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ARTICLE

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.

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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

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 supranational 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, ΔF508, 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 Pachecco, (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.

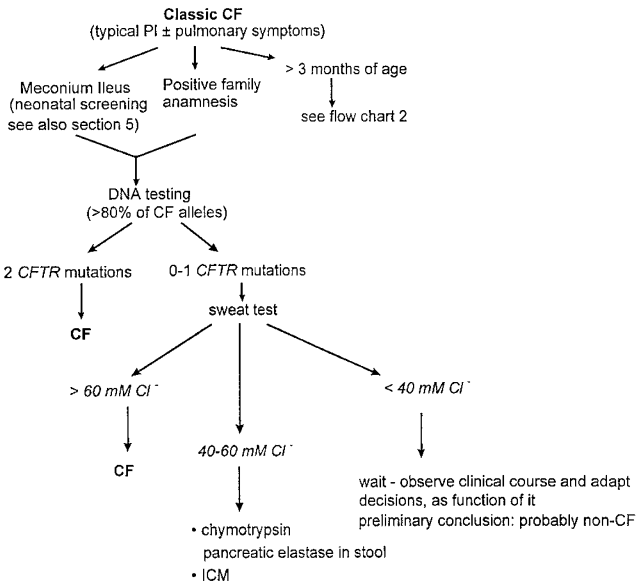

Figure 1 Classical CF in newborns.

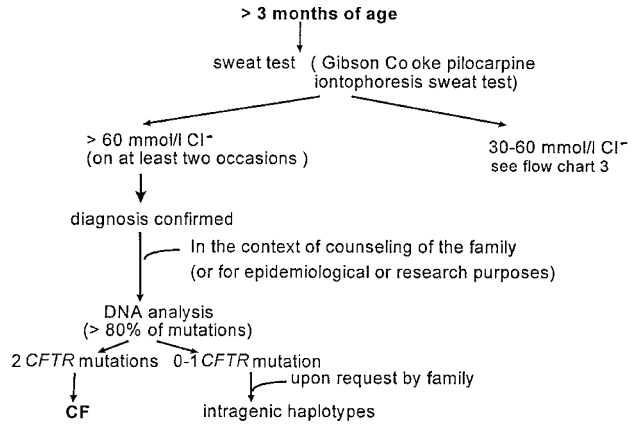

Figure 2 Classical CF in children, adolescents, or adults with typical CF symptoms.

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).

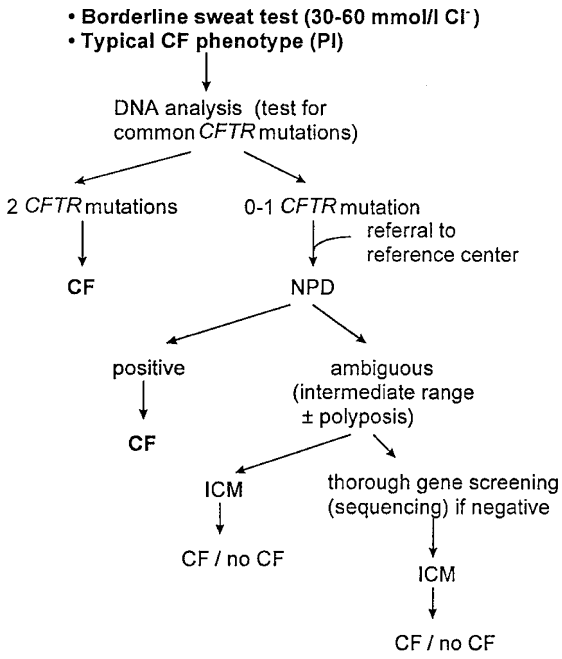

Figure 3 Children, adults with typical CF symptoms but borderline sweat test.

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 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 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.

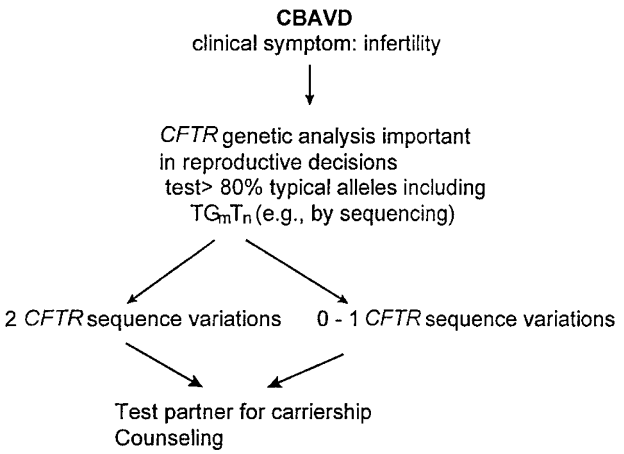

Figure 4 Congenital absence of vas deferens (CBAVD).

