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Evaluation of *CFTR* gene mutation testing methods in 136 diagnostic laboratories: report of a large European external quality assessment

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Within the framework of the European Concerted Action on Cystic Fibrosis (Biomed-2, BMH4-CT96-0462) a quality assessment was set up for 135 European and one Australian laboratory. Six DNA samples were sent to the various laboratories. These samples carried the following *CFTR* genotypes: dF508/N1303K; dI507/wild; dF508/G551D; dF508/621 + 1 GtoT; R553X/wild and 1717-1 GtoA/wild. Each laboratory was asked to process the samples as they routinely do, whether they checked for all mutations or not. More than 75% of the laboratories screened for at least six of these mutations. Heteroduplex analysis was the most frequently used primary testing method (47%), in many instances followed by restriction enzyme digestion. Only a minority of the laboratories made use of a commercial *CFTR* mutation detection kit. On average, 91% of the laboratories correctly typed both alleles of a given DNA sample. However, 35% of the laboratories incorrectly typed one or more alleles from a total of 12 alleles included in the trial. One laboratory even failed to identify four of the different alleles correctly. The genotyping error frequency tended to be lower in laboratories which perform more than 200 *CFTR* mutation analyses per year. The results of this quality control trial suggest that there are many laboratories (35%) which have a percentage of errors unacceptable in a routine testing setting. The development of a consensus testing strategy for routine diagnostic laboratories and centralised mutation analysis facilities for rare or country-specific mutations in a limited number of expert centres, in combination with regular training sessions and quality assessments, should further improve genotyping.

Keywords: cystic fibrosis; CFTR; quality control; mutation testing

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Introduction

Cystic fibrosis (CF) is one of the most common severe autosomal recessive disorders in Caucasians. It affects approximately 1 in 2500 live births, although the estimated prevalence shows substantial regional variation.[1]

Diagnostic or carrier testing for cystic fibrosis (CF) is performed by analysing the cystic fibrosis transmembrane regulator (*CFTR*) gene for mutations.[2–4] More than 600 mutations have been reported by members of the CF Genetic Analysis Consortium[5] and several *CFTR* mutation detection methods have been described for use in the diagnostic laboratory.[6] A first European quality assessment in 1994 revealed great diversity and lack of consensus for the best testing approach as well as a number of errors in the typing results.[7] The latter quality control trial included only 40 participating laboratories. The objective of the present study was to document more thoroughly the technical ability and testing strategies of laboratories which provide CF mutation testing services in Europe. Therefore a second quality control trial was organised in which 136 diagnostic laboratories from 21 European countries and one from Australia participated voluntarily. The results of this study provide extensive information on which *CFTR* mutations are routinely tested for, and on the methods that are routinely used in European diagnostic laboratories for mutation detection. There is still room for quality improvement in many laboratories.

Methods

Participants
The quality control trial was organised within the framework of the European Concerted Action on Cystic Fibrosis (Biomed-2, nr. BMH4-CT96-0462). In total, 136 laboratories, dispersed throughout Europe (and one from Australia), expressed the wish to participate in the trial (Figure 1). All these laboratories frequently perform *CFTR* mutation testing for diagnostic purposes (on average between 100 and 300 tests/year). An identification number was assigned to each of the participating laboratories before the start of the trial to protect the identify of the participants.

In the European Directory of DNA Laboratories,[8] which aims to maintain an inventory of DNA laboratories testing for specific disease mutations, 133 western European laboratories are listed as performing CFTR mutation testing. Of these laboratories, 89 (67%) participated in the present trial. In addition, 36 other western European, 10 central and eastern European, and one Australian genetic diagnostic laboratory, not listed in the EDDNAL database, elected to participate. It is reasonable therefore to assume that a large majority of European CF diagnostic laboratories is involved in the present study.

Set-up of the Quality Control Trial
The quality control trial included six DNA samples, labelled CF96-1 to CF96-6, which were derived from CF patients or CF mutation carriers with a CF family history. This collection of samples contained the most frequently found *CFTR* mutations in Europe: dF508/N1303K, dI507/wild, dF508/G551D, dF508/621 + 1 GtoT, R553X/wild, and 1717-1 GtoA/wild. The samples were diluted to a concentration of 300 ng/µl, aliquoted into sterile colour coded microcentrifuge tubes (10 µl per sample), and were sent to all participants. No clinical information on the patients from whom the six samples were derived was provided.

The aim of the quality control trial was to evaluate the accuracy of the methods routinely used for mutation testing by the participating laboratories and not the extent to which they could detect all the mutations present in the DNA samples. The laboratories were therefore asked to perform only their routine *CFTR* mutation testing strategy. The raw laboratory data, together with the conclusions drawn from these data, had to be returned to the coordinating centre within a period of 6 weeks. The laboratories were also asked to provide a list of the *CFTR* mutations which they tested for and a short description of the testing strategy used.

