Variation in Cystic Fibrosis Newborn Screening Algorithms in the United States

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Abstract

Rationale: Cystic Fibrosis (CF) newborn screening (NBS) algorithms in the USA vary by state. Differences in CF NBS algorithms could potentially affect the detection rate of CF newborns and lead to disparities in CF diagnosis amongst different racial and ethnic groups. *Objectives*: Generate a database of CF NBS algorithms in the USA and identify processes that may potentially lead to missed diagnoses or lead to health care disparities. *Methods*: We sent an online survey to state and regional CF and NBS leaders about the type and threshold of immunoreactive trypsinogen (IRT) cutoff used and methods used for *CFTR* gene variant analysis. Follow-up by email and phone was done to ensure a response from every state, clarify responses, and resolve discordances . *Results*: There was wide variation in the NBS algorithms employed by different states. Approximately half the states use a floating IRT cutoff and half use a fixed IRT cutoff. *CFTR* variant analysis also varied widely, with 2 states analyzing only for the F508del variant and 4 states incorporating *CFTR* gene sequencing. The other states used *CFTR* variant panels ranging from 23 to 365 *CFTR* variants. *Conclusions*: CF NBS algorithms vary widely amongst the different states in the USA, which affects the ability of CF NBS to diagnose newborn infants with CF consistently and uniformly across the country and potentially may miss more infants with CF from minority populations. Our results identify an important area for quality improvement in CF NBS.

Introduction

Cystic fibrosis (CF) newborn screening (NBS) for has been offered nationwide in the USA since 2010. The CFF has recommended nationwide screening, but it deferred to individual states for planning and screening protocol (algorithm) selection[1]. All CF NBS algorithms employ measurement of immunoreactive trypsinogen (IRT) as the first step[2]. Because IRT can be elevated in infants without CF, one of two different strategies can be used to improve the specificity of NBS. In the first strategy (IRT/IRT), a repeat IRT is obtained in approximately 2 weeks, and if still elevated sweat testing is performed. The other strategy (IRT/DNA) utilizes CF transmembrane conductance regulator (*CFTR*) gene variant analysis, and infants with 1 or 2*CFTR* variants are referred for sweat testing. Over time, the IRT/DNA strategy has been found to be more sensitive and faster than the IRT/IRT strategy, and as of 2020 all states have employed an IRT/DNA approach or incorporated DNA testing into their IRT/IRT algorithm (IRT/IRT/DNA)[3-5].

The methods used to define an elevated IRT and to select *CFTR*variants for DNA analysis can affect the ability of CF NBS to identify infants with CF. States can either use a fixed cutoff value to define an elevated IRT, but this does not account for variability in IRT measurement reagents and seasonal variation in IRT due its heat lability[6, 7]. To account for these factors, other states utilize a floating cutoff determined by a

rolling daily average. The specific *CFTR* variants selected for DNA analysis as well as the total number of variants analyzed can affect the sensitivity of CF NBS, and since infants from minority populations are less likely to have common CFTR variants they may potentially be missed by NBS depending upon the variants selected.

The demographics of the CF population in the USA have changed markedly in the last 20 years[8]. In 2000, 9.2% of patients in the CF Foundation Patient Registry were African-American or Hispanic; as of 2020, this has increased to 14.3%. Analysis of the last 10 years of NBS data shows that about 20% of newly diagnosed patients were from racial or ethnic minority populations[9]. These demographic changes, along with the issues of IRT cutoffs and *CFTR* variant selection[10, 11], raise concerns about the ability of different state CF NBS algorithms to identify newborns with CF, especially those from minority populations. Somewhat surprisingly, a comprehensive database of CF NBS algorithms by state in the USA has never been report. With that background in mind, the objective of our work was to fill this knowledge gap by using an online survey to acquire detailed data on CF NBS algorithms in every state in the USA and to identify areas for improving diagnosis of infants with CF, especially those from minority populations.