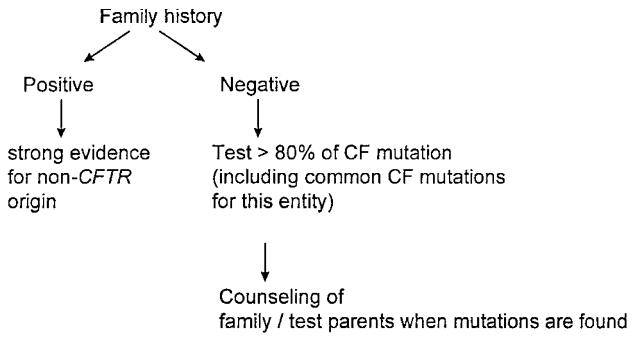

Figure 5 Pancreatitis in children.

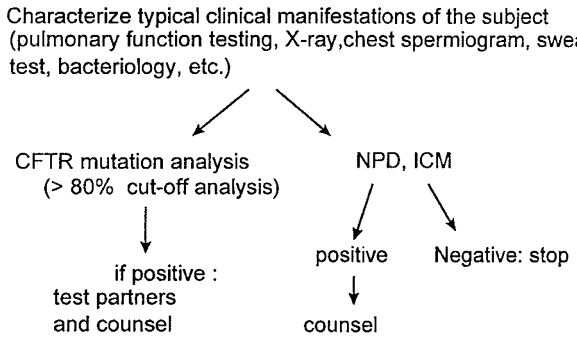

Figure 6 Other diseases with increased frequency of mutation (COPD, infertility, etc).

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 (Immonoreactive 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 calculation[9] 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 consanguinty. 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 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

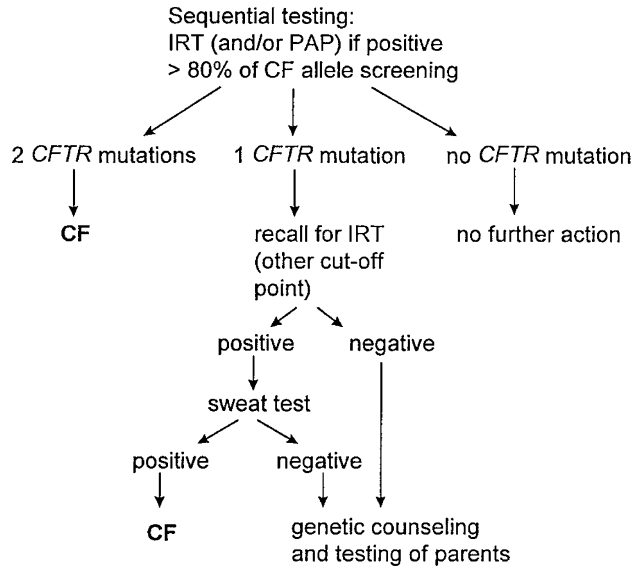

Figure 7 Neonatal screening.

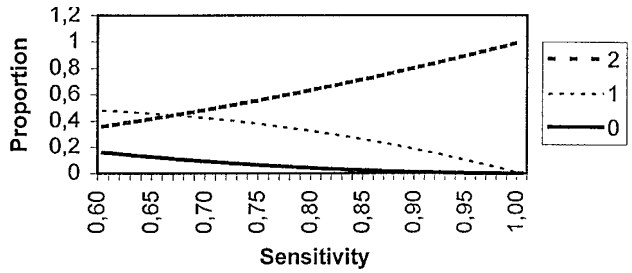

Figure 8 Proportion of all CF patients in a given population in which a mutation can be identified on both mutant *CFTR* genes (2), on only one mutant *CFTR* gene (1), and in which no mutation can be identified on any *CFTR* gene (0), all in function of the sensitivity of the test. Formula for 2 mutations 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.

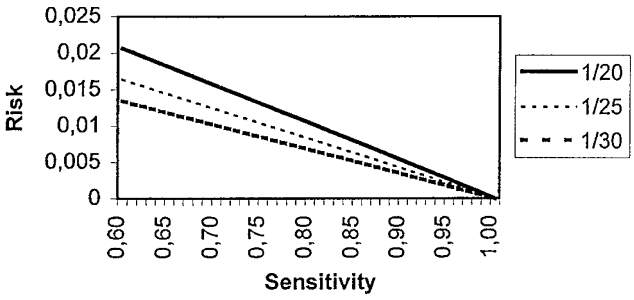

Figure 9 Risk for an individual of being a carrier when no mutation is identified with the test, as function of the sensitivity of the test. The risk is given for carrier frequencies of 1/20, 1/25 and 1/30 in a given population. $R = q(1-S)/[q(1-S) + (1-q)]$ (proportion of individuals that test negative 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 $R = q\ (1–S)$.

