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I. Development of a Best IRT/DNA Practices Protocol (BIPP) for Newborn Screening for Cystic Fibrosis

After the CDC encouraged states in October 2004 to proceed with planning newborn screening (NBS) programs for cystic fibrosis (CF)[1], an unprecedented nationwide implementation effort ensued resulting in an increase over three years from 4 to 8 states screening all newborns for this disease. The most up to date information on states that perform NBS can be found at http://genes-r-us.uthscsa.edu/nbdisorders.htm. There are currently 33 states that have universal CF NBS and another 2 states in which this is offered to select populations or universally offered but not required. The only comparable expedited NBS implementations occurred during the 1960s and 70s when many states proceeded with screening newborns for phenylketouria (PKU) and congenital hypothyroidism (CH). However, just as many errors occurred with PKU and CH screening, the same types of problems have developed with CF NBS. An improvement and sustained quality project for CF screening began as a collaborative effort between Wisconsin and the Newborn Screening Branch of the CDC. There are three sequential components to this project: 1) comprehensive review/description of Wisconsin's CF NBS program; 2) identification of process improvement opportunities; and 3) creation of a BIPP based upon insight gained with site reviews in two states.

A comprehensive review and description of the Wisconsin CF NBS program began in September of 2006. All steps in the NBS process, from prenatal education to follow-up of patients at the CF clinic, were reviewed and described in detail in a Wisconsin best practices protocol.

The next step involved application of methods for process improvement perfected in the automobile industry to the CF NBS process. This was done as a collaborative effort from experts in Wisconsin, Colorado, and Massachusetts whose state programs have a long-running tradition of excellence. The specific method applied is known as Process Failure Mode Effects Analysis (PFMEA).

To begin a PFMEA, a PFMEA team is selected from experts on the topic which included NBS lab directors, physicians who treat patients with CF, people in charge of follow-up after positive newborn screens and others. Once the team was assembled, we developed a flow chart illustrating every step in the NBS process starting with prenatal care and birth through care at CF clinics. Upon completion of the diagram, we brainstormed possible failure modes or potential problems at each point in the flow chart. For example, when communicating what NBS is to a new mom, the following failures could occur: the hospital fails to tell the mother about NBS, the hospital uses an unknown language for the mother, the hospital uses inappropriate materials to share with the mother, the caregiver does not fully understand the law, regulation or program, or the mother does not comprehend the information provided. Then each of these potential failures were ranked on a 10-point scale for their severity, occurrence, and ability to

detect. These three numbers are combined to create a risk priority number (RPN) which are then used to rank potential failures from needing the most attention to needing the least attention. Action plans were then developed for the potential failures with the highest RPNs.

All of this information was then combined with insight gained from the Wisconsin, Colorado and Massachusetts CF NBS programs to create this best practices protocol.

A. Introduction

1. Background

Regionalized newborn screening began in the United States during the 1960s after Guthrie[2] developed a screening system for PKU: the dried blood specimen technique, a test for PKU, and an applied intervention before clinical signs were apparent. "Newborn screening may be defined as a population-based public health service program applying preventive medicine systematically in defined regions to reduce newborn morbidity and mortality from certain biomedical and genetic disorders by using presymptomatic detection/diagnosis with dried blood specimens analyzed in central laboratories and employing automated procedures and linked to clinical follow-up programs".[3] When applied correctly, NBS can detect multiple debilitating childhood disorders a few days after birth, preventing the tragic consequences of delayed diagnosis.

On the other hand, NBS may cause harm—especially psychosocial stress in false positive families who outnumber true positives by approximately one hundered-fold.[3,4] Each state's newborn screening program includes six main objectives: education, screening, follow-up, diagnosis, management, and evaluation with exact protocols varying from state to state.[5] NBS programs emphasize early detection and treatment, as well as counseling for those parents whose infant has been diagnosed with a disorder. Currently, the state of Wisconsin mandates that all newborns be tested (unless a parent or guardian objects for religious reasons) for a wide range of disorders, including congenital hearing impairment, PKU and 37 other hereditary metabolic diseases, congenital hypothyroidism, congenital adrenal hyperplasia, hemoglobinopathies such as sickle cell disease, and cystic fibrosis (CF). Although there is variation in the NBS panels of states, a recent recommendation[5] urges uniformity with a core panel of 29 disorders included throughout the United States.

CF, a potentially debilitating and even lethal disorder caused by the mutations in the CF transmembrane conductance regulator (CFTR) gene, leads to salt loss in sweat, pancreatic insufficiency causing intestinal malabsorption and malnutrition, progressive lung disease and other organ failure if left untreated. However, through early detection via the NBS system, children have shown improved growth and nutritional status[7] and the potential for improved pulmonary function[8]. The traditional method of CF diagnosis depends on performing a sweat chloride test after signs/symptoms are recognized by a primary care physician; however, with this strategy the average age of diagnosis is four years.[9] NBS for CF became scientifically feasible in 1979 after Crossley et al. showed

high levels of immunoreactive trypsinogen (IRT) in infants with the disorder.[10] Today, IRT testing is still used in all three NBS CF protocols: IRT/IRT, IRT/DNA ΔF508, and IRT/DNA multi-mutation. Wisconsin uses the third protocol of IRT/DNA multi-mutation analysis from dried blood spots collected by a heel stick of the newborn shortly after birth.

2. History of CF Newborn Screening

Population-based NBS for CF was made possible in 1979 after Crossley and colleagues[22] demonstrated the elevation of immunoreactive trypsinogen concentrations in children with CF. The IRT level was able to be determined from the dried blood spot of the Guthrie card. However, since the method was new and lacked data regarding sensitivity, specificity and outcomes of children diagnosed before symptoms, two randomized controlled trials were conducted to investigate. One, conducted in the United Kingdom from 1985 to 1989, randomly allocated children to screened and unscreened groups based on week of birth[23]. Another trial, conducted in Wisconsin, randomly assigned infants to a screened group and a control group (screened but not reported) between 1985 and 1994. Infants in the screened group had their screen reported and sweat tests conducted by 4 to 6 weeks of age. Infants in the control group presented either with symptoms or a family history prior to 4 years of age, but then had their screen results released after 4 years of age to fully identify all cases of CF[24]. In the United States, Colorado was the first state to begin routine screening in 1982(?), with Wisconsin following suit upon the completion of their randomized controlled trial in 1994. Currently, 33 states have universal CF NBS and 2 states offer CF NBS to select populations or universally but is not required.

B. Prenatal and Perinatal Parental Education (Based on reviews at Meriter Hospital, Madison WI)

The first potential point of contact of the NBS system with expectant mother is during prenatal classes offered by many hospitals. While another potential point of contact could be during prenatal visits to a PCP, this would add to the burden of duties required of the PCP and is not likely to be accepted. Thus, women taking prenatal classes are an optimal population in which to begin education regarding NBS. Education is the key to preventing confusion and misunderstanding among parents, especially for parents receiving false positive results, is essential to the mission of doing more good than harm in NBS. However, since only approximately 60% of pregnant women attend prenatal childbirth classes at Madison, Wisconsin's Meriter Hospital, for example, this cannot be the only point of education in the NBS process.

