European survey of newborn bloodspot screening for CF: opportunity to address challenges and improve performance

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Short Title: Newborn screening for cystic fibrosis in Europe (48/55 characters incl. spaces)

Key words: cystic fibrosis, newborn bloodspot screening, IRT, PAP, *CFTR* gene analysis, CFSPID, carriers

Counts:

Abstract: 148 / 150 words

Main text: 3545 / 3000 words

Tables & Figures: 2 Figures & 4 Tables / 5 items

References: 25 / 30 References

ABSTRACT

Background: The aim of this study was to record the current status of newborn bloodspot screening (NBS) for CF across Europe and assess performance.

Methods: Survey of representatives of NBS for CF programmes across Europe. Performance was assessed through a framework developed in a previous exercise.

Results: In 2022, we identified 22 national and 34 regional programmes in Europe. Barriers to establishing NBS included cost and political inertia. Performance was assessed from 2019 data reported by 21 national and 21 regional programmes. All programmes employed different protocols, with IRT-DNA the most common strategy. Six national and 11 regional programmes did not use DNA analysis.

Conclusions: Integrating DNA analysis into the NBS protocol improves PPV, but at the expense of increased carrier and CFSPID recognition. Some programmes employ strategies to mitigate these outcomes. Programmes should constantly strive to improve performance but large datasets are needed to assess outcomes reliably.

INTRODUCTION

Newborn bloodspot screening (NBS) for Cystic fibrosis (CF) is a well-established, cost-effective public health strategy with international standards, increasingly adopted across Europe.[1-3]

The Neonatal Screening Working Group (NSWG) was established by the European CF Society (ECFS) to support implementation of NBS for CF, compare protocols to optimize effectiveness, reduce harm and establish consensus on all issues arising in NBS.[4-10] The first survey of the NSWG in 2005 identified 26 NBS programmes for CF across Europe, of which two were nationally co-ordinated (France and Austria).[4] In 2010, the NSWG published «best practice» guidelines for CF screening and acknowledged, in light of factors including geography, ethnicity and healthcare resources, that complete harmonisation of protocols was unlikely and probably not appropriate.[5] In 2016, the NSWG reported on NBS for CF in Europe identifying 17 national programmes, 4 countries with regional programmes and 25 countries not screening.[6] The approach to screening varied considerably across programmes. Although most were achieving the ECFS standards[2;3] with respect to timeliness, sensitivity and specificity, results were often poor and areas for improvement numerous. The NSWG recognised that clearer definitions were required for screening outcomes, to improve consistency in data collection and enable valid comparison of the performance of different protocols. As a consequence, 20 parameters were determined to calculate 8 key outcomes.[11]

In this study, we provide an up-to-date evaluation on NBS for CF across Europe in 2022 and a comparison of performance of national and regional programmes from data collected during the year 2019 using the parameters above.

METHODS

We conducted a cross-sectional survey of performance of NBS for CF across Europe. We recorded national and regional programmes in Europe in 2022. We obtained protocol detail and analysed outcome data for 2019, in order to compare the performance of different approaches to screening. We selected this year as it was pre-pandemic and enabled sufficient time for collection of false negative data (children diagnosed with CF who had a negative NBS result in 2019).
We contacted representatives of national and regional CF screening programmes in 44 European countries and seven countries considered transcontinental.[6] When there was no identified representative for a European country in our working group, we used the contact person from the ECFS registry.

Participants filled out a questionnaire (see supplementary material) based on 20 parameters established by the ECFS NSWG to calculate 8 key performance outcomes.[11] Data included: name of the country or region, year of commencement of screening and when the programme was established. Protocol detail was recorded for 2019 and the following parameters; number of live births, infants screened, infants with an inadequate dried blood spot (DBS) samples, infants with a positive tier 1 screening test, infants with a positive NBS result referred for diagnostic assessment (including sweat testing), infants who screened positive and who had a confirmed CF diagnosis; number of *CFTR* variants (2, 1, 0) in each infant with a confirmed diagnosis of CF; number of infants with a CFSPID designation, a pending conclusion (infants screened positive for whom the CF physician needs additional information to conclude CF, no CF, CFSPID), infants who did not complete the screening algorithm (lost to follow-up including death) and infants identified as carriers; number of children false negatives with, and without, meconium ileus (MI); number of children diagnosed with CF including false negatives; number of infants with a true negative

screening result, number of infants with a positive screening result but not diagnosed as CF or CFSPID; and age in days (mean, median, SD), where the date of birth is day 0 and when infant is first assessed by CF clinician for CF and CFSPID.

Infants screened positive for NBS are those referred for diagnostic assessment including sweat testing. In some programmes families receive a carrier result which does not lead to a diagnostic assessment (and in this exercise these infants do not contribute to the calculation of PPV).

Data were collected for the year 2019 only. The questionnaire was available as a paper-based document, online-document or survey-based tool (online platform Research Electronic Data Capture REDCap).

Programmes were classified as national or regional. A programme was considered national if the same protocol was employed across the whole country with central co-ordination. Regional programmes include either programmes that cover only a part of a country or different regional programmes that cover the entire country.

Questionnaire results were returned to the ECFS NSWG coordinator or entered directly onto the REDCap survey platform by participants. Data queries were sent to contributors and the final tables checked for accuracy by the authors. We excluded returns with less than 50% of the necessary data from the evaluation.

Data analysis

Data were presented graphically. Positive predictive value (PPV) was calculated as the proportion of infants diagnosed with CF by NBS out of all cases with a positive NBS result referred for diagnosis to the CF centre (sweat testing). A positive NBS result was defined as an infant referred for clinical and diagnostic assessment (sweat testing) to a CF centre. Sensitivity was calculated as the proportion of infants diagnosed with CF by NBS out of all CF cases diagnosed by NBS plus children born that year with false negative missed by NBS without MI. Infants who presented

clinically with MI but had a false negative NBS result were not included in the sensitivity calculation, on the basis that this presentation does not delay diagnosis and care.

We modelled the impact of programme size, determined by the number of infants diagnosed with CF, on the validity of the sensitivity outcome using a variety of potential sensitivity values. For example, evaluating the impact of one additional false negative case on the sensitivity. The model demonstrates that for programmes recognising less than 40 cases diagnosed with CF, the variance associated with an extra false negative case had a disproportionate impact on the sensitivity result. Sensitivity outcomes for programmes that recognise less than 40 CF cases per year should be considered with caution and we excluded them from comparison of performance.