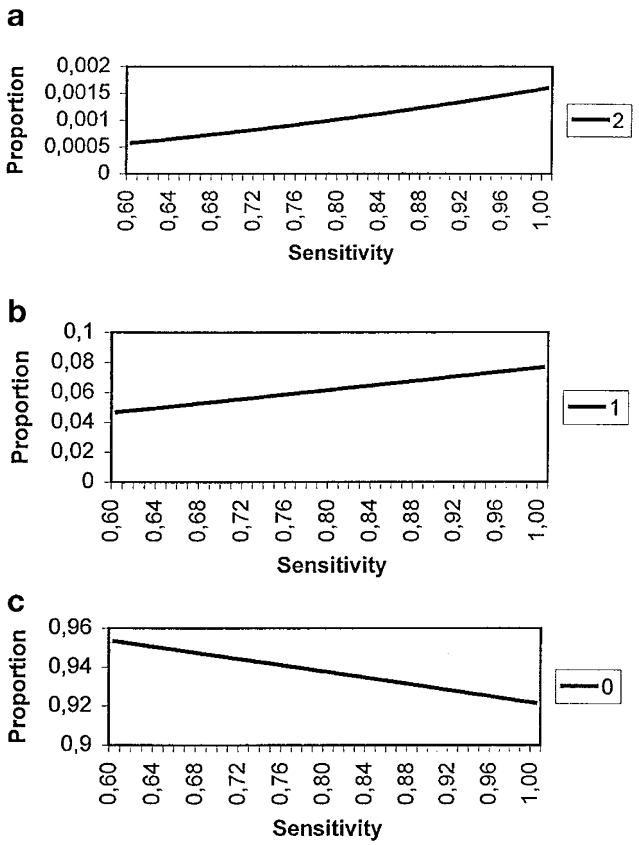

Figure 10 Proportion of couples in a given population, assuming a carrier frequency of 1/25, where (2) both partners will test positive for a mutation in function of the sensitivity of the test; (1) only one partner will test positive for a mutation; (0) both partners will test negative, in function 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).

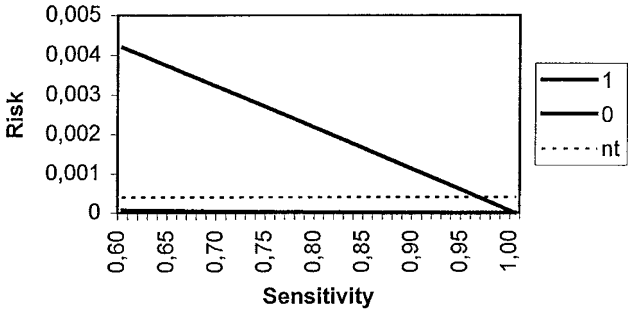

Figure 11 Risk for the children in a population with a carrier frequency of 125, in which only one partner tests positive for a mutation (1) both partners test negative (0). When both partners test positive for a mutation, the risk is 1/4. The risk is also shown when no test (nt) is performed.
Formula one partner positive:

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

(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:

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

(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

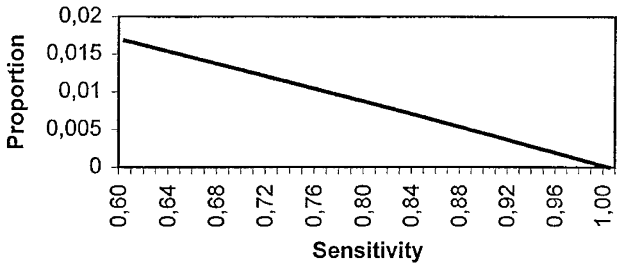

Figure 12 Proportion of couples, with only one partner who tests positive for a mutation, that have a real risk of having CF children, ie the couples where the partner that is negative for the test is still a CF carrier. A carrier frequency of 1/25 is assumed.

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

(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).