Mutation Detection Methods
Information about the *CFTR* mutation detection methodology used was provided by 132 of the 136 participating laboratories. All the methods mentioned had already been described in detail elsewhere. They all start with the amplification of a specific DNA fragment by the polymerase chain reaction (PCR), followed by heteroduplex analysis,[9,10] restriction enzyme digestion, reverse dot blot,[11] amplification refractory mutation system (ARMS),[12] single-strand conformation polymorphism (SSCP),[13,14] denaturing gradient gel electrophoresis (DGGE),[15,16] sequencing, restriction fragment length polymorphism (RFLP), or allele-specific oligonucleotide hybridisation (ASO).

Data Analysis
The analysis of the results of the quality control trial had to take into account that not all laboratories tested for the seven different mutations which were present in the six DNA samples. Therefore, samples were assigned as 'correctly' typed when the reported result was correct but also when a laboratory did not test for this particular mutation. In case an incorrect mutation assignment was made to a DNA sample, the sample was reanalysed by reverse dot blot.

Results

CFTR Mutation Testing Strategies in Diagnostic Laboratories

Based on the information provided by 132 of the participating laboratories, an overview of the currently applied *CFTR* mutation detection methods in the great majority of European diagnostic laboratories could be obtained (Table 1). The routine *CFTR* testing strategy
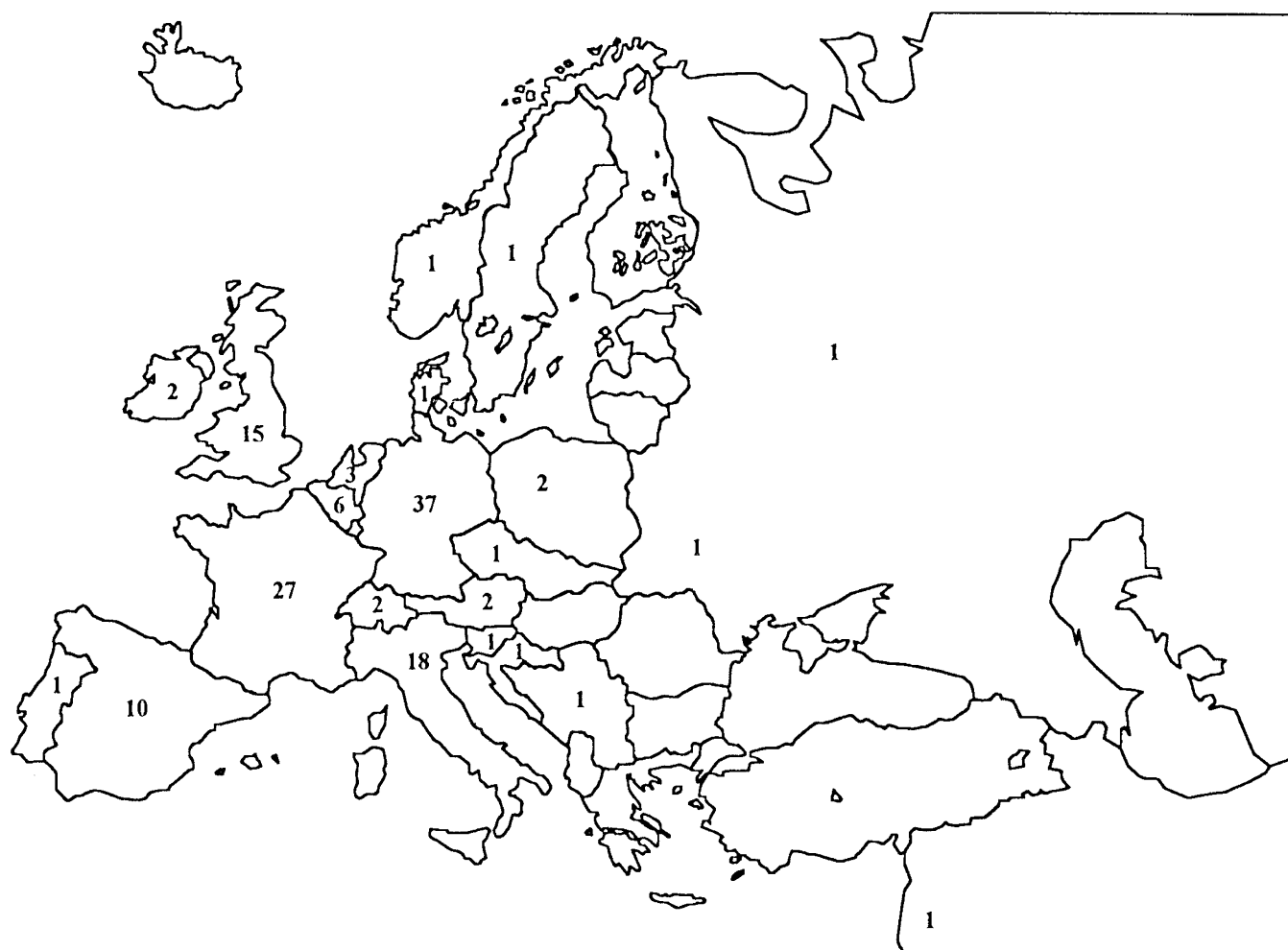
Figure 1 *Distribution of the laboratories participating in the quality control trial in European countries. The number of participants within each country is indicated.*