We used Excel and Stata, V.17.0 (Stata Corporation, Austin, Texas, USA) for data analysis, and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting in cross-sectional studies.[12]

Data were collected anonymously representing programme performance with no individual identifiers and ethics approval was not required.

RESULTS

Across Europe in 2022, we identified 22 national programmes and 34 regional programmes (in four countries) compared to 17 and 29 in 2016 (**Figure 1**). New national programmes have been established in Germany, Luxembourg, North Macedonia, Latvia and Belgium. Eight countries are considering establishing a programme, in various stages of preparation (**Figure 1**). In countries without NBS, the main reported barrier to establishing a programme was cost or political issues. Protocol detail and outcomes for 2019 were reported by 21 national programmes and 21 regional programmes (**Table 1a&b**).

Characteristics of 2019 NBS programmes for CF

All programmes employed measurement of IRT from a dried blood spot (DBS) sample taken in the first few days of life (IRT-1) as the initial stage of the protocol (**Figure 2**). From this initial step, there continues to be a wide variety of approaches to second and third tier testing to assess samples with a high IRT-1 value. Four programmes measured pancreatitis associated protein (PAP) as a second tier to IRT-1 and a combination of these were used to determine a positive second tier result. Six national (29%) and 11 regional programmes (52%) did not use DNA analysis of the *CFTR* gene, most progressing to a second IRT measurement (IRT-2) taken on day 14-21 of life. The majority of national programmes (12) used DNA analysis as a second tier with panels identifying between 4 and 680 *CFTR* variants. Four programmes used extended gene analysis (EGA) as a third or fourth tier of testing after a positive result from initial limited DNA testing (**Figure 2**). These countries used next generation sequencing (NGS) for all *CFTR* exons, some reported a preselected panel of variants, others reported all variants (**Table 1a**).[13-16]

Two programmes did not report carriers because of national law. In one programme (Norway), this was following negative EGA, which was considered sufficient to exclude a CF

diagnosis. The other programme (Germany) used more limited DNA analysis and did not report carriers as requested by national legislation. Denmark (after EGA) is reporting carrier status directly to the parents and not performing a sweat test (this carrier report is not considered a positive NBS result). Most programmes (39) do report carrier status although in the majority this requires sweat testing to exclude CF (i.e. the NBS result is positive). Three programmes (England, Northern Ireland and Scotland) used a third tier of IRT testing and, if the IRT-2 value, taken on day 21, is low, report the infant as a "probable carrier" (i.e., negative NBS result as no referral for clinical assessment and sweat testing).

A safety net, defined as further testing undertaken on infants with a very high IRT-1 but negative DNA or PAP testing, was reported by 21 programmes. For most (16) this involved referral for sweat testing. For five programmes, this involved an IRT-2 measurement on a DBS sample taken on day 21 and if below the cut-off reported as a negative NBS result.

Performance of 2019 NBS programmes

We received data sets for 42 programmes (21 national and 21 regional) for 2019 (**Table 2 a&b**). Six programmes had more than 40 true positive cases, but one country has not been analysed for sensitivity performance as it did not report the number of false negatives without MI (**Table 2a**). Programmes were classified as 1) No DNA analysis in protocol, 2) DNA analysis using variant panels and 3) protocols using EGA. The Dutch programme employs a unique combination of IRT-PAP-DNA and EGA.

Coverage of the NBS programme

Programmes reported good coverage of available infants into NBS, with the caveat than in some countries one infant may be recorded as being screened on more than one occasion (especially

pre-term infants). For national programmes, from 5,678,417 live births, 5,601,796 screening results were reported (98.7%). Incidence, as determined by the total number of CF diagnosis per live births, varied for national programmes from 1 in 2,201 to 1 in 10,928 (**Table 2a**).

Number of infants taken to second tier testing

Most countries provided a fixed cut-off for the IRT-1 value; three countries reported a floating cut-off. The IRT-1 cut-off ranged from the 90.0th to the 99.5th centile.

Number of CF cases diagnosed by NBS and infants with a CFSPID designation

Across all programmes, 1026 infants with a positive NBS result had a diagnosis of CF and 181 were given a designation of CFSPID (**Table 3**). The overall ratio of CF:CFSPID from the 15 national programmes that reported CFSPID cases was 6.1:1 (range from 31:1 to 1.3:1), and for 19 regional programmes was 1.6:1 (range 0.3:1 to 10:1). For the six large programmes, use of more extensive gene sequencing was associated with a low ratio of CF:CFSPID. Programmes not using DNA analysis had minimal reports of CFSPID cases.

Carrier recognition and reporting

In the 13 national programmes that reported carriers, 958 were recorded compared to 494 CF cases diagnosed by NBS, and the overall ratio of CF:carriers was 1:1.9. For the 16 regional programmes that reported carriers, the ratio of CF:carriers was 1:3.2.

Positive predictive value

The PPV varied from 4% to 91% for national programmes. For 7 programmes not using DNA analysis the average PPV was 17% (95% CI: 6-28%). For 14 programmes using DNA analysis, the average PPV was 43% (95% CI: 28-59%).

Sensitivity

For the five large programmes with data available, the sensitivity ranged from 94.5% (95% CI: 88-98%) in Russia to 99.2% (95% CI: 94-100%) in France (**Figure 2a**). We aggregated data from all programmes and demonstrated a lower sensitivity in countries that used no DNA analysis (mean 90% (95% CI: 80-100%)) compared to those that used DNA panels (mean 95% (95% CI: 90-100%)) or EGA (mean 97% (95% CI: 95-100%)) (**Table 2**).

Timeliness (age at initial visit to a CF centre)

For national programmes reporting this outcome, the median number of days when the newborn was first assessed by the CF team ranged from 12 to 37 (mean days: 26 (95% CI: 23-30)) and 88% (14/16) programmes achieved the ECFS standard of maximum 35 days (**Table 4**). For the regional programmes, the range was 15 to 60 days (8 regions (53%) did not achieve the ECFS standard).

