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Recommendations for quality improvement in genetic testing for cystic fibrosis

European Concerted Action on Cystic Fibrosis

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These recommendations for quality improvement of cystic fibrosis genetic diagnostic testing provide general guidelines for the molecular genetic testing of cystic fibrosis in patients/individuals. General strategies for testing as well as guidelines for laboratory procedures, internal and external quality assurance, and for reporting the results, including the requirements of minimal services in mutation testing, the nomenclature for describing mutations, procedures to control false-positive amplification reactions and to validate tests, and guidelines to implement a quality system in a molecular diagnostic laboratory are reviewed. *European Journal of Human Genetics* (2000) 8, S1–S24.

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Introduction

To perform a molecular genetic diagnostic test is a complex process. Errors in one of the steps in this process may affect the results and therefore the conclusions. Because the results of a genetic test can have serious implications for an individual and possibly for his relatives, it is important that the error rate of genetic tests should be reduced to an absolute minimum. Therefore good internal quality control systems for the whole procedure, from blood sampling to the delivery of the written report, should be worked out in each genetic diagnostic laboratory. This may require the implementation of good laboratory practice (GLP) procedures and some form of accreditation of laboratories able to demonstrate that they master all the parameters which affect results.

Obviously, genetic testing should be done in the context of appropriate genetic counselling. Laboratories offering genetic testing should work in close association with clinical geneticists and cystic fibrosis (CF) experts to ensure that the appropriate tests and the appropriate information are provided to the patients requesting these services.

The purpose of the present document is to define strategies and principles which increase the likelihood of CF testing in Europe being provided accurately and precisely. This document was prepared within the framework of the European Concerted Action on Cystic Fibrosis (BMH4-CT96-0462) and is based on the experience gathered by this concerted action on the facilities, procedures and modus operandi in the different countries (for membership of ECCACF see Appendix 2). More than 150 laboratories participated in this project; 90 actually formally approved this document; the others did not voice their approval or dissent. Although these recommendations focus on genetic testing for cystic fibrosis, the major issues of the recommendations are also applicable to other genetic disorders and are based on a series of

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previously published documents. They also provide the basis for the organisation of an internal quality control system in a genetic laboratory. The implementation of these guidelines will require specific efforts from individual laboratories. In addition, it is proposed that regional, national or supra-national agreements are made between laboratories and health authorities in order to structure these services optimally to the benefit of the population serviced.

Recommendations on the strategy for CF testing

Minimal services in mutation testing

It is advisable to organise the services on two levels in a particular region or country. There are very large numbers of CF mutations in the European population. Whilst it would be desirable to have a mutation detection rate superior to 95%, the molecular heterogeneity of *CFTR* gene defects in Europe and the variation in frequency from one population to another make this goal unachievable for all countries with the current technology. For this reason, it is important to know the ethnic or geographic origin of the patient under investigation and, if possible, of the parents and grandparents.

Tables 1 and 2 (see Appendix 2) give a summary of the data currently available in the literature. Table 1 does not give accurate frequencies for all regions and all mutations. It is not advisable to use these mutation frequencies for risk calculation. The data from the local population should be used. In several countries (Denmark, Sweden, Switzerland and Israel) a detection rate of 90% of the mutations (with a limited set of mutations, each with a frequency of over 1% in the population) is achieved.

In another seven countries (Belgium, Czech Republic, Estonia, Ireland, The Netherlands, Ukraine, UK), 80 to 90% of the mutations (each with a frequency of over 1% in the population) can be routinely detected.

Pilot studies have been carried out in several countries where the spectrum of CF mutations is well known (Bulgaria, France, Greece, Italy and Spain). For these countries, by extending mutation analysis to mutations with relative frequencies less than 1%, detection levels of over 80 or 90% are achieved. In countries where these levels of detection have not yet been reached we suggest:

- a pilot study to determine the most frequent mutations in the respective national populations and/or in their various regions;
- once this is known, test for these mutations or at least for the mutations with a frequency of more than 1% in that population.

To this end, international collaboration should be sought.

In addition to be able to detect *CFTR* mutations with a frequency of over 1% (including deletions), a molecular genetics laboratory should type the 5T allele (IVS8-6) if

patients are investigated for infertility.¹ Also, each CF laboratory should be able to perform segregation analysis using intragenic polymorphic markers (Table 3).

Analysis of other phenotypes possibly related to *CFTR* mutations (Azoospermia, CBAVD, disseminated bronchiectasis, pancreatitis, nasal polyposis, etc) is not encouraged at present but if done should not be restricted to the set of mutations used for typical cystic fibrosis.

Practical organisation in level 1 and level 2 laboratories should ideally be decided in consensus with the various laboratories of a particular region or country. If a consensus cannot be reached, the regional or national authorities could mediate to achieve a consensus. A network of the two testing levels would comprise:

- Level 1 (local), at which rapid, standardised, and cheap assays should be performed, with the emphasis on tests which are relevant for a diagnostic or therapeutic decision;
- Level 2 (national or European) extends the analyses towards more detail and organises training.

At level 2, databases with patient and mutation information could be set up, if desired. More sophisticated and new techniques could be explored, evaluated and validated for implementation in level 1 testing laboratories. External quality assessment trials should focus on level 1 activities and be supported by level 2.

Criteria for testing laboratories

The following minimal criteria should be met for a laboratory to attain **level 1** status:

- provision of an 80–90% mutation detection level for its region;
- quality accreditation by an independent official organisation, or at least able to demonstrate that it follows good laboratory practice (GLP) rules;
- proof of regular participation in specialised training sessions by all its personnel;
- regular participation (at least once a year) in external quality assessment schemes;
- appropriate turn-round time to provide results to a clinician (eg maximum 5–10 working days for prenatal and neonatal diagnosis).

A level 2 laboratory should be active in *CFTR* research, and its excellence recognised at the international level. It should be part of a network of recognised European centres.

The tasks of a level 2 laboratory are to:

- perform additional mutation screening for less common mutations on incompletely characterised samples

(a reference laboratory should have the capacity to detect any *CFTR* mutation);

- help level 1 laboratories perform the pilot studies;
- implement and update novel technologies for mutation detection;
- maintain and make available reagents, control DNAs and cell lines;
- train personnel in *CFTR* gene mutation analysis;
- organise and co-ordinate quality assessment schemes.

National authorities are encouraged to identify these facilities.

A European network of diagnostic laboratories involved in CF mutation analysis would benefit the overall service. Such a network would create a number of interesting opportunities: expertise and expensive infrastructure could be centralised, the overall effect would cut the cost per sample, and a large number of different diagnostic methods could be offered.

To become a level 2 laboratory the following additional criteria should be met:

- demonstrate that enough scientific expertise and the necessary infrastructure is available on site;
- work according to good laboratory practice (GLP) procedures and be quality accredited at national and/or European level to perform genetic tests.

At level 2, the mutation detection rate should be increased to a level at which virtually all mutations can be detected. This should be achieved using additional mutation-specific methods and/or generic methods, followed by confirmation of the mutation by sequencing. Moreover, functional testing of the patient (rectal chloride transport (ICM), nasal potential difference (NPD)), or at the protein and RNA expression level, could be organised at level 2, as the network will comprise laboratories able to perform these complex tests.

At present the organisation of genetic services differs from country to country. A good overview of medical genetic services in 31 countries is given by R Harris,² an updated version for France is in preparation (M Goossens (1999), personal communication).

Methods

A wide range of techniques are used to identify mutations and polymorphisms in the *CFTR* gene. There is no gold standard for routine testing. All available methods have disadvantages and require considerable skill and experience to perform. There is no standardisation or general preference as to which method(s) should be used, but laboratories should be aware of the limitations of the methods applied and know which mutations are not identifiable by the method used. This means that individual laboratories need to

choose a method which is suited to their experience and project in hand.³ It is also important to know the cost of a specific technique. Table 4 gives an overview of the total time required and actual costs for materials of the most used *CFTR* mutation detection methods. This information is based on a cost comparative study organised by the European Concerted Action on CF.

It is not practicable and usually irrelevant to try to force genetic laboratories to use one or more specific methods. More specifically with regard to CF testing, apart from the most prevalent mutation, $\Delta F508$, most of the alleles are rare or even private. Fewer than 10 of the known *CFTR* mutations each account for 1–3% of the carriers in the European population; other mutations are relatively more common in other ethnic groups. Therefore, analysis of numerous mutations is required to obtain satisfactory carrier detection levels.⁴ Based on published manuscripts^{5–7} and the results of a survey of the various CF molecular genetic laboratories (European Concerted Action BMH4-CT96-0462, unpublished data (M Macek Jr, C Deltas, P Pacheco, (1999), personal communication)) we recommend a minimal number of mutations be assembled for each ethnic population (or region) to ensure detection of disease mutations in at least 80% of all carriers and patients (Tables 1 and 2).

