Testing Guidelines for molecular diagnosis of Cystic Fibrosis.

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1. Nomenclature and Gene ID:

Cystic Fibrosis; CF; mucoviscidosis; OMIM 219700
Cystic fibrosis transmembrane conductance regulator gene; *CFTR;* OMIM 602421
Chromosome location 7q31.2

2. Introduction

These guidelines for cystic fibrosis genetic analysis are the consensus of discussions at a number of meetings of molecular geneticists and clinicians, beginning with the UK Best Practice Meeting in Manchester in October 2006. They are intended to reflect what is generally accepted as the best current practice in cystic fibrosis molecular analysis, interpretation and reporting, as well as offering guidance in areas where there may be differences in practice.

With regard to mutation nomenclature, in general the Human Genome Variation Society (HGVS) guidelines are followed based on CFTR ref seq NM_000492.3. The common abbreviation form of each mutation is given in parenthesis after each HGVS version. However in some sections, for convenience, the common abbreviation is used alone.

3. Description of the Disease

Cystic fibrosis (CF) is the most common autosomal recessive inherited disease in Caucasians and affects approximately 1 in 2500 individuals. It occurs with lesser frequencies in other populations. It is a complex multi-system disorder, that may affect the following organ systems:

- Pulmonary
- Pancreatic
- Gastro-intestinal
- Reproductive

The pathological processes affecting these systems arise from mutations in the *CFTR* gene which encodes the cystic fibrosis transmembrane conductance regulator, a membrane chloride channel located in the apical membrane of secretory epithelia. The CFTR protein is a cyclic-AMP dependent channel: increasing levels of c-AMP inside a secretory epithelial cell trigger activation of protein kinase A which binds the phosphorylation site on the (regulatory) R-domain of the CFTR protein thus opening the channel (Collins, 1992). The CFTR chloride channel essentially works as an electrostatic attractant by drawing intracellular and extracellular anions toward positively charged transmembrane domains inside the channel. The CFTR protein has 12 transmembrane (TM) domains. Two of these (TM1 and TM6) attract and bind chloride (and/or bicarbonate) ions. As the chloride ions bind to these sites in the pore, the mutual repulsion accelerates expulsion of the ions from the cell (Linsdell, 2006). When normally functioning CFTR is activated, chloride ions are secreted out of the cell. However, in addition to chloride ion secretion, the epithelial sodium channel (ENaC) is also inhibited by CFTR (Konig *et al*, 2001) and less sodium is absorbed into the cell, leaving a greater combined ionic gradient to allow water to leave the cell by osmosis providing fluid for epithelial tissue secretions. In cystic fibrosis these mucus secretions become hyperviscous and it is this which accounts for the principal features of cystic fibrosis.

There are more than 1600 individual *CFTR* mutations currently reported to cause CF (http://www.genet.sickkids.on.ca/cftr/app and http://www.hgmd.cf.ac.uk/ac/index.php).

These are inactivating (loss of function) mutations and include deletions, insertions, splice site mutations, nonsense mutations as well as more than 650 missense mutations. The severity and presentation of the disease may depend on the type of mutation and many studies have investigated the association between the genotype and the phenotype. In order to classify the main types of mutations, Vankeerberghen *et al* (2002) showed that although complete absence of the CFTR protein in exocrine tissues dramatically affected the viscosity and therefore transport of secretions, other *CFTR* mutations did not always correlate well with the observed CF phenotype and this lack of correlation may be a result of environmental factors and/or other compounding genetic factors. They divided the differing types of mutation into five classes, demonstrated in figure 1 (Welsh *et al* 1996).

I. Biosynthesis of CFTR protein is affected; mRNA processing is interrupted by the introduction of premature translation termination signals or by aberrant splicing. It is estimated that a half of all *CFTR* mutations fall into this class, p.Gly542X (G542X) being the most common in the UK.

