Improving the Sensitivity and Positive Predictive Value in a Cystic Fibrosis Newborn Screening Program Using a Repeat Immunoreactive Trypsinogen and Genetic Analysis

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Objective To evaluate the performance of a new cystic fibrosis (CF) newborn screening algorithm, comprised of immunoreactive trypsinogen (IRT) in first (24-48 hours of life) and second (7-14 days of life) dried blood spot plus DNA on second dried blood spot, over existing algorithms.

Study design A retrospective review of the IRT/IRT/DNA algorithm implemented in Colorado, Wyoming, and Texas.

Results A total of 1 520 079 newborns were screened, 32 557 (2.1%) had abnormal first IRT; 8794 (0.54%) on second. Furthermore, 14 653 mutation analyses were performed; 1391 newborns were referred for diagnostic testing; 274 newborns were diagnosed; and 201/274 (73%) of newborns had 2 mutations on the newborn screening *CFTR* panel. Sensitivity was 96.2%, compared with sensitivity of 76.1% observed with IRT/IRT (105 ng/mL cut-offs, $P < .0001$). The ratio of newborns with CF to heterozygote carriers was 1:2.5, and newborns with CF to newborns with *CFTR*-related metabolic syndrome was 10.8:1. The overall positive predictive value was 20%. The median age of diagnosis was 28, 30, and 39.5 days in the 3 states.

Conclusions IRT/IRT/DNA is more sensitive than IRT/IRT because of lower cut-offs (~97 percentile or 60 ng/mL); higher cut-offs in IRT/IRT programs (>99 percentile, 105 ng/mL) would not achieve sufficient sensitivity. Carrier identification and identification of newborns with *CFTR*-related metabolic syndrome is less common in IRT/IRT/DNA compared with IRT/DNA. The time to diagnosis is nominally longer, but diagnosis can be achieved in the neonatal period and opportunities to further improve timeliness have been enacted. IRT/IRT/DNA algorithm should be considered by programs with 2 routine screens. *(J Pediatr 2016;175:150-8)*.

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Cystic fibrosis (CF) newborn screening (NBS) has been universally offered to all newborns in the US since December 2009, and almost universally throughout Europe, Australia, and New Zealand.[1-3] Immunoreactive trypsinogen (IRT) is persistently elevated in the circulating blood of newborns with CF and is the first biochemical evidence that a child is at increased risk of having CF, leading to its use as the marker to be used on the first dried blood spot (DBS), collected in the first days of life.[4,5] The variability of IRT in newborns with CF is considerable, and is known to be associated with age, *CFTR* genotype, and the presence of meconium ileus at birth.[6] However, it is still not possible to adequately differentiate between newborns with and without CF just on the basis of IRT alone even after controlling for these factors.[6] Although NBS programs use an initial determination of IRT in their algorithms for NBS for CF, 1 IRT measurement alone results in unacceptable numbers of false positive screens, therefore, additional tiers are required to improve the specificity of the screen.[2]

Currently, there are 2 basic algorithms employed in the US, IRT/IRT and IRT/DNA, and variations on these algorithms have been implemented in order to improve sensitivity and specificity of the initial IRT assay. The IRT/IRT algorithm was first implemented in the US in 1982 and is based on the repeat measurement of IRT on 2 DBS collected on 2 separate occasions (first and second screens); the first is typically collected within the first 24-48 hours of life and the second between 10 days and 2 weeks.[7] The IRT/DNA algorithm tests all DBS for IRT and specimens with elevated IRT concentrations are subsequently screened for a fixed panel of *CFTR* mutations on the same specimen.[8] The increased specificity provided by the *CFTR* mutation analysis allows laboratories to lower the IRT cut-off, resulting in increased sensitivity compared

CF	Cystic fibrosis
CFFNPR	CF Foundation National Patient Registry
CRMS	CF-related metabolic syndrome
DBS	Dried blood spot
IRT	Immunoreactive trypsinogen
NBS	Newborn screening
PPV	Positive predictive value

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Table I. State specific IRT/IRT/DNA screening algorithms