Discussion

This comprehensive survey of NBS for CF across Europe demonstrates continued expansion but limited evidence of improved performance. In 2022, NBS for CF is now undertaken in 26 countries in Europe. Since our last survey in 2016, five new national screening programmes have been established in Germany, Luxembourg, North Macedonia, Latvia and Belgium (**Figure 1**). We also identified 34 regional programmes in 4 countries, two of them (Spain and Serbia) covering the whole country, Italy 19 of 20 regions and Ukraine only the region of West Ukraine. There continues to be a wide variety of approaches, but only 5 national programmes are still using an IRT-IRT protocol. While 4 programmes have introduced PAP as a second tier, the majority of national programmes are now using DNA analysis as a second tier with panels identifying between 4 and 680 *CFTR* variants, and 4 programmes have incorporated extended gene analysis (EGA) in their algorithm. (**Figure 2**).

Many programmes are still not achieving previously agreed minimal ECFS standards.[2;3] Compared to data from 2014, there is a small improvement in sensitivity but a remarkable deterioration in achieving a sufficient PPV (**Table 4**). Only 75% of national and 70% of regional programmes achieved an aimed sensitivity ≥95% in 2019. Although some programmes are achieving good PPV through a number of strategies, 57% of national and 83% of regional programmes did not achieve the ECFS standard (PPV >30%). Strategies to improve PPV included EGA and use of a second IRT measurement at day 21. In Denmark (PPV, 91%), after EGA excludes a second variant, a letter to parents explains the presumed carrier result so they can opt for genetic counselling. This is not considered a positive NBS result, as infants are not referred for clinical assessment and sweat testing.[16] Similarly in Norway, EGA is used to exclude a second variant, but in this programme parents are not informed of that result.[15] The high PPV in the

Netherlands is explained by the 4-tier protocol, where the initial biochemical step (IRT-PAP) reduces the number of samples referred for DNA testing and EGA.[14] Infants with one variant recognized on EGA are referred for clinical assessment and sweat testing, but the numbers are relatively small. In England, for infants with one variant recognized on a more limited DNA panel, a second IRT measurement is undertaken on a DBS sample from day 21. If the IRT-2 is low, the parents are informed of the "probable CF carrier" result and again this is not considered a positive NBS result as there is no referral for clinical assessment and sweat test.[17]

Performance of smaller programmes is difficult to assess reliably, being vulnerable to small changes in parameters, such as false negative cases and outliers that can skew the overall results. Collection of accurate data is important, but challenging in all countries and regions, especially those with no central co-ordination and challenging geography and health resources (**Table 2a**). Although national programmes were successfully established in Turkey and Russia, and have impacted positively on CF care, the national infra-structure means that many families are "lost to follow up", i.e., that a positive result at any point of the algorithm is not further tested and the protocol remains incomplete. In Germany, many NBS results are "lost to follow up" as the legislative rule requires that families with a positive NBS result to seek specialist advice on their own to exclude or confirm a CF diagnosis.

This survey also demonstrates that many programmes are achieving acceptable performance with timely recognition of infants with CF. Timeliness was better in the national compared to regional programmes (88% of national programmes achieving the national standard compared to 47% of regional programmes), but overall performance has not improved significantly since 2014 (**Table 4**). There is increasing evidence from registry and long-term cohort studies that this will have a positive impact on survival and health.[18;19] As new transformational therapies for CF emerge, the importance of early recognition is thrown into even sharper

perspective.[20] Whilst the evidence to support NBS for CF seems incontrovertible, this must be undertaken with a mind to minimising negative impacts and ensuring as efficient a protocol as possible.

To accurately compare different screening approaches, outcome data need to be collected consistently and accurately. It has been shown that different definitions of parameters significantly impact the calculation of global screening metrics such as specificity, PPV and sensitivity.[21] Metrics are dependent on the way tests and cases are counted unless definitions are clearly harmonized and they are the cornerstone for both quality assessment and improvement of programmes. The NSWG published a framework with clear parameters to determine consistent NBS performance outcomes in 2021[11], but it may be that this survey has been undertaken at too early a stage to benefit from this clarification, despite providing clear forms and guidance (including on line) to participants. Another limitation is that there is still no standardised recording of false negative cases and we know little about their age range of diagnosis, demographics or *CFTR* variants that are not in DNA panels representing the most common variants in a country. For future surveys, this system will be better established to collect the 8 key outcomes, and the NSWG will explore collecting more data on false negative cases. Hopefully data acquisition will be more complete, although challenges remain or can emerge as central co-ordination of programmes is discontinued. For example, due to health service restructure, France and Germany have concern that data might be incomplete on several levels. The lack of centralized feedback alone could explain the lower PPV for both countries and the higher sensitivity in France compared to previous evaluations.

In addition to recording the status of screening in 2022, we collected data for the year 2019 to compare the performance of approaches that regions and countries have used for the screening protocol. Again, this survey confirms previous results, that the use of more extensive

DNA analysis is associated with increased recognition of infants with CFSPID. The outlier for this finding is the Dutch programme which uses a combination of IRT-PAP, limited DNA analysis and then extended gene analysis to minimise the number of samples referred for sweat testing.

A significant number of programmes use a "safety net" to evaluate infants with a very high IRT-1 result but negative second tier testing. Approaches to the safety net vary, from referral for sweat testing to obtaining a second DBS sample.[14;22-24] The safety net strategy potentially facilitates the recognition of infants with rarer *CFTR* gene variants, but at the expense of reducing PPV. Future surveys will explore this aspect of NBS for CF in more detail.

In contrast to previous NSWG surveys, data were successfully reported from a number regional programmes (including 18/18 regions in Italy and 3/13 in Spain). Italy has a centralised data collection system, which supported the high return rate from that country. Implementation of similar systems in countries with regional CF NBS would facilitate more reliable annual data collection, and support collection of data from consecutive years. This would improve the assessment of performance in programmes with a relatively small number of screened babies, both regional and national.

Eight countries are considering establishing a programme, in various stages of preparation (**Figure 1**), but as new national programmes are established, it is timely to reflect on why a number of countries and regions have not yet initiated screening in this population. For the most part, replies suggest that cost is now a consideration, although for some regulatory issues are significant. Some health authorities (most notably in Sweden) continue to question scientific justification for screening and whether NBS for CF fulfils the criteria developed by Wilson and Junger to appraise new programmes.[25] This may be a position, which contrasts strongly to the health appraisal of other countries, but given the potential for harm, for example the acute stress of a false positive result or the longer-term unsettled nature of an unclear diagnosis (termed

CFSPID), it is important that NBS programmes strive to improve their performance and achieve the minimum ECFS standards.