Table 1 Overview of the methods used in 132 European diagnostic laboratories for *CFTR* mutation testing

Method	Total		Primary testing		Secondary testing	
	n	*%*	*n*	*%*	*n*	*%*
Heteroduplex	63	47.7	63	47.7	–	–
Reverse dot blot	50	37.9	36	27.3	14	10.6
non commercial	19	14.4	12	9.1	7	5.3
Inno-Lipa CF2	31	23.5	24	18.2	7	5.3
ARMS	29	21.9	19	14.4	10	7.5
non commercial	14	10.6	6	4.5	8	6
CF(4)	8	6	8	6.1	–	–
CF(12)	7	5.3	5	3.8	2	1.5
Restriction enzyme analysis	86	65.1	2	1.5	84	63.6
Sequencing	16	12.1	1	0.75	15	11.4
DGGE	14	10.6	3	2.3	11	8.3
SSCP	12	9.1	3	2.3	9	6.8
Other methods	25	18.9	5	3.8	20	15.1

Table 2 Number of different methods which are routinely used for *CFTR* mutation testing in 132 European diagnostic laboratories

Method no.	No. of laboratories	% of all laboratories
1	18	13.6
2	72	54.5
3	36	27.2
4	5	3.8
5	1	0.8

of the large majority (87%) of the laboratories involves the combination of two or more of these methods (Table 2). The most commonly used methods are heteroduplex analysis (48% of all laboratories) and digestion of a PCR-amplified DNA fragment with a specific restriction enzyme (65% of all laboratories) (Table 1). One or both of these methods is used by 79% of the laboratories. Heteroduplex analysis is performed essentially as primary testing tool, whereas restriction enzyme digestions are mainly used as a secondary method to identify specific mutations. Reverse dot blot and the amplification refractory mutation system (ARMS) are used by 38% and 22% of the laboratories respectively. However, only a minority of the participating laboratories makes use of the commercially available mutation detection kits: 23% and 11% respectively for the reverse dot blot kit (Inno Lipa CF2, Innogenetics n.v., (Gent, Belgium) and the ARMS kits (CF(4)m and CF(12)m, Johnson & Johnson Clinical Diagnostics Ltd, Amersham, U.K.). Together with heteroduplex analysis, reverse dot blot and ARMS analysis are the most frequently used primary testing methods of new DNA samples (used in up to 88% of all laboratories). All other methods are less frequently used for primary testing (only in 10.5% of the laboratories). Approximately 10% of the laboratories routinely sequence (parts of) the *CFTR* gene, or perform denaturing gradient gel electrophoresis (DGGE) or single-strand conformation polymorphism (SSCP). A minority of the European diagnostic laboratories uses a wide variety of other mutation detection methods, including restriction fragment length polymorphism (RFLP), allele-specific oligonucleotides (ASO), temperature gradient gel electrophoresis (TGGE), allele-specific PCR (AS-PCR), or other in-house developed methods.

More than 600 different mutations within the *CFTR* gene have already been identified.[5] Based on the relative frequencies of the most common mutations in their target population, the diagnostic laboratories routinely test for a limited number of mutations (4 to

20) (Figure 2). Fifty percent of the laboratories test for 8 to 11 mutations. The laboratories which test for more *CFTR* mutations usually apply indirect mutations detection assays such as DGGE or SSCP. Figure 3 schematically shows which of the 15 most frequently occurring *CFTR* mutations in Europe are tested for in the laboratories.[17] Sixty-nine percent of all laboratories test for the five most common *CFTR* mutations (with a frequency higher than 1% in Europe). A large variation in testing frequency is observed for all other mutations.

Genotyping Results of the Quality Control Trial

One Australian and 135 European diagnostic laboratories which provide CF mutation testing returned their results in this quality control trial. In total, nine out of the 12 alleles in the samples harboured a mutation in the *CFTR* gene. The seven mutations were frequently occurring (frequency above 0.24%) *CFTR* mutations (Figure 3). More than 75% of all the laboratories routinely tested for six of these mutations; only 46% of the laboratories tested for mutation 621 + 1 GtoT, present in sample CF96-4. Mutation dF508, present in three independent samples, and the most frequently observed mutation (Figure 3), was the only mutation which was tested for by all laboratories. Only one laboratory incorrectly typed this mutation in one of the samples (CF96-4). Mutation dI507 obtained the lowest correct score of all alleles actually tested (85% of the laboratories gave a correct result Table 3). For the
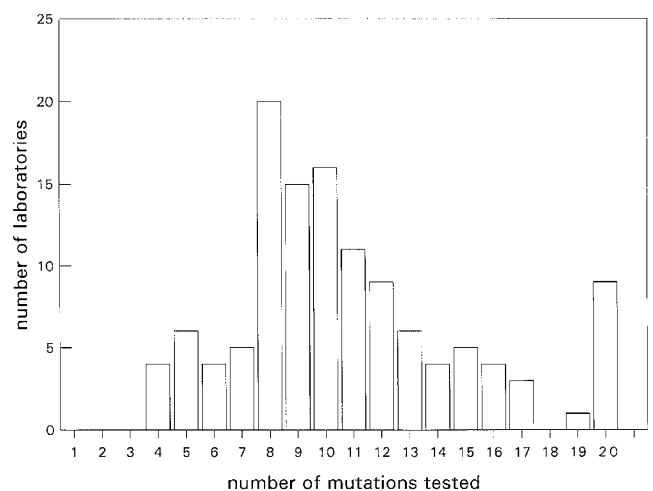

