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Screening Newborns for Cystic Fibrosis

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SCREENING NEWBORNS FOR CYSTIC FIBROSIS

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Background

Definition of the disease

Cystic fibrosis (CF) is one of the most common autosomal recessive disorders among Caucasian children.[1-4] This genetic life-threatening multi-organ disease in children and young adults is caused by mutations of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, leading to defective chloride channel functioning and the classic clinical triad of pancreatic insufficiency, chronic pulmonary disease, and elevated sweat chloride concentrations.[1-3,5]

In approximately 15% to 20% of children with CF, the first symptom is meconium ileus, an intestinal obstruction present at birth that is highly specific to CF and usually requires corrective surgery.[6,7] Potentially lethal protein-energy malnutrition develops in some infants. By four years of age, about 85% of children have some degree of mal-absorption.[5] The most common symptoms among children with CF and without meconium ileus include recurrent cough, wheezing, chronic abdominal pain, loose stools, and failure to thrive.[6]

Newborns initially have normal respiratory function but within a few months, the defective epithelia chloride channel may lead to abnormal respiratory secretions, broncho-pulmonary infection, and airway obstruction.[5] Most children with CF develop chronic infections with unusual respiratory pathogens such as staphylococcus aureus and pseudomonas aeruginosa (Pa).[5] Pulmonary disease is the most important cause of severe disability and premature death in children with CF. Recent evidence suggests that pulmonary inflammation in infants with CF occurs as early as four weeks of age.[4]

There is great heterogeneity in the clinical manifestations of CF.[8] The majority of children suffer from classic (or typical) CF, whereas some children suffer from non-classic (or atypical) CF. It is very important to distinguish these categories in order to avoid unnecessary and burdensome treatment and making incorrect assumptions about the prognosis for the individual child.[8]

Epidemiology

An estimated incidence of CF in Caucasians throughout the world has been reported to be between 0.25 to 5 % per 10,000 live births.[4] CF can be found in virtually every ethnic group but is most prevalent among children of Northern or Central European descent. Prevalence varies considerably across different ethnic groups, ranging from 1 in 2000 in Ireland to around 1 in 500,000

in Japan.[9] The heterogeneous nature of the condition to some extent reflects the large number of mutations that can affect the CFTR gene and the impact of other gene modifiers.[10]

CF is the second most common life-shortening, childhood-onset inherited disorder in the United States (US). On the basis of US data from newborn screening programs, birth prevalence is 1 in 2500 to 3500 births among non Hispanic whites, 1 in 4000 to 10,000 births among Hispanics, and 1 in 15,000 to 20,000 births among non-Hispanic blacks.[6] In 2000, the overall birth prevalence of CF in the US was approximately 1 in 3700 births.[6] Approximately 1000 children and adults in the US are diagnosed with CF each year.[6]

According to the Report of the Canadian Cystic Fibrosis Foundation Patient Data Registry,[11] in 2002, there were 3453 individuals with CF in Canada, with 120 individuals being newly diagnosed. Caucasians account for 95% of these cases, whereas the proportions of affected individuals among other ethnic groups (Black, Asiatic, American Indian and other) ranged from 0.5% to 1.5%.[11]

Analysis based on the Canadian Cystic Fibrosis Foundation Patient Data Registry showed that between 1971 and 1987, the overall CF birth rate was 1 in 2714, with no increasing or decreasing trend.[12] A decline in CF birth rates was observed from 1998 to 2000, yielding an estimated birth rate for year 2000 of 1 in 3608, a 25% decrease over the 12-year period.[12] No Alberta data are available.

Early identification

Clinical suspicion of CF arises in the following situations: family history of CF (it generally occurs in about 20% of cases), pattern of meconium ileum at birth, and pattern of intestinal mal absorption or chronic obstructive pulmonary disease. In the event of clinical suspicion, CF is confirmed by the gold standard diagnostic test, the measurement of sweat chloride concentrations.[1,13,14]

In general, sweat chloride test results are reported as positive ($>$ 60 mmol/l), borderline (40 to 60 mmol/l), or negative ($<$ 40 mmol/l).[15,16] A sweat chloride level above 60 mmol/l in the absence of CF is rare, although it has been reported in a number of unusual clinical conditions that can readily be distinguished from CF.[8]

CF has many characteristic signs and symptoms, but establishing a timely diagnosis remains a major challenge.[5] Early clinical recognition of CF on the basis of symptoms (without meconium ileus) is desirable but difficult because the majority of symptoms are not specific to CF. Consequently, affected

children often initially receive a diagnosis of food allergies, celiac disease, asthma, or bronchitis rather than CF.[6] Misdiagnosis can result in multiple office visits, unnecessary diagnostic tests and hospitalizations, considerable costs to the health-care system, and anxiety for parents.

Delays in obtaining a sweat test are common. With conventional methods of identification (the presence of pulmonary disease or pancreatic insufficiency and a sweat chloride level greater than 60 mmol/l), children with CF often have symptoms for months and years before definitive therapy is begun.[5,17] About half of these children have severe malnutrition or chronic lung disease at diagnosis.[5] The average age of diagnosis is about three years of age in the US 5 and 3.5 years of age in Canada (from 2000 data).[11]

Recognizing the limitations of sweat chloride testing and the potential value of early diagnosis, attempts to establish newborn screening programs for CF was initiated as early as 1970.[5]

Early treatment

Treatment options for CF include treating pulmonary infection and management of malnutrition;[18-20] however, there is currently no curative treatment for CF.[10,13] With the classic (typical) severe phenotype, clinical outlook without treatment is poor, with death frequently occurring in the first decade of life.[10] The severity of pulmonary disease generally determines prognosis.[5]

Over the past 30 years there has been a marked increase in the life expectancy of children and adults with CF.[4] The majority of children with CF in the US now survive into adulthood, with median predicted survival of 34 years. A decline of 45% to 70% in CF mortality rates for children 2 to 15 years of age during 1985 to 1999 have been reported.[7]

However, the attribution to this improvement is still under debate. Some believe that most of the increase in life expectancy has been attributed to improvements in therapy. It has been argued that therapeutic interventions administered before the onset of signs or symptoms may have the greatest long-term benefit.[4]

Given the recognized cohort effect in CF mortality (and presumably morbidity), comparisons between earlier unscreened cohorts with later screened cohorts may incorrectly attribute the better outcome in screened cohorts to earlier diagnosis. Comparisons based on non-experimental studies used concurrent controls may alsobe biased as non-random assignment of centres or regions to screening may be confounded with social factors or access to medical care which may influence outcome.[4]

Although improvement of survival rates and outcomes seem to be associated with the introduction of more successful treatment regimens provided by specialized centres, it is still uncertain whether other factors, such as diagnosis of milder cases, contributed to this observed improvement. Therefore, considerable doubt remains about whether treatments started before symptoms appear and lead to a CF diagnosis really influence the course of CF beneficially and alter the outcome.[21]