In conclusion, this survey demonstrates some areas of good practice but there is considerable scope for improvement in the quality of NBS for CF across Europe. Integrating DNA analysis into the NBS protocol improves PPV, but at the expense of increased carrier and CFSPID recognition which is a concern and should be monitored. There is a drive for more extensive gene analysis and our survey shows that this can be incorporated into a programme in a manner to improve performance whilst minimising negative impacts. The framework of the 20 parameters to calculate the 8 key outcomes established by the NSWG should be part of any annual report of a CF NBS programme. This can improve future international surveys and enable more valid comparison of protocol performance, but this depends on continued high-quality data collection preferably through a central coordinated system.

ACKNOWLEDGEMENT

We would like to thank all the staff of the NBS laboratories and national evaluation centres who helped us to check all parameters and gave us feedback. We also thank Eva SL Pedersen (Institute of Social and Preventive Medicine, University of Bern, Switzerland) for statistical support.

FUNDING

The survey was funded by the European CF Society.

CONFLICT OF INTEREST

There are no conflicts of interest.

LEGENDS

Figure 1: **The status of NBS for CF in Europe 2022**

National programmes are coloured dark green and regional programmes, light green. Countries considering or planning NBS for CF are coloured light orange and those with no plans, light grey.

Figure 2: **Algorithm used for CF-NBS in 2019**

National programmes are written in black font and regional programmes in grey font

Table 1a: **The structure of 21 national and NBS programmes for CF in Europe in 2019**

Abbreviations: DNA, Deoxyribonucleic acid analysis; IRT, immunoreactive trypsinogen; EGA: extended gene analysis; FEIA, Fluorescent Enzyme-Immunassay; PAP, pancreatitis associated protein; NR not reported

Table 1b: **The structure of 21 regional programmes for CF in Europe in 2019**

Abbreviations: DNA, Deoxyribonucleic acid; IRT, immunoreactive trypsinogen; ML, meconium lactase, PAP, pancreatitis-associated protein; DNA, deoxyribonucleic acid; ML, Meconium lactase

Table 2a: **The performance of 20 national NBS programmes for CF in 2019**

Abbreviations: IRT, immunoreactive trypsinogen; NBS, Newborn screening; CFSPID, CF screen positive, inconclusive diagnosis; PPV, positive predictive value; MI, meconium ileus

Table 2b: **The performance of regional NBS programmes for CF in 2019.**

Abbreviations: IRT, immunoreactive trypsinogen; NBS, Newborn screening; CFSPID, CF screen positive, inconclusive diagnosis; PPV, positive predictive value; MI, meconium illeus

Table 3: **Summary of key outcomes for national and regional programmes**

Table 4: **Comparison of the performance of national and regional programmes in 2014 and 2019 with ECFS standards**

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Figure 1: European countries and regions performing newborn screening for CF in 2022