CF mutation detection methods can be divided into two groups: mutation detection (test DNA sample for presence or absence of one specific mutation), and mutation scanning methods (screen sample for any deviation from the standard sequence). The features of all *CFTR* mutation detections currently applied are summarised in Table 5. The list is not exhaustive and will need updating when new technology becomes available. Moreover, new methods will need validation. An overview of mutations tested by available commercial kits is provided in Table 6.

Indications for testing in cystic fibrosis and related disorders

Whilst the quality of the laboratory method is a prerequisite for accurate testing, setting the correct indication is an integral part of the successful test. The *CFTR* gene is large (230 kb) with numerous mutations (>900) and potentially functionally important polymorphisms (>300).⁸ Nevertheless, strategies are available now which allow one to arrive at a reliable diagnosis or to rule out with high probability the presence of disease or of heritable mutations.

Combination of analysis of the *CFTR* gene for mutations, which in all cases should aim to identify mutations on more than 80% of chromosomes, by sweat chloride tests, nasal potential difference (NPD) measurements and measurements of rectal chloride transport (ICM) provide in the majority of cases reliable diagnostic tools. Nevertheless, clinicians may frequently establish a diagnosis of CF by sweat testing alone.

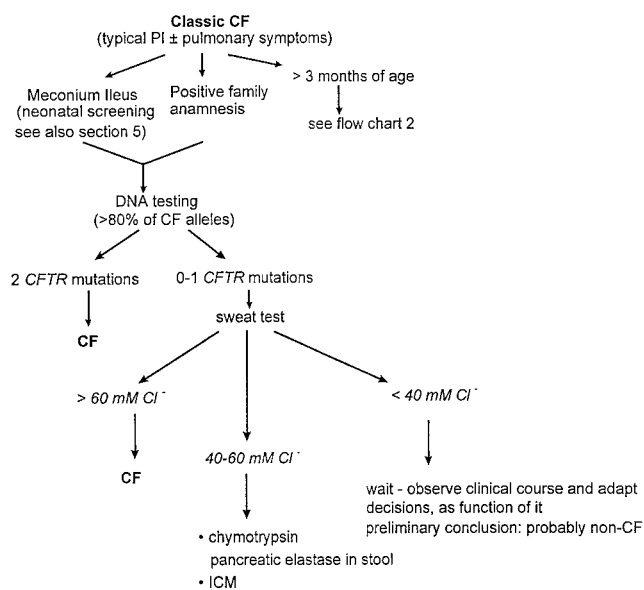


Figure 1 Classical CF in newborns.

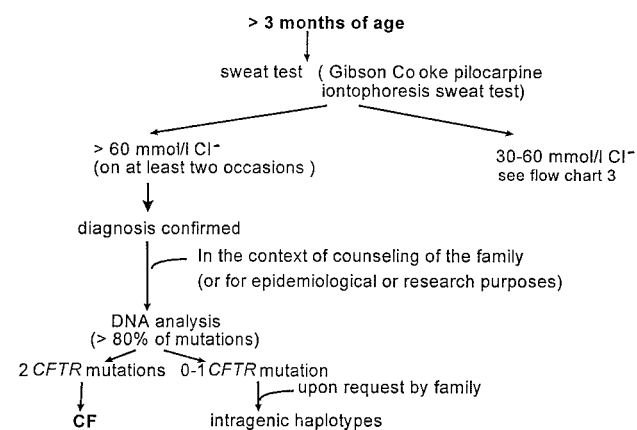


Figure 2 Classical CF in children, adolescents, or adults with typical CF symptoms.

Since facilities for some or all of these assays may not be available in every region or country in Europe it is recommended that health authorities provide the means to set up or develop centres which can provide reliable and accurate testing for CF, including at least one centre for NPD and/or ICM measurements.

In the following pages strategies and decision procedures are represented in the form of flow charts for diagnosing typical and atypical CF cases efficiently and justifiably, as well as the approach to be followed for relatives, individuals, or couples in the general population.

Classic CF The decision tree for classic CF diagnosis distinguishes between testing children younger than 3 months (Figure 1) older than 3 months and adults with classic CF symptoms (Figure 2), and those with borderline sweat test results (Figure 3).

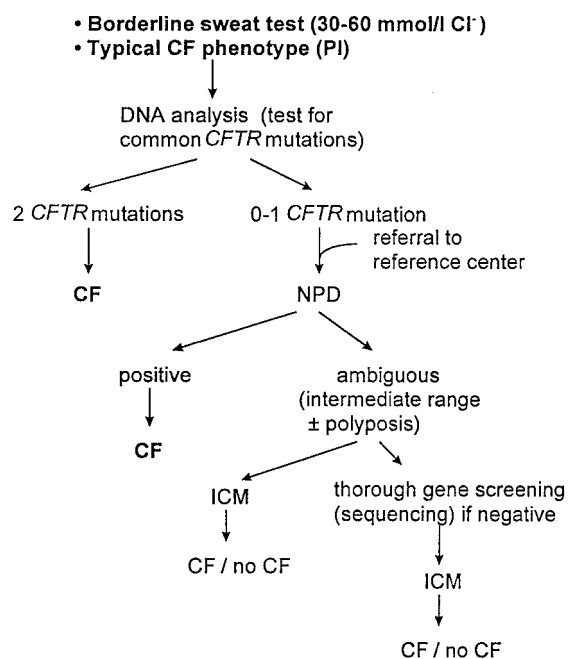


Figure 3 Children, adults with typical CF symptoms but borderline sweat test.

Exceptional cases of cystic fibrosis would be:

- *de novo* mutation; (in fact 6 cases are known with a new mutation of paternal origin. Also cases of non-paternity have been identified.
- complex alleles (to be confirmed by pedigree analysis) composed of different polymorphisms with functional repercussions;
- uniparental disomy;
- genetic heterogeneity cannot be excluded (there is probably $\leq 1\%$ of patients with typical clinical CF including positive sweat test).

Atypical CF A series of diseases is associated with an increased frequency of CF mutations. The more frequent are described here with the current most appropriate procedure for *CFTR* testing.

Clinically atypical CF would be:

- pancreas sufficiency (1–20% in different populations);
- highly variable clinical manifestations such as atypical asthma, nasal polyposis, CBAVD (Figure 4), bronchiectasis, pancreatitis in children (Figure 5) and adults, liver cirrhosis, and diffuse panbronchiolitis (Japan, China).

In adults of reproductive age with pancreatitis no *CFTR* mutation analysis is advised at present because of insufficient knowledge of mutations, and ascertainment of risk is not possible from the available data. In the foreseeable future,

tests for the entity-associated *CFTR* sequence variations/ mutations and genetic counselling/testing of the family will be available. In adults >50 years of age definitely no diagnostic *CFTR* mutation analysis is advised (no correlation with the clinical symptoms), unless requested for genetic counselling in the family.

Other diseases with increased frequency of mutations (eg pulmonary diseases, infertility, etc) should be handled as in Figure 6.

Relatives of CF patients In addition to standard genetic counselling for genetic diseases the following approach is proposed:

- (1) Parents: when genetic counselling is requested, a test can be done for the mutations found in the index case; otherwise screen for common mutations (>80%)
- (2) Siblings (adults, sexually active sibs/relatives): ideally testing should be done only on request of the individual and not of the parent. Screening for common mutations or segregation of intragenic polymorphisms should be pursued if the mutations are not found. An exception may be in the case of siblings with mild or atypical CF symptoms, or with chest X-ray abnormalities. Sweat testing of sibs is routine in many

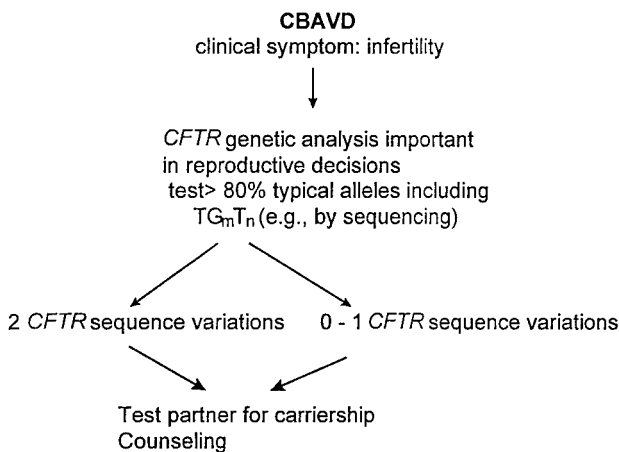


Figure 4 Congenital absence of vas deferens (CBAVD).

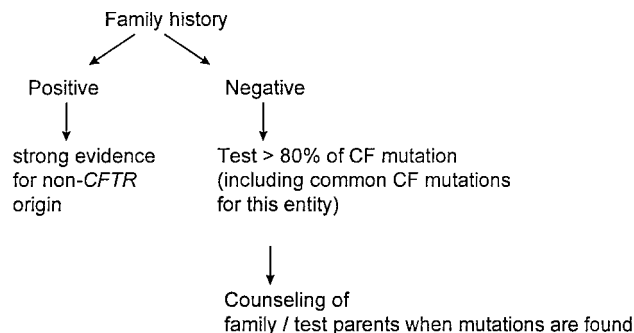


Figure 5 Pancreatitis in children.