	Colorado/Wyoming	Texas
IRT cut-offs employed for each of the 2 screens		
First DBS	60 ng/mL	60 ng/mL
Second DBS	60 ng/mL	60 ng/mL
Other considerations	N/A	Over 21 d of age: 46.5 ng/mL*
Second screen algorithm	Test second DBS only if first screen is ≥60 ng/mL, first DBS is unsatisfactory, or no matched screen is identified	All second DBS are tested
CFTR mutation analysis	All second DBS specimens with IRT greater than 60 ng/mL First DBS specimen tested if IRT greater than 60 ng/mL and no satisfactory second specimen submitted.	All second DBS specimens with IRT greater than 60 ng/mL First DBS specimen tested if IRT greater than 60 ng/mL and no satisfactory second specimen submitted.
Number of mutations	46 mutations (7/1/08-6/30/11) 44 mutations (7/1/11-12/31/12)	40 mutations
Ultrahigh algorithm with no mutations	Infants with IRT >150 ng/mL (on either screen) and 0 mutations are recalled for sweat test	Infants with IRT >150 ng/mL (on either screen) and 0 mutations are recalled for sweat test

N/A, not applicable; *CFTR*, Cystic Fibrosis Transmembrane Conductance Regulator.
Data presented from initiation of algorithm (Colorado/Wyoming: July 1, 2008, Texas: December 1, 2009) through December 31, 2012.
*Prior to December 12, 2012, the cut-off for older infants was 30 days of age.

with IRT/IRT programs.[9,10] Most IRT/DNA programs employ a cut-off for IRT at the 96th or 97th percentiles based on daily IRT distributions. The reported sensitivity of IRT/DNA algorithms using a multimutation panel is 95%-98%.[2,3,11,12] In addition to identifying children with CF, IRT/DNA programs identify heterozygote carriers that require genetic counseling following a negative sweat test to rule out a diagnosis of CF; a broader panel of *CFTR* mutations may increase both sensitivity and carrier detection rate.[11,12] Finally, some infants will have an inconclusive diagnosis, or CF-related metabolic syndrome (CRMS), as a result of any CF NBS program.[13] The frequency of identification of an inconclusive diagnosis is much higher in NBS programs using CF mutation analysis.[14]

Balancing the need for a more sensitive algorithm to identify newborns with CF in a timely manner with the desire to minimize the number of families impacted by false positive results, an IRT/IRT/DNA algorithm was introduced in the US in 2008.[15] Colorado, Wyoming, and Texas adopted the IRT/IRT/DNA algorithm in which DBS with elevated IRT on the first and second routine DBS are subsequently tested for *CFTR* mutations. The purpose of this study is to present the results of the multistate implementation of IRT/IRT/DNA algorithm and to compare this method with the IRT/IRT and IRT/DNA protocols that are commonly used in the US.

Methods

Records from each of the CF NBS programs were obtained from the public health laboratories within each state. Details of each individual NBS program are described below. The IRT/IRT/DNA algorithm began in Colorado and Wyoming in June 2008, and in Texas in December 2009 (**Table I**). Colorado performs the testing for the state of Wyoming, therefore, the 2 programs are presented together. Data from newborns born before December 31, 2012, are presented.

Colorado and Wyoming

The NBS laboratory in Colorado is under contract with the state of Wyoming to test all DBS from Wyoming. Each state has its own follow-up staff. Colorado requires each newborn to have an initial NBS specimen obtained as close as possible to discharge, but no later than 48 hours of age, and a second NBS specimen collected between 8 and 14 days after birth, but in no case less than 72 hours or greater than 30 days after birth. Wyoming requires every newborn to be screened 3-5 days following birth for full term children and 5-8 days for premature children. A second specimen is collected between 7-10 days after birth. DBS from both states are sent to the Colorado Department of Public Health and Environment Laboratory Services Division. IRT is measured through PerkinElmer AutoDELFIA instrumentation (PerkinElmer Inc, Waltham, Massachusetts). Each abnormal value (≥60 ng/mL) is repeated in duplicate; if the average of the 2 results is lower than 60 ng/mL, the result is considered normal. Colorado and Wyoming test second specimens for IRT only if the first DBS results in elevated IRT. The mutation analysis is performed using an Asuragen kit (Asuragen Inc, Austin, Texas) from July 1, 2008, to June 30, 2011, and using Luminex xTAG CF kit v 2 (Luminex Corporation, Austin, Texas) after June 30, 2011 (**Table II**; available at www.jpeds.com). Initial DBS with elevated IRTs from newborns without a matching DBS submitted for testing, or for whom a matching specimen cannot be identified, are sent for mutation analysis 28 days after testing of the initial sample; 744 first specimens were tested for mutations (677 in Colorado, and 67 in Wyoming), and no cases with CF were identified. Abnormal results are reported to the designated primary care clinician and follow-up testing is coordinated with the CF center. Confirmatory diagnosis is made at Children's Hospital Colorado and a clinical decision is based on results from genotyping from NBS, confirmatory *CFTR* mutation analysis, clinical symptoms, and sweat chloride testing. In