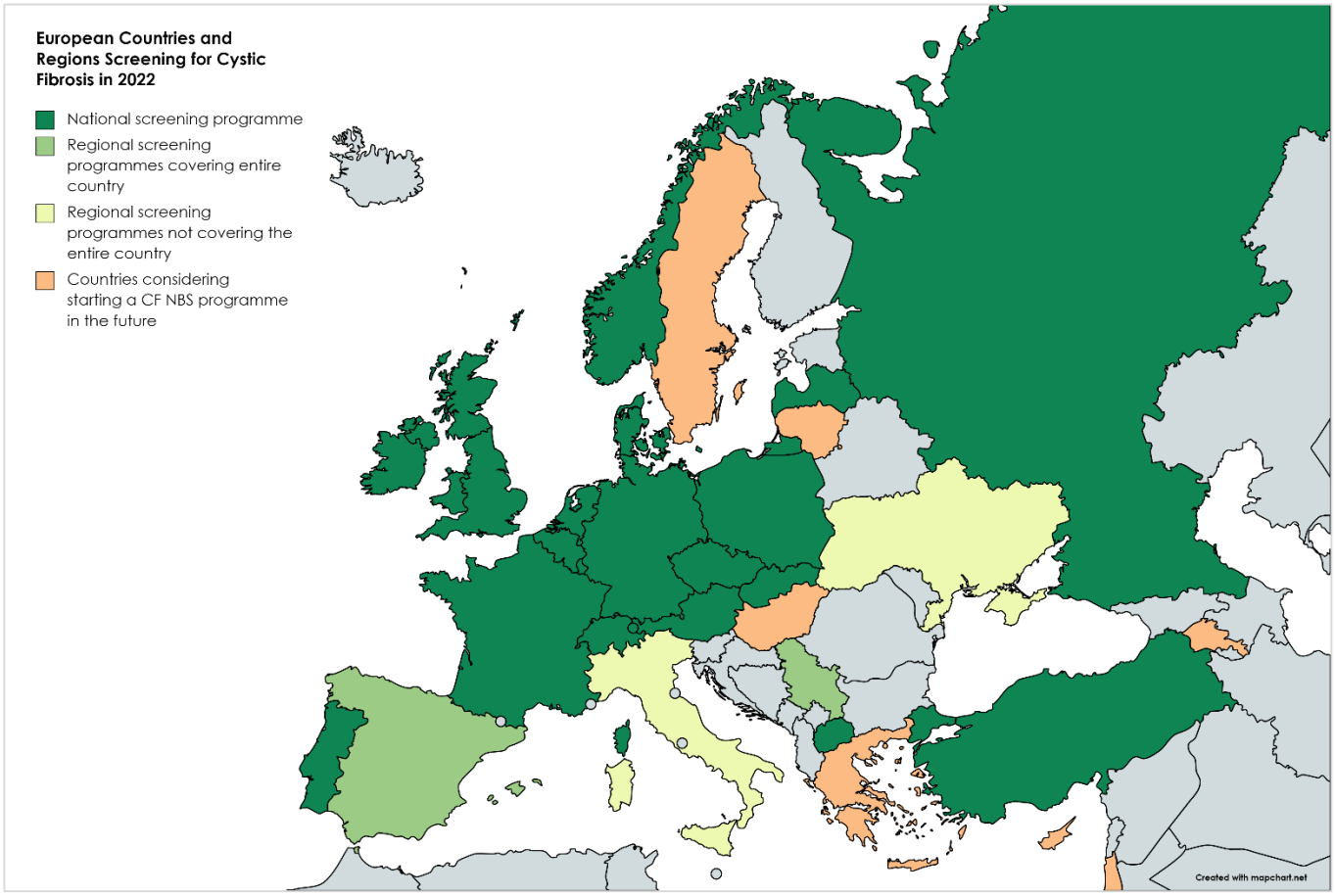
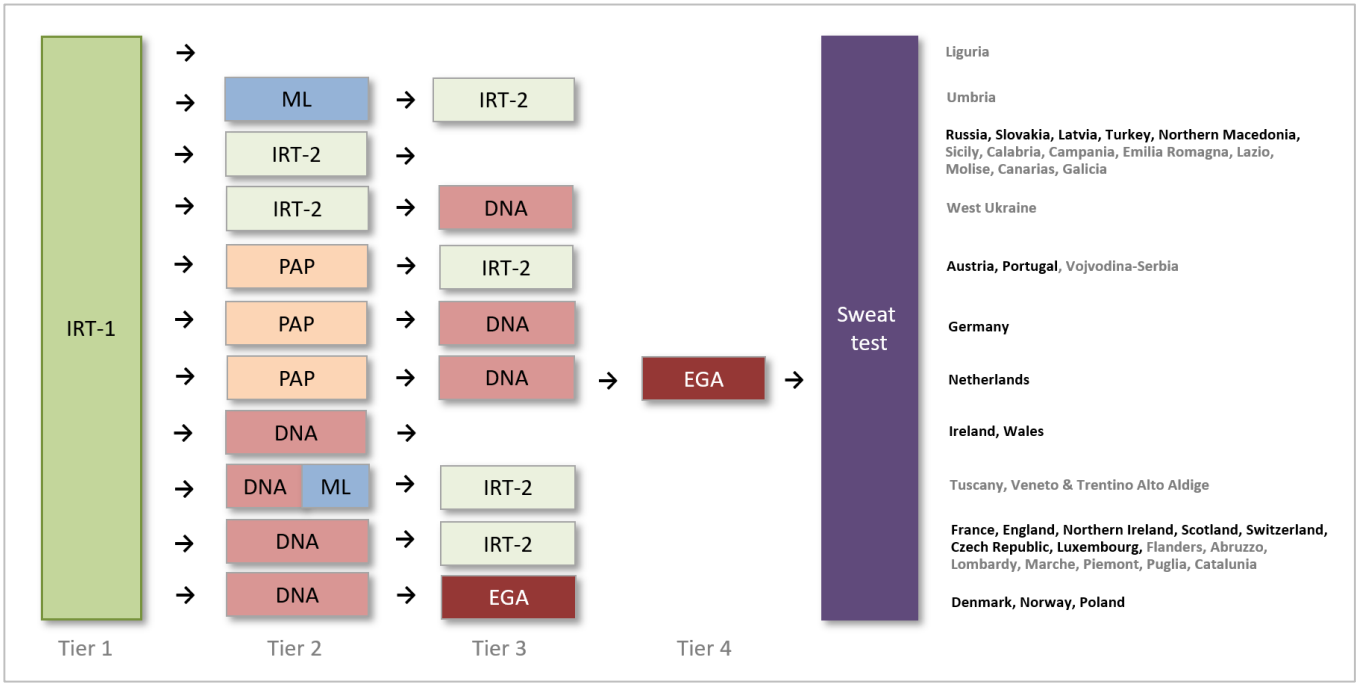


Figure 2: Algorithm used for CF newborn screening in 2019



Abbreviations: DNA, Deoxyribonucleic acid; IRT, immunoreactive trypsinogen; EGA, extended gene analysis; ML, meconium lactase; PAP, pancreatitis associated protein

Legend: National programmes, black font; Regional programmes, grey font

Table 1a: National CF newborn screening programme protocols in 2019

	Austria	Czech Republic	Denmark	England	France	Germany	Ireland	Latvia	Luxembourg	Netherlands
Year commenced	1997	2009	2015	2007	2002	2016	2011	2019	2018	2011
Screening algorithm	IRT / PAP / IRT	IRT / DNA / IRT	IRT / DNA / EGA	IRT / DNA / IRT	IRT / DNA / IRT	IRT / PAP / DNA	IRT / DNA	IRT / IRT	IRT / DNA / IRT	IRT / PAP / DNA / EGA
Tier 1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1
IRT-Method	AutoDELFIA	AutoDELFIA	GSP Neonatal IRT	GSP or AutoDELFIA	GSP or AutoDELFIA	GSP or AutoDELFIA	AutoDELFIA	FEIA Labsystems	AutoDELFIA	GSP Neonatal IRT
Tier 1 cut off IRT ng/mL (percentile cut-off)	65 (99.0)	65 (99.0)	50 (98.0)	GSP: 55, AutoDELFIA 65 (99.5)	GSP: 55 Auto DELFIA: 65 (99.5)	- (90.0)	58 (99.0)	70 -	60 (98)	60 (99.5)
Tier 2	PAP	DNA	DNA	DNA	DNA	PAP (MucoPAP-F), if IRT-1 between 90th and 99th centile	DNA	IRT-2	DNA	PAP
Tier 2 cut off	• 2.5 µg/L for IRT-1 65-100 • 1.3 µg/L if IRT-1 > 100 • IRT-1 x PAP > 170	50 variants	1 variant (F508del) • if homozygous: referral to CF centre • if heterozygous: EGS of CFTR gene	4 variants (50-100 variants if only 1 variant detected initially)	29 variants	2.1 µg/L	38 variants	70	50 variants	• ≥ 3 µg/L for IRT-1 60-100 • ≥ 1.2 µg/L if IRT-1 100-124 or • IRT ≥ 124
Tier 3	IRT-2	IRT-2	EGA	IRT-2	IRT-2	DNA if PAP > cut-off	-	-	-	DNA
Tier 3 cut off	65 ng/ml	• ≥ 50 ng/ml up to day 42, • ≥ 30ng/ml beyond day 42	All variants (whole CFTR gene)	GSP: 46, AutoDELFIA 52 IRT-2 if one variant identified on the 50-100 variant tier 2 panel	≥ 37 (GSP) ≥ 40 (autoDELFIA) IRT-2 if no written informed consent for DNA is available	31 variants	-	-	-	35 variants
Safety Net	If IRT-1 > 130 direct to sweat test	IRT-2 if no variant identified in tier 2 and IRT-1 ≥ 200 in Bohemia, ≥ 150 in Moravia	If IRT-1 > P 90.0 and NGS if no variant identified in tier 2 If IRT-1 >148 (P 99.9): EGS of whole CFTR gene	IRT-2 if no variant identified in tier 2 and IRT-1 >120	IRT-2 if no variant identified and IRT-1 ≥ 90 (GSP) or ≥ 100 autoDELFIA)	IRT-1 > 99.9th centile direct to sweat test	No	No	IRT-2 at day 21 if no DNA analysis was performed	EGA (all variants), if no variants identified in tier 3 and IRT-1 ≥ 100 (P 99.9)