Characterize typical clinical manifestations of the subject (pulmonary function testing, X-ray, chest spermiogram, sweat test, bacteriology, etc.)

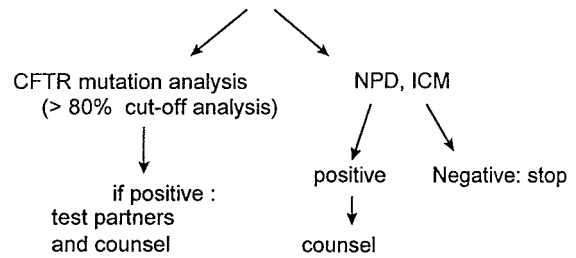


Figure 6 Other diseases with increased frequency of mutation (COPD, infertility, etc).

clinics. No information regarding carrier status should be communicated to third parties without consent of the testee.

- (3) Prenatal diagnosis (on request and after genetic counselling): it is essential to have an index case available in the extended family (high risk of carriership) so that the carrier status of the parents can be assessed. In case two mutations are not identified the CF haplotypes should be reconstructed. Prenatal diagnosis can be offered based on the confirmation of parental mutation/haplotype (including biochemical assays). Where only one parent is related to the index case and a mutation cannot be identified in the other parent, the various options available – no prenatal diagnosis, prenatal exclusion of the single mutation – should be discussed during counselling. If echogenic bowel is identified by foetal sonography, testing of the foetus for CF mutations should be considered.

Individuals without family history The purpose of carrier testing is to provide individuals with informed reproductive options:

- information should be provided to all couples, preferably before pregnancy starts about the frequency of CF carriers and the possibility of being tested for carriership;
- the provision of information should be recorded;
- on request by any adult or adolescent (potentially sexually active people) appropriate information about the disease and its genetic aspects should be provided, as well as the possibility and the limits of testing.

Individuals found to be carriers should be referred to an authoritative genetic or CF clinic.

Infertile couples For *in vitro* fertilisation (IVF) procedures and for sperm donors, genetic counselling and systematic screening for common CF mutations (>80%) should be considered.

Neonatal screening Various approaches or variants of the same procedure for neonatal testing exist in different regions of Europe. For the sake of completeness a standard procedure is given. As more data are obtained on the use of the PAP (Pancreas Associated Protein) test, its more general use should be considered in the future. This is an alternative or supplement to the IRT (Immunoreactive Trypsin) test (see Figure 7).

Risk Calculation

More than 900 mutations have been described in the *CFTR* gene. In a given population not all mutations can be identified by most diagnostic tests. Risk calculation⁹ may therefore be required in order to determine the remaining risk when no mutations are found.

It should be noted that frequencies of *CFTR* mutations found in CF carriers (*q*) of a given population might be different from the frequencies of *CFTR* mutations among CF patients in that population. Formulas and figures are presented only for individuals or couples with no family history of CF and no consanguinity. For each situation an extensive and a simplified formula is given. The simplified formulas are easier to use and the error, compared with the mathematically more correct ones, is very small. This error might be even smaller than the error of the input parameter *q* (*q* varies from 1/20 to 1/30 in the Caucasian population, and is not precisely known for the majority of the populations).

The sensitivity of the test determines the proportion of all CF patients in a given population who can be detected by the test (Figure 8). The higher the sensitivity of the test, the higher the proportion of CF patients in whom a mutation can be identified on both *CFTR* genes.

When a test with a sensitivity of less than 100% is used, a negative result does not necessarily mean that this individual

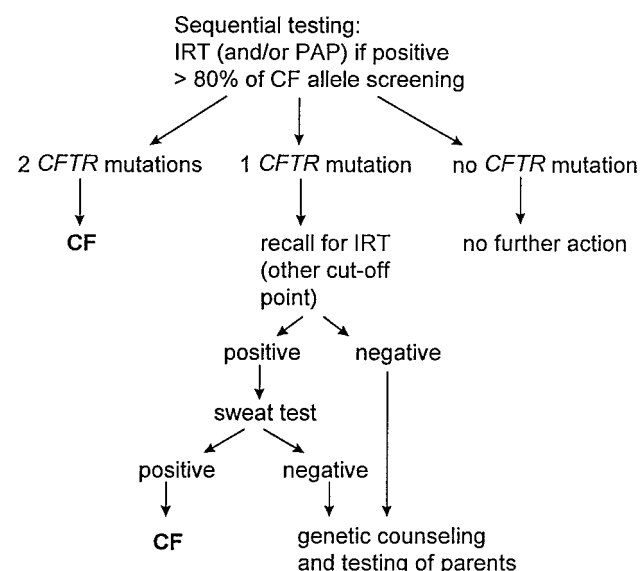


Figure 7 Neonatal screening.

is not a carrier. The risk for an individual of being a carrier when no mutation has been identified expressed in function of the sensitivity of the test, is given in Figure 9. The higher the sensitivity of the test, the lower the risk for an individual of being a carrier when no mutation is identified.

Only those couples comprising partners who are both carriers of a *CFTR* mutation, have a 1 in 4 risk of having CF children. A test with a sensitivity less than 100% will not detect all these couples (Figure 10).

The 1 in 4 risk is much higher than the risk for a random couple who have not been tested (Figure 11). When both partners test negative, the risk for any of their children is

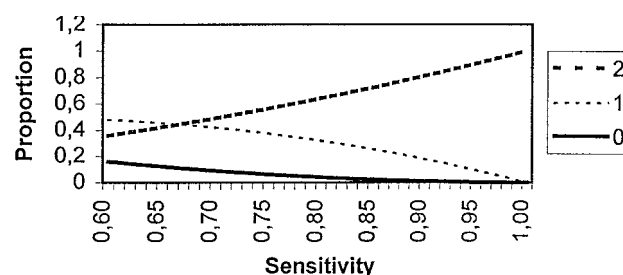


Figure 8 Proportion of all CF patients in a given population in which a mutation can be identified on both mutant *CFTR* genes (2), on only one mutant *CFTR* gene (1), and in which no mutation can be identified on any *CFTR* gene (0), all in function of the sensitivity of the test. Formula for 2 mutations found to be positive: $P = S^2$; Formula for 1 mutation found to be positive: $P = 2S(1-S)$; Formula for no mutation found to be positive: $P = (1-S)^2$; with P = proportion of CF patients; S = sensitivity of the test, ie proportion of mutant *CFTR* alleles in a given population that can be identified with the test.

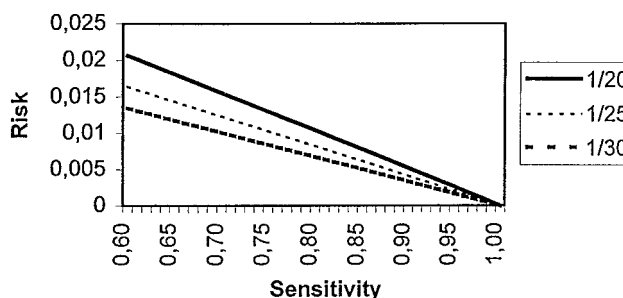


Figure 9 Risk for an individual of being a carrier when no mutation is identified with the test, as function of the sensitivity of the test. The risk is given for carrier frequencies of 1/20, 1/25 and 1/30 in a given population. $R = q(1-S)/[q(1-S) + (1-q)]$ (proportion of individuals that test negative but are in fact carriers compared to all individuals that test negative, both the carriers with a negative test result and individuals that test negative because they are truly no CF carriers) with R = risk of being a carrier; S = sensitivity of the test, ie proportion of mutant *CFTR* alleles in a given population that can be identified with the test; q = carrier frequency in a given population.

Risk of an individual being a carrier when no mutation is identified with the test; simplified formula is $R = q(1-S)$.