According to two systematic reviews,[4,7] only two randomized controlled trials (RCTs) on the effects of early treatments as a result of screening for CF have been undertaken to date, one in the United Kingdom (UK) and the other one in the US. The Cochrane review[4] based on the analysis of the RCT conducted in the US (Wisconsin) concluded that nutritional benefits of newborn screening are apparent. Screening provides a potential opportunity for better pulmonary outcomes. Confounding factors such as severe genotype, pancreatic status, and early acquisition of pseudomonas aeruginosa have influenced long-term pulmonary prognosis in this study.[4]

Based on the analysis of the two RCTs and several observational studies, Grosse and colleagues[7] concluded that early diagnosis and treatment of classic CF has been shown to result in improved nutrition and growth, but the evidence is less clear on potential benefits in onset of pulmonary disease and longer survival. The RCT conducted in the UK and three observational studies found that CF-related childhood mortality was 5% to 10% lower in screened cohorts and these differences were statistically significant. The RCT conducted in the US did not find any difference in child survival, although it was not powered to observe a significant difference in child mortality and data collection and analysis is still ongoing.[7]

The RCT conducted in the US found that age at time of diagnosis is an important factor impacting nutritional status and associated pancreatic insufficiency. A delayed diagnosis increased the risk of malnutrition in childhood. As far as pulmonary disease is concerned, although neonatal screening provides an opportunity to identify infants before lung disease is apparent, screened infants appear to be at greater risk of respiratory involvement.[22]

Newborn Screening (NBS) for CF

Screening is defined as the systematic application of a test or enquiry, to identify individuals at sufficient risk of a specific disorder to benefit from further investigation or direct preventive action, amongst individuals who have not sought medical attention on account of symptoms of that disorder.[23]

A successful screening program for CF refers to the ability of the program to appropriately identify and refer for care those with CF, while meeting the needs of those who do not have CF, particularly those infants identified by the screening program as carriers (individuals unaffected by CF but have a mutation in one of their CFTR genes).[24] Some measures of success might include: (1) how closely the screen approaches 100% sensitivity, (2) the ability of the screen to minimize intrusion on the lives of parents of unaffected infants, (3) the acceptability by the families regarding the integration of CF into existing screening programs, or (4) the acceptability and impact on primary care physicians who must deal with screening results and parental anxieties.[24]

Technology development

The possible advantages of early diagnosis and treatment of CF were recognized as early as 1970.[13] In 1979, development of a test to measure immunoreactive trypsinogen (IRT) in dried blood spots made universal newborn screening for CF feasible.[4,6] Increased IRT concentrations at birth, an indirect measure of pancreatic injury, are characteristic of newborns affected by CF, but can also be found in healthy infants or premature babies.[25] However, IRT values tend to remain elevated for several months in newborns with CF because pancreatic trypsinogen leaks back through interstitial fluid due to partial obstruction of pancreatic ducts,[2] whereas in false positives they usually return to normal within the first few weeks of life. Therefore, repeated measurement of IRT (IRT/IRT) can be used to distinguish the infants with CF from healthy infants. Measurement of IRT (single or repeated measurement) has been the primary CF screening tool used for screening newborns.

Since the discovery of the gene responsible for CF in 1989, more than 1300 CFTR mutations have been identified.[11] The most common mutation throughout the world, F508, presents in an estimated 20% to 80% of infants with CF depending on their ethnic origin[14, 26] and it accounts for 71% of CFTR mutations found among Canadian infants with CF. (see Appendix B: Table B1)[11] The frequency of F508 and other mutations varies across ethnic groups and geographic regions (see Appendix B: Table B2). Worldwide, the majority of mutations are rare, with most frequencies under 0.1%.[26]

Screening programs started to include DNA analysis in their protocols in the early 1990s.[6] However, no region or nation is currently employing DNA analysis alone, all programs include IRT measurements on first week samples as the first step in the protocol.[27]

NBS protocols

Multiple protocols and algorithms are used to screen newborns for CF. All protocols begin with measurement of IRT in dried blood spots.

Infants who have elevated IRT levels may be referred for:

- A sweat chloride test for confirmation of CF (single IRT test),
- Second IRT measurement (IRT/IRT),
- DNA test (IRT/DNA),
- Pancreatitis-associated protein (PAP) (IRT/PAP), or
- Lactase (LACT) test (IRT/LACT).

In addition, novel protocols have been developed based on different combinations of these tests, such as three-stage IRT/DNA/IRT protocol.[25] Each of these protocols have their own advantages and drawbacks.

IRT

This protocol involves measurement of IRT on a heel prick blood sample during the first week of life, followed by a sweat chloride test in infants with elevated IRT levels to confirm the diagnosis. This protocol has been used in the early years of newborn screening for CF.

Different laboratory kits for IRT produce varying distributions of IRT measures, and screening programs set cutoffs on the basis of evaluations of specimens from their own populations and the screening protocols and algorithms used.[6]

The specific value or a percentile (cutoff) is used to decide whether IRT is sufficiently elevated to warrant further testing. The choice of the IRT cutoff is a balance between sensitivity and specificity.[28] Lowering the cutoff point of IRT will increase sensitivity, but correspondingly increase false positives and thus decrease specificity.

Since elevated IRT levels during the first week of life are not only found in infants with CF but also in healthy infants, the major problem associated with this protocol is its low positive predictive value due to a high number of false positives.

IRT/IRT

In this protocol, if the initial IRT result is positive, a second blood sample is taken at about 2 to 4 weeks of age. At this age, elevated IRT values are more specific for CF because IRT values decrease with age in infants without CF. Infants with persistently elevated IRT levels are then referred for sweat chloride testing.[29,30] This was the initial protocol adopted by most screening programs in Australia, New Zealand, Europe, and the US.[25]

A major disadvantage of this protocol is that a second blood sample will be required from 1 in 200 to 250 of all babies screened. A considerable number of unaffected babies will be subjected to a sweat test, generating a great deal of parental anxiety.[25,29]

Another apparent issue noted particularly in the US is that obtaining a follow up blood sample is very difficult.[31]

IRT/DNA

The IRT/DNA (F508 only) protocol includes a second-tier DNA test for F508 from the same blood spot when an initial IRT elevation was observed. Infants with two copies of the mutation clearly have CF and are referred for treatment. Infants with one copy of the mutation are referred for sweat chloride testing to determine if they have CF or if they are carriers.[25]

This protocol is associated with increased sensitivity, specificity, and positive predictive value and has the advantages of an earlier diagnosis in infants homozygous for F508 and of eliminating the need for a second blood sample. A potential disadvantage of DNA analysis is unwanted carrier identification[10] which results in the increase in the number of unaffected infants referred for a sweat test.[29]

With the more recent availability of multiple mutation panels, some screening programs have substituted second tier multiple mutations analysis for F508 only to further increase screening sensitivity.[30] Most commercially available mutation tests include 23 to 97 mutations.[1] DNA mutation panels used by the French national screening program and in the primary studies included in this report are presented in Table B3.