Mothers who do elect to attend such classes are verbally instructed regarding the basic information of the NBS process. At the Meriter Hospital class, they are told that NBS is a state law, that the test is done when the baby is 24 hours old or older, and that the test screens for approximately 53 inherited conditions that are important to detect in the first weeks of life. They are also informed that the results will be sent to their baby's PCP, and the family will be contacted if any results are abnormal. At the class, the mother also receives an informational book called *First Weeks*. The section on newborn screening states the following:

> "When your baby is at least 24 hours old, he will have a blood test called the Neonatal Screen. The blood test screens for congenital conditions that, if not treated, can lead to serious health problems. All results of the screen are reported to your baby's doctor. If you are interested in the conditions that this test screens for, ask your health care provider or refer to the newborn screening brochure. If you go home before your baby is 24 hours old, arrangements will need to be made for the blood screen. Speak with your health care provider to help make arrangements."

This informational booklet is available in multiple languages including Spanish and Hmong.

A second potential point of contact is after delivery. This time is not optimal, however, due to low retention rates of information provided. After maternal delivery, a nurse briefly reviews the information that was presented in childbirth classes. However, if the mother did not take the class, this is the first time she will be told about the NBS program. In this situation, Wisconsin's NBS brochure is the principal source of information, and post-partum mothers are encouraged to read the document and ask any questions they may have about screening their baby.

If mothers do not give birth in a hospital, it is very difficult to contact them for education and execution of NBS. Efforts to reach these populations are key to having high rates of coverage for a NBS program. Matching birth certificate data to newborn screen data would aid in identifying these hard to reach populations.

C. Dried Blood Specimen Collection (based on national recommendations and review in Madison, WI)

1. Background

The newborn screening procedures for congenital disorders other than hearing all rely on an initial dried blood specimen which is typically collected in the birthing hospital by a clinical lab technician using a special blood spot collection card[2] also known as the "Guthrie card". Correct, efficient specimen collection is crucial to maintaining the quality and accuracy of a NBS program. Although blood collection may seem simple, it has been emphasized "that unacceptable and poor quality specimens place an unnecessary burden on the screening facility, cause unnecessary trauma to the infant and anxiety to the infant's parents, potentially delay the detection and treatment of the affected infant, and could contribute to a missed or late diagnosed case". [11] For these reasons, extra attention and training must be directed toward the specimen collection portion of the NBS process.

2. Specimen Collection

The national recommendations for collecting newborn dried blood specimens were published by the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS).[11] This publication gives detailed instructions on acceptable methods. Generally, those instructions are summarized on the back of each state's Guthrie cards. Two methods are

acceptable, namely 1) direction application of blood to the filter paper portion of the Guthrie card; and 2) indirect application using a capillary tube for blood collection. At Meriter Hospital, after each baby is delivered, the health unit secretary (HUC) receives birth information from the delivering nurse. The HUC then fills out a NBS card, removing the blue hearing screen portion to be filled out separately, and orders the blood collection from the hospital lab. The HUC also gives the mother a packet of information including the birth certificate and other important documents. This packet includes a brochure on NBS in Wisconsin. While the current brochure lacks specific information about which disorders are tested for, it does explain the purpose and process of the program. The HUC also verbally explains the basics of the NBS program, referring any specific questions to either a nurse or doctor.

A laboratory technician then comes to the unit to obtain the blood sample once the newborn is more than 24 hours old. The laboratory technician picks up the NBS card from the nursing station and goes to the mother's room to draw the blood. Before starting any portion of the heel stick blood collection procedure, the laboratory technician identifies the mother, baby and NBS card via name and barcode on their respective bracelets. The technician then warms the newborn's heel with a warm pack of no more than 42° C for three to four minutes prior to the stick. This technique increases blood flow to the heel, aiding in more efficient blood collection. A diaper with warm water added to the absorbent portion is most often used as a warm pack.

After warming, the technician selects a puncture site on either side of the heel per directions and diagrams on the reverse side of the Guthrie card. The puncture site should be selected from the medial or lateral position of the plantar surface of the heel. Previous puncture sites or the curvature of the heel must not be used. The site is first cleansed with 70% isopropanol and allowed to air dry. The technician then sticks the site with a disposable lancet with a point of 2.0 mm or less (or use of a special incision device) and allows blood to begin flowing while avoiding excessively "milking" the puncture site which may force out a disproportionately large amount of tissue fluids mixed with the blood. This could adversely affect laboratory results and is strongly cautioned against.

After the heel stick, the laboratory technician wipes away the first drop of blood with a sterile gauze pad, and then allows a large drop of blood to form on the heel. The technician then touches the blood drop to the preprinted circles on the filter paper part of the Guthrie card, taking care to not press the filter paper against the puncture site. If one large drop does not sufficiently fill in the circle on the Guthrie card (completely saturate the circle on both the front and back of the card), the technician waits for another large drop to form on the baby's heel. It is imperative that this drop is added to the card as quickly as possible so the blood does not have time to clot, which can alter laboratory tests. The technician repeats this process, moving in one direction across the Guthrie card, until all five circles are filled.

The lab collects the cards to let them dry for three hours, taking care to not close the biohazard flap onto the blood spots before they are fully dried. Prematurely closing the biohazard flap contaminates samples, leading to an unsatisfactory sample and the

necessity to re-stick the newborn. After the blood spots are completely dry, the hospital then sends them to the State Laboratory of Hygiene once daily.

D. Dried Blood Specimen Testing (based on reviews in Madison, WI and Denver, CO)

1. Background
The Wisconsin Newborn Screening Laboratory came into existence in May 1978 when the PKU statute was expanded to include three additional disorders (congenital hypothyroidism, galactosemia, and maple syrup urine disease). At that time, all testing was centralized to the Wisconsin State Laboratory of Hygiene. For the past 29 years, it has been the only laboratory performing newborn screening testing in Wisconsin. The NBS program is administered by the Maternal Child and Health Bureau of the Wisconsin Department of Health. Since 1990, the department has employed an advisory group system to determine which disorders will be screened for in Wisconsin. The system consists of an umbrella group that makes recommendations directly to the Wisconsin Secretary of Health and Human Services. The umbrella group relies on a series of sub-committees focusing on in particular disorders (endocrine, hemoglobinopathy, metabolic, and CF) to assist in making a decision about the addition of a particular disorder. Once a disorder or a group of disorders is approved for testing, the NBS laboratory is responsible for acquiring the appropriate technology, establishing reporting levels, reporting results, and short term follow-up of the abnormal test results. Specimens are received daily (Monday – Saturday) by the newborn screening laboratory and initial testing is completed within 24 hours of receipt. Repeat or second tier testing may delay the results anywhere from 24 to 96 hours. Most test results, especially for the more life-threatening disorders (i.e., galactosemia), are completed on the day of specimen receipt.