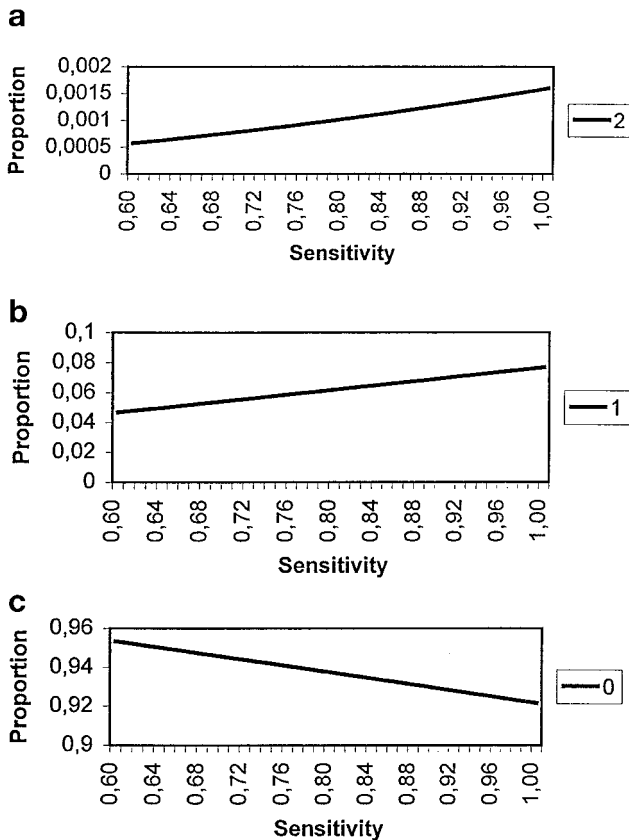


Figure 10 Proportion of couples in a given population, assuming a carrier frequency of 1/25, where (2) both partners will test positive for a mutation in function of the sensitivity of the test; (1) only one partner will test positive for a mutation; (0) both partners will test negative, in function of the sensitivity of the test. Formula both partners positive (Figure 10a): $P = (Sq)^2$; Formula one partner positive (Figure 10b): $P = (2Sq(q(1-S))/(q(1-S) + (1-q))) + 2Sq(1-q)$ (= proportion of couples where 'negative' partner is a carrier + proportion of couples where 'negative' partner is no carrier) Formula both partners negative (Figure 10c): $P = (q(1-S)/(q(1-S) + (1-q)))^2 + (2q(1-S)(1-q)/(q(1-S) + (1-q))) + (1-q)^2$ (= proportion of couples where both 'negative' partners are carriers + proportion of couples where one 'negative' partner is a carrier + proportion couples where both 'negative' partners are no carriers) with P = proportion of couples; S = sensitivity of the test, ie proportion of mutant *CFTR* alleles in a given population that can be identified with the test; q = carrier frequency in a given population. The proportion of couples in which both partners test positive (2) is small and varies from 1/1724 (sensitivity 0.60) to 1/625 (sensitivity 1.00).

Proportion of couples in a given population when only *one partner* tests positive, Simplified formulae is $P = 2Sq(1-S)q + 2Sq(1-q)$ (= proportion of couples where 'negative' partner is a carrier + proportion of couples where 'negative' partner is no carrier) Proportion of couples in a given population when *both partners* test negative, simplified formula is $P = ((1-S)q)^2 + 2(1-S)q(1-q) + (1-q)^2$ (= proportion of couples where both 'negative' partners are carriers + proportion of couples where one 'negative' partner is a carrier + proportion couples where both 'negative' partners are no carriers).

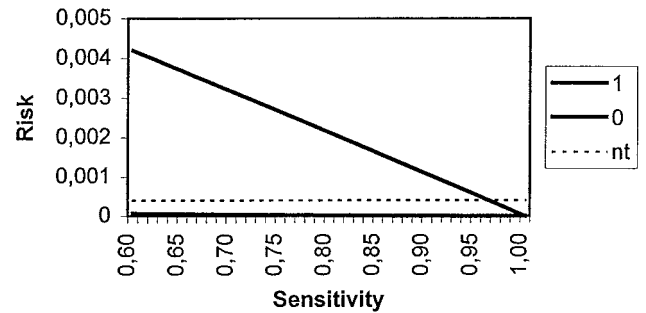


Figure 11 Risk for the children in a population with a carrier frequency of 1/25, in which only one partner tests positive for a mutation (1) both partners test negative (0). When both partners test positive for a mutation, the risk is 1/4. The risk is also shown when no test (nt) is performed.

Formula one partner positive:

$$P = \frac{1}{4} \left(\frac{2Sq^2(1-S)/(q(1-S) + (1-q))}{(2Sq^2(1-S)/(q(1-S) + (1-q))) + 2Sq(1-q)} \right)$$

(risk that both mutant *CFTR* genes are transmitted* proportion of couples in which one partner tests positive and the 'negative' partner is still a carrier compared with all couples in which one partner tests positive)

Formula both partners negative:

$$P = \frac{1}{4} \left(\frac{(q(1-S)/(q(1-S) + (1-q)))^2}{(q(1-S)/(q(1-S) + (1-q)))^2 + 2q(1-S)(1-q)/(q(1-S) + (1-q)) + (1-q)^2} \right)$$

(risk that both mutant *CFTR* genes are transmitted* proportion of couples where both partners test negative and are in fact carriers compared to all couples where both partners test negative).

Formula when no test (nt) is performed: $R = \frac{q^2}{4}$ (risk that both mutant *CFTR* genes are transmitted* risk that both partners are carriers (no test performed)).

With R = risk; S = sensitivity of the test, ie proportion of mutant *CFTR* alleles in a given population that can be identified with the test; q = carrier frequency in a given population. The risk for each of the children in couples where both partners test negative (0) is small and varies from 1/14190 (sensitivity 0.60) to 0 (sensitivity 1.00). When both partners test positive for a mutation, the risk is 0.25 (not shown).

Risk for each of the children where *one partner* tests positive, simplified formula is $R = (1-S)q/4$ (risk that 'negative' partner is still a carrier* risk that both mutant *CFTR* genes are transmitted) Risk for each of the children when *both partners* test negative. $R = ((1-S)q)^2/4$ (risk that both 'negative' partners are still carriers* risk that both mutant *CFTR* genes are transmitted).

lower than when no test is performed. If only one of the partners tests positive, again the risk will depend on the sensitivity of the test (Figure 11). Since the test can be negative either because the mutation is not detected or because the partner is not a true CF carrier, the risk for their children can be either lower or higher than the risk for a random non-tested couple. Only at very high sensitivities will the risk for couples, in whom only one partner tests positive, be lower than the risk for random untested couples. At lower test sensitivities, the risk will be higher than when

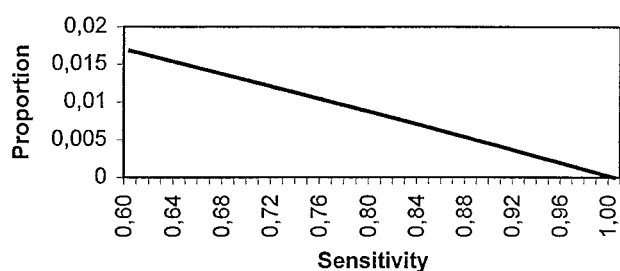


Figure 12 Proportion of couples, with only one partner who tests positive for a mutation, that have a real risk of having CF children, ie the couples where the partner that is negative for the test is still a CF carrier. A carrier frequency of 1/25 is assumed.

$$P = \frac{2Sq^2(1-S)/(q(1-S) + (1-q))}{(2Sq^2(1-S)/(q(1-S) + (1-q))) + 2Sq(1-q)}$$

(proportion of couples with one partner who tests positive but where the 'negative' partner is still a carrier compared to all couples with one partner who tests positive) with

P = proportion of couples, with only one partner who tests positive, that have a real risk of having CF children;
S = sensitivity of the test, ie proportion of mutant *CFTR* alleles in a given population that can be identified with the test;
q = Carrier frequency in a given population.

Proportion of couples, with only one partner testing positive, that have a real risk of having CF children, simplified formulae $P = 2Sq^2(1-S)/(2Sq^2(1-S) + 2Sq(1q))$ (proportion of couples with one 'positive' partner in which the 'negative' partner is still a carrier compared to all couples in which one partner tests positive).

no test is performed. For this reason routine screening of couples in the population might be problematic. Indeed, the proportion of couples with only one partner who tests positive is about 1/20–1/25. They will have an increased risk after testing, although only a minority of such couples has a real risk of having CF children. The proportion of these couples who have a real risk of having CF children is given in Figure 12. This proportion is again determined by the sensitivity of the test.

In the majority of couples, no mutation will be found (Figure 10). If the test has a 100% sensitivity, they will have no risk of having CF children. If the test has a lower sensitivity, there is still a very small risk of having CF children (Figure 13).