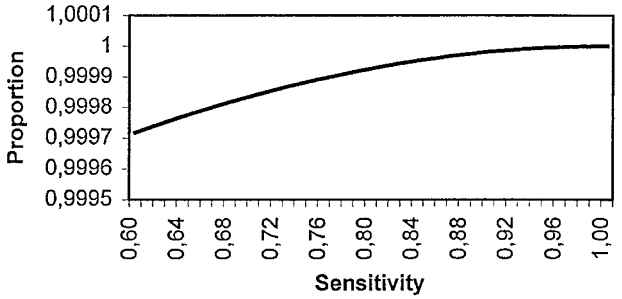

Figure 13 Proportion of couples, with both partners testing negative, that have no risk of having CF children, either because only one or both partners are truly no CF carriers. A carrier frequency of 1/25 is assumed.

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

(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).

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 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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References

1 Dörk T, Dworniczak B, Aulehla-Scholz C *et al*: Distinct spectrum of *CFTR* gene mutations in congenital absence of vas deferens. *Hum Genet* 1997; **100**: 365–377.
2 Harris R: Genetic services in Europe. *Eur J Hum Genet* 1997; **5** (supplement 2): 1– 220.
3 Cotton RGH: Mutation detection and mutation databases. *Clin Chem Lab Med* 1998; **36**(8): 519–522.
4 Grody W, Desnick R, Carpenter N, Noll W: Diversity of cystic fibrosis mutation screening practices. *Am J Hum Genet* 1998; **62**: 1252–1254.
5 Estivill X, Bancells C, Ramos C, the Biomed CF Mutation Analysis Consortium: Geographic distribution and regional origin of 272 cystic fibrosis mutations in European populations. *Hum Mut* 1997; **10**: 135–154.
6 Rending S, Calafelli F, Cappello N *et al*: Genetic history of cystic fibrosis mutations in Italy. I. Regional distribution. *Ann Hum Genet* 1997; **61**: 411–424.
7 Stuhrmann-Sprangenberg M, Aulehla-Scholz C, Dworniczak B, Reiss J, Berufsverband Medizinische Genetik: Qualitätssicherung, Leitlinien zur Molekulargenetischen Diagnostik der Cystischen Fibrose. *Med Genetik* 1997; **4**: 553–559.
8 The CF mutation database. 1999 http://www.genet.sickkids.on.ca/cfr
9 Bridge P: The calculation of genetic risk – worked examples in DNA diagnostic, Johns Hopkins University Press: Baltimore and London, 1997.
10 National Committee for Clinical Laboratory Standards: *Molecular Diagnostic Methods for Genetic Diseases; Proposed Guidelines*. NCCLS document MM1-P ISBN 1-56238-340-X. 1997.
11 Prence E: A practical guide for the validation of genetic tests. *Gen Test* 1999; **3**: 201–205.
12 OECD: *The OECD Principles of Good Laboratory Practices*, (revised 1997): OECD Environmental Health and Safety Publications. Series on Principles of Good Laboratory Practice and Compliance Monitoring 1998; No. 1: ENV/MC/CHEM (98)17.
13 Visviskis S, Schlenck A, Maurice M: DNA extraction and stability for epidemiological studies. *Clin Chem Lab Med* 1998; **36**(8): 551–555.
14 Madison L, Hoar DI, Holroyd CD, Crisp M, Hodes ME: DNA banking: the effects of storage of blood and isolated DNA on the integrity of DNA. *Am J Med Genet* 1997; **27**: 379–390.
15 Cotton R: *Mutation Detection*. Oxford University Press, Oxford, New York, Tokyo, 1997.
16 Rommes J, Kerem B, Greer W, Chang P, Tsui LC, Ray P: Rapid nonradioactive detection of the major cystic fibrosis mutation. *Am J Hum Genet* 1990; **46**: 395–396.
17 Taylor GR, Noble JS, Hall JL, Quirke P, Stewart AD, Mueller RF: Rapid screening for dF508 deletion in cystic fibrosis. *Lancet* 1989; **1345**.
18 Cuppens H, Buyse I, Baens M, Marynen P, Cassiman JJ: Simultaneous screening for 11 mutations in the cystic fibrosis transmembrane conductance regulator gene by multiplex amplification and reverse dot-blot. *Mol Cell Probes* 1992; **6**: 33–39.
19 Ferrie RM, Schwarz MJ, Robertson NH *et al*: Development, multiplexing and application of ARMS tests for common mutations in the *CFTR* gene. *Am J Hum Genet* 1992; **51**(2): 251–262.
20 Ravnik-Glavac M, Glavac D, Chernick M, di-Sant'Agnese P, Dean M: Screening for CF mutations in adult cystic fibrosis patients with a directed and optimized SSCP strategy. *Hum Mut* 1994; **3**(3): 231–238.
21 Ravnik-Glavac M, Glavac D, Dean M: Sensitivity of single-strand conformation polymorphism and heteroduplex method for mutation detection in the cystic fibrosis gene. *Hum Mol Genet* 1994; **3**: 801–807.
22 Fanen P, Ghanem N, Vidaud M *et al*: Molecular characterization of cystic fibrosis: 16 novel mutations identified by analysis of the whole cystic fibrosis conductance transmembrane regulator (*CFTR*) coding regions and splice site junctions. *Genomics* 1992; **13**: 770–776.
23 Costes B, Girodon E, Ghanem N *et al*: Psoralem-modified oligonucleotide primers improve detection of mutations by denaturing gradient gel electrophoresis and provide an alternative to GC-clamping. *Hum Mol Genet* 1993; **2**: 393–397.
24 Taylor G: *Laboratory Methods for the Detection of Mutations and Polymorphisms in DNA*. CRC Press, Boca-Raton, New York, London, Tokyo, 1996.
25 Dequeker E, Cassiman J-J: Evaluation of *CFTR* gene mutation testing methods in 136 diagnostic laboratories: report of a large European external quality assessment. *Eur J Hum Gen* 1998; **6**: 165–175.
26 Elles R: *Methods in Molecular Medicine. Molecular Diagnosis of Genetic Diseases*. Totowa, New Jersey 1996.
27 Huisman W: Quality system in the medical laboratory; the role of a quality manual. *Ann Biol Clin* 1994; **52**: 457–461.
28 Dybkaer R: A quality manual for clinical laboratory including the elements of a quality system. *Scand J Clin Lab Invest* 1993; **53** (suppl 21).
29 Eucromic Quality Assessment Group: Quality guidelines and standards for genetic laboratories/clinic in prenatal diagnosis on fetal samples obtained by invasive procedures: *Eur J Hum Genet*. 1997; **5**: 342–350.
30 Clinical Pathology Accreditation UK: EQA Scheme Accreditation. Company Registration no. 2675095 1996.