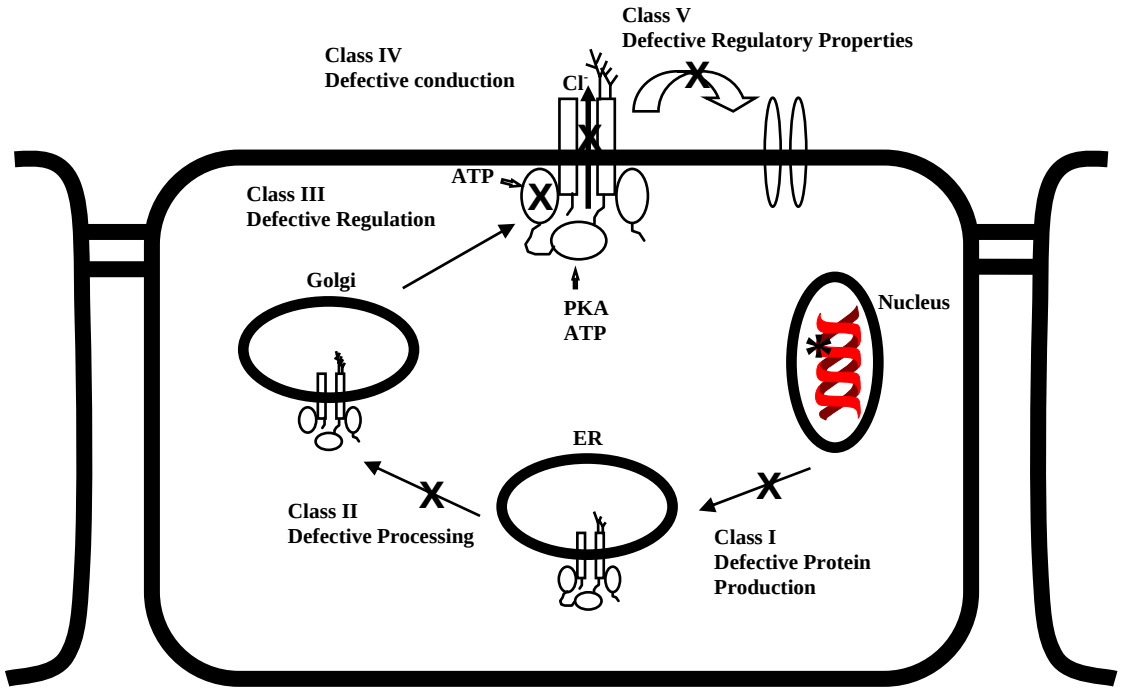
Nonsense mutations, frameshift or splice mutations are Class I.

II. CFTR is produced but does not fold correctly, giving rise to improper maturation (glycosylation) of the protein. This class includes the p.Phe508del (F508del) mutation which produces a defective protein which is destroyed by the Endoplasmic Reticulum (ER)-Associated Degradation (ERAD) pathway (Farinha *et al*, 2005) thereby reducing the amount of CFTR present at the cell surface.

III. These mutations affect chloride channel gating; CFTR is improperly activated as mutations affect binding and hydrolysis of ATP or phosphorylation of the R-domain. e.g. p.Gly551Asp (G551D), the most common missense mutation worldwide.

IV. CFTR does not allow proper chloride flux due to defective conduction through the pore, although some mutations cause lower chloride channel activity e.g. p.Arg117His (R117H), some may produce a higher current. The class also includes p.Asp1152His (D1152H) and is frequently associated with milder phenotypes.

V. These mutations affect the regulation of other ion channels such as the ENaC sodium channel and the Outwardly Rectifying Chloride Channel (ORCC).



***from* : Anne Vankeerberghen, KU Leuven**
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Figure 1: Schematic representation of the different classes of *CFTR* mutations.