Materials and Methods

Survey Design and Distribution

An online survey (SurveyMonkey,San Mateo, CA) was distributed by email by the US CF Foundation (CFF) (Table 1 in the online supplement) in August of 2021. Survey recipients included CF Care Center directors and members of the CFF Quality Improvement (QI) Consortium, a 75-member group of CF NBS leaders from every state in the USA. Information was also obtained about clustering of states (a practice in which a state sends their dried blood spot specimens to another state and outsourcing to a vendor for analysis). Periodic reminders were sent by the CFF to ensure responses from representatives of every state. In some cases, respondents provide data via email. The survey ended in December 2021.

In order to ensure receipt of responses from across the nation, respondents and other CF leaders from each state were contacted until finalized, confirmed accurate data were received. Following completion of data collection, the responses from within the same state were compared to one another in order to confirm consistency and ensure accuracy. In some cases, multiple representatives from the same state provided discrepant information. When this occurred, CF NBS leaders in those states were informed of the discrepancies and asked to confirm the true values. For some regions, respondents needed to be contacted on multiple occasions before we could ascertain accurate answers regarding either IRT cutoffs or *CFTR* panels. For three states, state NBS lab leaders were contacted to determine with certainty the algorithm that was being used in 2021.

Data Analysis

All initial survey responses were downloaded from SurveyMonkey and exported into Microsoft Excel and then REDCap. Data from email responses were entered by hand into the REDCap database. Responses were organized by IRT cutoff method and *CFTR* variant analysis method. The data available includes the 50 states of the USA as well as the District of Columbia.

Results

For the 25 states that utilized IRT fixed cutoffs, the distribution shown in Figure 1 was wide with the mean value being 63.7 and the median 60 ng/ml. For 12 states that employ a 2-specimen IRT/IRT/DNA algorithm (AZ, CO, DE, ID, MD, NV, NM, OR, TX, UT, WA, and WY) the IRT cutoff question (Table 1) was restricted to the initial dried blood spot (DBS) specimen since states rely on this value for decisions about proceeding further in the NBS algorithm. Even amongst states that use a fixed IRT cutoff, there is variation in when In some states, multiple samples are obtained at different points in time or the cutoff is dependent on the age of the baby at the time of sample collection. For our analysis, we used the initial cutoff value reported by the state.

For the 26 regions that utilized IRT floating cutoffs, the range is from the 95^{th} to the 98.8^{th} percentile with

a mean of 95.9th percentile and median at the 96th percentile. Texas uses a unique hybrid model of both floating and fixed cutoffs; the initial IRT has a floating cutoff and if it is above the 95th percentile, a fixed cutoff is applied to a second sample. For the purposes of this analysis, we considered Texas to be a state with a floating cutoff. West Virginia reported switching from a floating cutoff of the 90thpercentile to the 95th percentile in 2021 due to numerous false positives. The latter value was used for analysis in this publication.

There was tremendous variation in how states do *CFTR* variant analysis. Two states test for F508del only, and the other states and regions use panels that test between 23 to 365 *CFTR* variants. Four states (CA, NC, NY, and WI) incorporate *CFTR* sequencing into their algorithm. If the IRT is elevated, CFTR variant panel analysis is performed, and if 1 variant is detected CFTR sequencing is performed, either by the Sanger method (CA) or next generation sequencing (NC, NY, and WI).

Some states are clustered together, in which one state performs the analyses for one or more other states, and some states outsource their*CFTR* variant analysis to an outside laboratory (PerkinElmer Genomics, Pittsburgh, PA). These are described in Table 2. Although states that utilize outsourcing all utilize the same laboratory, they each set their own IRT cutoffs and select their own *CFTR* variant panel.

We noted a geographic distribution of which states that utilized fixed vs. floating IRT cutoffs, as shown in Figure 3. In general, western states tended to use fixed cutoff values, especially before Colorado (with Wyoming) switched to a floating cutoff near the end of 2021. In contrast, most of the eastern states began with a floating cutoff strategy and have retained it. The midwest and south are mixed with about half using each approach.

The free text portion of the survey provided valuable insight and feedback. Some respondents sought advice on newer methodologies such as next generation sequencing. A request was also submitted by one state's CF clinical leader for a virtual conference that would include the NBS lab leaders to discuss recent issues and clarify *CFTR* panel options. As a consequence of follow-up emails and telephone calls to respondents, communications ensued that provided learning experiences. In one case, a connection was made between a CF center director and NBS lab leader which stimulated a potential partnership. We also recognized from some comments that keeping up with evolving CF NBS algorithms was a challenge because CF clinical leaders may not be adequately informed when the state NBS lab changes the CF NBS algorithm. This situation is especially challenging when "border babies" are involved, i.e., when a woman residing in one state where she and her family receive healthcare has her infant delivered in an adjacent state where the NBS specimen is collected and analyzed.

