CF Sweat Test Improvement Collaborative Webinar

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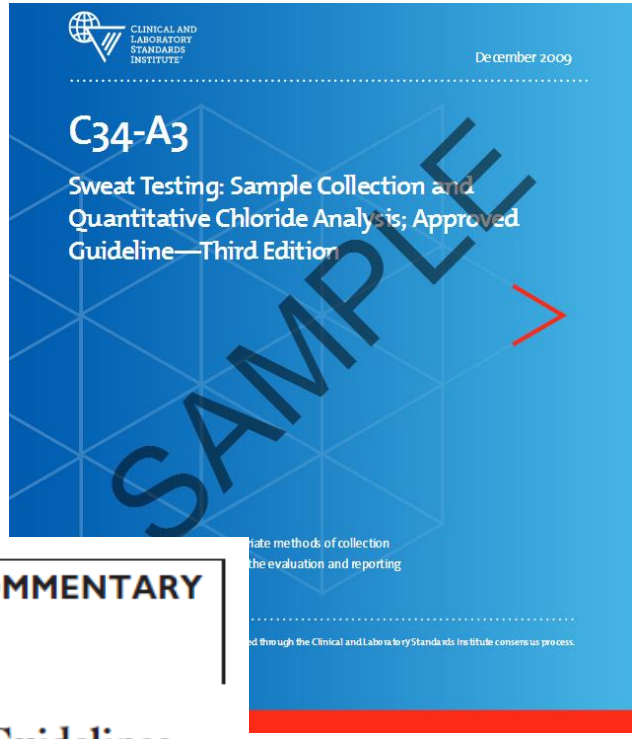
# Standardization of Sweat Testing

- Clinical Laboratory Standards Institute (CLSI)
  - Original 1994 (updated 2000, 2009)
- Cystic Fibrosis Foundation
  - 2007 Guidelines




COMMENTARY

## Diagnostic Sweat Testing: The Cystic Fibrosis Foundation Guidelines

VICKY A. LEGRYS, DRA, JAMES R. YANKASKAS, MD, LYNNE M. QUITTELL, MD, BRUCE C. MARSHALL, MD, AND PETER J. MOGAYZEL, JR, MD, PHD

The Cystic Fibrosis Foundation (CFF) accredits cystic fibrosis (CF) centers, located in teaching and community hospitals nationwide, which provide comprehensive diagnosis and treatment for people with CF. The CF centers are evaluated



From clsi.org; LeGrys VA et al. J Pediatr 151(!):85-9 (2007)

# CF Foundation Guideline 1

- The laboratory must perform quantitative pilocarpine iontophoresis sweat chloride testing according to the procedures outlined in CLSI document C34-A3 without modifications
  - Stimulation of sweat using QPIT
  - Collection of sweat into gauze or filter paper, or Macroduct coil
  - Evaluation of amount collected in weight or volume
    - Minimum 75 mg weight or 15 µL volume
  - Measurement of sweat $Cl^-$ concentration by coulometric titration (chloridometer) (or other outlined methods)



LeGrys VA et al. J Pediatr 151(!):85-9 (2007)

# Other Guidelines

- Minimum age for testing is 48 hours
- Sweat collection and analysis should be performed in duplicate
- The incidence of quantity not sufficient (QNS) samples should be ≤ 10% in patients ≤ 3 months and ≤ 5% in patients > 3 months of age
  - QNS samples can delay confirmation or rule-out CF diagnosis; also $$
- Insufficient samples should not be pooled
  - The minimum sample requirement is based on a physiologic sweat rate of > 1 g/m$^2$/min for the standard electrode size, stimulation area and collection time.
  - Pooling can lead to false-positive and false-negative results



LeGrys VA et al. J Pediatr 151(!):85-9 (2007)

# QNS: Gibson-Cooke vs. Wescor Macroduct™

| | Gibson-Cooke gauze/filter paper QNS % | Macroduct system with Coil QNS % | p value |
|---|---|---|---|
| Hammond KB, et al, J Pediatr 1994 (IL) | 0.7% | 6.1% | Not reported |
| LeGrys VA, McColley SA, et al, J Pediatr 2010 (CFF) | 6.5 % (< 3 mos)<br>3.7% (> 3 mos) | 7.9% (< 3 mos)<br>4.8 % (> 3 mos) | p = 0.321<br>p = 0.086 |
| Kleyn M, et al, Pediatr Pulmonol 2011 (MI) | 17% | 21% | NSD |
| Laguna TA, et al, Pediatr Pulmonol 2012 (MN) | 15.4% | 2.1% | p < 0.0001 |