3.1 Respiratory System

The respiratory system is particularly vulnerable to disease in cystic fibrosis. This is for two principle reasons. Firstly, the hyperviscous mucus blocks the airways resulting in a clinical picture similar to chronic obstructive airways disease. The patient develops a chronic productive cough, hyperinflated lungs and bronchiectasis at an early age requiring regular physiotherapy to expectorate mucus combined with mucolytics to assist mucus breakdown. Secondly, the defective CFTR protein predisposes the patient to secondary colonisation with pathogenic bacteria – particularly *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Haemophilus influenzae* (Cystic Fibrosis Foundation, 2001). Complications arising from these infections are frequently responsible for the reduction in lifespan of cystic fibrosis patients. Davies *et al* (1997) demonstrated experimentally that *Ps. aeruginosa* more avidly binds to epithelial cells in respiratory epithelium with mutated CFTR than in healthy tissue. They also showed that through restoration of normal CFTR function the process can be reversed leaving the way open for future genetic modulation therapy in the management of CF.

In addition, Worlitzsch *et al* (2002) found that in tissues affected by *CFTR* mutations, there was increased epithelial oxygen consumption as the result of increases in the airway surface liquid volume (ASL) absorption and mucus stasis leaving a hypoxic and thickened mucus prior to colonisation by an infecting organism. Opportunistic *Ps. aeruginosa* organisms were more able to penetrate this hypoxic mucus and this further exacerbated the hypoxia (Worlitzsch *et al,* 2002).

3.2 Pancreas

90% of cystic fibrosis patients exhibit pancreatic insufficiency (Ratjen and Doring, 2003). The defective CFTR protein causes the exocrine pancreas to produce digestive enzymes with reduced volume and fluidity. However, Choi *et al* (2001) showed CFTR to play an important role in the regulation of other transporters, notably the chloride-bicarbonate anion exchanger. This was noted because in mutant CFTR tissues, acidic rather than alkaline fluids are secreted from the epithelium and that in these tissues the cystic fibrosis *CFTR* mutations prevented HCO_3^- transport whereas those associated with pancreatic disease show reduced HCO_3^- transport. In the pancreas, this results in impaired HCO_3^- flux and near normal Cl^- flux out of the secretory epithelium further confirming the complexity of CFTR function (Choy *et al,* 2001). As a consequence of the increased viscosity of the secretions, digestive proenzymes become activated before reaching the duodenum thus causing damage to and inflammation of the pancreatic ductules (Ratjen and Doring, 2003). As there is reduced fluid leading to a chronically inflamed pancreas with decreased exocrine digestive enzyme secretory capability, the patient suffers from malabsorbtion of fats and protein, exacerbated by the increase in metabolic demand which accompanies endobronchial infection (Ratjen and Doring, 2003). Thus the patient has a higher metabolic demand and reduced absorption. Without pancreatic enzyme supplementation, this causes failure to thrive in children (often a presenting sign), weight loss, loss of muscle bulk and a further decreased ability to fight lung infections. Also, Lanng *et al* (1994) showed that although the Islets of Langerhans were initially spared in pancreatic disease associated with CF, the prevalence of diabetes mellitus increased with age as a result of chronic pancreatic damage. This finding demonstrates further that the pancreas becomes progressively diseased throughout the course of the condition, eventually affecting its endocrine function.

3.3 Gastrointestinal tract

Gastro-intestinal manifestations of disease in cystic fibrosis include reflux oesophagitis, oesophageal varices, meconium ileus, rectal prolapse and intussusception (Eggermont, 1996). In approximately 10% of neonates, meconium ileus is the presenting feature of CF (Eggermont, 1991). Eggermont *et al* (1996), reported that some gastro-intestinal complications such as oesophageal varices and peptic oesophagitis do not occur as a direct result of *CFTR* mutations (as this mucosa does not contain CFTR protein) but rather in the small bowel and to a lesser extent the large bowel (which contains relatively less CFTR) the pattern of reduced chloride and fluid leads to a thickened sticky mucus which reduces its absorptive capability. An added gastro-intestinal complication is that pancreatic supplementation has been associated with colonic strictures causing a 'cystic fibrosis fibrosing colonopathy' and thus leading to colonic and ileal obstruction (Eggermont, 1996).