Figure 2 *Frequency distribution of the number of laboratories and the number of* CFTR *mutations which are routinely tested in the laboratories participating in the quality control trial.*
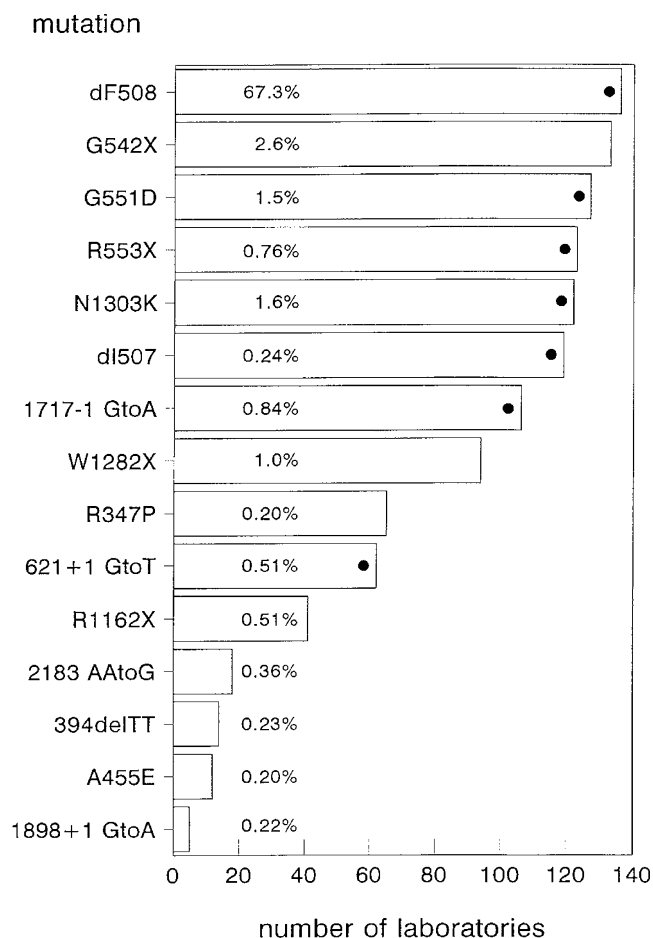
mutation

Figure 3 *Frequency distribution of the mutations tested by different laboratories, from the 15 most frequently observed CFTR mutations in Europe[22] The number of laboratories which routinely tests for a particular mutation is indicated by horizontal bars; the frequency of the mutation in Europe in %; the mutations present in the DNA samples included in the quality control trial by bullets.*

genotyping results of a sample (both alleles), the percentage of laboratories which correctly genotyped a sample varied between 81 and 98% for the six samples, with an average of 91%. Because not all laboratories tested for all the mutations, the percentage of laboratories which did 'not incorrectly' assign the alleles in a sample is also given. The percentage of laboratories which did 'not incorrectly' genotype a test sample varied between 83% and 99%, with an average of 94%.

The total number of alleles which were incorrectly genotyped in the six samples is shown in Table 4. Thirty-five percent of the laboratories did incorrectly type one or more of the *CFTR* alleles (on a total of 12 alleles). Table 5 shows the genotyping error frequency in

relation to the number of *CFTR* mutation analyses which are performed per year in the diagnostic laboratories. The genotyping error frequency tends to be lower (although not statistically significant) in laboratories which perform more than 200 *CFTR* mutation analyses/year in comparison with laboratories performing less than 200 mutation analyses/year.

Table 6 provides a detailed overview of the individual results obtained for each of the DNA samples included in the quality control trial. The mutation detection method which was used is indicated when a sample was incorrectly genotyped. A short description of these individual results and sources of the errors (if known) is provided below.