# Contributors to QNS

- Prematurity (< 37 weeks or < 39 weeks)
- Low birth weight (less than 2.5 kg)
- Low weight at time of testing (less than 3 kg)
- Race (higher QNS in African American race)
- Dehydration (acutely ill, inpatients)
- Technical errors
  - Variability in QNS rates within and across sites = evidence that CLSI-34 A3 is not followed exactly



Collins MN et al, AJCP 142:72-75 (2014); Laguna TA et al, Pediatr Pulmonol 47:736-42 (2012); Kleyn M et al, Pediatr Pulmonol 46:23-30 (2011); LeGrys VA et al, J Pediatr 157:1035-7 (2010);

# Recommendations to minimize QNS

- Set testing criteria for patient testing
  - Exceptions require approval by Laboratory or CF Center Director
- Avoid sweat testing infants <2 weeks of age or < 2 kg of weight and/or < 36 weeks corrected age (gestational + post delivery age)
  - Avoid repeated testing if acutely ill inpatients that are initially QNS
- Follow CLSI procedure and Manufacturer guidelines exactly
  - Placement of positive electrode and collecting material is critical
  - Secure the collecting material with adhesive bandage
  - Limit number of personnel performing testing; routinely perform competency testing
- Direct warming does not appear to increase collection volume nor do oral salt supplements



Courtesy of McColley and LeGrys, 2015 CF Sweat Test Improvement Collaborative Webinar;
LeGrys VA et al, J Pediatr 157:1035-7 (2010)

# Example of sweat test QI: Illinois Newborn Screening Quality Consortium

- CF Foundation Screening Improvement Project Grant (2011 - 2015)
  - PI: Susanna McColley, MD
- Goal: reduce QNS rates from 15 sites (13 IL, 2 MO)



Courtesy of S McColley

# Checklist for Wescor Macroduct™ system

## Sweat Test Collection Checklist for the Wescor Macroduct System

***This checklist supplements and does not substitute for manufacturer's instructions and published CLSI 34-A3 standards. Follow the CLSI 34-A3 standards exactly as written.***

**The technologist should wear powder-free gloves. Keep the room comfortably warm. Ensure that the infant is adequately hydrated.**

**STIMULATION**

- ❑ Prep skin appropriately: Wash patient's stimulation sites well using a gauze pad soaked with CLRW (distilled, deionized water). Leave the skin damp, or alternatively, place a drop of distilled, deionized water on each gel prior to securing it for stimulation, making sure the area is not so wet as to short out the current. Obtain the water fresh, every day of testing.
- ❑ Use the inner (volar) surface of the forearms for sweat collection; avoid use of thighs.
  - o If the legs must be used, wash them thoroughly with distilled, deionized water to remove any residual urine. Ensure that the collection site does not become contaminated with urine during collection.
- ❑ Perform stimulation (iontophoresis) sequentially, not simultaneously, on each forearm. Stimulate and collect sweat from **both** forearms.
- ❑ Place the red (+) electrode about half way between the elbow and wrist; avoid placing over the wrist tendon.
- ❑ Use Coban or another type of stretchy tape to secure the electrodes.

**COLLECTION**

- ❑ Following iontophoresis, ensure the collection area is rinsed well with distilled, deionized water and dried thoroughly before collection using 4x4 inch gauze.
- ❑ Keep the Macroduct collector in the plastic protector or on the patient's skin at all times.
- ❑ Ensure that the technologist's fingers are dry when handing the collector and patient skin; NEVER touch the underside of the collector.
- ❑ Place the macroduct collector **directly on the area of skin** that was stimulated.
- ❑ Apply the collector so that there is **full contact** with the forearm.
- ❑ Secure the collector coil with stretchy tape to hold it secure during collection.
- ❑ Collect sweat for no more than 30 minutes on each side. Use a dual channel timer, one for each side.
- ❑ Follow the procedure for removing the sweat sample from the coil exactly as written in the Wescor Macroduct procedure manual. Note: "Removing the complete device before detaching the tubing may create a vacuum that will draw the collected sweat from the tubing and seriously reduce the sample volume" from Wescor manual.
- ❑ Consider using a single use syringe with a blunt needle to remove the sweat from the collecting coils.
  - o If you use the plastic reusable squeeze device and it becomes contaminated with sweat, cleanse thoroughly with distilled, deionized water before using it again. Rinse and dry the nippers and the plastic squeeze dispenser with distilled, deionized water after each use.
- ❑ Use the 0.2 mL PCR tubes for specimen transfer.
- ❑ Label each sweat container with patient identification, to include L and R specimen sites.
- ❑ The minimum volume for analysis is 15uL. Verify by pipetting or weighing.
- ❑ Follow institutional infection control procedures for disinfecting equipment. Do not use blea