3.4 Reproductive tract in males

Most males with cystic fibrosis are infertile as a result of aspermia secondary to congenital bilateral absence of vas deferens (CBAVD) (Ratjen and Doring, 2003). This used to mean that virtually all cystic fibrosis males were effectively sterile. However, Oates *et al* (1992) were able to demonstrate a microscopic epididymal sperm aspiration (MESA) technique, which successfully extracted viable sperm from the testis of a CF affected patient (Oates *et al,* 1992). Interventions such as this allow renewed hope of fertility for CF patients.

4. Historical Context

When the *CFTR* gene was cloned and its major mutation, p.Phe508del (F508del) identified in 1989, it was widely believed that there might be another half dozen mutations that would account for the remaining 30% or so of CF mutations. Perhaps that was founded more on wishful thinking than on sound scientific evidence (and, after all, there were not that many genes which had been extensively characterised at the time); in fact, the current number of sequence variants in the *CFTR* gene exceeds 1600. This explosion in the number of variants has necessitated changes in molecular strategies as well as a more subtle approach to interpretation of molecular results. The 'black-and-white' days of the early nineties have gone and been replaced by a need to interpret many shades of grey and therefore these guidelines address not only classic CF but also *CFTR* related disease.

5. Reason for Referral and Indication for Testing

It is recommended that laboratories consider the testing criteria formulated and agreed by the UKGTN (at the time of writing we are still awaiting publication of these on the UKGTN website). In principle, referrals that do not meet the criteria should only be accepted after discussion with the referring clinician. Laboratories should ensure that they perform a sufficient level of analysis to answer the clinical question, but do not carry out inappropriate testing (Dequeker *et al,* 2008).

5.1 Query CF

In the absence of a clear clinical diagnosis of cystic fibrosis, but where symptoms indicative of CF are present, a limited test for the most common CFTR mutations is indicated.

This is the largest category of testing and is frequently used as an exclusion test effectively to rule out a diagnosis of CF. The advent of newborn screening is expected to reduce the number of such tests. In a newborn baby, symptoms indicative of CF include meconium ileus (a blockage of the small intestine with meconium, and a very strong indicator of CF), delayed passage of meconium (24-48 hours), and salty-tasting skin. In the infant, loose stools (indicative, but not diagnostic, of pancreatic insufficiency), chest symptoms, and general failure to thrive are all indicators of CF, albeit rather soft markers. In older children and young adults, possible indicators include chronic chest disease and bronchiectasis, signs of pancreatic insufficiency and obstructive azoospermia.

5.2 Confirmation of CF

When a diagnosis of CF has been made on clinical grounds, molecular testing is still strongly indicated. Firstly, identification of two mutant alleles allows a molecular confirmation of the diagnosis of CF (in the event of any residual doubt). Secondly, confirmation of the presence of the mutation(s) in the parents enables prenatal diagnosis to be offered in future pregnancies. Thirdly, it enables carrier testing amongst relatives to be offered. Finally, identification of the genotype is of academic and clinical value in establishing genotype:phenotype associations (http://www.genet.sickkids.on.ca/cftr/Team.html gives an up to date listing of CF mutations).

5.3 Relatives of CF patients

Relatives of CF patients are at increased risk of carrying a *CFTR* mutation and are therefore at increased risk of having a child with CF. Cascade testing of relatives is therefore indicated, once appropriate counselling has been given, both for the familial mutation and for common mutations (Roberts *et al,* 2003).

5.4 Partners of relatives, or opportunistic testing of individuals with no family history

Partners of relatives, especially if the relative is a carrier, should be tested if an accurate pregnancy risk is required. Other individuals at 'population risk' may also request testing. For example, sperm and egg donors may be required to undergo testing before IVF procedures are instigated. For this group a test of the most common mutations rather than mutation scanning is appropriate.