2. Specimen Receiving
Guthrie cards are collected from each birthing hospital in the state and delivered to the central testing lab(s) through their next-day delivery service. In Wisconsin, the NBS laboratory provides next day courier service, but about 15% of the birthing hospitals use their own courier service. As determined in Colorado, using a hospital courier service is encouraged to keep laboratory costs down. In Wisconsin, UPS guarantees delivery by 10:30 AM the following day, but special arrangements have been made so most specimens usually arrive at approximately 8:30 AM making it possible to start the testing on the day of receipt. Specimens arriving after 10:30 AM are set aside to be tested the following day.

Upon delivery, specimens are removed from their UPS envelope packaging. The Guthrie cards are then inspected thoroughly for irregularities that could affect testing procedures. First, the yellow biohazard cover sheet is removed and inspected for blood. If any blood is present and it appears to come directly from the sample spot (for instance, the biohazard flap is closed before the blood is completely dry), the spot is deemed unsatisfactory. Unsatisfactory spots are crossed out with a red Sharpie marker and not used for testing. Other irregularities leading to an unsatisfactory status include the

following: blood not completely soaked through to opposite side of card, dark/shiny spots within circle which suggest blood clotting, and filter paper damaged from scratching by capillary tube from which the blood came if the indirect collection method was used. At least three spots of the total five spots on the filter paper must be acceptable for screening or the entire card is deemed unsatisfactory. If less than three satisfactory circles of blood are acheived, the top sheet receives a large red X and is set aside. The hospital is then contacted and required to repeat the blood collection. In Wisconsin, an average of 1.5 % of Guthrie cards per year are unsatisfactory. Effort to have less than 2% of samples deemed unsatisfactory is crucial for capturing as many children as possible in a timely manner.

After all mailing envelopes have been opened, the specimens are barcoded for use with Specimen Gate™ or another laboratory information management system. Such systems aid in positive identification of samples, specimen tracking throughout the laboratory environment, follow-up, statistical reporting, and performance monitoring.

The "top slip" of the multi-parted forms are removed from the card and sent to the data entry staff who then create a computer record for each blood specimen, entering the demographic information (name, date of birth, mother, hospital, etc.) into the record stored in the NBS database. Data entry is double checked by one person reading aloud the top slip information and another person comparing it to the information entered into the database. Once the testing is completed, the laboratory test results are added to the computer records. By using laboratory information systems, it is eventually the goal to link hospital records to NBS records and eliminate the data entry step. However, technology does not currently allow for this linkage.

A pack of specimen cards is then taken to an automated punch machine where 1/8 inch disks are punched from the blood circles and delivered to a designated well of a 96 well microplate. The punch format is previously established for each test and programmed into the punch machine. The specimen number is displayed on the computer screen, and the operator manually confirms that the specimen in the machine is the same number as that on the computer screen. Paper format sheets are also available to identify which specimen number is placed into each well of the microplate.

Once the pack of cards is punched, the microplates fan out to the different testing stations in the laboratory. The punched specimen cards are put into plastic bags and left in the sample receiving area at room temperature for 2 days, after which they are transferred to a walk-in cooler (4°C) for long-term storage. After one year, the cards are destroyed by a private company which radiates the blood spots, shreds them, then disposes of the remains in a landfill. Some states elect to save specimens indefinitely; however, this practice is limited by availability and cost of storage space.

3. Specimen Testing

The laboratory performs five basic types of technology to accomplish all the testing: tandem mass spectrometry, immunochemistry, basic chemistry, electrophoresis, and mutation analysis. The technologies used in screening for the disorders are as follows:

Tandem mass spectrometry (MS/MS) simultaneously screens for 35 hereditary metabolic disorders by measuring 34 blood acylcarnitines and five amino acids. The basic principle is that the compounds (acylcarnitines and amino acids) of interest are first ionized and then enter into the first mass spectrometer which is electronically set by mass/charge ratio to identify the compounds of interest. The compounds then pass into a nitrogen collision chamber where the compound is systematically broken apart. Next, the fragments enter the second mass spectrometer where the fragment of interest is measured, directly relating to the compound concentration in the blood. Many of the metabolic disorders detected by tandem mass spectrometry have unique abnormal acylcarnitine profiles.

Hemoglobinopathy testing is a two-tiered process. The first tier is a type of electrophoresis called isoelectric focusing. With this technology, the various hemoglobin proteins (fetal, adult, sickle, C, D, or E hemoglobin) can be separated on an agarose gel based upon their individual isoelectric points. A visual check of the gel can identify the appropriate hemoglobins. Specimens with hemoglobins other than fetal and adult (normal) are re-analyzed by isoelectric focusing and by high performance liquid chromatography to both confirm what was visually seen on the gel and also quantify the relative amounts of hemoglobins present to distinguish traits from compound heterozygotes for an abnormal hemoglobin and beta thalassemia. The NBS laboratory also provides confirmatory testing on whole blood for babies identified through screening to have a hemoglobin disease. If results are judged to be affected by a transfusion, the results are reported as inconclusive, and a second sample is requested at a later date. However, the laboratory does report abnormal results along with the inconclusive report if abnormal results are obtained. The NBS laboratory also provides confirmatory testing on whole blood for babies identified through screening to have a hemoglobin disease

Endocrine disorders (hypothyroidism and congenital adrenal hyperplasia) are detected through immunochemistry techniques. In this technology, 96 well microplates are coated with an antibody specific for a certain antigen (TSH or 17-OHP). As the antigen elutes from the blood spot it either binds directly (TSH) to the bound antibody and a second labeled antibody or competes with a labeled antigen (17-OHP) for antibody sites. After incubation the excess fluorescent label is removed and the bound fluorescence is measured in a fluorometer. In the TSH "sandwich" assay, the more fluorescence, the higher the concentration in the blood. In the 17-OHP competitive assay, the more fluorescence, the lower the blood concentration. The basic chemistry technology consists of eluting the analyte (biotinidase or galactosemia enzyme) from the blood disk, reacting it with a substrate, and then measuring the product of that reaction either colorimetrically or fluorometrically.

i) Screening for Cystic Fibrosis

There are currently four different protocols used for screening for CF in the United States. While all four begin with an IRT assay, the second step varies by protocol. The protocols are the following: IRT/IRT, IRT/DNA, IRT/IRT/DNA, and the California method.

The first step in any of the four protocols consists of analyzing all specimens received for immunoreactive trypsinogen (IRT) by the time-resolved fluoroimmunoassay (DELFIA). A 1/8 inch sample disk is punched from the dried blood spot and placed in a 96 well microplate as described previously. The IRT assay is a sandwich assay where the wells of the 96 well microplate are coated with an anti-IRT IgG antibody. A solution containing europium labeled anti-IRT IgG antibody is added to the 96 well microplate containing the blood spots. As the IRT molecules elute from the blood spot, they bind with both the labeled and unlabeled antibody (i.e., sandwiched in between the two). After incubation, the blood spot and excess europium-labeled antibody are removed. An enhancement solution is added to separate the europium-labeled antibody from the bound antibody, and fluorescence is measured in a fluorometer. The fluorescence response for each specimen is compared against a set of IRT calibrators (specimens with known IRT levels), with results calculated in ng/dL of whole blood. The assay steps, including calculations, are completed by an automated instrument called an AutoDELFIA[13].