Sample CF96-1 (dF508/N1303K) This was correctly typed in 134 of 136 laboratories, ie 99%. Two of these laboratories reported that they could not distinguish the dF508 and dI507 mutations based on the method used (heteroduplex analysis), and were therefore also scored as correct. Two laboratories did not detect the N1303K mutation, although their routine testing strategy included this mutation. The first indicated a wild type for allele 2 instead of N1303K. The raw data of the reverse dot blot (Inno Lipa CF2) showed that the hybridisation with either wild-type and mutant-type of N1303K failed. In their comments, they admitted that a conclusion should not have been made based on the quality of their test. The poor quality of the test was probably caused by the application of a hybridisation temperature different from the temperature indicated in the protocol book of the Inno Lipa CF2 strips. The second laboratory indicated a homozygous dF508/dF508 result for sample CF96-1. Their assays included heteroduplex analysis and non-commercial ARMS. Although the correct dF508/N1303K could have been derived from their raw data, the laboratory concluded differently. No comments were given afterwards.

Sample CF 96-2 (dI507/wild) This was correctly typed in 113/136 laboratories, ie 83%. Seventeen laboratories incorrectly interpreted the dI507 allele as a dF508. Thirteen of these laboratories used a heteroduplex assay. After sending the correct data, three of them commented that they could not distinguish dI507 and dF508 solely based on this method. The four other laboratories which typed the sample as dF508/wild used respectively allele-specific PCR, GeneScan analysis, and ASO reverse dot blot assay. None of these laboratories retyped the sample or gave comments on

Table 3 Schematic overview of the results of the quality control trial

			Allele A		Allele B		Total sample	
							Both alleles correctly assigned	Not incorrectly assigned[b]
			Testing labs[a]	Correct assignment	Testing labs[a]	Correct assignment		
Sample	Allele A	Allele B	n (% of total)	n (% of testing labs)	n (% of total)	n (% of testing labs[a])	n (% of testing labs[a])	n (% of all labs)
CF96–1	dF508	N1303K	136 (100)	136 (100)	122 (90)	120 (98)	120 (98)	134 (99)
CF96–2	dI507	wild	119 (87)	101 (85)	136 (100)	131 (96)	96 (81)	113 (83)
CF96–3	dF508	G551D	136 (100)	136 (100)	127 (93)	123 (97)	123 (97)	131 (96)
CF96–4	dF508	621+1 GtoT	136 (100)	135 (99)	62 (46)	54 (87)	53 (85)	129 (95)
CF96–5	R553X	wild	123 (90)	119 (93)	136 (100)	134 (99)	117 (95)	130 (96)
CF96–6	1717–1 GtoA	wild	106 (78)	101 (92)	136 (100)	133 (98)	98 (92)	129 (95)

[a]'Testing labs': the labs that effectively tested for the particular mutation.
[b]'Not incorrectly assigned': the mutation(s) in the sample were correctly detected, or the laboratory did not test for this mutation and assigned it as wild type.

their results. As a result of an administrative error, one laboratory indicated a genotype wild/wild although their raw data clearly showed the correct genotype dI507/wild. Another laboratory found, based on a home-made line probe assay, the genotype dI507/N1303K for sample CF96-2. No comments on probable causes for this error were given afterwards. Finally, four other laboratories reported the genotype dI507/dF508 based on their results with the commercial Inno Lipa CF2 kit. Three of these labs did not send their raw data or comments on the results. The raw data of the fourth laboratory showed indeed a very weak signal for dF508, but the quality of the other signals on the strip was also poor. A conclusion based on this reverse dot blot strip should therefore not have been made.

Sample CF96-3 (dF508/G551D)　This was correctly typed in 131 of 136 laboratories, ie 96%. One of these laboratories could not distinguish the G551D from the R553X mutation. They tested for the presence of R553X by digestion of a multiplex PCR product (exons 9, 10 and 11) with *Hinc* II. On the data sheet,

Table 4 Number of incorrectly typed *CFTR* alleles by the diagnostic laboratories in the quality control trial

No. of alleles incorrectly typed (in a total of 12)	No. of laboratories (total 136)	% of all laboratories
0	88	64.7
1	35	25.7
2	12	8.8
3	0	0
4	1	0.7

they indicated that R553X could only be distinguished from G551D in homozygotes but not in heterozygotes. From the laboratories which typed incorrectly, two reported R553X instead of G551D, based on restriction endonuclease digestions of PCR products with *BgtN* I and *Hinc* II, or *Msp* I. Two other laboratories reported dF508/R553X and G551D as the genotype for CF96-3. The methods used were restriction endonuclease digestion (*Hae* III) in the first laboratory, and RFLP in the second laboratory. Finally, one laboratory reported the genotype dF508/2183 AAtoG, based on the results of an ASO dot blot procedure.[18] No raw data or other comments were given.

