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Implementation of the first worldwide quality assurance program for cystic fibrosis multiple mutation detection in population-based screening☆

Marie C. Earley [a,*], Anita Laxova [b], Philip M. Farrell [b], Rena Driscoll-Dunn [a], Suzanne Cordovado [a], Peter J. Mogayzel Jr. [c], Michael W. Konstan [d], W. Harry Hannon [a]

[a] Centers for Disease Control and Prevention, Atlanta, Georgia 30341, USA
[b] University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin 53726, USA
[c] Cystic Fibrosis Center, Johns Hopkins Medical Institutions, Baltimore, Maryland 21287, USA
[d] Rainbow Babies and Children's Hospital and Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, USA

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ABSTRACT

Background: CDC's Newborn Screening Quality Assurance Program collaborated with several U.S. Cystic Fibrosis Care Centers to collect specimens for development of a molecular *CFTR* proficiency testing program using dried-blood spots for newborn screening laboratories.
Methods: Adult and adolescent patients or carriers donated whole blood that was aliquoted onto filter paper cards. Five blind-coded specimens were sent to participating newborn screening laboratories quarterly. Proficiency testing results were evaluated based on presumptive clinical assessment. Individual evaluations and summary reports were sent to each participating laboratory and technical consultations were offered if incorrect assessments were reported.
Results: The current CDC repository contains specimens with 39 different *CFTR* mutations. Up to 45 laboratories have participated in the program. Three years of data showed that correct assessments were reported 97.7% of the time overall when both mutations could be determined. Incorrect assessments that could have lead to a missed case occurred 0.9% of the time, and no information was reported 1.1% of the time due to sample failure.
Conclusions: Results show that laboratories using molecular assays to detect CFTR mutations are performing satisfactorily. The programmatic results presented demonstrate the importance and complexity of providing proficiency testing for DNA-based assays.

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1. Introduction

The U.S. Centers for Disease Control and Prevention (CDC) Newborn Screening Quality Assurance Program (NSQAP) is a comprehensive program serving newborn screening (NBS) programs in the U.S. and around the world. For over 31 years NSQAP has provided quality control (QC) and proficiency testing (PT) materials in the dried-blood spot (DBS) matrix as well as training, consultations, and filter paper evaluations. As of December 31, 2009, NSQAP provided QC and/or PT materials for 33 analytes covering all primary and most secondary targets on the U.S. Secretary's Advisory Committee for Heritable Disorders in Newborns and Children recommended uniform screening panel [1]. These materials were sent to 440 laboratories in 61 countries, including all U.S. NBS laboratories [2]. Most NBS assays utilize biochemical methods to detect a variety of genetic disorders; however cystic fibrosis (CF) screening often incorporates a second-tier molecular test that identifies region appropriate CF-causing mutations in the CF transmembrane conductance regulator (*CFTR*) gene. Thus, it was essential that NSQAP develop new strategies to provide a proficiency testing program for NBS laboratories that can accommodate the variable CF multi-mutation assays.

CF is an autosomal recessive, life-threatening disease with an average incidence of approximately 1:4,000 in Western Europe, North America, and Australasia. NBS to detect CF first became feasible after 1979 when researchers in New Zealand discovered that DBS specimens from newborns that were later diagnosed with CF by a sweat test were shown to have high levels of immunoreactive trypsinogen (IRT) [3]. Soon thereafter, countries such as Australia and the U.S. state of Colorado implemented IRT/IRT testing, which involved an initial IRT

Abbreviations: ACMG, American College of Medical Genetics; CFF, Cystic Fibrosis Foundation; *CFTR*, Cystic Fibrosis Transmembrance Conductance Regulator gene; DBS, dried-blood spot; IRT, Immunoreactive trypsinogen; NBS, Newborn screening; NSQAP, Newborn Screening Quality Assurance Program.

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* Corresponding author at: Centers for Disease Control and Prevention, 4770 Buford Highway, MS F-19, Atlanta, GA 30341. Tel.: +1 770 488 7828; fax: +1 770 488 7459.
E-mail address: MEarley@cdc.gov (M.C. Earley).

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measurement followed by analysis of a second specimen at approximately 2 weeks of age to determine if the hypertrypsinogenemia was persistent enough for diagnostic referral [4,5].

However, because of concerns about suboptimal sensitivity of the initial IRT cutoffs and observations revealing that repeat (recall) specimens of some CF patients showed precipitous decreases in IRT, Wisconsin developed the two tier IRT/DNA method. This method used a lower IRT cutoff followed by detection of the p.Phe508del mutation in the *CFTR* gene [6]. Wisconsin's IRT/DNA screening results gave better sensitivity than their IRT/IRT program and clinical data indicated significant benefits from early diagnosis, resulting in Wisconsin's implementation of routine IRT/DNA screening in 1994 [7]. In 1999, the New England Newborn Screening Program began using a multiple mutation panel for *CFTR* mutations in order to increase their sensitivity further, capturing other mutations seen in the Massachusetts population [8]. This mutation detection expansion increased the test's sensitivity, as well as the number of *CFTR* mutation carriers detected.