In IRT/IRT protocols, this step is repeated for all children when a second sample is received at two weeks of age. However, approximately 5% of children are lost to follow-up at this step in programs with routine second samples. This potential loss of an undiagnosed CF case can be avoided by using the IRT/DNA or California method because all assays are completed on one specimen. To be recommended for a sweat test using the IRT/IRT method, a child must have IRTs over 100 ng/mL and 70 ng/mL for their first and second specimens respectively.

The IRT/IRT/DNA method is similar to the IRT/IRT method due to its reliance on a second sample for a subsequent IRT at two weeks of age, but differs because second samples are not analyzed for children who had an IRT below 70 ng/mL in the first sample. If both IRTs are over the cutoffs of 70 ng/mL and ? ng/mL, the sample is then analyzed for DNA.

Quality control of the IRT assay consists of analyzing four specimen-like materials with known IRT concentrations in duplicate in each 96 well microplate. The Westgard philosophy is applied during this test for quality assurance of data. Two of the control levels are supplied with the reagent kit and two others are prepared in-house from commercially available trypsinogen, plasma and blood cells.

In the IRT/DNA, IRT/IRT/DNA and California protocols, the samples are tested for CF-causing mutations in the DNA. Previously, many labs only tested for the most common ΔF508 allele. However, Wisconsin implemented CFTR multi-mutation analyses when Massachusetts demonstrated[12] significant advantages of multi-mutation testing compared to ΔF508 detection. Multi-mutation panels vary by the product used and should be chosen based on the racial composition of the population being screened. For example, Colorado is electing to use a panel with multiple mutations common in Hispanic populations due to the high number of Hispanics living in their state.

After all of the 96 well IRT microplates have been analyzed and the results calculated, the highest 4 percent of the daily IRT results are referred for the second tier DNA testing.

The DNA testing consists of retrieving the specimen cards that require the second tier test from the packs of 76 specimens. After the appropriate specimens have been removed from the packs, a second analyst checks to make sure the correct specimens have been retrieved. A 1/8 inch disk is then manually punched into an appropriately labeled microcentrifuge tube. Special precautions are taken to avoid cross contamination between the specimens during punching. The DNA from each blood spot is eluted using a modification of the Gentra Generation DNA Purification and elution kit (Minneapolis, MN). The modification consists of adding sterile water and heating to 99°C for 20 minutes. After the DNA is eluded from the blood spot, 10 μL of the eluate is added to polymerase chain reaction (PCR) tubes that are held in a 96 well microplate. The PCR master mix which contains the primers for each of the CF alleles is then added to each tube along with the other required reagents for PCR. The 96 well microplate is then placed in a thermocycler (ABI 9700) and thermocycling for 30 cycles commences, which lasts for approximately 2 hours.

Upon completion of the thermocycling, the CF alleles are detected by a commercial detection kit (CF-Gold Lap, Roche Molecular Systems). This is a strip technology where the conjugates for the wildtype (typical form of the allele) and mutant alleles are imbedded in a paper strip. There is one strip for each specimen analyzed which is placed in the appropriate well of a 40 well tray. A volume of 200 μL of the PCR product for each specimen is added to the appropriate well (strip). After the addition of hybridization, wash, and staining solutions, the wild type and any mutations will appear on the strip as a horizontal blue line. A calibrated reference guide is used to identify the appropriate wildtypes and mutants CF alleles.

Quality control of the DNA mutation assay consists of analyzing a reagent blank (no DNA), a ΔF508 homozygote, and a ΔF508 heterozygote specimen with each run. For acceptance, the reagent blank must not show any DNA bands, the ΔF508 homozygote must show only the mutant band and no wildtype band, and the ΔF508 heterozygote must show both the wild type and mutant bands. If any of these above conditions are not met, the run is repeated. In addition to these control results, each normal and heterozygote specimen analyzed must show the wild type for each of the 25 mutations. The lack of DNA in the blood may cause some allele bands to not appear or be very faint. In this case, those specimens are repeated, often increasing the DNA concentration by punching a 3/16 inch spot instead of the standard 1/8 inch. The last quality assurance activity is to repeat those specimens with a mutant allele detected. In this case, a control with the suspected mutation is also analyzed along with the specimen in question. Repeating the DNA analysis on such specimens confirms that the correct specimen was punched initially and that the correct mutation was identified.

Blind quality control (QC) samples are also intermittently added to the daily samples received for analysis. In this case, the laboratory director slips in a Guthrie card with a known IRT and DNA status with the cards upon receipt from the delivery service. The blind QC then continues through the normal testing protocol until results are obtained and reported to the director of the laboratory. At this point, laboratory scientist learns that the sample is actually a blind QC and not a new CF diagnosis.

E. Communication with Primary Care Providers and Parents

Reporting of NBS test results for CF and other disorders is performed by the Newborn Screening Laboratory staff according to specific rules. The physician contacted, generally the infant's primary care provider (PCP), is the doctor listed on the Guthrie card. If test results are normal, a letter/report is mailed to the specimen submitter, who notifies the PCP who then tells the parent(s)—ideally in person during the first well baby visit at two weeks of age. If the test results are abnormal, there are three types of communications:
1) Specimens with IRT results ≥ 170 ng/dL (99.9th percentile) and no mutations are reported as a possible abnormal. This is a single page (blue) report that has a banner that reads "Possible Cystic Fibrosis Report". This report states that no mutation was detected that sweat testing is recommended only if there is a family history of CF or has clinical signs of CF.
2) Specimens with one mutant CF allele are reported on a single page report (blue) with a banner that also reads "Possible Cystic Fibrosis Report". It lists the IRT results, the mutation detected, and a message that a sweat test is required at a Cystic Fibrosis Foundation certified center. Single allele reports are faxed to the PCP and a regional CF center. Friday reports are held until the following Monday.
3) Specimens with two mutant CF alleles are reported on a single (yellow) report with a banner that reads "Definite Cystic Fibrosis Report". It lists the IRT value, the genotype and a message that a sweat test is required at a Cystic Fibrosis Foundation certified center. Specimens that have a CF genotype (two mutations) are also called to the PCP and faxed to the regional CF center. There is no standard method of communicating with parents (i.e., PCPs are not given instructions on how to communicate the information arising from the NBS tests.)

When a laboratory scientist calls a PCP to inform them of an abnormal result, a script or outline detailing the main topics of the communication is helpful technique for minimizing communication errors between the laboratory scientist and PCP.