Discussion

In this comprehensive analysis of CF NBS algorithms in the USA, we found substantial variations in IRT cutoffs and *CFTR* variant analysis. These results have important clinical implications for the effectiveness of different states' CF NBS programs in identifying newborns with CF, and they provide compelling evidence of the need for a nationwide QI effort to make CF NBS more consistent across the country and aligned with best practices.

Although the Centers for Disease Control and Prevention and the Association of Public Health Laboratories (APHL) are two federally-supported organizations with national responsibilities for NBS, they have never shared regional NBS methodologies and outcomes. The only other study that presented data on state CF NBS algorithms was published by Pique, et al in 2015[12]. However, data for that study was acquired at a time when many states were using a *CFTR* variant analysis kit (Hologic, Marlborough, MA) that was subsequently withdrawn from the market due to manufacturing defects. Furthermore, at the time of their study, many states had not yet progressed to a DNA-based algorithm. Our study presents comprehensive, up-to-date data on CF NBS in the USA at a time when all states are using IRT/DNA algorithms.

Our results have important implications for equity in CF NBS. For example, the two states analyzing only for F508del in the high IRT specimens cannot exceed 85% sensitivity in a typical population of racially and ethnically diverse American infants, However, it has been known for decades[11, 13] that African American

and Hispanic infants have variants other than those in commonly used *CFTR* panels. In fact, the American College of Medical Genetics[14, 15] variant lists were developed primarily for the white population. Although some biotechnology companies have expanded their *CFTR* panel kits to provide broader racial and ethnic coverage, even with the use of larger CFTR variant panels, e.g. 139 variants, more infants from racial and minority groups will be missed compared to white infants (M McGarry, unpublished observations). Ultimately *CFTR* sequencing methods[16, 17] are needed to better address the equity challenges.

Our results show that an infant's probability of being diagnosed early through CF NBS depends on where the birth occurs and also potentially on when a baby is born, i.e., the season of the year. These differences are attributable to both the DNA/*CFTR* tier and IRT cutoff values and whether they are fixed or floating. Assessment of IRT has demonstrated conclusively that it is a heat-labile biomarker[3, 7] that is also affected by kit-related variations[3, 18]. Every state now shows ambient temperature swings that are being increased by climate change. Although the impact of high temperatures in lowering IRT levels can potentially be mitigated by expedited courier delivery of NBS specimens, the kit-to-kit variations persist. In fact, IRT levels as low as 40 ng/ml have been recorded in states with a 95^{th}percentile cutoff value [3]. As Martiniano et al reported from analysis of 14 years of monthly IRT data, these variations have been evident since at least 2006 and IRT levels show a downward drift in recent years [18]. That study also revealed the clinical impact of IRT levels slightly below fixed IRT cutoff values as they cause false negative results and "missed cases." Consequently, while our survey was underway, Colorado, after critically reviewing a large database of over 800,000 babies screened, changed from a fixed threshold at 60 ng/ml to a floating cutoff at the 96^{th} percentile. This is an example of the type of large database driven QI effort needed in many states to reduce the number of false negative results rather than relying on short term, small data sets for evaluations and cutoff conclusions—the apparent *modus operandi* of most states. Our results demonstrate that many states are not utilizing the optimal method of determining IRT cutoff by continuing to use fixed cutoffs.

It should be emphasized that none of the NBS tests for other genetic conditions show such great variation in cutoff values. Even though the tests used in screening for congenital hypothyroidism have shown seasonal and kit-related variations[19], their impact has apparently not altered sensitivity but does lower the positive predictive value in colder months. The issue of fixed and floating cutoffs has been described in detail in a document published by APHL[20]. In a section on CF, it is stated that "The IRT cutoff is floating and/or fixed. A floating cutoff is recommended because IRT is subject to seasonal variations and lot-to-lot variability of the reagents." In addition to a higher likelihood of achieving equity, the floating cutoff provides the advantage of a predictable number of samples for DNA/CFTR analyses in the second tier.