Sample CF96-4 (dF508/621 + 1 GtoT)　This was correctly typed in 129 of 136 laboratories, ie 95%. Of the incorrect laboratories, four reported dF508/wild, although their testing methodology included mutation 621 + 1 GtoT. The mistake of the first of these laboratories was based on an administrative error. Retyping of the DNA sample afterwards by SSCP gave the correct 621 + 1 GtoT mutation. The second laboratory used the RFLP technology, but no data were available which could explain the incorrect assignment. The third laboratory used a home-made reverse dot blot. Upon reanalysis of the reverse dot blot, a signal on the 621 + 1 GtoT mutant probe was observed, but the difference with the background level was very small. As a consequence, this laboratory decided to remove mutation 621 + 1 GtoT from the set of CF mutations detectable with their home-made reverse dot blot. The fourth laboratory used restriction enzyme analysis to check for 621 + 1 GtoT but did not provide raw data or

Table 5 Number of laboratories with genotyping error(s) in relation to the number of *CFTR* mutation analyses performed yearly

Number of tests per year	Total number of laboratories	Percentage of all laboratories	Number of laboratories with genotyping error(s)	Percentage of laboratories with genotyping error(s)
<100	16	27	6	37
100–200	18	31	8	44
200–300	13	22	3	23
>300	12	20	3	25
Total	59	100	20	34

For this analysis, only a subset of 59 laboratories provided adequate information, but this subgroup is likely to be representative for the whole group, since the overall percentage of laboratories which made genotyping error(s) in the subgroup is 34%, in comparison with 35% for the whole group.

feedback comments. Three other laboratories did not routinely test for mutation 621 + 1 GtoT, but found other mutations. Two laboratories reported dF508/R553X and the third the genotype dF508/R553X-3849 + 4AtoG. These results were based on analyses by reverse dot blot, SSCP or restriction enzyme analysis. Contamination of sample CF96-4 with sample CF96-3 before distribution by the coordinating centre is very unlikely since each series of quality control samples was prepared and aliquoted on separate days and only three of 136 laboratories found this mutation in CF96-4. None of the latter three laboratories retyped the samples.

Samples CF96-5 (R553X/wild) This was correctly typed in 130 laboratories, ie 95.6% and incorrectly by the other six laboratories. Two laboratories wrote G553X instead of R553X for allele 1 on the data sheet, and apologised for the typing error afterwards. Two other laboratories reported the genotype wild/wild, although testing for mutation R553X was performed. The first used the new ARMS kit CF(12)m-PCR from Johnson & Johnson. Although a very weak signal for the R553X mutation could be observed on the raw data, they interpreted the sample as wild type. The second laboratory which typed the sample as wild/wild performed RFLP and restriction endonuclease digestion of the PCR products with *Hinc* III and *Mbo* I. One laboratory reported the genotype R553X/621 + 1 GtoT, which can only be explained if two samples were pooled. The last laboratory reported genotype R553X/3849 + 10 kb LtoT, but they required larger amounts of DNA to confirm their results in a new test. Finally, it should be noted that five laboratories reported not to be able to amplify sample CF96-5. This sample was provided in a blue tube, and the 10 μl DNA was therefore difficult to see in this tube. Centrifugation of the tube was necessary to recover the complete sample.

Sample CF96-6 (1717-1 GtoA/wild) This was correctly typed in 129 laboratories, ie 94%. Three laboratories reported the genotype 1717-1 GtoA/1717-1 GtoA. All three laboratories used the Inno Lipa CF2 kit, but no raw data or feedback comments were sent. One laboratory reported a genotype W1717/wild. This laboratory used SSCP, but did not specifically test for the 1717-1 GtoA mutation. A fifth laboratory reported the presence of S549R instead of 1717-1 GtoA, based on the results of DGGE with GC-clamps, and confirmation by sequencing. Finally, two laboratories reported a genotype wild/wild for sample CF96-6. The first of these laboratories used a multiplex ARMS kit, and indicated on the data sheet that a testing for 1717 + 1 GtoA was performed instead of a testing for 1717-1 GtoA. The raw data did not show the 1717-1 GtoA mutation. No comments were given and no retyping was done. The second laboratory which reported a wild/wild genotype used restriction enzyme analysis. Subsequently, they provided copies of the raw cycle sequencing data, but the copies were not clear enough for interpretation.

Discussion

The results from this study provide information both on currently used mutation detection strategies in diagnostic laboratories and on the technical quality of the genetic analyses in these laboratories. It is clear that at present a wide variety of different mutation analysis methods is being used in European diagnostic laboratories. In general, most laboratories apply a two-step strategy.

The most popular primary CF mutation testing method is heteroduplex analysis, which is being used by

Table 6 Detailed genotyping results of the DNA samples included in the quality control trial