The sensitivity of a given DNA mutation panel varies according to race and ethnicity, thereby including mutation specific panels for particular racial and ethnic minority populations can improve detection of CF among these populations.[6]

The advantages of moving from IRT/DNA (F508) to IRT/DNA (multiple mutation panel) include determining the actual genetic diagnosis of most infants, genotyping infants at diagnosis, and facilitation of managing false-positive results for infants with one mutation.[32]

IRT/DNA/IRT

To address the drawbacks with the IRT/DNA protocol, the IRT/DNA IRT protocol, which includes a second IRT measurement after DNA testing, significantly reduced the number of the second IRT tests compared to the IRT/IRT protocol and, also, the number of negative sweat tests.[25]

Other protocols

A protocol that combines IRT measurement and pancreatitis-associated protein (PAP) test is used in France.[25] PAP is a stress protein that is absent in a healthy pancreas. It is synthesized in high amounts by a diseased pancreas and is therefore elevated in the blood of newborns with CF.[33,34]

Another uncommonly used protocol is to add meconium lactase testing to the IRT or IRT/DNA protocols. Only one study conducted in Italy used this protocol.[25] The rational for the use of lactase, a proteolytic labile protein produced in the intestinal mucosa, is due to its high meconial levels in most infants with CF.[35]

Potential harmful effects of screening

Early detection and treatment as a result of newborn screening has potentially harmful side effects. These are related to therapies (e.g. drug resistance and toxicities) and earlier exposure (through person-to-person transmission from older children with CF) to bacteria associated with chronic airway infection.[6]

Adverse psychosocial effects are more controversial for the families of heterozygous infants who gain no apparent benefit from screening.[5] When IRT/DNA screening identifies a single CFTR mutation, some parents may develop anxiety over the uncertainty of the diagnostic outcome or while waiting for the sweat test results.[5] Anxiety and grief reactions associated with carrier-state diagnoses are thought to place families at risk for impaired parent-child bonding, disrupted relationships, personality problems, and the development of psychogenetic symptoms or some variant of the vulnerable child syndrome.[5]

The potential psychosocial risks of screening include factors associated with: 1) false positives (e.g., unnecessary testing and possibly unnecessary treatment for the child, undue parental anxiety, and desensitization of providers); 2) false-negatives (e.g., potential delay in diagnosis and false reassurance for patients); 3) carrier reporting (e.g., possibly unwanted information and fear of stigmatization or insurance discrimination); and 4) misinformation (e.g., errors in communication or misunderstanding of results).[6]

Current Status of Screening Programs for CF

Newborn screening tests have been available for CF for over three decades, but few national screening programs exist. Reasons for this include uncertainty over the long term benefits of implementing newborn CF screening and the lack of a definitive screening test.[27]

According to a review published in 2003, Australia, New Zealand, and France currently have a national newborn screening program for CF. Some countries such as Austria, Belgium, Germany, Italy, Spain, the United Kingdom, and the United States have local or regional screening programs, whereas no screening programs currently exist in Canada, Denmark, Finland, Netherland, Norway, and Switzerland.[27]

Australia and New Zealand

Australia has comprehensive screening coverage. In 1998, over 90% of Australian newborns were screened for CF.[27] Newborn screening programs for CF started in Australia and New Zealand in 1981 and an IRT/DNA-based screening program is currently being used.[28]

Europe

NBS programs for CF were introduced in Europe in the beginning of the 1970s.[21] In France, a national NBS program for CF was introduced in 2003[27] using the IRT/DNA/IRT protocol.[10] In the UK, implementation of a national population screening program using the IRT/DNA/IRT protocol began in 2004[36] and is expected to be complete by September 2007 (personal communication, Dr. Kevin Southern, UK, March 2007).

United States

There are two major types of CF programs in the Untied States: mandatory state-based and hospital-based programs.[37] Protocols vary by state but the primary screening test in each program is an IRT measurement. The second screening is usually either IRT confirmation alone or reflex DNA screening for specific CF gene mutations.[37] In 2000, approximately 400,000 (10%) children born in the US were screened for CF.[6]

Canada

No national newborn screening programs for CF currently exist in Canada.[27] There has been a pilot project in the Calgary Health Region since June 2005 (personal communication, Ms. Irene Mazurenko, Alberta Health and Wellness, March 2007). Alberta will be launching cystic fibrosis screening as part of the newborn metabolic screening program as of April 2, 2007 (available at website: http://www.health.gov.ab.ca/key/devscreensvc.html#Newborn). Ontario will begin screening for cystic fibrosis in late 2007 (available at website: http://www.health.gov.on.ca/english/providers/media/news_releases/archives/nr_06/nov/nr_112306.pdf).

Regulatory Status

Health Canada issued a medical device license to DELFIA NEONATAL IRT ASSAY (Manufacturer WALLAC OY) on Oct 31, 1999.[38] No information regarding the regulatory status of other IRT tests was found on the Health Canada or US Food and Drug Administration websites.

No information regarding the regulatory status of the PAP test (MucoPAP; DYNABIO S.A., Marseille, France) were found from the Health Canada website. The first author of the only study that reported on PAP[34] and the manufacturer (DYNABIO S.A., Marseille, France) were contacted but no response has been obtained to date.

Evidence on the Diagnostic Validity of NBS

Two systematic reviews[13,39] and six primary studies[29,31,34,35,40,41] met the inclusion criteria (see Appendix A for Methodology). Three of the six primary studies published in 1997[29,31,35] were included in the two systematic reviews and therefore were not included for further analysis. A number of primary studies that only reported on a single protocol rather than compared two different protocols were not included in this report. Details regarding study objectives, population, screening protocols, and results of diagnostic validity from the primary studies are tabulated in Appendix C.

Evidence from systematic reviews

The systematic review by the **Catalan Agency for Health Technology Assessment** (CAHTA) (published in 2000) had two objectives: (1) to evaluate the scientific evidence on the diagnostic validity of screening strategies with IRT and/or the study of specific mutations of DNA (F508 and others), and (2) to evaluate the scientific evidence on the efficacy/effectiveness of an early intervention in newborns with CF. This review identified 12 screening programs in different states or countries in Europe, North America, and Australia.

The review found that strategies that use IRT present a good diagnostic validity so that a sensitivity of 87.5% has a 99.6% level of specificity. These values may vary depending on the cut-off point chosen to classify the newborns as positive or negative, but they are good indicators of the capacity of IRT to discriminate between those with and without the disease. Despite this, the positive predictive value of IRT alone is relatively low (Table 1 and Table 2).

The diagnostic validity and predictive values improve when IRT is used jointly with a second IRT measurement in a second blood sample or when used in combination with a specific mutation panel on the same initial blood sample. The improved diagnostic validity is particularly relevant to specificity since in

view of the low prevalence of the disease, a small improvement in specificity means a substantial reduction in the number of false positives. The results from the meta-analysis show that an approximate improvement in specificity by 0.3% is statistically significant and also clinically relevant.

When the IRT/IRT protocol was compared with the IRT/DNA protocol, no statistical or clinically relevant differences were found between them. In other words, the IRT/IRT protocol and the IRT/DNA protocol are equivalent in terms of diagnostic validity (Table 1) and predictive value (Table 2). The IRT/DNA protocol offers the advantage of requiring only one blood sample, which avoids a second visit. This is important since screening strategies that require a second visit reported losses to follow-up in ranges between 2% to 22%.