Courtesy of McColley and LeGrys, 2015 CF Sweat Test Improvement Collaborative Webinar

# Checklist for gauze or filter paper collection

## Sweat Test Collection Checklist for Gauze/Filter Paper

***This checklist supplements and does not substitute for published CLSI 34-A3 standards. Follow the CLSI 34-A3 standards exactly as written.***

**The technologist should wear powder-free gloves. Keep the room comfortably warm. Ensure that the infant is adequately hydrated.**

**STIMULATION**

- ❑ Prior to initial weighing, label the weighing vials or specimen containers and lids with sufficient information to prevent misidentification. The labeling system should include the date of the testing, and the designation "left" and "right". Weigh the labeled vials containing the collecting gauze or filter paper prior to testing.
- ❑ Validate the stability of sweat weight if a delay occurs between weighing the gauze or filter paper and placing it on the patient. The gauze should be placed on the patient within 30 minutes following weighing.
- ❑ Keep the pre-weighed collection vial inside a plastic bag at all times after initial weighing and prior to reweighing after collection. Do not handle the vial or collecting material directly with ungloved fingers.
- ❑ Prep skin appropriately: Wash patient's stimulation sites well using a gauze pad soaked with CLRW (distilled, deionized water). Obtain the water fresh, every day of testing. Dry thoroughly with gauze pads.
- ❑ Use the inner (volar) surface of the forearms for sweat collection; avoid use of thighs.
  - If the legs must be used, wash them thoroughly with distilled, deionized water to remove any residual urine. Ensure that the collection site does not become contaminated with urine during collection.
- ❑ Perform stimulation (iontophoresis) sequentially, not simultaneously, on each forearm. Stimulate and collect sweat from **both** forearms.
- ❑ Use 1 ½ x 1 ½ inch electrodes on all patients. Visually inspect the electrodes prior to use for pitting, scratches or irregularities that may cause burns.
- ❑ Use a 2 x 2 inch gauze pad for stimulation.
- ❑ Collect on a 2 x 2 inch gauze pad or 2 x 2 inch filter paper.
- ❑ Use pilocarpine nitrate under the positive electrode.
- ❑ Do not use saline as the electrolyte solution under the negative electrode.
- ❑ Do not exceed 4.0 mA during iontophoresis. In general, a current of 2.5-4.0 mA for 5 minutes is adequate.
- ❑ Place the red (+) electrode about half way between the elbow and wrist; avoid placing over the wrist tendon.
- ❑ Use Coban or another type of stretchy tape to secure the electrodes.

**COLLECTION**

- ❑ Following iontophoresis, ensure the collection area is rinsed well with distilled, deionized water and dried thoroughly before collection using 4x4 inch gauze.
- ❑ Quickly place the pre-weighed gauze or filter paper **directly on the area of skin** that was stimulated. Cover with plastic wax film and secure with stretchy tape.
- ❑ Collect sweat for no more than 30 minutes on each side. Use a dual channel timer, one for each side.
- ❑ Carefully blot back condensate that forms during the 30 minute collection into the gauze or filter paper prior to removal from the arm.
- ❑ Reweigh the collection vials with the gauze or filter paper immediately following collection
- ❑ Sweat samples should be analyzed soon after collection on the same day. In unusual circumstances, reweigh promptly, and store with a tightly fitting lid. Under these conditions sweat is stable for up to 72 hours at 4 ° C.
- ❑ The minimum volume for analysis is 75 mg.
- ❑ Follow institutional infection control procedures for disinfecting equipment. Do not use bleach.