Conclusion

The recommendations described here are an attempt to provide written suggestions for the quality improvement of CF testing in Europe. While it may be difficult for a number of laboratories to fulfil all the criteria for a level 1 or 2 laboratory in a short time, the approval of these recommendations by the many laboratories involved in CF testing suggests that at least the awareness exists that quality assessment is possible and necessary. Guidelines, as the word indicates, attempt to be a guide to better quality, not laws

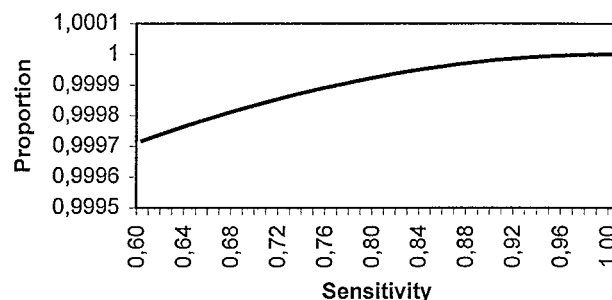


Figure 13 Proportion of couples, with both partners testing negative, that have no risk of having CF children, either because only one or both partners are truly no CF carriers. A carrier frequency of 1/25 is assumed.

$$P = \frac{(2q(1-S)(1-q)/(q(1-S) + (1-q))) + (1-q)^2}{(q(1-S)/(q(1-S) + (1-q)))^2 + 2q(1-S)(1-q)/(q(1-S) + (1-q)) + (1-q)^2}$$

(proportion of couples where both partners test negative with one or both partners truly none carriers compared to all couples where both partners test negative) with P = proportion of couples, where both partners test negative, that truly have no risk of having CF children; S = sensitivity of the test, ie proportion of mutant *CFTR* alleles in a given population that can be identified with the test; q = carrier frequency in a given population.

Proportion of couples, where both partners test negative, and who truly have no real risk of having CF children, simplified formulae:

$$P = \frac{(1-q)^2 + 2(1-S)q(1-q)}{(1-q)^2 + 2(1-S)q(1-q) + ((1-S)q)^2}$$

(proportion of couples in which both partners test negative in which one or both partners are truly no carriers compared to all couples in which both partners test negative).

which must be respected and adhered to at the risk of punishment. The Steering Committee of the Concerted Action also hopes that by publishing these recommendations many laboratories will be able to use the document to obtain improved facilities and equipment from their authorities, and that regional, national or supra-national exchanges and discussions will lead to the evolvement of a free collaborative network for CF testing to the benefit of patients, families and the general population.

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Appendix 1

More detailed guidelines about laboratory methods, organisation and reporting are given here. These guidelines are based as much as possible on existing internationally available documents. Aspects which particularly pertain to CF have been highlighted or have been added.

Methods

There are three essential steps in mutation screening: the DNA extraction, DNA banking, and the mutation testing method.

DNA extraction A number of methods exist for the preparation of nucleic acid samples for molecular genetics analysis. Since this stage can have a significant impact on the quality of the final results, care should be exercised to ensure that a validated protocol is followed, independent of whether the extraction method was developed in-house, obtained from the literature, or purchased as a kit from a manufacturer. Protocols that have been either developed in-house or as a modification of a manufacturer's kit should be validated (see further). Protocols that follow exactly a validated published method should undergo thorough performance verification.^{10,11} Written procedures of the methods used for DNA purification, including the sources of all components used, should be kept. Complete references should be included in standard operating procedure manuals. Changes in any of the procedures or source of components should be documented and approved by the laboratory, with date and initials recorded.¹²

The ideal method of DNA isolation in a molecular diagnostic laboratory must be simple, fast, safe and economical, but also precise and reliable. It must yield a high quality and quantity of high molecular weight DNA.

DNA banking Isolated material must be stored at 4°C or frozen. Excess DNA sample material should be stored at a temperature not higher than 0–5°C to ensure long-term stability. Concerning DNA stability and storage we refer to the literature.^{13,14} An optimal storage procedure would involve aliquoting of the DNA solution in one primary stock solution frozen at –80°C, and multiple portions for subsequent analyses stored at 4°C and/or –20°C. This procedure avoids repeated freeze/thaw cycles and minimises the possibility of DNA contamination.

Cystic fibrosis mutation testing methods A wide range of mutation testing methods^{15–24} is currently used in diagnostic laboratories.²⁵ The most frequently used mutation detection methods for cystic fibrosis are heteroduplex analysis, restriction enzyme analysis, reverse dot-blot, the commercial kits INNO-LiPA CF2 (Innogenetics nv, Gent, Belgium), Elucigene CF4 and CF12 (AstraZeneca Diagnostics, Abingdon, Oxfordshire, UK), and OLA Cystic Fibrosis Assay (PE Applied Biosystems, New Jersey, USA). Single strand conformation

polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), two-dimensional DNA electrophoresis, and sequencing are mostly used as CF mutation screening methods.²⁶ The ideal method for mutation testing in a molecular diagnostic laboratory must be rapid and cheap, allow automatisation, 100% efficiency and avoid the use of radioactivity and toxic reagents.

Whatever the methods used in a diagnostic laboratory, it is important that they are thoroughly validated (see test validation and characterisation). Written standard operating procedures, including the sources of all components, should be kept. Changes in any of the procedures or source of components should be documented and approved, with date and initials recorded.¹²

Laboratory organisation

Quality system A quality system for a molecular genetics laboratory should be directed at all fundamental aspects of its function. This means the setting-up of a quality system according to a molecular genetic translation of the criteria of good laboratory practices as developed by the OECD, the EN 45001 standard (the general criteria for the operation of testing laboratories) or ISO 17025, which are accepted in the European Union as the present standard. A quality system also includes the obligation to join external quality assessment schemes, and to make use of their results. Information about the requirements for a quality system is described in the literature.^{10,27–29}

Because the methodology of molecular biological diagnostics is constantly changing, no general standard can at present be defined. We suggest the guidelines formulated by the National Committee for Clinical Laboratory Standards (NCCLS) and the European Concerted Action (BMH-CT93-1673) (Eucromic)³⁰ be followed. A guide to fundamentals of quality assessment of molecular amplification methods in clinical diagnostics has recently been published by M Neumaier.³¹ These guidelines describe in detail reagent quality control, equipment calibration and maintenance, proficiency testing, training of technical staff, internal and external quality control.

Specimen types, specimen identification and access Collection and identification of the specimens are (often) carried out by non-laboratory personnel. Written procedures for proper collection, packing, shipment and handling of specimens are recommended by all the guidelines,^{7,10,32,33} documents, and quality assessment authorities consulted.^{12,34} Based on a review of these guidelines, we propose implementation of the following items.

(1) Specimen types

Since genetic analysis in cystic fibrosis is PCR-based, a minimal amount of DNA is required in the vast majority of cases. Unless extensive analysis is expected or planned, a mouthwash sample (or a buccal scrape sample in the

case of a baby) is sufficient. Otherwise a small volume of blood (2–5 ml) in EDTA will provide enough DNA for extensive analysis.

Prenatal diagnosis of cystic fibrosis is usually carried out on a chorionic villus sample taken during the first trimester of pregnancy. The samples should be checked by microscopic inspection and dissected immediately. Ideally they are independently prepared samples, which should all be tested. This minimises the potential problem of maternal cell contamination of the sample.

Amniocytes can also be used for molecular genetic analysis – either directly spun down from amniocentesis sample or after 10–14 days culture. Direct analysis should be carried out with caution, as the foetal cells are invariably contaminated with maternal cells. Tissue culture of the cells can either remove/reduce maternal complication or result in overgrowth of maternal fibroblasts. The analysis of a few polymorphic markers (CA repeats or STR) can identify the presence of contamination when compared with maternal DNA.

(2) Specimen identification

The specimen container should be clearly marked with a unique patient identifier, such as a hospital patient identification number. In most situations, the patient's name is not sufficient, although the combination of patient name and birth date is generally enough to prevent identification errors. The container should also be labelled with the date and time that the specimen was acquired.

(3) Request forms

All specimens should be accompanied by a request form which contains as much of the following information as possible

- Patient's name
- Date of birth
- Date of collection
- Gender
- Ethnicity (if applicable)
- Place of birth of patient, parents and grandparents
- Unique identifier found on the specimen container
- Specimen type (blood, amniotic fluid, etc)
- Reason for requesting the test, based on clinical information
- Relevant clinical or laboratory information, including sweat test results
- Pedigree (recommended for all cases)
- Referring physician or health professional
- Weeks of gestation (for prenatal diagnosis)
- Biling information (if applicable)

(4) Criteria for rejecting specimens¹⁰

It is recommended that each laboratory has written criteria for acceptance or rejection of specimens.

Rejection of specimens is strongly recommended if either the specimen or the request form lacks sufficient information for the laboratory or clinician to uniquely identify the specimen, or lacks other information necessary to determine if the specimen or test requested is appropriate for answering the clinical question. It is also strongly recommended that improperly handled or transported specimens should be rejected.

Other conditions for accepting or rejecting specimens are left to the discretion of the laboratory. Given the power of gene amplification techniques, it is difficult to set an arbitrary minimum cellular content or volume for specimen acceptance, but it is recommended that each laboratory develops its own standards. It is recommended that prenatal specimens, especially chorionic villus samples, be assessed for maternal cell contamination; this is done morphologically and by PCR-based DNA analysis.

(5) Accessing specimens

Each specimen should be assigned a unique laboratory identifier when accepted for testing. This identifier should be linked with the unique patient/family identifier and with other identifiers, such as those for individual electrophoretic gels or blots that may be used in the laboratory. The unique laboratory identifier should differentiate between specimens from different patients, between different specimens submitted from the same patient, and between specimens, from different patients of the same family.