Table 1: Estimation of overall sensitivity and specificity

Protocol	Sensitivity		Specificity	
	Global Estimation	95% CI*	Global Estimation	95% CI*
IRT	85.73%	79.67 – 90.20%	99.66%	99.53 – 99.80%
IRT/IRT	93.64%	88.95 – 96.42%	99.95%	99.94 – 99.97%
IRT/DNA	94.00%	87.43 – 97.24%	99.93%	99.90 – 99.96%

Adapted from Serra-Prat 2000[13]
*CI: confidence interval

Table 2: Predictive values of different newborn screening protocols for CF

Protocol	Prevalence	Positive predictive value	Negative predictive value
IRT	1/4000	5.92%	99.996%
	1/2000	11.21%	99.993%
IRT/IRT	1/4000	31.88%	99.998%
	1/2000	48.35%	99.997%
IRT/DNA	1/4000	25.13%	99.998%
	1/2000	40.17%	99.997%

Adapted from Serra-Prat 2000[13]

The review concluded that at present there are screening techniques available for CF with good diagnostic efficacy. That is, they have a good capacity to discriminate between affected and unaffected newborns. While in recent years life expectancy of children with CF has increased substantially, this improvement cannot be ascribed directly and solely to earlier detection and treatment. The scientific evidence currently available on the efficacy of newborn screening for CF is limited and inconclusive.

The systematic review prepared by the **NHS R&D HTA** program (1999) covered a wide range of aspects about screening for CF, but there is one chapter that focused on newborn screening for CF. This review included 20 newborn screening programs from different countries. The pooling of the results from all studies showed that, in total more than five million newborns were screened with a low false-positive rate (0.5 per 1000), acceptable detection rate (90%), and favorable positive predictive value (33%). These numbers varied depending on the protocol used as shown in Table 3.

Table 3: Summary performance indicators according to protocol

Protocol	False positive rate	Positive predictive value	Detection rate
IRT	2.1 per 1000	10%	90%
IRT/IRT	0.3 per 1000	45%	90%
IRT/DNA	0.7 per 1000	25%	97%
IRT/DNA/IRT	0.6 per 1000	27%	97%

Adapted from Murray et al. 1999[39]

The two systematic reviews are consistent in that a single IRT test is associated with low positive predictive value. The CAHTA review[13] found no difference in terms of diagnostic validity and predictive value between IRT/IRT and IRT/DNA protocols, whereas the NHS systematic review[40] demonstrated that positive predictive value is substantially higher for the IRT/IRT protocol. As shown in Table 3, there appears to be no difference in diagnostic validity and predictive value between IRT/DNA and IRT/DNA/IRT protocols.

Evidence from primary studies

Three primary studies[34,40,41] published after the two systematic reviews were included in this report. Of the three studies, one study[41] was conducted in the US, one[40] in Italy, and the other one[34] in France. No Canadian study was

found. Two studies[40,41] used the protocols only including IRT and/or DNA test, whereas the other study adopted a protocol that included a PAP test,[34] which is rarely used. Results from these studies are presented according to these two categories of screening protocols.

Screening protocols that only include IRT and/or DNA testing

IRT/IRT vs. IRT/DNA/IRT

In a study conducted in Italy, Narzi and colleagues[40] compared the IRT/IRT protocol with the IRT/DNA/IRT protocol. Two hundred thousand newborns were screened in this study. The distribution of the ethnic origin of the screened population was not reported. In the IRT/DNA/IRT protocol, the second IRT was performed regardless of the results from the DNA testing.

The author concluded that comparison of the two screening protocols in terms of sensitivity in detecting CF patients demonstrated that the IRT/DNA/IRT protocol is more effective because it is able to detect a higher number of CF infants and CF carriers than the IRT/IRT protocol.

IRT/DNA (F508 only) vs. IRT/DNA (multiple mutation)

In a study conducted in the US , Comeau and colleagues[41] compared the predictive values of screening with single mutation (F508 only) with those of multiple CFTR mutations. A total of 323,506 newborns were screened in this study. The screened infants came from different ethnic origins, such as White non-Hispanic, Hispanic, Black non-Hispanic, Asian, and others.

This study used a modified two-tier IRT/DNA protocol – failsafe protocol, whereby infants who have the highest IRT values and do not have CFTR mutations that were detected are still referred to sweat chloride measurement to maximize CF detection among racial and ethnic populations whose mutations are not represented on common mutation panels.

The study demonstrated that multiple CFTR mutation testing increased diagnostic sensitivity, was better for predicting post-screening risk of CF, but resulted in 26% more carrier identifications and referrals. The study also demonstrated the utility of including a "failsafe" provision for referral for sweat chloride testing to maximize CF detection among ethnic populations whose mutations are not represented on common mutation panels.

Screening protocols that include other uncommon tests

In a study conducted in France, Sarles and colleagues[34] compared the IRT/DNA/IRT protocol that is used in the French national newborn screening program with the proposed IRT/PAP protocol.

The analysis showed that a protocol in which newborns with IRT > 100 ng/ml plus PAP > 1.0 ng/ml and those with IRT > 50 ng/ml plus PAP > 1.8 ng/ml were recalled for sweat testing would have equal or better sensitivity than that of the IRT/CFTR mutation protocol. The expected false-positive rate (<0.25%) is considered acceptable.

The main advantage of the IRT/PAP protocol is that it does not require DNA analysis, thereby avoiding all of the drawbacks of molecular biology (need for informed consent, unwarranted detection of carriers, and detection of borderline forms of CF). It is cheaper and easier to implement than the current IRT/DNA protocol.

Clinical Guidelines and Position Statement

In November 2003, the Centres for Disease Control and Prevention (CDC) and the US Cystic Fibrosis Foundation co-sponsored a workshop to review new evidence relating to NBS for CF published after a previous meeting held in 1997. The CDC then issued a report in October 2004, which stated that "the results support the efficacy of newborn screening in reducing morbidity from CF. In particular, the benefits of improved growth are now more clearly established than in 1997, and the implications of growth retardation for other clinical outcomes in CF are better understood. In addition, benefits in terms of improved patient-oriented outcomes, including cognitive outcomes, hospitalizations, and survival, have been reported in recently published studies. Results from studies do not demonstrate clear benefits on other important measures, including HRQoL and pulmonary outcomes." The CDC suggested that "... on the basis of evidence of moderate benefits and low risk of harm, CDC believes that newborn screening for CF is justified. States should consider the magnitude of benefits and costs and the need to minimize risks through careful planning and implementation, including ongoing collection and evaluation of outcome data".[6] The recommendation was made based on inconsistent or limited-quality patient-oriented evidence.[6]

The CDC report also highlighted some important issues associated with the implementation of newborn screening programs and suggested that:

- NBS programs should collect follow-up data in collaboration with CF care centres and analyze this information to monitor and improve the quality of CF newborn screening.
- NBS programs should be accompanied by rigorous infection control practices to minimize the risk to children with CF detected at an early age of acquiring infectious organisms associated with lung disease from older children.