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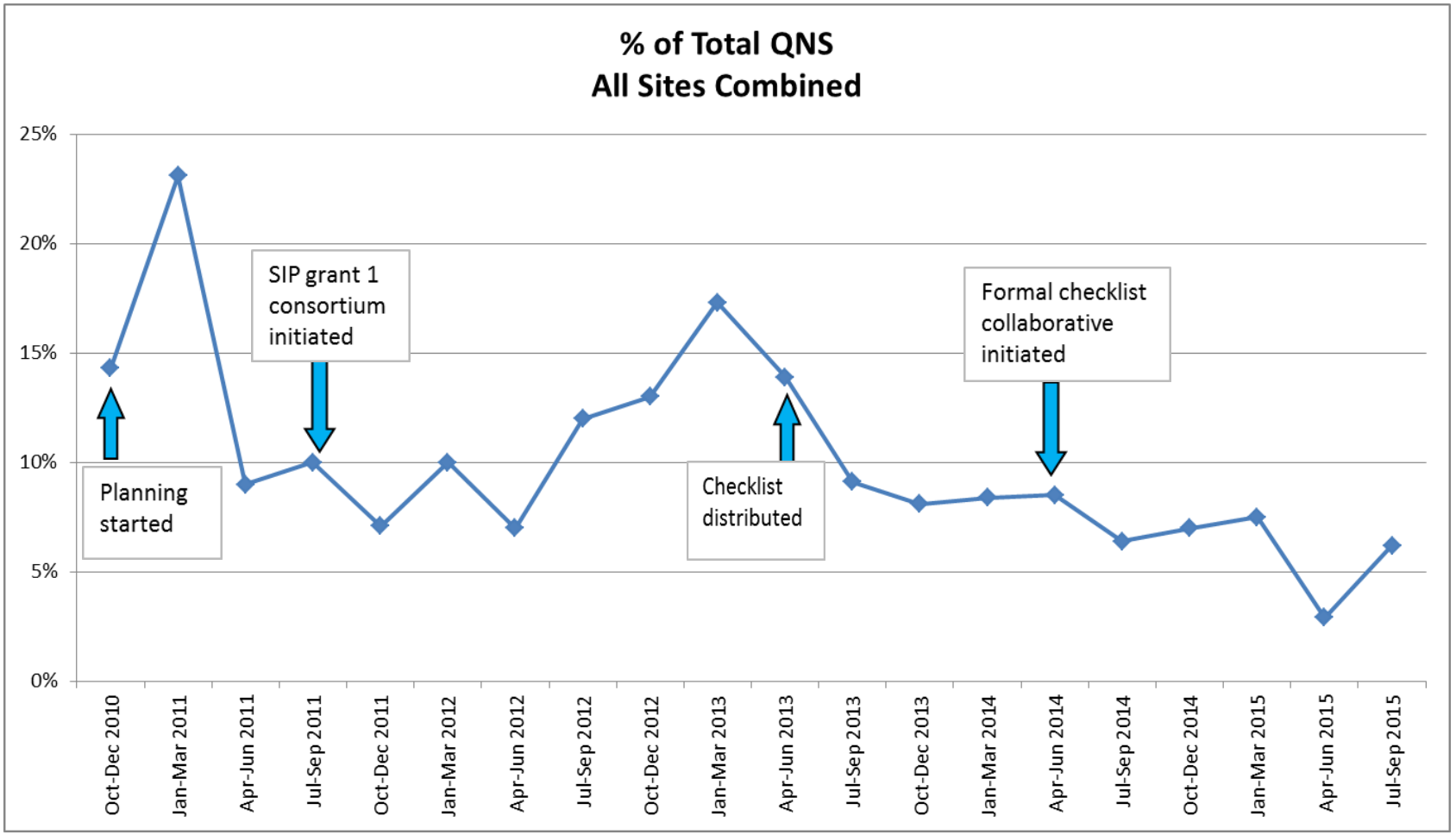
# Illinois QI Results






Courtesy of McColley and LeGrys, 2015 CF Sweat Test Improvement Collaborative Webinar

# Summary

- Sweat testing is the gold standard confirmatory test for CF
- Either the Gibson-Cooke quantitative pilocarpine iontophoresis method (collected with gauze or filter paper) or the Wescor Macroduct™ system should be used for sweat collection
- Sweat testing methods have improved over time but are still require technical expertise and strict adherence to the CLSI guidelines
- Reducing QNS rates relies on both adherence to guidelines as well as individual CF Center analysis and site specific QI efforts

Questions?