It has become increasingly clear that the various CF NBS algorithms are not equivalent in sensitivity and efficiency/timeliness. False negative results are more likely with higher IRT cutoff values[3, 18] and fewer *CFTR* variants[12]. This raises the question of how such wide variations in CF NBS algorithms arose. A review of the historical evolution of CF NBS tests provides possible answers. In the case of*CFTR* variant analysis, the likely explanation is that DNA biotechnology has evolved faster than NBS labs have been able to keep up with opportunities to expand their panels, while adding costs as the new options were marketed in association with greater knowledge of*CFTR* pathogenic variants of CF patients[10, 21].

As for the variations in IRT cutoff values, and whether they are fixed or floating, it seems likely that the answer also lies in an historical perspective. Originally, before the discovery of the *CFTR* gene in 1989, all CF NBS algorithms were IRT/IRT and required what we now regard as relatively high cutoff values to ensure that screening was practical. The CFF raised questions about this and other aspects of IRT-based screening in an influential 1983 position paper[22]. However even after the IRT/DNA or IRT/IRT/DNA algorithms were implemented, the 2-sample states continued with fixed cutoffs through 2020 with the exceptions of Texas and more recently Colorado.

The limitations of this study include our focus on a narrow window of time, i.e., the second half of 2021, during a period in which algorithm changes were occurring. For example, we learned during the analysis of data that Oregon is changing to a floating IRT cutoff and the following states are transforming to next generation sequencing: Florida, Kentucky, and Utah. CF NBS algorithms are constantly changing in every

state, and it is likely that regular surveys like our will need to be conducted in order to maintain accurate and current information on CF NBS practices in the USA. In addition, on a national basis we only evaluated the initial IRT cutoff value in the 2-specimen states that employ IRT/IRT/DNA. However, very little research has been done on the optimal IRT cutoff value for the second specimen, and this can be a source of false negative results also[23].

The wide variations in CF NBS algorithms are unique among newborn screening protocols, and involve both IRT cutoffs and CFTR variant analysis. The only consistency is that all states now use a 2-tier strategy beginning with IRT and then, if it is out-of-range, progressing to *CFTR* variant analysis. Although CF NBS has been offered in the USA now for over a decade, our results demonstrate the need for continued improvement and modification of CF NBS algorithms in order to optimize detection of CF newborns and achieve equity and inclusion in CF NBS.