Sample	Allele 1	Allele 2	No. of laboratories	Method used
CF96–1 dF508/N1303K	dF508	N1303K	119	
	dF508	–	13	
	dF508 or dI507	N1303K	1	
	dF508 or dI507	–	1	
	dF508	wild	1	Inno-Lipa CF2
	dF508	dF508	1	Heteroduplex Analysis/ARMS
CF96–2 dI507/wild	dI507	wild	93	
	–	wild	17	
	dI507 or dF508	wild	3	
	dF508	wild	17	13 ⋅ Heteroduplex analysis 1 ⋅ allele specific PCR 1 ⋅ GeneScan analysis 1 ⋅ ASO reverse dot blot
	wild	wild	1	administration error (Inno-Lipa CF2)
	dI507	N1303K	1	home-made line probe assay
	dI507	dF508	4	3 ⋅ Inno-Lipa CF2 1 ⋅ method not specified
CF96–3 dF508/G551D	dF508	G551D	120	
	dF508	–	7	
	dF508	G551D or R553X	1	
	dF508/dI507	G551D	2	
	broken tube		1	
	dF580	R553X	2	Restriction enzyme analysis
	dF508	R553X and G551D	2	1 ⋅ Restriction enzyme analysis 1 ⋅ RFLP
	dF508	2183AAtoG	1	ASO dot blot
CF96–4 dF508/621+1GtoT	dF508	621+1 GtoT	53	
	dF508	–	73	
	dF508 or dI507	621+1 GtoT	1	
	dF508 or dI507	–	1	
	wild	621+1 GtoT	1	
	dF508	wild	4	1 ⋅ SSCP 1 ⋅ RFLP 1 ⋅ home-made reverse dot blot 1 ⋅ Restriction enzyme analysis
	dF508	R553X	2	1 ⋅ SSCP 1 ⋅ Reverse dot blot
	dF508	R553X/3849+4 AtoG	1	1 ⋅ Restriction/enzyme analysis
CF96–5 R553X/wild	R553X	wild	115	
	–	wild	8	
	R553X or G551D	wild	2	
	No amplification		5	
	G553X	wild	2	administration error
	wild	wild	2	1 ⋅ ARMS 1 ⋅ Restriction enzyme analysis
	R553X	621+1 GtoT	1	RFLP
	R553X	3849+10kb LtoT	1	Restriction enzyme analysis/ sequencing
CF96–6 1717–1 GtoA/wild	1717–1 GtoA	wild	99	
	–	wild	30	
	1717–1 GtoA	1717–1 GtoA	3	3 ⋅ Inno-Lipa CF2
	W1717	wild	1	SSCP
	wild	wild	1	Restriction enzyme analysis
	S549R	wild	1	DGGE
	1717+1 GtoA	wild	1	ARMS administration error

almost half of all laboratories. The presence of specific mutations is usually examined by restriction enzyme analysis. Because the dF508 mutation accounts for more than 50% of all mutations in most northern European countries,[17,19–22] a large proportion of diagnostic laboratories (47%) first test for the presence of this mutation. It should be noticed that mutations dF508 and dI507 can only be distinguished in heterozygotes when heteroduplex analysis is done using PCR primers C16B and C16D.[6] The results of the quality control trial indicate that this is not adequately verified in several laboratories, since 13 erroneously genotyped sample CF96-2 as dF508/wild instead of dI507/wild. The most frequently used alternative primary testing methods are the reverse dot blot method and the ARMS. Remarkably, fewer than one in three laboratories uses a commercial kit (Inno Lipa CF2, ARMS CF(4), or ARMS CF(12)) as primary testing tool, although this could be expected to improve the protocol standardisation and hence the reproducibility and error rate, especially in laboratories which are less experienced in mutation analysis testing.

The choice of a routinely applicable secondary testing method largely depends on the information which was obtained from the primary test. Restriction enzyme analysis is by far the most used method, probably because it is easy to perform and to interpret. Some of the participating laboratories routinely check for more than 20 different mutations with restriction enzyme analysis. However, a comparative study of the actual cost and hands-on time for different CF mutation detection methods, shows that restriction enzyme analysis is very time consuming and thus expensive when a large set of different mutations must be tested [unpublished data]. Many other methods are being used for secondary testing, although none of these methods is widely used. Apart from the technical accuracy and ease of use, the choice of method according to some laboratories is also based on its cost per analysis. In this regard, it should be noted that a rather expensive method such as sequencing is performed in more than 10% of the diagnostic laboratories as a method of choice for secondary testing. This study also revealed that more than 30% of the laboratories routinely apply three or more different methods for *CFTR* mutation analysis. Such strategy is probably less cost-effective in comparison with strategies involving only one or two different assays. A systematic comparative cost–benefit analysis is in progress and should help in clarifying this issue.

The results also indicate that both the methods used and the selection of mutations which are tested largely depend on the laboratory which is responsible for the CF mutation testing service in a particular European region. This implies that, even within one country, different sets of mutations are tested in different laboratories. We wonder whether it would not be more efficient and more beneficial for the patients or carriers to use – at least in the first phase – a generally accepted consensus strategy in all diagnostic laboratories. Testing for less frequent or country-specific mutations could then be centralised in a limited number of reference laboratories. The introduction of such an approach is supported by the results from the present quality control trial: no less than 35% of the laboratories incorrectly genotyped at least one of the 12 *CFTR* alleles. It appears that a tendency to genotyping errors in laboratories which less frequently do *CFTR* mutation analyses favours a first phase testing strategy for diagnostic laboratories. In addition this would facilitate the transferability of results between different centres.