F. Initial Follow-up Assessment at the Pediatric CF Clinic, University of Wisconsin Hospitals and Clinics in Madison, WI

1. Diagnosis

Of the following five groups of newborn IRT/DNA test results, groups 3, 4, and 5 with a family history of CF are referred to the Pediatric CF Clinic:

1. IRT < 96% - normal report
2. IRT > 96% with no mutation – normal report
3. IRT > 96% with two mutations – definite CF report
4. IRT > 96% with one mutation – possible CF report
5. IRT > 99.8% with no mutation – possible CF report

Groups 4 and 5 are considered possible cases of CF; however, while group 5 is a possible case, the chance is remote. As a possible CF case, newborns are brought in for sweat testing and genetic counseling in one visit. Prior to 2000, families were told to wait until their baby was four weeks old to be brought in for follow-up testing. However, it was determined that a delay of four weeks caused high anxiety for family members and could be associated with malabsorption and malnutrition in CF infants. Now it is practice that the baby has a sweat test as early as fourteen days after birth. The sweat test method used is the quantitative pilocarpine iontophoresis test (QPIT) developed originally by Gibson and Cook[15] with measurement of chloride concentration. This procedure should be performed according to CLSI guidelines.[16] To increase consistency and accurateness of results, training a limited number of laboratory technicians to perform sweat tests is advised. Through this practice, variation in the results by administrator of the sweat test is decreased. Interpretation of sweat chloride concentrations is as follows:

1. Cl ≥ 60 mEq/L → definite CF case
2. Cl = 40-59 mEq/L → probable CF case
3. Cl = 30-39 mEq/L → possible CF case

While waiting approximately one hour for the results of the sweat test, parents receive genetic counseling. If the sweat test is negative, the families receive counseling regarding CF carrier status. In this session, it is clearly outlined that their child does not have CF and will never develop it later in life. However, it is emphasized that the infant is a carrier for a CF mutation, meaning at least one of the parents is a carrier. Parents of children that are carriers also receive a brochure regarding carrier status along with the genetic counseling session. The brochure illustrates the transmission pattern for the mutations and outlines implications of carrier status. This time while the parents are waiting for the results of the sweat test is optimal for communication as well as relationship building with the nurse in the CF center. Along with easing parental stress and anxiety regarding test results, a strong relationship facilitates transition to the clinic for positive sweat test results.

Group 3 newborns are considered definite cases of CF (i.e., they have been presumptively or genetically diagnosed with CF from the newborn screen). A sweat test is scheduled for the newborn within ten to fourteen days after birth. However, if a sibling has already been positively diagnosed with CF, the newborn does not need a sweat test and is still definitively diagnosed with CF.

If a sweat test is positive and confirms CF diagnosis, the CF center specialists tell the family. For consistency of information given during this session, giving the specialists specific talking points is useful.

To confirm that the appropriate number of CF cases are diagnosed through NBS, one can compare incidence rates of CF in the population to detection rates via NBS. If the numbers vary dramatically, a portion of the NBS process has gone awry and must be examined more closely.

2. Treatment

After positive CF confirmation from the sweat tests, the education and therapy portion of the first clinical visit begins. During this first visit, education is key. While the family is undoubtedly upset about the diagnosis of a potentially life-shortening disease, explanation and education regarding the disease itself and its effects on the body systems are crucial during this visit. Important topics covered in this session include the administration of salt supplements to prevent hyponatremic dehydration, especially during the summer months. Principles of infection control are also covered in this first visit. For example, families are taught to restrict their child's interaction with other, unrelated children with CF because of the threat of bacterial transmission. If there is any evidence that the baby has pancreatic insufficiency, the family is taught the enzyme supplement regimen and begins the administration of it immediately. University of Wisconsin doctors begin the enzyme regimen prior to analyzing the stool for elastase content. This conservative approach is key to preventing the malnutrition often associated with CF. Along with beginning enzymes, the newborn is started on CF specific fat-soluble vitamins. The Madison CF Center has decided that it is more beneficial to start fat-soluble vitamins immediately than to wait for the blood tests to determine if the newborn's levels are low. These two conservative approaches are used with the hope of preventing any malnutrition or failure to thrive and are based on the following expectation from research elsewhere: half of CF patients will have fat malabsorption during the first month of life, two-thirds by six months, and about 90% by one year of age.

During the second visit, scheduled six weeks later, the pediatric pulmonologists teach the family the chest physiotherapy regimen. At the Madison clinic, doctors have concluded that attempting to teach physiotherapy during the first visit is overwhelming and retention of the technique is low. Therefore, waiting until the second visit allows the families to adjust psychologically to the CF diagnosis and leads to greater ability to remember the chest physiotherapy regimen. Also during the second visit, the baby receives their first throat swab culture for respiratory pathogens, a practice which is continued at every visit after. During the first year of life, clinic visits are scheduled every six weeks; after the child reaches one year of age, the visits are every three months.

G. Monitoring of Nutrition and Pulmonary Status

By six months of age, the child has a variety of blood tests performed. This gives doctors information regarding the child's complete blood count as well as levels of liver enzymes, vitamin A and E, bilirubin, and blood glucose. At approximately age four years, the child begins using the spirometer to test for pulmonary function test (PFT). While it is often the case that the PFTs will not be interpretable until about six years of age, starting at four allows the child to become familiar with the routine and practice proper technique.

Beginning at ages 8 to 10 years, children start preparing for the transition to adult CF clinic. While the actual transition does not usually occur until they are 18 to 21, the transition process begins much earlier. The University of Wisconsin Hospitals and

Clinics uses a unique booklet outlining the transition process and what the clinic expects from the child at each step along the way. For example, at ages 8 to 10, children are expected to be able explain what it means to have CF, begin to understand how their lungs work, and help their parents remember their enzymes, among other tasks. By age 14, the patient is expected to begin clinic visits without their parents and by 18 to 21, transition to the adult CF clinic. Along with the booklet is an insert with pictures and names of the doctors and nurses associated with the adult CF clinic to aid in the transition to the unknown. Oftentimes, many young adults will tour the adult clinic and get to know the staff prior to their care transition. The close association with the two clinics helps with the difficult transition period.

H. Quality Assurance

1. Newborn Screening Quality Assurance Program (NSQAP)
In an effort to increase technical proficiency and maintain confidence in laboratories' performances while processing large volumes of specimens, the CDC has undertaken the challenge of implementing a global Quality Assurance (QA) Program for NBS for a variety of disorders including CF (http://www.cdc.gov/labstandards/nsqap.htm). This voluntary program has grown substantially in the past ten years to encompass 372 NBS laboratories in 55 countries with 35 laboratories participating in the CF portion as of February 2007. The NSQAP, headquartered at the CDC and co-sponsored by the Association of Public Health Laboratories, consists of two branches: quality control (QC) materials for periodic use and quarterly proficiency testing (PT). The QC materials are intended to supplement and aid in monitoring the long-term consistency of their assay of choice. The PT portion provides laboratories with unidentified dried blood specimens to give the lab an independent external assessment of their performance.