In October 2004, CDC published a pivotal recommendation that U.S. states begin CF NBS based on results from a randomized controlled trial which showed better nutritional and growth outcomes among screened children, as well as risk–benefit analyses that evaluated experienced CF NBS programs [9]. With the inclusion of CF on the U.S. recommended uniform newborn screening panel in 2006, states began to implement CF screening. By December 2009, all states and the District of Columbia were screening for CF using an IRT/IRT algorithm (N = 16) or an IRT/DNA algorithm (N = 35).

As more U.S. states began CF NBS, quality assurance became increasingly important. NSQAP began offering IRT PT materials in 2002 and QC materials in 2006. With the increase in the number of laboratories using multi-mutation *CFTR* assays in CF NBS, NSQAP recognized the need for a *CFTR* mutation PT program. This program presented unique challenges for several reasons: (1) there are a multitude of different mutation panels being used in the DNA component of the IRT/DNA algorithm; (2) there are many commercially available and in-house methods being used to detect these different *CFTR* mutation panels; and (3) there is limited availability of suitable challenge specimens. Hence, NSQAP, in collaboration with U.S. CF care centers, implemented a PT program that provides NBS laboratories with blind-coded DBS specimens prepared from volunteer donors that cover the most common *CFTR* mutations including but not limited to the American College of Medical Genetics (ACMG) recommended 23 mutations [10,11]. This study describes the design, implementation, and encouraging outcomes of this PT program for DNA-based methodologies used in NBS for CF detection. This is the first application of a worldwide multiple mutation DNA PT program for population-based screening.

2. Materials and methods

2.1. Overall design of the pilot PT program

The overall development of this program is described in Fig. 1.

2.2. Specimen collection

Whole blood was collected voluntarily from adult or adolescent CF patients and/or parents into 10 mL EDTA tubes (Becton Dickinson, Thomas Scientific, Swedesboro, NJ) and mixed well by inversion. Tubes were labeled with a specimen number and genotype, and then shipped overnight to CDC with cold packs. Upon arrival, the hematocrit of the blood was adjusted to 50–55% and 75 μL aliquots of blood were spotted onto Whatman 903 filter paper cards (Piscataway, NJ). Cards were dried overnight at room temperature and stored at −20 °C with desiccant and humidity indicator cards. [12] The non-CF DBS specimens were made from a unit of whole blood collected in EDTA tubes from a donor who

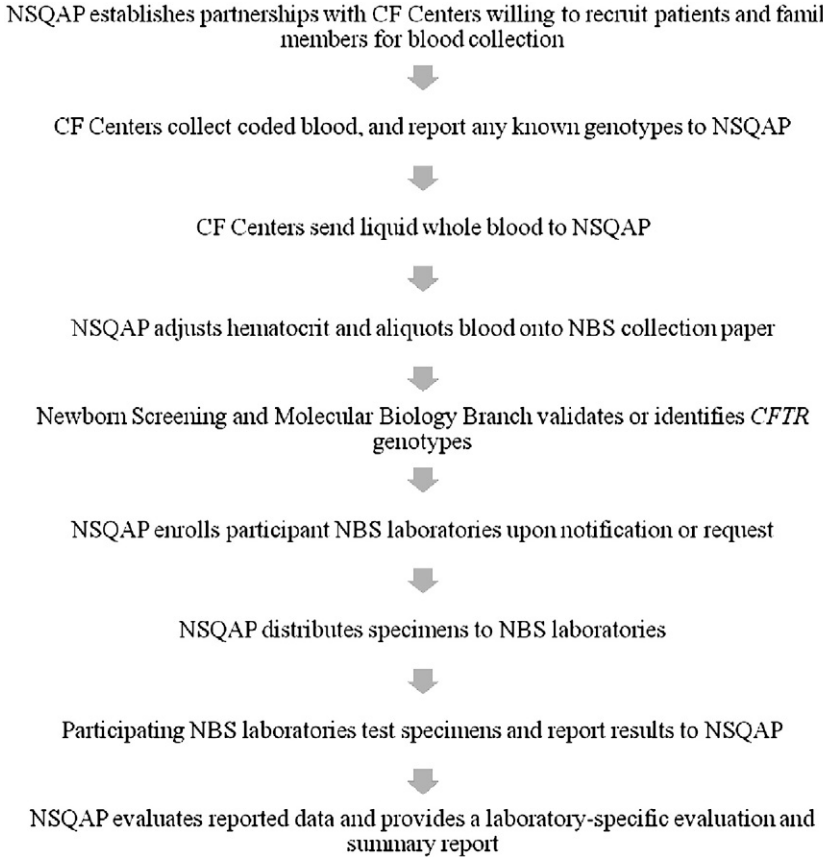

Fig. 1. Flow chart of development for proficiency testing program.