- NBS programs should ensure parental and provider education and communication of screening results to primary-care providers in a manner that will ensure prompt referral to diagnostic centres. There should be centres skilled in providing both sweat tests to young, pre-symptomatic children with CF and accurate and effective counseling to families, including those with infants identified as carriers.

Discussion

Diagnostic validity of screening protocols

Overall, evidence about the diagnostic validity of each of the screening protocols is inconclusive. The two systematic reviews agreed in that IRT alone is associated with low positive predictive value. One systematic review[13] found no differences in sensitivity, specificity, and positive predictive value between the IRT/IRT protocol and the IRT/DNA protocol.

Evidence from one primary study[40] suggests that, when compared with the IRT/IRT protocol, the IRT/DNA/IRT protocol demonstrated higher sensitivity, with reduced false positives and therefore the number for confirmatory sweat chloride test.

Only one published study[34] was identified that attempted to compared the proposed IRT/PAP protocol with the IRT/DNA/IRT protocol that is used by the French national screening program. This study found that two proposed thresholds (IRT > 100ng/ml plus PAP > 1.0ng/ml and IRT > 50ng/ml plus PAP > 1.8ng/ml) would have equal or better sensitivity than that of the IRT/DNA protocol. It was suggested that the use of the IRT/PAP protocol can bypass all the drawbacks of a DNA test and it is less expensive and easier to implement. This protocol is not found to be used elsewhere.[25]

Implementation issues

Population

All primary studies on newborn screening for CF was conducted outside of Canada. Only one US study[41] reported the distribution of ethnic origin of the screened population. This distribution is quite different from that of the Canadian population.

In general, populations of different ethnic origin may differ in terms of the incidence of CF, levels of IRT in normal infants, and the frequency distribution of the CFTR mutations, all of which will influence the sensitivity and specificity of the screening protocols. For example, Africa-American infants

without CF have higher IRT levels, but with lower incidence of CF. Using the IRT/IRT protocol in this population will have higher false positive rates (but this is dependent on the IRT cutoff), which can lead to lower positive predictive value and specificity.

Similarly, the use of a screening protocol that includes DNA analysis in a population with a diverse distribution of CFTR mutations (i.e., higher rates of uncommon mutations) will decrease the sensitivity of the IRT/DNA (F508 only or a few mutations) protocol.

There is currently no information available on the incidence of CF, levels of IRT in normal infants, or the frequency distribution of the CFTR mutations across different Canadian populations, such as the Aboriginal (First Nations and Inuit) population, generalizing the research findings to the Canadian (and Albertan) population is of question.

Issues with IRT measurement

Timing for initial/second IRT test

All currently used screening protocols measure IRT levels initially during the first week of life. However, this is not the best time from a diagnostic validity perspective.[13] The levels of trypsinogen are distributed in a similar fashion in affected and unaffected newborns in the first ten days of their lives. After the first 20 days of life the levels of IRT drop considerably in unaffected infants, while remaining high in infants with CF. Therefore, the best time for measuring IRT from the standpoint of diagnostic precision and more specifically in terms of specificity, is during the second month of life (between day 20 and 69).[13] The time period however for taking routine blood samples from infants for NBS programs for other diseases is during the first few days of life.

The timing for the second IRT measurement varied in the included studies, ranging from 21 days to the 5th week of life. In the three included primary studies, the timing for the first and/or second IRT tests was reported only for the whole population screened. Whether the timing was different for ill or premature infants was not mentioned.

Cut-off points for initial/second IRT test

Cut-off points (absolute values or percentiles) for the first and second IRT measurements varied across the included studies (see Table C1). The main reasons for this variation may include use of different laboratory IRT kits and the consideration of the IRT levels in the screened population. Choosing appropriate cut-off values for local screening programs depends on the mix of ethnic origin and prevalence of CF (family history of CF).

Selection of DNA panels

A decision with respect to the CFTR mutations used in the second tier DNA analysis would need to consider the distribution of the common CFTR mutations among the populations to be screened. The protocol with a single F508 may be enough for a population with high frequency of this mutation. However, in populations that have large subpopulations with high frequencies of a particular mutation, inclusion of other mutations to the second tier screen may be warranted. On the other hand, as some authors pointed out, since most mutations are rare, expanding the panel of screened mutations may achieve only marginal gains in sensitivity.[16] The selection of mutation panels based on the frequency in the CF population should also recognize that sometimes the frequency of a mutation in the general populations is different from that in the CF population[31]

Only one US study[41] compared the protocol using a single mutation (F508) with the protocol that includes multiple mutations. This study found that the protocol including multiple mutations increased diagnostic sensitivity, but resulted in more unwanted carrier detection and referrals for the confirmatory sweat chloride test. The insertion of a second IRT test after the DNA test would reduce the number of false positives.

Ethical, social, and legal issues

Most included studies did not provide any information regarding ethical issues around DNA testing, management of identification of a carrier, social or legal issues, or specialized counseling for parents. In all the studies that used DNA analysis in their screening protocols, informed consent was obtained from parents.

Balancing potential harms of newborn screening for CF with the benefits is challenging. First, it involves balancing physical benefits with psychological harms, which are complex qualitative comparisons. Second, the harms (false positives) do not occur in the same population as the benefits. Finally, technical decisions about the screening methods to increase the sensitivity of the program will increase the false positive rate.[42]

In policy terms, the false positive rate may be more important as these infants will need further investigation and are potentially at risk of misdiagnosis and unnecessary treatment. While failure to detect cases through screening may have medico-legal implications, the proportion of false positives may have important human and economic dis-benefits.[23] Some authors suggest that, given the ambiguous and contested nature of the benefits of CF newborn screening and the evidence of harm to false positive families, it may be morally preferable to "avoid harm" by minimizing false positives and to be "tolerant" of missed CF cases.[42]

Conclusion

CF is a one of the most common autosomal recessive disorders among Caucasians, with the incidence highly varying across different ethnic groups. In 2000, one in every 3608 babies born in Canada was diagnosed with CF. The clinical manifestations of this life threatening genetic disease include meconium ileus, pancreatic insufficiency, chronic pulmonary disease, and failure to grow.

There is currently no cure for CF. There continues to be lots of controversy in terms of the long-term benefits of the current treatment strategies. Evidence from two randomized controlled trials included in the two systematic reviews indicated that there are nutritional and growth benefits from early treatment, but not in pulmonary improvement.

Two systematic reviews and three primary studies were included in this report; the findings from these studies are inconclusive. Furthermore, economic studies comparing the various protocols, which would help to inform the cost issues, were not located using this search strategy. The two systematic reviews found that IRT alone is associated with low positive predictive value. One systematic review found no differences in sensitivity, specificity, and positive predictive value between the IRT/IRT protocol and the IRT/DNA protocol with prevalence rates of CF from 1/2000 to 1/4000.