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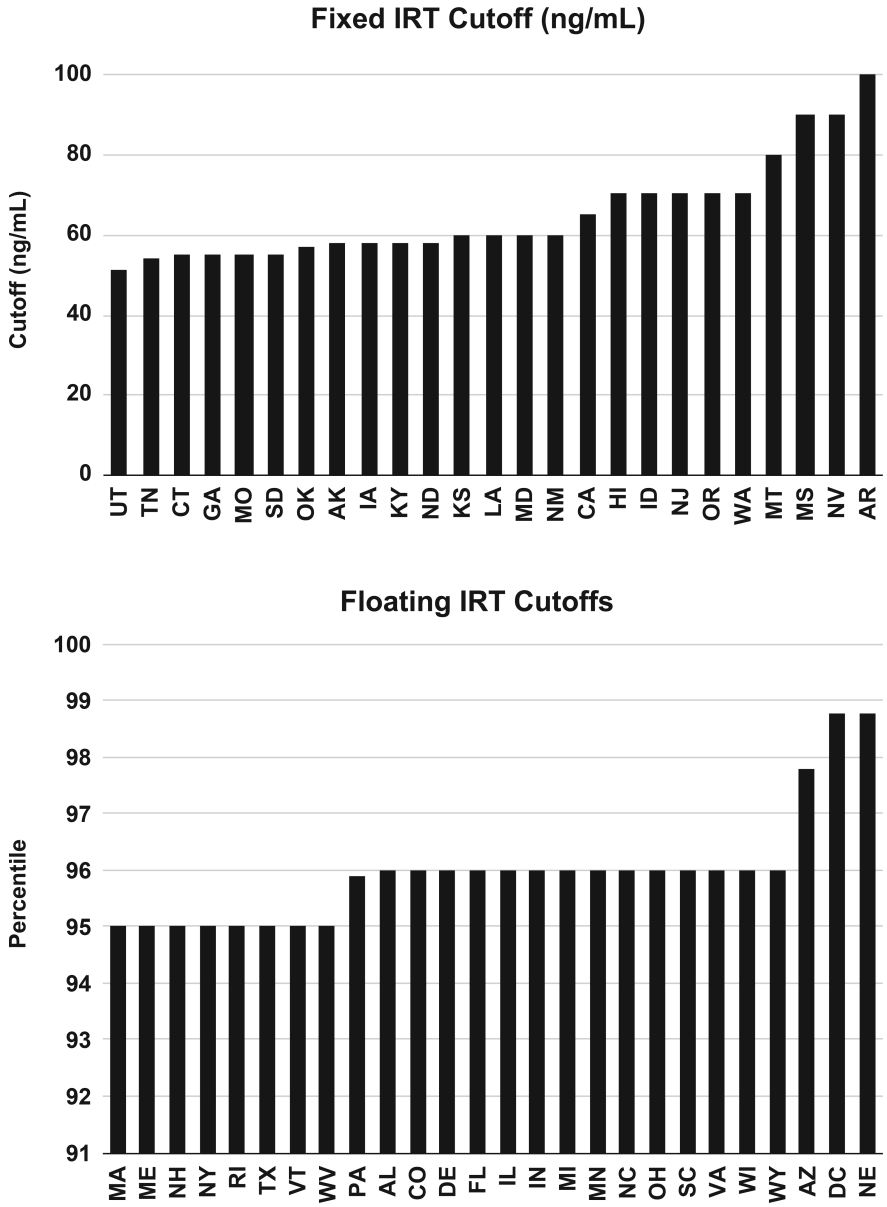
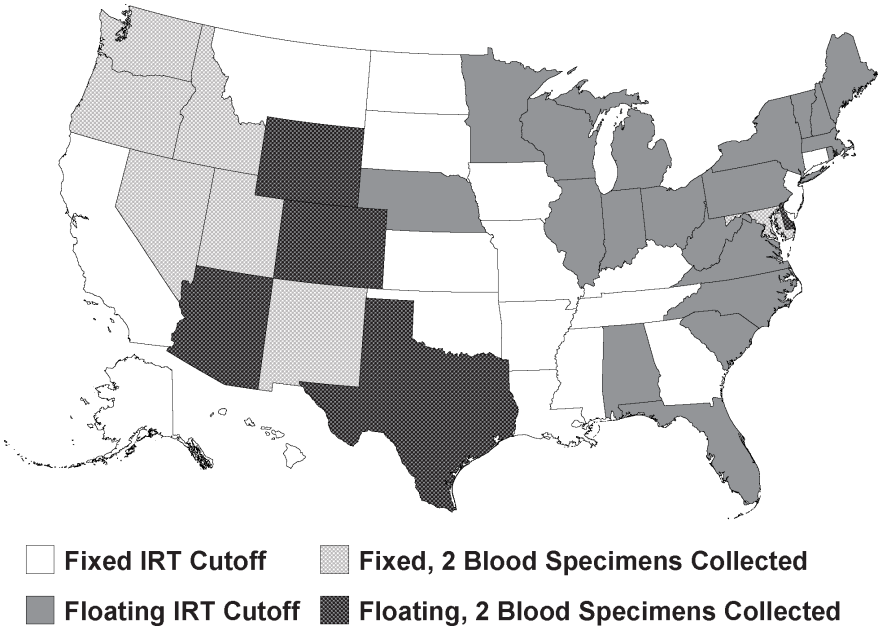
Figure legends.

Figure 1. Fixed IRT cutoff values by state. These results are for the first (initial) dried blood spot specimen.

Figure 2. Floating IRT cutoff values by state. All states but Texas and Colorado collect a single dried blood spot specimen. In the case of Texas and Colorado, the value shown is for the first specimen only.

Figure 3. Geographic distribution of fixed and floating IRT cutoff states. The stippled states collect two dried blood spot specimen and use a IRT/IRT/DNA.





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