With respect to CF, there are three specific programs that are available: IRT/DNA PT, CF Mutation Detection Pilot PT, and IRT QC. The IRT/DNA PT program challenges both tiers of CF screening allowing specimens to follow the laboratory's algorithm of initial IRT testing and confirmatory mutation analysis. The specimens with high IRTs are prepared with ΔF508 homozygous or heterozygous lymphoblasts that have been immortalized with Epstein Barr Virus and grown in culture. Consequently, these specimens can be used for mutation detection. Participants may report IRT only or both IRT and mutation results. There are 29 domestic participants and 66 foreign laboratories participating in this program.

The second PT program, the CF Mutation Detection Pilot PT program, began in February 2007. The specimens are prepared from anonymous adult or adolescent CF donors and are not tested for IRT. Therefore, only the second tier of testing is challenged. This allows NSQAP to assess a laboratory's ability to detect a variety of mutations, not only ΔF508. There are 14 domestic participants and 13 foreign participants. We expect this program to grow quickly due to the number of laboratories adding CF to their testing panel and using mutation analysis.

IRT QC, the final CF program, has 29 domestic participants and 68 foreign participants. There is no fee for participation in any of the NSQAP programs; laboratories are only asked to report their results once they are obtained. By using external and internal assessments of performance, maintenance of high standards of accuracy is obtained in NBS programs for CF.[17]

2. Program Evaluation and Assessment Scheme (PEAS)

Developed as a cooperative agreement between the Health Resources and Services Administration Maternal and Child Health Bureau, Genetic Services Branch and the National Newborn Screening and Genetics Resource Center, Department of Pediatrics at the University of Texas Health Science Center at San Antonio, the Program Evaluation and Assessment Scheme (PEAS) was designed as a tool to aid programs in self-evaluation and give direction for quality assurance measures. The PEAS recognizes that there are six distinct components to the NBS process: education, screening, follow-up, diagnosis, management, and evaluation, and that specific sets of criteria should be used to analyze each portion of the process. These criteria are provided by the PEAS, comprised as a uniform set of questions that can be used as a self-assessment tool (see appendix III). Although the PEAS committee members acknowledge that NBS programs can be successful in a variety of ways, these criteria provide a standard platform on which to begin.[18]

Appendices

I) Ten Steps to Conduct a PFMEA (source?)

Step 1: Review the Process—use a process flowchart to identify each process component
Step 2: Brainstorm potential failure modes—review existing documentation and data for clues
Step 3: List potential effects of failure—there may be more than one for each failure
Step 4: Assign Severity rankings—based on the severity of the consequences of failure
Step 5: Assign Occurrence rankings—based on how frequently the cause of the failure is likely to occur
Step 6: Assign Detection rankings—based on the chances the failure will be detected prior to the customer finding it
Step 7: Calculate the RPN—Severity X Occurrence X Detection
Step 8: Develop the action plan—Define who will do what by when.
Step 9: Take action—implement the improvements identified by your PFMEA team
Step 10: Calculate the resulting RPN—re-evaluate each of the potential failures once improvements have been made and determine the impact of the improvements

II) Current IRT Cutoff Values for Domestic and Foreign Laboratories[19]

Laboratory		IRT cutoff ng/mL	Method	Mean	Mode
Domestic	1	90	AutoDelfia	95	100
	2	105	AutoDelfia		
	3	70	Delfia		
	4	70	AutoDelfia		
	5	170	AutoDelfia		
	6	90	AutoDelfia		
	7	100	AutoDelfia		
	8	>95 percentile	Delfia		
	9	170 + top 4% of daily run	AutoDelfia		
	10	100	AutoDelfia		
	11	100	Delfia		
	12	90	AutoDelfia		
	13	170	ICN		
	14	63	AutoDelfia		
	15	65	AutoDelfia		
	16	100	AutoDelfia		
	17	100	AutoDelfia		
	18	105	AutoDelfia		
	19	100	AutoDelfia		
	20	63	AutoDelfia		
	21	90	Delfia		
	22	90	ICN		
	23	70	Delfia		
Central and South America	1	55	AutoDelfia	72	90
	2	70	AutoDelfia		
	3	70	AutoDelfia		
	4	60	Delfia		
	5	32	AutoDelfia		
	6	90	AutoDelfia		
	7	90	Delfia		
	8	90	Delfia		
	9	90	AutoDelfia		
Europe	1	60	Delfia	67	70
	2	65	AutoDelfia		
	3	70	AutoDelfia		
	4	60	AutoDelfia		
	5	120	AutoDelfia		
	6	75	DiaSorin		
	7	70	Other		
	8	60	AutoDelfia		
	9	70	AutoDelfia		
	10	70	AutoDelfia		
	11	70	AutoDelfia		
	12	70	AutoDelfia		
	13	65	AutoDelfia		
	14	65	AutoDelfia		

	15	65	AutoDelfia		
	16	60	AutoDelfia		
	17	50	ELISA-home made		
	18	68	AutoDelfia		
	19	55	AutoDelfia		
	20	60	Delfia		
	21	70	Delfia		
	22	70	AutoDelfia		
	23	50	BioRad Quantase		
	24	70	AutoDelfia		
Asia and South Africa	1	75	Delfia	65	50
	2	65	Delfia		
	3	60 or top 2% of assay	AutoDelfia		
	4	50	Bioclone		
	5	78	Delfia		
	6	86	BioRad Quantase		
	7	50	BioRad Quantase		
	8	60	BioRad Quantase		
	9	70	Delfia		
	10	70	Delfia		
	11	50	BioRad Quantase		

III) Self-Reported Unacceptable Specimens[20] in 2005

State/ Territory	Total number of specimens received	Number of specimens unacceptable	% of samples unacceptable
Alabama		0	0.00%
Alaska	19,924	250	1.25%
Arizona			
Arkansas	39,805	402	1.01%
California	546,380	2453	0.45%
Colorado	156,256	5368	3.44%
Connecticut	42,193	79	0.19%
Delaware	23,989	65	0.27%
District of Columbia			
Florida	314,401	14932	4.75%
Georgia	183,174	4175	2.28%
Hawaii	17,881	57	0.32%
Idaho	41,794	604	1.45%
Illinois	182,799	686	0.38%
Indiana	107,983	798	0.74%
Iowa	41,041	450	1.10%
Kansas	40,657	2718	6.69%
Kentucky			
Louisiana	63,979	3296	5.15%
Maine	13,912	124	0.89%
Maryland	133,798	6182	4.62%

Massachusetts	86,549	1553	1.79%
Michigan	133,680	3094	2.31%
Minnesota	73,857	561	0.76%
Mississippi	42,877	991	2.31%
Missouri	88,498	1563	1.77%
Montana			
Nebraska	26,288	159	0.60%
Nevada	69,473	1018	1.47%
New Hampshire	14,842	84	0.57%
New Jersey	123,283	844	0.68%
New Mexico	53,762	2074	3.86%
New York	261,567	4059	1.55%
North Carolina	137,700	3777	2.74%
North Dakota	10,255	126	1.23%
Ohio	151,193	315	0.21%
Oklahoma	54,437	532	0.98%
Oregon	92,050	670	0.73%
Pennsylvania	148,189	1824	1.23%
Rhode Island	14,088	63	0.45%
South Carolina	55,208	680	1.23%
South Dakota	12,728	56	0.44%
Tennessee	97,213	5278	5.43%
Texas	751,855	10807	1.44%
Utah	103,466	678	0.66%
Vermont	6,513	80	1.23%
Virginia	114,848	1856	1.62%
Washington	152,599	1430	0.94%
West Virginia	29,739	2088	7.02%
Wisconsin	74,872	940	1.26%
Wyoming	6,399	357	5.58%