The organisation of European quality control trials appears to be useful for several reasons. A quality control trial organised by an independent body provides the laboratories with the possibility to evaluate their performance and to compare it with that of other European laboratories. If genotyping errors are found in the quality control trial, the laboratories can identify the source of their mistake(s). This feedback might identify errors which would not have been detected if quality control assessment were based solely on internal controls. This is clearly illustrated by the mistyping of sample CF96-2 by several laboratories using heteroduplex analysis.

The ultimate aim of these quality control trials is to improve the quality of routine mutation analysis tests. It is therefore of interest to compare the results of the present trial with those of the previous one.[7] From a subset of 29 laboratories which participated in both quality control trials, 28 scored better or as well in the second trial as in the first. This suggests that regular organisation of international quality control trials by an independent body should be encouraged.

The type of error made by the different laboratories ranged from administrative (typing) errors, to misinterpretation of the data, and erroneous technical results. This further stresses the point that diagnostic laboratories should improve validation of their technical procedures. Indeed, methods of detecting human errors at every stage of the procedure, from patient to

final report, are necessary. Since most diagnostic tests are done in laboratories which focus (largely) on research, non-technical administrative aspects of the procedure tend to be less well organised.

In conclusion, the results of this quality assessment are encouraging but at the same time raise several questions about the overall quality available in genetic diagnostic laboratories in Europe. They are encouraging in respect of the numbers of laboratories which participated voluntary, while disappointing in the number and nature of the errors. Based upon the results of this study, we therefore recommend that

1 all diagnostic laboratories would regularly participate in external quality assessment schemes,

2 the personnel involved would be encouraged to participate regularly in specialised training sessions,

3 consensus strategies for diagnostic testing would be introduced, and

4 centralised mutation analysis facilities would be identified for rare mutations.

We are convinced that the introduction of such measures has the potential to reduce future genetic misdiagnosis rates to a more acceptable level. Finally, it is clear that the findings from this CF quality control trial are also likely to apply to mutation analyses involving other genes and diseases. The results should make all the laboratories more conscious of the important role the quality of testing can play in the genetic services provided to the population.

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References

1 Welsh MJ, Tsui LC, Boat TF, Beaudet AL: Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D. *The Metabolic Basis and Molecular Bases of Inherited Disease*, 7th edn. McGraw-Hill Health Professions Division, New York, 1995, vol III, ch 127, pp 3799–3876.

2 Kerem B, Rommens JM, Buchanan JA *et al*: Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989; **245**: 1073–1080.

3 Riordan JR, Rommens JM, Kerem B *et al*: Identification of the cystic fibrosis gene: cloning and characterisation of complementary DNA. *Science* 1989; **245**: 1066–1073.

4 Rommens JM, Iannuzzi MC, Kerem B *et al*: Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989; **245**: 1059–1065.

5 The CF mutation database. 1997 http://www.genet.sickkids.on.ca/cftr

6 Schwarz M, Malone G: Methods for testing in cystic fibrosis. In: Rob E (ed). *Molecular Diagnosis of Genetic Diseases* Humana Press, Totowa, 1996, Ch 5, pp 99–119.

7 Cuppens H, Cassiman JJ: A quality control study of CFTR mutations screening in 40 different European laboratories. The European Concerted Action on Cystic Fibrosis. *Eur J Hum Genet* 1995; **3**: 235–245.

8 EDDNAL, http://www.eddnal.com/

9 Rommens 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.

10 Taylor GR, Noble JS, Hall JL, Quirke P, Stewart AD, Mueller RF: Rapid screening for dF508 deletion in cystic fibrosis. *Lancet* 1989; 1345.

11 Cuppens H, Buyse I, Baens M, Marynen P, Cassiman JJ: Simultaneous screening for 11 mutations in the cystic fibrosis transmembrane conductane regulator gene by multiplex amplification and reverse dot-blot. *Mol Cell Probes* 1992; **6**: 33–39.

12 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**: 251–262.

13 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 optimised SSCP strategy. *Hum Mut* 1994; **3**: 231–238.

14 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.

15 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.

16 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.

17 Estivill X, Bancells C, Ramos C and 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.

18 Castaldo G, Rippa E, Sebastio G *et al*: Molecular epidemiology of cystic fibrosis mutations and haplotypes in southern Italy evaluated with an improved semi-automated robotic procedure. *J Med Genet* 1996; **33**: 475–479.

19 The Cystic Fibrosis Genetic Analysis Consortium: Worldwide survey of the delta F508 mutation. Report. *Am J Hum Genet* 1990; **47**: 354.

20 European Working Group on CF Genetics (EWGCFG): Gradient of distribution in Europe of the major CF

mutation and of its associated haplotype. *Hum Genet* 1990; **85**: 436–445.

21 Romeo G, Devoto M: Population analysis of the major mutation in cystic fibrosis. *Hum Genet* 1990; **85**: 391.

22 Estivill X, Chillton M, Casals T *et al*: Delta F508 gene deletion in cystic fibrosis in Southern Europe. *Lancet* 1989; **2**: 1404.