31 Neumaier M, Braun A, Wagener N: Fundamentals of quality assessment of molecular amplification methods in clinical diagnosis. *Clin Chem* 1998; **44**(1): 12–26.
32 M Schwarz and Clinical Molecular Genetics Society: Cystic Fibrosis: Guidelines. 1996. http://www.molgenqa.demon.co.uk/cf.htm.
33 The American College of Medical Genetics: *Standard and Guidelines: Clinical Genetics Laboratories*. 1994.
34 College of American Pathologists: Commission on Laboratory Accreditation. 1993; 60093-2750.
35 Kwok S, Higuchi R: Avoiding false positives with PCR. *Nature* 1989; **339**: 237–238.
36 Kitchin P, Szotyori Z, Fromholc C, Almond N: Avoidance of false positives. *Nature* 1990; **344**: 201.
37 Persing DH: Polymerase chain reactions: trenches to benches. *J Clin Microbiol* 1991; **29**: 1281–1285.
38 Koch D, Peters T: Selection and evaluation of methods with an introduction to statistical techniques. In: Burtis C, Ashwood E (eds). Fundamentals of Clinical Chemistry. Saunders: Philadelphia, 1996, pp 170–181.
39 Shultz E: Clinical interpretation of laboratory procedures. In: Burtis C, Ashwood E (eds). Fundamentals of Clinical Chemistry. Saunders: Philadelphia, 1996, pp 192–199.
40 Ad Hoc Committee on Mutation Nomenclature: Update on nomenclature for human gene mutation. *Hum Mut* 1996; **8**: 197–202.
41 Antonarakis S and The Nomenclature Working Group: Recomendations for a nomenclature system for human gene mutations. *Hum Mut* 1998; **11**: 1–3.
42 Beaudet A, Tsui LC: A suggested nomemclature for designating mutations. *Hum Mut* 1993; **2**: 245–248.
43 Beutler E, McKusick V, Motulsky A, Scriver C, Hutchison F: Mutation nomenclature: nicknames, systematic names and unique identifiers. *Hum Mut* 1996; **8**: 203–206.
44 Beutler E: The designation of mutation. *Am J Hum Genet* 1993; **53**: 783–785.
45 Dörk T, Neumann T, Wulbrand U *et al*: Intra- and extragenic marker haplotypes of *CFTR* mutations in cystic fibrosis families. *Hum Genet* 1992; **88**: 417–425.
46 Cuppens H, Heng H, Raeymaekers P, De Boeck C, Cassiman JJ: *CFTR* haplotype backgrounds on normal and mutant *CFTR* genes. *Hum Mol Genet* 1994; **3**: 607–614.
47 Morral N, Estivill X: Multiplex PCR amplification of three microsatellites within the *CFTR* gene. *Genomics* 1992; **13**: 1362–1364.