IV) Recommended Core Mutation Panel for General Population CF Screening[21]

DF508	DI507	G542X	G551D	W1282X	N1303K
R553X	621+1G®T	R117H	1717-1G®A	A455E	R560T
R1162X	G85E	R334W	R347P	711+1G®T	1898+1G®A
2184delA	1078delT	3849+10kbC®T	2789+5G®A	3659delC	I148T
3120+1G®A					

V) A Sample of the PEAS[17] Criteria

III. ANALYTICAL CONSIDERATIONS

A. TESTING PROCESS - The testing processes must undergo rigorous quality checks in order to ensure accurate screening results. Up-to-date procedure manuals that meet the requirements of CLIA '88 are required, including relevant updates, and the laboratory must have a CLIA/CAP certificate for operation. There must be an overall quality assurance plan with defined corrective actions to be taken when quality control indicators detect problems. Testing must be accurate and precise, and testing must occur within a time period sufficient to accomplish the goal of early detection and treatment as a preventive measure. *(Note: All terms and definitions within this section are those used in CLIA'88.)*

PERFORMANCE INDICATOR
FINDINGS

	Yes	No	In Progress
1. Procedures Manual:			
a. There is a CLIA compliant laboratory procedure's manual(s).	☐	☐	☐
b. The procedure's manual defines the process for establishing analytical ranges for the various screening tests.	☐	☐	☐
c. The procedure's manual(s) is updated as procedural changes occur.	☐	☐	☐
d. All procedural changes are appropriately documented with date and person changing.	☐	☐	☐
e. Discontinued procedures are archived with documentation of the date the procedure was begun, the date discontinued, and the person archiving.	☐	☐	☐
f. There is a documented review of the procedure's manual(s) by the laboratory technical supervisor at least annually.	☐	☐	☐
g. A working procedure's manual(s) is readily available to all technical personnel.	☐	☐	☐
h. There is a written course of emergency action to be taken if a test system fails.	☐	☐	☐
i. There is an accountability process that ensures that personnel follow the procedures included in the procedure's manual(s).	☐	☐	☐
2. Quality Assurance Program:			
a. There is a written quality assurance plan for the laboratory.	☐	☐	☐
b. There is a periodic review of the quality assurance plan.	☐	☐	☐
c. There is routine documentation and review of all quality assurance events.	☐	☐	☐
d. The quality assurance plan defines a quality control process in the laboratory.	☐	☐	☐
e. The quality assurance plan specifies a process for:			
i. Sharing quality assessment findings among staff members in order to resolve problems.	☐	☐	☐
ii. Reviewing the effectiveness of corrective actions taken with appropriate staff.	☐	☐	☐
iii. Preventing recurrence of identified problems.	☐	☐	☐
iv. Obtaining information to assess clinical validity of screening tests.	☐	☐	☐
v. Comparing screening test results with confirmatory testing results and resolving any discrepancies.	☐	☐	☐
vi. Documenting corrective actions for any discrepancies found.	☐	☐	☐
f. The methods for using quality control materials specify:			
i. The number, type and frequency of testing controls for each different analytical procedure.	☐	☐	☐
ii. An identical analytical process for analysis of controls and patient's specimens.	☐	☐	☐
iii. Acceptability criteria with corrective actions if a test system is "out of control."	☐	☐	☐
iv. A protocol for detecting/correcting errors resulting from a test system failure.	☐	☐	☐
v. A protocol for detecting/correcting errors caused by operator performance.	☐	☐	☐

vi. Documentation of corrective actions in the event of error detection. ☐ ☐ ☐

g. The use of control materials complies with CLIA '88 including:
   i. A procedure for validating the concentration of control materials. ☐ ☐ ☐
   ii. A procedure for establishing acceptable accuracy limits for controls. ☐ ☐ ☐
   iii. A procedure for establishing acceptable precision limits for controls. ☐ ☐ ☐
   iv. A process for monitoring assay accuracy based on control results. ☐ ☐ ☐
   v. A process for monitoring assay precision based on control results. ☐ ☐ ☐
   vi. Documentation of corrective actions in the event of accuracy or precision noncompliance. ☐ ☐ ☐

h. Validation of assay calibrators/standards complies with CLIA '88 including:
   i. A process for validating calibrator/standard concentrations before routine use. ☐ ☐ ☐
   ii. A process for documenting all calibrator/standard validation activity. ☐ ☐ ☐
   iii. A process for actions to be taken when performance of controls indicates a deviation from the anticipated results. ☐ ☐ ☐
   iv. Criteria for establishing acceptable assay performance (linearity, CV, etc.) ☐ ☐ ☐
   v. A process for documenting acceptable assay performance. ☐ ☐ ☐
   vi. A process for action(s) when assay performance is unacceptable. ☐ ☐ ☐
   vii. Documentation of corrective actions when unacceptable assay performance is noted. ☐ ☐ ☐

i. The use of commercial assay kits and kit components complies with CLIA '88 including:
   i. A protocol for reagent kit validation (both accuracy and precision). ☐ ☐ ☐
   ii. Documentation of kit validation prior to routine use. ☐ ☐ ☐
   iii. Adherence to manufacturer's protocol and/or documentation of all deviations ☐ ☐ ☐
   iv. Documenting acceptable kit performance indicators on a routine basis. ☐ ☐ ☐
   v. Where appropriate, validating/documenting proper instrument operation. ☐ ☐ ☐
   vi. Documenting comparative evaluations of new lots of kit components with lots currently in use. ☐ ☐ ☐
   vii. Validation/documentation of methodology for those kits not FDA approved. ☐ ☐ ☐

j. There is a procedure for validating reagent performance including**:**
   i. Comparison to stated precision/accuracy specifications (commercial reagents). ☐ ☐ ☐
   ii. Establishing performance specifications (prepared reagents). ☐ ☐ ☐
   iii. Documenting performance characteristics. ☐ ☐ ☐

k. The laboratory satisfactorily participates in dried blood spot and other CLIA compliant, external proficiency testing activities. ☐ ☐ ☐

l. There is a comprehensive written procedure for handling analysis, review and reporting of proficiency testing results. ☐ ☐ ☐

m. The analytical procedure for analyzing proficiency and patient specimens is identical. ☐ ☐ ☐

n. To the extent possible, proficiency samples are run for each analyte in the laboratory. ☐ ☐ ☐

o. Personnel who routinely perform testing test proficiency specimens. ☐ ☐ ☐

p. Proficiency testing records are accessible on site for at least two years. ☐ ☐ ☐

q. There is a written procedure for investigating, documenting and correcting problems identified as a result of unacceptable proficiency testing results. ☐ ☐ ☐

r. There is documentation of appropriate review of proficiency testing results by the technical laboratory supervisor. ☐ ☐ ☐

s. There is documentation of corrective actions taken in the event of a proficiency testing error. ☐ ☐ ☐

VI) Standard Operating Procedure (SOP) for IRT/DNA testing procedure in Wisconsin

IRT:

1. After specimens have been removed from their packaging, inspected, and deemed satisfactory, punch 1/8 inch disks from the dried blood circles and put in a 96 well microplate.
2. Add solution containing europium labeled anti-IRT IgG antibody to each well of the 96 well microplate.
3. Incubate.
4. Add enhancement solution to each well.
5. Use AutoDELFIA to read fluorescence values and send the top 4% of daily values or values over 170 ng/mL for the next tier of testing.