	Northern Ireland	Northern Macedonia	Norway	Poland	Portugal	Russia	Scotland	Slovakia	Switzerland	Turkey	Wales
Year commenced	1984	2019	2012	2009	2015	2007	2003	2009	2011	2015	1996
Screening algorithm	IRT / DNA / IRT	IRT / IRT	IRT / DNA / EGA	IRT / DNA / EGA	IRT / PAP / IRT	IRT / IRT	IRT / DNA / IRT	IRT / IRT	IRT / DNA / IRT	IRT / IRT	IRT / DNA
Tier 1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1
Method	AutoDELFIA	DELFIA	GSP Neonatal IRT	Luminometry (IBL)	AutoDELFIA	DELFIA	AutoDELFIA	GSP Neonatal IRT	FEIA Labsystems	FEIA Trimaris	AutoDELFIA
Tier 1 cut off IRT ng/mL (percentile cut-off)	65 (99.4)	70 (99.5)	40 (96)	- (99.4)	65 (99)	Variable cut-off across regions aiming for 99.5 centile	62 (99.5)	60 (99)	70 (99.2)	90 (99.1)	52
Tier 2	DNA	IRT-2	DNA	DNA	PAP	IRT-2	DNA	IRT-2	DNA	IRT-2	DNA
Tier 2 cut off	4 variants (50 variants if only 1 variant detected initially)	45 ng/ml	152 variants (MiSeq139+13)	680 variants	> 1.6 µg/L	Variable cut-off across regions	4 variants (50-100 variants if only 1 variant detected initially)	55 ng/ml	18 variants	70 ng/ml (97.7)	8 variants
Tier 3	IRT-2	-	EGA	EGA *	IRT-2	-	IRT-2	-	IRT-2	-	-
Tier 3 cut off	GSP: 46, AutoDELFIA 52 IRT-2 if one variant identified on the 50-100 variant tier 2 panel	-	All variants (whole CFTR gene: Sanger confirma-tion of variants before report)	1220 variants	50 ng/ml	-	GSP: 46, AutoDELFIA 52 IRT-2 if one variant identified on the 50-100 variant tier 2 panel	-	70 ng/ml (99.2)	-	-
Safety Net	IRT-2 if no variant identified in tier 2 and IRT-1 > 120	No	EGA if no variants identified in tier 2 panel and IRT-1>120	No	No	No	IRT-2 if no variant identified in tier 2 and IRT-1 > 120	No	IRT-2 if no variants identified in tier 2 and IRT-1 > 100	No	IRT-1 > 170 and no variants identified in tier 2, direct to sweat test

Abbreviations: DNA, Deoxyribonucleic acid analysis; IRT, immunoreactive trypsinogen; EGA, extended genome analysis; FEIA, Fluorescent Enzyme-Immunassay; PAP, pancreatitis associated protein; NR, not reported

***** In Poland all exons of the gene are sequenced but the programme does not report all variants

Table 1b: Regional CF newborn screening programme protocols in 2019

	Abruzzo (Italy)	**Calabria (Italy)**	**Campania (Italy)**	**Emilia Romagna (Italy)**	**Lazio + Molise (Italy)**	**Liguria (Italy)**	**Lombardy (Italy)**	**Marche (Italy)**
Year commenced	2016	2004	2014	1984	2000	1997	1983	1995
Screening algorithm	IRT / DNA / IRT-SN	IRT / IRT	IRT / IRT	IRT / IRT	IRT / IRT	IRT	IRT / DNA / IRT-SN	IRT / DNA / IRT-SN
Tier 1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1
IRT method	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT
Tier 1 cut off IRT ng/mL (percentile cut-off)	48 (98)	50 (97.5)	48 (99)	55 (98.4)	47 (97.5)	70 (98.8)	60 (98)	42 (97)
Tier 2	DNA	IRT	IRT	IRT	IRT	-	DNA	DNA
Tier 2 cut off ng/mL	67 variants 85% detection rate	35 ng/mL	37	35	43	-	186 variants 94% detection rate	67 variants 85% detection rate
Tier 3	IRT-2	-	-	-	-	-	IRT-2	IRT-2
Tier 3 cut off ng/mL	40	-	-	-	-	-	50	31
Safety Net	IRT-2 if no variant identified in tier 2 and IRT-1 >65	No	No	No	No	No	IRT-2 if no variant identified in tier 2 and IRT-1 >85	IRT-2 if no variant identified in tier 2 and IRT-1 >48

Abbreviations: DNA, Deoxyribonucleic acid; IRT, immunoreactive trypsinogen; ML, meconium lactase, PAP, pancreatitis associated protein; NR not reported