Evidence from one primary study suggested that the IRT/DNA/IRT protocol demonstrated higher sensitivity, with reduced false positives and therefore the number referred for the confirmatory sweat chloride test was lower when compared with the IRT/IRT protocol. Furthermore, the DNA test could be performed using the same blood spot collected for the initial IRT test. The second IRT test would require a follow-up visit.

Limited evidence from one US study suggests that including more CFTR mutations in the DNA analysis increased sensitivity and has the advantage of avoiding second blood sample collection, but resulted in higher unwanted carrier detection.

The IRT/PAP protocol has the advantage of bypassing all the drawbacks of the DNA test, the PAP is less expensive and this protocol is easier to implement. There are several ethical issues surrounding DNA testing. However, no definitive conclusions can be made at this time because of very limited evidence on the IRT/PAP protocol, which is currently only used in France.

Generalizing research evidence from current literature to the Canadian (or Alberta) context is challenging as no Canadian study has been published to date. Collecting and analyzing local data is important when planning regional or provincial newborn screening programs in Alberta. Information on the incidence of CF, prevalence of CF in the various ethnic sub populations, and

IRT levels among normal infants in Alberta will help to inform the IRT test characteristics to maximize screening accuracy. The choice of protocol (IRT/IRT, IRT/DNA, IRT/DNA/IRT, or IRT/PAP) needs to account for several factors, such as tolerance for high false positive and referral rates, ethical challenges, and parental anxiety.

Appendix A: Methodology

Search

Literature searches were conducted by the Alberta Heritage Foundation for Medical Research (AHFMR) librarian for the period between 1996 and 2006. Searches were limited to humans and English language.

Medical Subject Headings (MeSH) related to the topic used: Infant; Infant, newborn; Neonatal screening; Cystic fibrosis. Variations of subject headings and keywords were used alone or in combinations in the following electronic databases and websites.

Table A1: Search strategy

Database, Platform and URL	Version or Search Date	Search Terms
Core Databases		
The Cochrane Library http://www.thecochranelibrary.com	2006-12-12 Issue 4, 2006	(infan* OR neonat* OR newborn* OR newborn*) AND ("cystic fibrosis" OR CF) AND screen* in Title, Abstract or Keywords
PubMed www.pubmed.gov	2006-12-12	#1 (infan* OR neonat* OR newborn OR newborns OR new-born OR new-borns) AND (cystic fibrosis) AND (screening[Text Word]) #2 #1Limits: English, Publication Date from 1996, Humans #3 #1 AND (in process[sb] OR publisher[sb] OR pubmednotmedline[sb]) #4 #2 OR #3
CRD Databases (DARE, HTA & NHS EED) http://www.york.ac.uk/inst/crd/crddatabases.htm	2006-12-12	(infan* OR neonat* OR newborn* OR newborn*) AND screen* AND cystic fibrosis
Web of Science- SCI and SSCI Licensed Resource (ISI Interface)	2006-12-12 (up to 2006-12-09)	TS=((infan* OR neonat* OR newborn* OR new-born*) AND ("cystic fibrosis") AND screen*)

Table A1: Search strategy (continued)

Database, Platform and URL	Version or Search Date	Search Terms
Core Databases (continued)		
CINAHL Licensed Resource (OVID Interface)	2006-12-12 (Up to December Week 1 2006)	1 exp Cystic Fibrosis/ 2 screen$.mp. 3 (neonat$ or infan$ or newborn$ or newborn$).mp. 4 1 and 2 and 3 limit 4 to (english language and yr="1996- 2007")
EMBASE Licensed Resource (OVID Interface	2006-12-12 (up to 2006 Week 49)	
PsycINFO Licensed Resource (OVID Interface)	2006-12-12 (Up to December Week 1 2006)	1 exp Cystic Fibrosis/ 2 screen$.mp. 3 (neonat$ or infan$ or newborn$ or newborn$).mp. 4 1 and 2 and 3 limit 4 to (english language and yr="1996 - 2007")
Library Catalogues		
NEOS (Cenral Alberta Library Consortium) http://www.library.ualberta.ca/catalogue	2006-12-12	(infan$ OR neona$ OR newborn$ OR newborn$) AND screen$ AND cystic fibrosis
Guidelines		
AMA Clinical Practice Guidelines http://www.albertadoctors.org	2006-12-12	Browse titles
CMA Infobase http://mdm.ca/cpgsnew/cpgs/index.asp	2006-12-12	cystic fibrosis
National Guideline Clearinghouse www.ngc.gov	2006-12-18	cystic fibrosis AND screen*
Canadian Task Force on Preventive Healthcare heep://www.ctfphc.org	2006-12-18	Browse the website
Coverage/Regulatory/Licensing Agencies		
Alberta Health and Wellness http://www.health.gov.ab.ca	2006-12-18	cystic fibrosis
Health Canada http://www.hc-sc.gc.ca	2006-12-18	cystic fibrosis screening
Medical Devices Active Licence Listing http://www.mdall.ca/	2006-12-18	cystic fibrosis

Table A1: Search strategy (continued)

Database, Platform and URL	Version or Search Date	Search Terms
Coverage/Regulatory/Licensing Agencies (continued)		
US Food and Drug Administration www.fda.gov	2006-12-18	cystic fibrosis screening
Aetna Clinical Policy Bulletins http://www.aetna.com/about/cov_det_ policies.html	2006-12-19	Cystic fibrosis
BlueCrossBlue Shield http://www.bluecares.com/tec/index. html	2006-12-19	Cystic fibrosis
Grey Literature Sources		
NeLH (National electronic Library for Health http://www.nelh.nhs.uk/	2006-12-18	cystic fibrosis screening
Other HTA Resources		
AETMIS http://www.aetmis.gouv.qc.ca	2006-12-18	cystic fibrosis screening
CADTH www.cadth.ca	2006-12-19	cystic fibrosis
Institue for Clinical and Evaluative Sciences (ICES), Ontario http://www.ices.on.ca/	2006-12-19	cystic fibrosis
Health Technology Assessment Unit At McGill http://www.mcgill.ca/tau/	2006-12-19	Browse website
Medical Advisory Secretariat http://www.health.gov.on.ca/english/providers/program/mas/mas_mn.html	2006-12-19	Browse website
ECRI Licensed Resource www.ecri.org	2006-12-19	cystic fibrosis AND screen*

Table A1: Search strategy (continued)

Database, Platform and URL	Version or Search Date	Search Terms
Other HTA Resources (continued)		
Health Quality Council, Saskatchewan http://www.hqc.sk.ca/	2006-12-19	cystic fibrosis
CCE http://www.med.monash.edu.au/healthservices/cce/	2006-12-19	"cystic fibrosis" screening cystic fibrosis" screening site:.ca
Metabrowsers/Search Engines		
Google http://www.google.com	2006-12-19	"cystic fibrosis" screening cystic fibrosis" screening site:.ca

Note:

Truncation: The * symbol is a truncation character that retrieves possible suffix variations of the root word e.g. surg* retrieves surgery, surgical, surgeon, etc. Semicolons are used to separate terms that were searched separately.

Limits: Searches were limited to Humans; Publication dates: 1996 and on, and English language. These limits are applied in databases where such functions are available.