(6) Specimen transport and storage

Each laboratory should establish criteria based on its own experience in successful extraction of analysable DNA from the various sample types. In general, frozen tissue should be transported on dry ice, fresh tissue on wet ice, and fixed or dried tissue at room temperature. Amniotic fluids should be transported at room temperature which allow the establishment of cell cultures. DNA can be extracted from whole blood stored at room temperature for a week or more.¹⁴ Frozen tissue can be stored at -70°C indefinitely; fixed or dried samples can be stored at room temperature for variable periods depending on the laboratory's experience.

(7) Specimen retention

A primary issue regarding specimen retention involves ethical and legal considerations such as specimen ownership, confidentiality, and informed consent.

Until universal recommendations are adopted or regulations are implemented, each laboratory should establish its own policy regarding specimen retention and the use of archived specimens or stored DNA (anonymisation

when used in research etc). Such policies will need to be in compliance with institutional regulations that may exist with federal and/or state regulations as they become implemented.

Controlling false-positive nucleic acid and target amplification reactions A significant challenge facing the diagnostic amplification of nucleic acids is the occurrence of false-positive results due to contaminating nucleic acids. The ability of amplification techniques to produce large numbers of copies of a sequence from minute quantities of nucleic acid necessitates that extreme care should be taken to avoid false-positive results due to transfer of DNA between samples.

(1) Laboratory design

Ideally, three physically separate areas of the laboratory should be available for reagent preparation, specimen preparation, and amplification and product detection. The reagent preparation area, for those laboratories using only commercially available kits, is considered to be at the site of manufacture. In laboratories where enzymatic or chemical means of inactivating amplified products are used, the demands for physical separation of pre- and postamplification procedures may be somewhat reduced, but good laboratory practice should still be diligently exercised.

(a) Workflow

Specimens should be processed in an area of the laboratory that is isolated from amplification and detection areas. Ideally, the specimen preparation area should be under positive pressure to other areas of the laboratory. If the specimen preparation area cannot be maintained at a positive pressure to other areas of the laboratory, specimen preparation should be performed in a class II biological safety cabinet to prevent contamination. The pre- and postamplification laboratories should be served by separate ventilation systems. Also, the postamplification area should be under negative pressure. Traffic of personnel should be from the specimen preparation area, with a change of laboratory coats, to the pre- and postamplification areas. Laboratory coats should be dedicated to specific areas and changed when going in and out of each area. With the introduction of commercially licensed tests and new methods, some of these requirements may be reduced. However, if contamination becomes a problem, then separate areas of the laboratory should be devised to accommodate these different processing stages.

(b) Containment devices

In the event of separate laboratory space not being available to segregate pre- and postamplification

activities, a class II biological safety cabinet should be used as a containment device for specimen preparation. Class I safety cabinets do not provide protection for material contained within them. Dead-air boxes with ultraviolet light attachments can provide a clean bench area for specimen preparation in a dedicated specimen preparation laboratory; UV lamps lose energy efficiency over time.

(2) Laboratory practice

Specific laboratory practice should be implemented to minimise the occurrence of false-positive results. These include the preparation of reagents and solutions, the correct use of pipettes, the use of laboratory coats and gloves and procedures for manipulation of reaction tubes. In addition, special care should be taken in the use of appropriate controls.^{10,35-37}

(3) Selection and preparation of controls

A *positive control* that amplifies weakly but consistently should be selected. The use of dilute positive controls prevents the unnecessary generation of large amounts of amplified product that can result in contamination.

Applicable *reagent controls* should be included with each amplification batch run. These controls contain all necessary components of the reaction without the addition of template nucleic acid or human DNA.

Negative controls should be dispensed last so that they reflect the state of the reagents added.

Assays based on presence or absence of PCR products must include known control primers yielding a positive result to check for proper amplification and sizing of the PCR products and to ensure that a negative result is accurate. This should include a positive result with control primers detecting a spiked additive or a constitutive component.

When specimens are analysed for *sequence variation*, controls containing all alleles to be detected must be included.

Assays in which the result is based on *fragment size* must include size markers (sequencing ladders, etc) covering the range of expected results during gel electrophoresis.

Assays based on changing of electrophoretic mobility must include appropriate controls to ensure correct interpretation. New samples should be confirmed by alternative methods. Any unexpected result requires a repeat of the assay. Procedures for the analysis of possible new mutations should be available.

Test validation and characterisation For most tests performed in general clinical laboratories the process of test validation is fairly straightforward and can be done according to well established guidelines.³⁸ However, the unique aspects

of genetic testing make the validation of genetic tests a challenge in many instances. Validation of a clinical laboratory test should be on both analytical and clinical levels. Analytical validation involves determination of the various parameters themselves, such as accuracy, precision, analytical range, sensitivity, specificity, detection limit, interferences, and recovery.³⁸ Clinical validation refers to determination of the predictive value of the test, or the probability that a person with a positive test result will have or will develop the disease.³⁹

Test validation should be conducted before a new test is introduced for clinical use. The test should be subjected to literature review and to analytical and laboratory/clinical correlation studies. This means characterisation of the detected mutations, establishing the performance properties of the test to ensure the test's ability to provide consistent and reliable results, establishing the clinical utility of the test, defining aspects of the procedure which should be carefully regulated to maintain test performance, and defining the limitations of the test.

Such validation is necessary to assure the safe and effective application of a genetic test for its intended use. Each laboratory should develop its own validation protocol. We recommend the recently published practical guide for the validation of genetic tests by E Prenc¹¹ be followed and used.

Safety Training and use of safe laboratory practices are essential for the protection of all personnel. *Good Laboratory Practices*¹² and the Commission on Laboratory Accreditation of the College of American Pathologist³⁴ recommend a general safety file in each laboratory. This document should describe safety measures concerning infective samples, dangerous chemicals, radiation and electronic danger, and instructions for proper cleaning of the laboratory.

Reports

Reports of the results Genetic test results should be communicated to the referring physician or genetic professional and to any physician designated by the patient. Reports of test results should be issued in a standardised form, clearly intelligible to the non-specialist. In general, the laboratory should not directly report on the results to the patient: the laboratory should ensure that the clinician reporting to the patient has a full understanding of the results and the underlying clinical meaning of the result. The laboratory report should include:

- collecting date
- nature of the sample
- name of the individual
- date of birth/place of birth
- laboratory identification number of patient and sample
- date of report

reason for testing

the genotype and/or haplotype established for the individual

interpretation of the data (should relate to the reason for testing prenatal diagnosis, carrier testing, the sensitivity of the test, etc)

the signature of the laboratory director or other authorised individual and his/her name

It is recommended that along with this information, the following be included: the family number (if it has been assigned), and a pedigree with the genotype information indicated if applicable (eg linkage study).^{10,33}

Nomenclature for designation of mutations A systematic common nomenclature is essential for the deposition of mutations into computerised databases and their subsequent accessibility to the research and clinical community. Databases of mutations in genes are required for efficient access by clinicians and researchers. Clinical geneticists can identify and study patients with the same mutations and perhaps provide prognostic information. Researchers can readily determine whether a specific mutation has been described.¹⁵

It is obvious that the most unambiguous nomenclature system is based on genomic DNA. However, length polymorphisms may create a problem in the numbering of nucleotides. Therefore a reference sequence standard needs to be established. We recommend the nomenclature system suggested by the Ad Hoc Committee on Mutation Nomenclature⁴⁰ and Antonarakis *et al.*⁴¹

Thanks to the electronically available database set up by L-C Tsui,⁸ the nomenclature of cystic fibrosis mutations is universally accepted. Although the nomenclature for CF is simple, results of the European quality control trials (Dequeker and Cassiman²⁵) demonstrated that a significant number of laboratories violated the nomenclature rules. Based on earlier published documents,^{7,10,32,41-44} we describe in the following the major issues of the CF mutation nomenclature system.

(1) General recommendations

A single letter code is used for designating amino acids. Nucleotides are designated as DNA bases, not as RNA bases. Nucleotides are designated in the sense strand (eg ATG for a methionine codon). With this nomenclature system, mutation designations starting with a letter refer to an amino acid and the number refers to a codon position. In contrast, mutation designations starting with a number refer to a nucleotide position in the coding sense strand, and subsequent letters refer to DNA bases. Nucleotide changes are indicated by arrows. Superscripts and subscripts should not be used, and there should be

no spaces between the numbers and letters in a mutation designation.

(2) Missense and nonsense mutations

Missense and nonsense mutations are described in terms of the change in the gene product. A missense mutation is designated by the number of the amino acid position and the single-letter abbreviations of the amino acids involved. The abbreviation for the normal amino acid precedes the number, and the mutant amino acid follows the number, with no spaces between. For example, G551D indicates that the glycine residue at position 551 in the protein has been replaced by an aspartic acid residue. If different nucleotide substitutions lead to the same amino acid substitution, such as that occurring at S549 in the *CFTR* gene, the mutation designation should include the nucleotide change, within parenthesis, immediately following the designation for the amino acid substitution. Using the cystic fibrosis gene example, this would be S549R(1777 A/C), or S549R(1778 T/G).