	Piedmont + Valle D'Aosta (Italy)	Puglia + Basilicata (Italy)	Western Sicily (Italy)	Eastern Sicily (Italy)	Tuscany (Italy)	Umbria (Italy)	Veneto + Trentino Alto Aldige (Italy)
Year commenced	2002	2016	1993	1999	1984	2006	1984
Screening algorithm	IRT / DNA / IRT-SN	IRT / DNA / IRT-SN	IRT/ IRT	IRT / IRT	IRT / DNA+ML/ IRT	IRT / ML / IRT	IRT / DNA+ML/ IRT-SN
Tier 1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1
IRT method	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT	GSP Neonatal IRT	AutoDELFIA
Tier 1 cut off IRT ng/mL (percentile cut-off)	60 (98.6)	59 (95)	53.2 (99)	50 (98.5)	49 (99)	49 (99)	62 (98.8)
Tier 2	DNA	DNA	IRT-2	IRT-2	DNA + ML	ML	DNA + ML
Tier 2 cut off	388 variants 90% detection rate	not reported	40	40	ML >0.5 U/g 336 variants 90% detection rate	ML >0.5 U/g	ML >1U/g 67 variants 93% detection rate Veneto 95% detection rate Alto Adige
Tier 3	IRT-2	IRT-2	-	-	IRT-2	IRT-2	IRT-2
Tier 3 cut off	45	35	-	-	23	23	40
Safety Net	IRT-2 if no variant identified in tier 2 and IRT-1 >79	IRT-2 if no variant identified in tier 2 and IRT-1 >100	No	No	IRT-2 if no variant identified in tier 2 and IRT-1 >57	IRT-2 if no variant identified in tier 2 and IRT-1 >57	IRT-2 if no variant identified in tier 2 and IRT-1 >120

	Cataluña (Spain)	Canarias (Spain)	Galicia (Spain)	Flanders (Belgium)	Vojvodina (Serbia)	West Ukraine (Ukraine)
Year commenced	1999	2016	2003	2019	2009	2019
Screening algorithm	IRT / DNA / IRT	IRT / IRT	IRT / DNA	IRT / DNA / IRT	IRT / PAP / IRT	IRT / IRT / DNA
Tier 1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1	IRT-1
IRT method	AutoDELFIA	NR	NR	GSP Neonatal IRT	DELFIA	NR
Tier 1 cut off IRT ng/mL (percentile cut-off)	60 (98.2)	60	70	- (99)	70	60 (99.5)
Tier 2	DNA	IRT-2	DNA	DNA	PAP	IRT-2
Tier 2 cut off	50 variants	40	277 variants	12 variants	• 2.5 μg/L for IRT-1 65-100, • > 1.33 μg/L if IRT-1 100-130	(99.5)
Tier 3	IRT-2	-	-	IRT-2	IRT-2	DNA
Tier 3 cut off	35	-	-	- (99)	50	32 variants
Safety Net	IRT-2 if 1 or no variant identified in tier 2 and IRT-1 > 150	No	No	IRT-2 if no variant identified in tier 2	IRT-2 if IRT-1 >130	No

Table 2a: Performance of the national screening programmes 2019

		England #	France #	Germany #	Poland #	Russia [+] #	Turkey #	Austria	Czech Republic	Denmark	Ireland	Latvia	Luxembourg	Netherlands	Northern Ireland	Northern Macedonia	Norway	Portugal	Scotland	Slovakia	Switzerland	Wales
Screening population	Live births	625642	764049	778090	374954	1027537	1183652	84952	112231	62667	59796	18786	7231	171195	22447	19812	54495	87426	50282	57054	86172	29947
	Total number screened	615809	763706	769421	373719	1009832	1153238	86456	113144	62843	59591	10458	7231	170065	22432	15033	55499	87364	50223	57165	88774	29793
NBS results	IRT 1 > cut off	3458	4373	8892	2586	9138	12701	230	1031	2155	759	148	73	871	182	60	2404	1200	336	198	774	95
	Positive NBS result, referred to CF centre	248	514	799	339	1388	2949	79	118	11	70	17	16	38	14	25	8	24	20	72	98	17
Outcome	Carrier	137	240	NR *	218	NA	NA	NA	78	153	44	2	4	6	9	NA	NR *	NA	5	NA	57	5
	CF by NBS	160	126	144	65	104	116	15	27	10	25	2	4	31	3	8	7	8	11	7	24	6
	CFSPID	23	7	7	50	NA	NA	NA	10	0	1	0	0	1	2	NA	1	NA	0	NA	4	0
	Ratio CF:CFSPID	7 : 1	18 : 1	20.6 : 1	1.3 : 1	NA	NA	NA	2.7 : 1	NA	25 : 1	NA	NA	31 : 1	1.5 : 1	NA	7 : 1	NA	NA	NA	6 : 1	NA
	False negatives with MI	3	3	3	1	2	NR	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
	False negatives without MI	4	1	3	2	6	NR	1	0	0	2	0	0	0	1	0	0	0	0	1	0	0
	Total False negatives	7	4	6	3	8	55	1	0	0	2	0	0	0	1	1	0	0	0	1	1	0
	Total CF (all cases from NBS + total FN)	167	130	150	68	112	171	16	27	10	27	2	4	31	4	9	7	8	11	8	25	6
	Lost to follow up	8	93	300	51	395	222	6	1	0	0	3	0	0	4	0	0	0	2	NR	2	2
Performance	PPV (%)	64.5	24.5	18.0	19.2	7.5	3.9	19	22.9	90.9	35.7	11.8	25	81.6	21.4	32	87.5	33.3	55	9.7	24.5	35.3
	Sensitivity without MI (%)	97.6	99.2	97.9	97.0	94.5	NA	93.7	100	100	92.6	100	100	100	75%	100	100	100	100	87.5	100	100
	Incidence of CF (95% CI)	1:3746 (3220-4359)	1:5877 (4948-6978)	1:5187 (4421-6086)	1:5514 (4348-6993)	1:9174 (7622-11038)	1:6922 (5959-8039)	1:5310 (3253-8666)	1:4157 (2851-6061)	1:6267 (3372-11641)	1:2215 (1519-3229)	1:9393 (2349-37594)	1:1808 (679-4814)	1:5522 (3883-7849)	1:5611 (2106-14948)	1:2201 (1145-4230)	1:7785 (3712-16340)	1:10928 (5464-21834)	1:4571 (2532-8251)	1:7132 (3566-14265)	1:3447 (2329-5102)	1:4991 (2242-11111)
	Median days seen in CF centre																					
	CF	22	32	24	37	36	NR	35	31	28	20	NR	29	19	19	28	12	NR	24	24	16	26
	CFSPID	27	31	28	50	NA	NA	NA	54	NA	21	NA	NA	19	21	NA	34	NA	NA	NA	17	NA

Countries with more than 40 children diagnosed with CF per year

[+] Data from Russia for 50 regions out of 85 total (72% of the population)

* according to the country's law

Abbreviations: ST, sweat test; IRT, immunoreactive trypsinogen; CF, Cystic Fibrosis; CFSPID, Cystic Fibrosis Screen Positive Inconclusive Diagnosis; PPV, positive predictive value; MI, meconium ileus; NR, not reported; NA, not applicable