Study selection

Inclusion criteria

Studies were included if they met all of the following criteria:

- Study design: systematic review, or primary study that compared two or more newborn screening protocols
- Population: newborn (neonate)
- Technology: newborn screening tests for CF
- Outcome measures: at least one of the following: sensitivity, specificity, false positive rate, false negative rate, positive predictive value, negative predictive value

Exclusion criteria

Studies were excluded if they met any of the following criteria:

- Conference abstracts, editorials, comments, letters
- Primary studies that only reported the results of a single screening protocol
- Population other than newborns (e.g., adolescents, adults)

Data extraction

Data from the included systematic reviews and primary studies were extracted by one researcher (BG) according to the pre-determined data extraction protocol. Methodological quality of the included studies was not assessed in this report.

Data extraction from systematic reviews

- Author, publication date
- Focus of the review (Newborn or other population)
- Quality appraisal of included studies
- Main findings from included studies (comparison of difference protocols in terms of test accuracy)
- Conclusions

Data extraction from primary studies

- Authors, country, year of publication
- Objective
- Population:
  - Total number
  - Ethnic origin
  - Incidence of CF
- Screening protocol
  - Protocols (test, sequences) used for comparison
  - Timing for first and/or second IRT
- Cutoff point for initial and/or second IRT
  - CFTR mutation panels included in the DNA analysis
  - Criteria for positive screening
- Result
  - Diagnostic validity and predictive value (sensitivity, specificity, false negative, false positive, PPV, NPV)
  - Ethical issues associated with DNA analysis
  - Implementation issues
- Conclusion

Appendix B: CFTR mutations and DNA panels

Table B1: Top 25 mutations found in Canadians with CF

Mutations	Percent %
1. ΔF508 or ΔI507	71.1
2. 621+1G->T	2.9
3. G542X	1.6
4. G551D	1.5
5. A455E	1.0
6. 711+1G->T	1.0
7. N1303K	0.9
8. M1101K	0.8
9. R117H	0.6
10. W1282X	0.6
11. G85E	0.6
12. I148T	0.5
13. L206W	0.5
14. R553X	0.4
15. 1717-1G->A	0.4
16. 3849+10kb->T	0.3
17. IVS8-5T	0.3
18. R560T	0.3
19. Y1092X	0.2
20. 2789+5G->A	0.2
21. P67L	0.2
22. 3659delC	0.2
23. R347P	0.2
24. R1162X	0.2
25. 3905insT	0.1
Unidentified	9.6

Source: Adapted from Canadian Cystic Fibrosis Foundation 2002[11]

Table B2: Most common CFTR mutations in the world[44]

Name of Mutation	Frequency	(%)	Population with the highest prevalence
ΔF508	28,948	(66.0)	
G542X	1,062	(2.4)	Spanish
G551D	717	(1.6)	English
N1303K	589	(1.3)	Italian
W1282X	536	(1.2)	Jewish-Askhenazi
R553X	322	(0.7)	German
621+1G->T	315	(0.7)	French-Canadian
1717-1G->A	284	(0.6)	Italian
R117H	133	(0.3)	
R1162X	125	(0.3)	Italian
R347P	106	(0.2)	
3849+10kbC->T	104	(0.2)	
ΔI507	93	(0.2)	
394delTT	78	10-30%*	Nordic, Finnish
G85E	67		
R560T	67		
A455E	62		
1078delT	57		
2789+5G->A	54		Spanish
3659delC	54		
R334W	53		
1898+1G->T	53		
711+1G->T	49		French-Canadian
2183AA->G	40		Italian
3905insT	38	6-17%*	Swiss; Amish; Acadian
S549N	30		
2184delA	29		
Q359K/T360K		87.5%*	Jewish-Georgian

Table B2: Most common CFTR mutations in the world[44] (continued)

Name of Mutation	Frequency	(%)	Population with the highest prevalence
M1101K		69%*	Hutterite
Y122X		48%*	French, Reunion Island
1898+5G->T		30%	Chinese, Taiwan
3120+1G->A		11%	African-American
I148T		9.1%	French-Canadian

The source of data is obtained from the CF Genetic Analysis Consortium (1994).
The frequency is based on the screening of 43,849 CF chromosomes, although not all of them have been tested for the indicated mutations. The mutations are found in patients of Caucasian origin, except indicated otherwise. The geographic location (or ethnic group) with the highest prevalence is indicated for some of the mutations. A rough relative frequency (expressed in %,*) is given for those mutations studied in relatively small-size samples or in the indicated populations only.

Table B3: Different DNA panels

NBS programs	DNA panels
Panels used by national screening program	
France National NBS program[44]	**A panel of 30 mutations:** ΔF508, G542X, N1303K, 1717-1G>A, G551D, W1282X, R553X, I507del, 1078delT, 2183AA>G, 3849+10kbC>T, R1162X, 621+1G>T, R334W, R347P, 3659delC, R117H, S1251N, E60X, A455E, 2789+5G>A, 394delT, G85E, 1811+1,6kbA>G, Y122X, 711+1G>T, W846X2, Y1092X C>A, 3272-26A>G, 3120+1G>A 320pb
Panels used in the primary studies	
Narzi et al. 2002[40] Lazio Region, Italy A panel of 31 world most common mutations	See Table B.2
Comeau et al. 2004[41] Massachusetts, US	**A panel of 16 mutations:** ΔF508, R117H, G551D, G542X, W1282X, N1303K, R334W, 621+1G>T, R553X, ΔI507, 1717-1G>A, R347P, R560T, 3849+10kbC>T, A455E, S549N **A panel of 27 mutations:** 16 mutations mentioned above except S549N plus 3120+1G>A, 3659delC, A559T, R1162X, S1255X, 405+3A>C, 711+1G>T, 2789+5G>A, G480C, 2307insA, G85E, 1078delT
Sarles et al. 2005[34] France	**A panel of 20 mutations:** 20 CFTR mutations (CF20 Elucigene Kit; Orchid Biosciences Inc, Abingdon, UK) (names of individual mutations were not reported).

Appendix C: Diagnostic validity of different screening

Table C1: Summary of primary studies

Study	Population	Types of protocol
Narzi et al. 2002[40] Italy **Objective:** to compare the sensitivity of the IRT/IRT and IRT/DNA/IRT protocols	**Time period:** 1992 to 2000 **No. of newborns screened:** 200 000 51844 using IRT/DNA/IRT **Ethnicity:** NA **Incidence of CF in this population:** 1: 2982	**Protocol used:** IRT/IRT (RIA kit Sorin, Sluggia, Italy) compared with IRT/DNA/IRT **Timing for IRT test** 1st IRT test: between 2nd and 5th day of life 2nd IRT test: between w3rd and 5th weeks of life Timing for ill or premature infants: NAw **IRT cutoffs:** 1st IRT: > 80 ng/ml (until 1998) and average of all the first test values + 3SD (after 1998) 2nd IRT: > 60 ng/ml (until 1998) and average of all the second test values + 2SD (after 1998) **DNA analysis:** 31 most common worldwide mutations of CFTR gene (2nd IRT performed regardless of the results of DNA test) **Criteria for positive screening:** At least one mutation was found or both IRT values higher than the cutoffs. **Confirmatory test:** clinical assessment, sweat chloride test, pancreatic function test, and genetic test for subjects not previously characterized.