Nonsense mutations are designated similarly, except that X represents a termination codon. For example, G542X indicates that the glycine residue at amino acid position 542 has been replaced by a termination codon.

(3) Insertions and deletions

Insertion or deletion mutations are designated by a nucleotide number of the sense strand, followed by ins (for insertion) or del (for deletion). The nucleotide position is the one preceding an insertion, or the first that is deleted. The exact nucleotides are specified if only one or two are involved. For example, 441delA indicates the deletion of deoxyadenylic acid at nucleotide position 441. The 'name' 241delAT indicates the deletion of deoxyadenylic acid at nucleotide position 241, and the deletion of deoxythymidylic acid from nucleotide position 242. Mutations involving both substitution and a small insertion or deletion can be designated by the first altered base, followed by the nucleotide change. For example, 2183AA-G indicates replacement of AA at nucleotide positions 2183 and 2184 in the normal sequence by G in the mutant allele.

(4) In-frame deletions

Deletions of single amino acids result from deletions of three bases and are represented by a Δ followed by the single-letter code of the amino acid and its position, eg Δ F508 is the deletion of phenylalanine (F) at position 508. An acceptable alternative is 'Delta', 'delta' or 'del'. It has been proposed to write amino acid deletions in the future as amino acid codon del e.g. F508 del.⁴¹

(5) Complex deletions/insertions

There are several mutations which involve the deletion or insertion of four or more bases and these are usually named as the number of bases which are deleted or inserted, eg 1461ins4 which is the insertion of four bases (in this case AGAT) after base 1461, and 1949del84 which is the deletion of 84 bases from base 1949. There are some much larger deletions which remove one or more complete exons, eg *CFTR*del2 which is the deletion of exon 2.

(6) Splicing mutations

These are (usually) the substitution of a base in the splice acceptor site (an AG dinucleotide at the 3' end of the intron) or the splice donor site (a GT dinucleotide at the 5' end of the intron), both of which are highly conserved in human genomic DNA. The position of the mutated base is numbered from the first or last base in the exon, as intronic bases are not themselves numbered. Thus the mutation 621 + 1G > T is the substitution of a guanine by a thymidine at the first base in intron 4 (the last base of exon 4 being numbered 621 and the first base of intron 4 being 621 + 1). Similarly, 621 + 2T > C is a substitution at the second base in intron 4. Numbering of the acceptor site bases is from the 5' end of the exon, eg 1717-1G > A is the substitution of the last base of intron 10 (a guanine) by an adenine. Some splicing mutations are quite distant from intron/exon boundaries, eg 3849 + 10 kbC > T.

(7) Other nomenclature rules

Nomenclature rules suggested for larger deletions and insertions, splicing mutations, mutations in the noncoding sequence and the more complex mutations are addressed by the Ad Hoc Committee on Mutation Nomenclature.⁴⁰

Appendix 2

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Table 1 Mutations found at a frequency higher than 1% in Europe⁵⁻⁷

Country									
Albania	dF508 (70.0)								
Austria	dF508 (63.7)	G542X (2.1)	R1162X (1.9)	G551D (1.1)					
Belarus	dF508 (63.0)	N1303K (2.7)	G542X (2.1)	W1282X (1.7)					
Belgium	dF508 (75.5)	N1303K (2.9)	G542X (2.7)	W1282X (1.5)	S1251N (1.3)	1717-1G>A (1.1)			
Bulgaria	dF508 (61.0)	N1303K (409)	G542X (3.6)	R347P (2.0)	1677delTA (2.0)	R1070Q (1.5)	W1282X (1.3)	G1244V+S912L (1.0)	
Croatia	dF508 (64.5)	N1303K (3.6)	G542X (3.3)	G551D (1.1)					
Cyprus	dF508 (46.7)	L346P (16.7)	1677delTA (6.7)						
Czechia	dF508 (71.6)	G551D (4.0)	N1303K (3.0)	G542X (2.2)	1898+1G>A (2.0)	2143delT (1.2)	CFTRdele2,3(21kb) (4.6)		
Denmark	dF508 (87.2)	394delTT (1.9)	N1303K (1.0)						
Estonia	dF508 (54.0)	394delTT (15.0)							
Finland	dF508 (46.2)	394delTT (28.8)							
France	dF508 (66.8)	G542X (3.1)	1717-1G>A (1.6)	N1303K (1.4)					
Germany	dF508 (73.2)	R553X (2.7)	R347P (1.3)	G551D (1.3)	N1303K (1.2)	G542X (1.2)	3849+10kbC>T (1.2)	CFTRdele2,3(21kb) (1.5)	
Greece	dF508 (52.2)	621+1G>T (4.5)	G542X (3.9)	N1303K (3.3)	2183AA>G (1.8)	2789+5G>A (1.8)	E822K (1.6)	R117H (1.2)	R344W (1.2) 3272-26A>G (1.0) R1158X (1.0) G85E (1.0)
Hungary	dF508 (54.7)	G542X (2.2)	N1303K (1.0)						
Ireland	dF508 (72.7)	G551D (6.9)	R117H (2.0)	G542X (1.0)					
Israel	dF508 (32.2)	W1282X (36.2)	G542X (5.4)	3849+10kbC>T (4.6)	405+1G>A (3.8)	N1303K (3.0)	Q359K+T360K (1.9)	S549R (1.1)	
Italy	dF508 (51.1)	G542X (4.8)	N1303K (4.8)	2183AA>G (2.7)	R1162X (2.4)	1717-1G>A (2.1)	W1282X (1.2)	R553X (1.2)	
Latvia	dF508 (54.2)	3849+10kbC>T (12.5)	N1303K (8.3)	W1282X (4.2)					
Lithuania	dF508 (30.9)	R553X (4.8)	N1303K (2.4)						
Macedonia	dF508 (56.0)	G542X (3.3)	N1303K (2.6)	621+1G>T (1.9)	3849+10kbC>T (1.9)	457TAT>G (1.3)	V1397E (1.3)		
North Africa	dF508 (32.0)	N1303K (10.2)	W1282X (8.2)	711+1G>T (7.5)	G542X (4.8)	R1162X (2.7)	L227R (1.4)	S549R (1.4)	S549N (1.4) S549I (1.4) G551D (1.4) S945L (1.4)
Northern Ireland	dF508 (68.0)	G551D (5.1)	R117H (4.1)	R560T (2.9)	G542X (2.2)	621+1 G>T (2.2)	dI507 (1.7)		
Norway	dF508 (66.7)	394delTT (4.2)	R117H (3.0)	G551D (1.2)					
Poland	dF508 (52.9)	3849+10kbC>T (2.6)	G542X (2.5)	N1303K (1.7)	1717-1G>A (1.7)	R553X (1.0)	CFTRdele2,3(21kb) (2.0)		
Portugal	dF508 (52.3)	R1066C (3.5)	G542X (2.3)	R334W (2.0)	A561E (2.0)	3272-26A>G (1.5)	N1303K (1.5)		
Romania	dF508 (27.0)								
Russia P	dF508 (45.0)	W1282X (2.1)	N1303K (1.6)	1677delTA (1.6)	2143delT (1.0)	CFTRdele2,3(21kb) (7.5)			
Russia M	dF508 (54.5)	N1303K (2.6)	2143delT (2.0)	2184insA (2.0)	W1282X (2.0)	G542X (1.8)	3849+10kbC>T (1.8)	CFTRdele2,3(21kb) (8.4)	
Slovakia	dF508 (59.4)	G542X (5.6)	R553X (3.4)	N1303K (3.0)	R347P (1.7)	CFTRdele2,3(21kb) (4.6)			
Spain	dF508 (54.4)	G542X (7.7)	N1303K (2.5)	1811+1.6kbA>G (1.5)	R1162X (1.3)	712-1G>T (1.1)	1609delCA (1.0)		
Sweden	dF508 (73.3)	394delTT (9.7)	3659delC (3.0)	175insT (2.4)	T338I (1.2)				
Switzerland	dF508 (43.2)	R553X (24.2)	3905insT (14.7)	G542X (3.2)	1717-1G>A (2.1)	K1200E (2.1)			
The Netherlands	dF508 (74.4)	A455E (3.3)	1717-1G>A (1.5)	G542X (1.3)	R553X (1.2)				
Turkey	dF508 (34.8)	N1303K (6.4)	1677delTA (2.8)	E92X (2.8)	R347H (2.8)	G542X (2.8)	K68N (1.4)	2043delG (1.4)	2183AA>G (1.4) 2789+5G>A (1.4)
Ukraine	dF508 (50.0)								
United Kingdom	dF508 (75.3)	G551D (3.1)	G542X (1.7)						
Yugoslavia	dF508 (66.3)	G542X (5.3)							

Sources: Deltas C., Doerk T., Graham C., Macek M. Jr., Pacheco. P. (Personal communication, January 1999).