Table 2b: Performance of the regional screening programmes 2019

		Abruzzo (Italy)	Calabria (Italy)	Campania (Italy)	Emilia Romagna (Italy)	Lazio + Molise (Italy)	Liguria (Italy)	Lombardy (Italy)	Marche (Italy)	Piemonte + Valle D'Aosta (Italy)	Puglia + Basilicata (Italy)	Western Sicily (Italy)	Eastern Sicily (Italy)	Tuscany (Italy)	Umbria (Italy)	Veneto + Trentino Aldige (Italy)	Cataluña (Spain)	Canarias (Spain)	Galicia (Spain)	Flanders (Belgium)	Vojvodina (Serbia)	West Ukraine (Ukraine)
Screening population	Live births	8500	14491	46731	30922	40812	8747	73117	9667	28813	31258	NR	NR	23451	5577	43029	61691	13830	15718	63721	17000	53343
	Total number screened	8671	13948	51330	32709	42235	8537	74051	9557	29468	32579	18381	20385	24101	6110	42885	62041	13526	15994	62986	17000	51004
NBS results	IRT 1 > cut off	107	429	733	183	860	100	1265	388	292	295	195	405	209	49	469	624	267	110	528	112	390
	Positive NBS and referred for ST	12	131	175	32	429	100	352	78	45	112	25	44	32	7	96	128	34	18	36	10	12
Outcome	Carrier	7	9	16	2	23	NA	113	21	30	36	NA	0	21	NA	41	4	3	15	0	NA	NR
	CF by NBS	2	0	7	8	10	2	21	2	10	8	3	4	2	0	10	6	1	3	12	4	8
	CFSPID	1	3	7	0	1	1	26	6	1	3	0	2	3	0	8	8	1	0	3	1	0
	Ratio CF:CFSPID	2 : 1	NA	1 : 1	NA	10 : 1	2 : 1	0.8 : 1	0.3 : 1	10 : 1	2.7 : 1	NA	2 : 1	1:1.5	NA	1.3 : 1	0.8 : 1	1 : 1	NA	4: 1	4 : 1	NA
	False negatives with MI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	NR	1
	False negatives without MI	1	0	1	0	0	0	3	0	0	1	0	0	0	0	1	0	0	0	1	0	0
	Total False negatives	1	0	1	0	0	0	3	0	0	1	0	0	0	0	2	0	0	0	3	0	1
	Total CF (all cases from NBS + total FN)	3	0	8	8	10	2	24	2	10	9	3	4	2	0	12	6	1	3	15	4	9
	Lost to follow up	1	0	5	7	25	0	0	3	2	12	0	0	2	4	22	44	4	0	NR	1	2
Performance	PPV (%)	16.7	NA	4	25	2.3	2	6	2.6	22.2	7.1	12	9.1	6.3	NA	10.4	4.7	2.9	16.7	33.3	40	66.7
	Sensitivity without MI (%)	66.7	NA	87.5	100	100	100	87.5	100	100	88.9	100	100	100	NA	90.9	100	100	100	92.3	100	100
	Incidence	1:2830	NA	1:5841	1:3865	1:4081	1:4374	1:3047	1:4834	1:2881	1:3473	NA	NA	1:11725	NA	1:3586	1:10282	1:13830	1:5239	1:4248	1:4250	1:5927
	Median days seen in CF centre																					
	CF	39	NA	45	44	60	15	15	36	40	NR	28	33	55	NR	28	27	NR	36	21	30	NR
	CFSPID	43	NR	60	NA	NR	43	22	36	NR	NR	NA	NR	42	NR	30	29	NR	NA	23	30	NA

Comments: The Spanish regions Valencia, Navarra and Murcia regions are not included in the table as there are > 50% data missing.

Abbreviations: ST, sweat test; IRT, immunoreactive trypsinogen; CF, Cystic Fibrosis; CFSPID, Cystic Fibrosis Screen Positive Inconclusive Diagnosis; PPV, positive predictive value; MI, meconium ileus; NR, not reported; NA, not applicable

Table 3: Summary of key outcomes for national and regional programmes

	Total numbers (N=42)	**National programmes** (N=21)	**Regional programmes** (N=21)
Total number screened	6,239,294	5,601,796	637,498
CF diagnosis by NBS	1026	903	123
CFSPID	181	106	75
Carriers	1299	958	341
Lost to follow up	1223	1089	134
False Negatives with MI	102	90	12
False Negatives without MI	29	21	8
Sensitivity without MI (mean (95% CI), range)	91% (89-93%) 67 – 100%	91% (89-93%) 75 – 100%	91% (86-96%) 67 – 100%
PPV (mean (95% CI), range)	12% (10-14%) 2 – 91%	13% (11-15%) 4 – 91%	6% (2-11%) 2 – 67%
Timeliness in days (mean (95% CI), range of median days)	32 (27-36) 12-60	26 (23-30) 12-37	37 (29-45) 15-60

Abbreviations: MI, meconium ileus

Table 4: Comparison of the performance of national and regional programmes in 2014 and 2019 with ECFS standards

ECFS standards [+]	2014 National programmes * (n=13)		2019 National programmes (n=21)		2019 Regional programmes (n=21)	
	Achieving standards	Range of performance	Achieving standards	Range of performance	Achieving standards	Range of performance
Positive predictive value (PPV) > 30%	62% (8/13)	3 – 75%	43% (9/21)	4 – 91%	17% (3/18)	2 – 67%
Sensitivity ≥ 95%	69% (9/13) °	81 – 100%	75% (15/20) °	75 – 100%	70% (14/20) °	67 – 100%
Timeliness (seen in CF centre by 35 days)	92% (12/13)	15 – 53 days	88% (15/17)	12 – 37 days	47% (7/15)	15 – 60 days

+ ECFS (European Cystic Fibrosis Society) Standards of Care: Best Practice guideline. *J Cyst Fibros* 2014; 13(Suppl 1):S23-S42 (Reference 2). ECFS best practice guidelines: the 2018 revision. *J Cyst Fibros* 2018; 17(2):153-178 (Reference 3)

* Data from the 2014 survey (*J Cyst Fibros* 2017;16:207-13 (Reference 6)

° Sensitivity without meconium ileus used for calculation in EU survey 2014 and 2019