	Diagnostic validity	Author's conclusion
	Sensitivity: IRT/IRT: 83% to 86% IRT/DNA/IRT: 97% **Specificity:** NA **Positive predictive value:** NA	Comparison of the two screening protocols in terms of sensitivity in detecting CF patients demonstrated that the IRT/DNA/IRT protocols is more effective because it is able to detect a higher number of CF patients and CF carriers than the IRT/IRT protocol.

Table C1: Summary of primary studies (continued)

Study	Population	Types of protocol
Comeau et al. 2004[41] US **Objective:** to compare the predictive values and sweat-test referral patterns of screening with single mutation (ΔF508 alone) with those of screening with multiple-CFTR mutations and to evaluate the utility of the provision for sweat testing of infants with very high IRT values in the absence of a detected CFTR mutations	**Time period:** 1999 to 2003 **No. of newborns screened:** 32 506 **Ethnicity:** White non-Hispanic 74.3%; Hispanic 11.4%; Black non-Hispanic: 7.2%; Asian 5.6%; other 1.3%; unknown 0.2% **Incidence of CF in this population:** 1:2888	**Protocol used:** IRT (Wallac DELFIA kits, Turku, Finland) /DNA (ΔF508) compared with IRT/DNA (multiple mutations) **Timing for IRT test** 1st IRT test: NA 2nd IRT test: not relevant **IRT cutoffs:** 1st IRT: initially IRT concentration > 90th percentile and changed to 95th percentile 9 months later 2nd IRT: not relevant Timing for ill or premature infants: NA **DNA analysis:** initially included a panel of 16 CFTR mutations and increased to 27 mutations 21 months later. **Criteria for positive screening:** (1) IRT ≥ 95th percentile + 2 mutations (2) IRT ≥ 95th percentile + 1 mutation (3) IRT ≥ 99.8th percentile + 0 mutation **Confirmatory test:** positive sweat testing (Cl ≥ 60mEq/L) or by a CF specialist according to the criteria set by the US Cystic Fibrosis Foundaton.

	Diagnostic validity	Author's conclusion
	Sensitivity: IRT/DNA (ΔF508): not clearly presented IRT/DNA (multiple mutations): not clearly presented **Specificity:** NA **Positive predictive value:** IRT/DNA (ΔF508): 9.8% IRT/DNA (multiple mutations): 8.2% **Negative predictive value:** IRT/DNA (ΔF508): 99.9% IRT/DNA (multiple mutations): 99.9%	The study demonstrated that multiple-CFTR-mutation testing increased diagnostic sensitivity, was better for predicting post-screening risk of CF, but resulted in 26% more carrier identifications and referrals. The study also demonstrated the utility of including a "failsafe" provision for referral for sweat testing to maximize CF detection among ethnic populations whose mutations are not represented on common mutation panels.

Table C1: Summary of primary studies (continued)

Study	Population	Types of protocol
Sarles et al. 2005[34] France **Objective**: to compare , in the same population of newborns, the performance of the CF screening protocols currently in use in France (IRT/CFTR mutation analysis) with that of a protocol using IRT and PAP test	**Time period:** 2002 to 2003 **No. of newborns screened:** 204 749 **Ethnicity:** NA **Incidence of CF in this population:** 1/4266	**Protocol used:** IRT (CIS-Bio International, France or Perk in-Elmer, Finland)/DNA/IRT compared with IRT/PAP (MucoPAP, DYNABIO S.A., Marseille, France) **Timing for IRT test** 1st IRT test: day 3 2nd IRT test: day 21 Timing for ill or premature infants: NA **IRT cutoffs:** 1st IRT: > 50 ng/ml and changed to > 65 ng/ml 3 months later 2nd IRT: > 45 ng/ml **DNA analysis:** 20 mutations **PAP:** performed in all newborns **Criteria for positive screening:** (1) 1 or 2 mutations were found or (2) 2nd IRT > 45 ng/ml if no mutation found **Confirmatory test:** sweat chloride test ≥ 60 mEq/L

*a & *b: The PPV can be calculated considering that "affected babies" are newborns with 2 CFTR mutations and/or abnormal sweat test and "suspected babies" are either (a) those requiring sweat testing for diagnosis (1 or 0 mutations and elevated IRT at day 21) or (b) those whose parents had some sort of notification on an abnormal test results, that is, newborns with 1 mutation and those recalled for blood collection at day 21

CF: cystic fibrosis; CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; Cl: chloride; DNA: deoxyribo nucleic acid; IRT: immunoreactive trypsinogen; MI: meconium ileus; NA: not available; PAP: pancreatitis-associated protein; SD: standard deviation

	Diagnostic validity	Author's conclusion
	Sensitivity: not clear **Specificity**: not clear **Positive predictive value:** IRT/DNA/IRT: 21.4%*a or 4%*b IRT/PAP: 8.6% (when the 1st IRT was performed once) and 13% (when the 1st IRT was performed twice)	The proposed two thresholds, that is, IRT greater than 100 ng/ml plus PAP greater than 1.0 ng/ml, and IRT greater than 50 ng/ml plus PAP greater than 1.8 ng/ml, has equal or better sensitivity than that of the IRT/CFTR mutation protocol. The expected false-positive rate (<0.25%) is considered acceptable. Its main advantage is that it does not require CFTR mutation analysis, thereby all of the drawbacks of molecular biology (need for informed consent, unwarranted detection of heterozygotes, and detection of borderline forms of CF) are avoided.

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IHE Publications

- Cost-effectiveness in the detection of syphilis
- The use and benefit of teleoncology services
- Screening newborns for hearing
- Screening newborns for cystic fibrosis
- The use of nitric oxide in acute respiratory distress syndrome
- Pre-operative routine testing – is it necessary?
- Consensus Statement on Self-monitoring in Diabetes
- Consensus Statement on How to Prevent Low Birth Weight

Request

This report was prepared in response to a request from Alberta Health and Wellness on the published evidence of newborn screening protocols for cystic fibrosis. This report focuses on the published scientific evidence over the last 10 years on the diagnostic validity of different screening protocols. Practical issues associated with the implementation of screening programs, particularly with protocols that include DNA testing, are addressed whenever information is available.

A successful screening program for CF refers to the ability of the program to appropriately identify and refer for care those with CF, while meeting the needs of those who do not have CF, particularly those infants identified by the screening program as carriers (individuals unaffected by CF but have a mutation in one of their CFTR genes).[24] Some measures of success might include: (1) how closely the screen approaches 100% sensitivity, (2) the ability of the screen to minimize intrusion on the lives of parents of unaffected infants, (3) the acceptability by the families regarding the integration of CF into existing screening programs, or (4) the acceptability and impact on primary care physicians who must deal with screening results and parental anxieties.[24] This report reviews the evidence in the field.

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