DNA multi-mutation analysis:

1. Add DNA purification solution to a 1.5 mL microfuge tube, aspirate; add DNA purification solution again, aspirate.
2. Add DNA elution solution, aspirate.
3. Add water; aspirate.
4. Heat samples for 15 minutes in dry heating block.
5. Vortex samples briefly.
6. Short spin in microfuge.
7. Prepare the PCR Master Mix and samples in the following manner
    a. 10X PCR buffer 10 µL
    b. CF-Gold Primer Mix 10 µL
    c. 100 µM dATP 0.3 µL
    d. 100 µM dCTP 0.3 µL
    e. 100 µM dGTP 0.3 µL
    f. 100 µM dUTP 0.6 µL
    g. nuclease free water 37.0 µL
    h. uracil deglycosylase 3.0 µL
    i. Taq polymerase 3.5 µL
8. In a PCR tube, 65 µL volume of Master Mix is added to 25 µL of 32 mM MgCl2 and 10 µL of DNA extract for a total volume of 100 µL.
9. Add samples to the Thermocycler and allow to cycle in the following way: 95° for 30 seconds: denaturing of double stranded DNA.
10. 60° for 30 seconds: annealing of DNA and primers.
11. 72° for 1 minute: extending of DNA from primers.
12. Repeat process for 32 cycles or approximately 2 hours.
13. After thermocycling, wash products in 5 mL of wash solution for 3 minutes at room temperature.
14. Incubate 200 µL of product at 50° C for 25 minutes.
15. Aspirate; wash.
16. Aspirate; add 5 mL of conjugate solution and incubate at 50° C for 20 minutes.
17. Aspirate; wash for 5 minutes at room temperature.
18. Aspirate; add 5 mL of citrate buffer and incubate for 7 minutes at room temperature.
19. Aspirate; add 5 mL of substrate solution and incubate for 15 minutes at room temperature.
20. Aspirate; wash with 3 mL of deionized water.

21. Read CF GOLD 1.0 Reference Guide within 30 minutes of finishing procedure.

References

1. Grosse SD et al. Newborn Screening for Cystic Fibrosis Evaluation of Benefits and Risks and Recommendations for State Newborn Screening Programs. *Morbidity and Mortality Weekly Report*. 2004; 53(RR-13): 1-39.
2. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketouria in large populations of newborn infants. *Pediatrics*. 1963; 32: 338-343.
3. Farrell PM, Elias. 2006. (due to be published this month)
4. Kwon C, Farrell PM. The magnitude and challenge of false-positive newborn screening test results. *Arch Pediatr Adoles Med*. 2000; 154: 714-718.
5. Newborn screening: toward a uniform screening panel and system. Department of Health and Human Services. *Federal Register*. 2005; 70: 11247.
6. Therrell BL, Lloyd-Puryear MA, Mann MY. Understanding newborn screening system issues with emphasis on cystic fibrosis screening. *Journal of Pediatrics*. 2005; 147:6-10.
7. Farrell MH, Farrell PM. NBS for cystic fibrosis: ensuring more good than harm. *Journal of Pediatrics*. 2003; 143:707-712.
8. Farrell PM, Li Z, Kosorok MR, Laxova A, Green CG, Collins J, et al. Bronchopulmonary disease in children with cystic fibrosis after early or delayed diagnosis. *American Journal of Respiratory Critical Care Medicine.* 2003; 168:1100-1108.
9. Mischler EH, Farrell PM. Newborn screening for cystic fibrosis. *Adv Pediatr*. 1992; 39: 31-64.
10. Crossley JR, Elliott RB, Smith PA. Dried-blood spot screening for cystic fibrosis in the newborn. *Lancet.* 1979; i:472-474.
11. Hannon et al. Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard-Fourth Edition. *NCCLS*. 2003; 17:1-5.
12. Comeau AM, Parad RB, Dorkin HL et al. Population-based newborn screening for genetic disorders when multiple mutation DNA testing is incorporated: a cystic fibrosis newborn screening model demonstrating increased sensitivity but more carrier detections. *Pediatrics*. 2004; 113: 1573-1581.
13. AutoDELFIA Neonatal IRT manual. PerkinElmer Life and Analytical Sciences. Wallac Oy, Mustionkatu 6, Turku, Finland.
14. Linear Array CF Gold 1.0 manual. Roche Molecular Systems. Branchburg, NJ. Version 4; 2003.
15. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat for cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. *Pediatrics*. 1959; 23: 545-549.
16. LeGrys VA et al. Sweat Testing: Sample Collection and Quantitative Analysis; Approved Guidelines-Second Edition. *NCCLS*. 2000. 20(14): 1-40.
17. "Newborn Screening Quality Assurance Program: 2006 Annual Report". Centers for Disease Control and Prevention and the Association of Public Health Laboratories; 2007.

18. “Program Evaluation and Assessment Scheme (PEAS)”. Health Resource and Services Administration Maternal and Child Health Bureau, Genetic Services Branch and National Newborn Screening and Genetics Resource Center, Department of Pediatrics at the University of Texas Health Science Center at San Antonio. Aug 25, 2006.
19. “Current IRT Cutoff Values for Domestic and Foreign Laboratories”. Newborn Screening Division of the CDC. Jun 2007.
20. “Laboratory Specimen Information”. National Newborn Screening Information System: http://www2.uthscsa.edu/nnsis/.
21. Grody W, Cutting G, Klinger K, Richards CS, Watson M, Desnick R. Laboratory Standards and Guidelines for Population-based Cystic Fibrosis Carrier Screening. *Genetics in Medicine*. 2001; 3:149-154.
22. Crossley JR, Elliott RB, Smith PA. Dried-blood spot screening for cystic fibrosis in the newborn. Lancet 1979; 1:472-474.
23. Chatfield S, Owen G, Ryley HC, et al. Neonatal screening for cystic fibrosis in Wales and the West Midlands: clinical assessment after 5 years of screening. Arch Dis Child 1991; 66:29-33.
24. Fost N, Farrell PM. A prospective randomized trial of early diagnosis and treatment of cystic fibrosis: a unique ethical dilemma. Clin Res 1989; 37:495-500.