Table 2 Overview of total sensitivity for mutations found at a frequency higher than 1% (January 1999)

Country	Total sensitivity %	No. mutations	% mutations not found
Albania	70	1	28.1
Austria	68.8	4	35
Belarus	69.5	4	29.5
Belgium	85	6	8.4
Bulgaria	77.3	8	14.5
Croatia	72.5	4	25
Cyprus	90	8	10
Czechia	88.6	7	4.9
Denmark	90.1	3	4
Estonia	69.4	2	13.5
Finland	75	2	21.2
France	72.9	4	17.5
Germany	83.6	8	16.4
Greece	74.5	12	13.7
Hungary	57.9	3	41.1
Ireland	82.6	4	13.9
Israel	88.2	8	8.4
Italy	70.3	8	22.7
Latvia	79.2	4	20.8
Lithuania	38.1	3	66
Macedonia	68.3	7	50
North Africa	73.8	12	24.5
Northern Ireland	86.2	7	13.8
Norway	75.1	4	23.8
Poland	64.4	7	31.1
Portugal	64.8	7	27.7
Romania	27	1	57.3
Russia (sint Petersburg)	58.8	6	52.8
Russia (Moscow)	75.1	8	
Slovakia	77.7	6	21
Spain	69.5	7	17.9
Sweden	89.6	5	7.3
Switzerland	89.5	6	9.5
The Netherlands	81.7	5	13.5
Turkey	58	10	41.1
Ukraine	50	1	49.1
United Kingdom	80.1	3	13.9
Yugoslavia	71.6	2	26.4

Table 3 Intragenic polymorphisms in the *CFTR* gene

Polymorphic locus	Reference
IVS6a-GATT	Dörk <i>et al.</i> ⁴⁵
1001+11C→T	Cuppens <i>et al.</i> ⁴⁶
IVS8-CA	Morral <i>et al.</i> ⁴⁷
Tn (5T-7T-9T)	Cuppens <i>et al.</i> ⁴⁶
TUB9 (intron 9 1525-61 A or G)	Dörk <i>et al.</i> ⁴⁵
M470V	Dörk <i>et al.</i> ⁴⁵ , Cuppens <i>et al.</i> ⁴⁶
T854T	Dörk <i>et al.</i> ⁴⁵ , Cuppens <i>et al.</i> ⁴⁶
IVS17B-TA	Morral <i>et al.</i> ⁴⁷
IVS17B-CA	Morral <i>et al.</i> ⁴⁷
TUB18 (intron 18 3601-65 A or C)	Dörk <i>et al.</i> ⁴⁵
TUB20 (intron 20 4006-200 A or G)	Dörk <i>et al.</i> ⁴⁵
Q1463Q	Cuppens <i>et al.</i> ⁴⁶

Table 4 Cost comparative study for the most frequently used *CFTR* mutation detection methods (January 1999)

Mutation detection method	Time scheme			Price of material and supplies for analysing 5 samples	No. mutations
	Working time	Waiting time	Total time		
INNO-LiPA CF2	40min-2h	6h-8h	6h40min-10h	157.3-200 Euro	8 mutations
Elucigene CF12	30min-2h	5h30min-6h	6h-8h	157.3-200 Euro	12 mutations
OLA cystic fibrosis assay	1h30min-3h	4h30min-10h30min	7h-12h30min	237.5-275 Euro	31 mutations
Restriction enzyme analysis	1h15min-2h	5h-10h	7h-13h	16.2-21.3 Euro	1 mutation
Heteroduplex analysis	30min-1h	6h-8h	7h-12h15min	10-12.5 Euro	1 or 2 mutations
DGGE	1h30min-2h	6h-20h	8h-22h	20-22.5 Euro	1 exon or multiplex
SSCP	2h30min-3h	10h-22h	12h30min-25h	16.2-18.7 Euro	1 exon

These results were obtained from the comparative study of the actual cost and hands-on time for different CF mutation detection techniques. The data obtained from the workshop on DNA extraction and CF mutation detection technique held in Leuven, September 1996 were compared with the information obtained from a detailed questionnaire which was sent to laboratories which participate in the European Quality control trial 1998 (except for the OLA kit).

For each technique, the total working time and the costs were evaluated. The total time for the different techniques was divided into the working time i.e. the time needed for one person to do the practical work, and the waiting time, i.e. the time when the samples are in a machine or during a chemical reaction without any handling by a person. The costs for the *CFTR* mutation detection techniques were costs for material and supplies. Material and supplies were valued on the basis of replacement prices (Western European prices).

Table 5 Features of the most common CF mutation detection techniques (January 1999)

<i>Mutation specific methods</i>	
Heteroduplex Analysis	Useful for small deletions/insertions Mostly used for dF508/dI507 Other insertions/deletions eg 394delTT, 1461insAGAT Visualisation with either ethidium bromide or silver staining Cheap, easy to use
Restriction Enzyme Analysis	Ideally used for confirmation, verification or mutations detected by other methods Can be used for local specific mutation Testing of relatives for 'family' mutations Used in restriction generating PCR Specificity not absolute Relatively expensive
ARMS	Allele-specific PCR, useful for isolated (local specific) mutations Result may rely on a absence of PCR product Can be useful in determining maternal contamination May be applied to pooled samples, in a research setting only! Difficulty in designing primers Cheap once primers have been designed
RG PCR	Useful for isolated (local specific) mutations May be useful for certain applications
Reverse/Normal Dot Blots	Developed according to local population frequencies Useful for large numbers of samples Visualisation/labelling methods Difficult to establish initially May require radioactivity
INNO-LiPA	See Table 6 for mutations tested INNO-LiPA <i>CFTR</i> 12 and INNO-LiPA <i>CFTR</i> 17+Tn (latter includes polyT) INNO-LiPA will distinguish heterozygotes (Wild-type probes included for every mutation detected) No special equipment required, except for a shaking waterbath More robust with respect to methodology High specificity Sensitivity generally high (European-wide) but not locally variable Relatively expensive (although not per mutation) Labour costs reduced Automatable with the auto-LiPA
Elucigene	See Table 6 for mutations tested Elucigene does not generate normal alleles for mutations except dF508 More robust with respect to methodology Elucigene may be sensitive to DNA sample type High specificity Sensitivity generally high (European-wide) but not locally variable Relatively expensive (although not per mutation) Labour costs reduced
OLA	See Table 6 for mutation tested OLA will distinguish heterozygotes Requirement fo expensive equipment ABI 310 or 377 More robust with respect to methodology High specificity Sensitivity generally high (European-wide) but not locally variable Relatively expensive (although not per mutation) Labour costs reduced
<i>Generic methods</i>	
SSCP DGGE	Cheap, once established Difficult to set up (primer design) High pick-up rate, especially when multiplexed
Sequencing	For absolute confirmation of new findings Exclusion of polymorphisms
<i>Polymorphisms</i>	
For Prenatal Diagnosis where one or no mutations have been found For assessment of maternal cell contamination PolyT interpretation – TG _m For confirmation of zygosity Exclusion of uniparental disomy	

Table 6 Mutations detected by commercial kits
(November 1999)

INNO-LIPA	
CF2:	dF508, dI507, G542X, 1717-1G>A, G551D, R553X, W128X, N1303K
CFTR12:	dF508, dI507, G542X, 1717-1G>A, G551D, R553X, W1282X, N1303K, S1251N, R560T, 3905insT, Q552X
CFTR17+Tn:	394delTT, G85E, 621+1G>T, R117H, 1078delT, R347P, R334W, E60X, 2183AA>G, 2184delA, 711+5G>A, 2789+5G to A, R1162X, 3659delC, 3849+10kbC>T, 2143delT, A455E, (5T/7T/9T)
Elucigene	
CF4:	dF508, 542X, G551D, 621+1G>T
C12	dF508, G542X, G551D, N1303K, W1282X, 1717-1G>A, R553X, 621+1G>T, R117H, R1162X, 3849+10kbC>T, R334W
CF20:	1717-1G>A, G542X, W1282X, N1303K, dF508, 3849+10kbC>T, 621+1G>T, R553X, G551D, R117H, R1160X, R334W, A455E, 2183AA>G, 3659delC, 1078delT, dI507, R345P, S1251N, E60X
CF Poly-T	5T/7T/9T
OLA	
CF OLA assay:	dF508, F508C, dI507, Q493X, V520F, 1717-1G>A, G542X, G551D, R553X, R560T, S549R, S549N, 3849+10kbC>T, 3849+4A>G, R1162X, 3659delC, W1282X, 3905insT, N1303K, G85E, 621+1G>T, R117H, Y122X, 711+1G>T, 1078delT, R347P, R347H, R334W, A455E, 1898+1G>A, 2183AA>G, 2789+5G>A