5.5 Suspicion of a CFTR-related disease

There are a number of clinical presentations in which there is an increased risk of disease causing *CFTR* mutations. These include disseminated bronchiectasis, congenital absence of the vas deferens (CBAVD), chronic idiopathic pancreatitis and other less common presentations such as allergic bronchopulmonary aspergillosis. In some cases, molecular analysis may aid in the diagnosis and patients frequently have one typical *CFTR* mutation, together with one 'mild' mutation (See tables 5 and 6 below for some of the typical genotypes associated with *CFTR*-related diseases). Identification of a CF-causing mutation warrants the offer of cascade testing to relatives after appropriate genetic counselling has been given.

5.6 Fetal Echogenic Bowel (FEB)

The presence of echogenic bowel on fetal ultrasound scan may be associated with an increased risk of CF. The referral sample may be parental blood (or mouthwash) samples or a prenatal sample (cultured or direct). This is a complex referral and is dealt with separately (section 7).

5.7 Consanguinity

Consanguinity increases the chances of autosomal recessive disease in the offspring, particularly if there is known to be a mutation in the family or there is a common mutation in the population, for example p.Phe508del (F508del). Couples who are first cousins may be referred for *CFTR* mutation screening and certain related couples may be at higher risk if there has been a tradition of cousin marriages in their family for a number of generations.

5.8 Carrier testing in children

Guidelines from the Clinical Genetics Society recommend that carrier testing is not carried out in minors under the age of 16 except in exceptional circumstances. Such referrals should only be accepted from, or after review by, a clinical geneticist. (Information about carrier status consequent to newborn screening may lead to increase in demand for testing other siblings). For further information see:- 16 (iv)

5.9 Newborn screening

The UK CF Newborn Screening Programme is responsible for the implementation of a national CF screening programme throughout the UK under the umbrella of the UK Newborn Screening Programme. Guidelines are already available for this and can be found at http://www.newbornbloodspot.screening.nhs.uk/cf/index.htm

6. Strategies for Molecular Testing

6.1 Methodology

Although there is no gold standard for routine testing, initial analysis of a sample is usually by means of a commercially available kit, which will analyse approximately 30 sequence variants, accounting for more than 90% of CF disease-causing mutations (depending on local figures); although some laboratories use alternative methods. The mutations tested should identify at least 80% of mutations in the UK population e.g. at least p.Phe508del (F508del), p.Gly551Asp, (G551D), p.Gly542X (G542X) and c.489+1G>T (621+1G>T). Reports should specify the proportion of mutations identified by the test in the population of origin of the patient; they should also state that further testing is available if no mutation or only one mutation is identified and a clinical diagnosis of CF is made.

Subsequent analysis will depend on the reason for referral and might involve whole gene screening or testing for particular mutations. Whether commercial kits or 'in-house' methods are employed, laboratory personnel should be proficient in performing the test and interpreting the raw data. Furthermore, laboratories should be aware of the limitations of their chosen method e.g. which mutations are not identified, if there is the possibility of false negative or false positive results, and the general robustness of the test.

Methods used in *CFTR* testing can be divided into two groups: those targeted at known mutations (*i.e.* testing DNA samples for presence or absence of specific mutation(s), and scanning methods (*i.e.* screening samples for any deviation from the standard sequence). These now include searching for large unknown *CFTR* rearrangements, including large deletions, insertions and duplications, by semi-quantitative PCR experiments, *i.e.* Multiplex Ligation-dependant Probe Amplification (MLPA) or Quantitative Fluorescent Multiplex PCR. Such rearrangements, which can escape detection using conventional amplification assays, have

been shown to occur in up to 2% of alleles in CF patients and 1% in CBAVD patients.

Even though commercial kits may be CE-marked *in vitro* diagnostic devices (IVDD), assay performance should always be verified by laboratories before diagnostic use. The combined use of all these techniques cannot guarantee detection of the two disease-causing mutations (in *trans* – *i.e.* on both parental alleles) in all patients; 1-5% of alleles remain undetermined in CF patients with the classical form and even more in patients with atypical presentations.

Moreover, the percentage of undetected mutations increases from Northern-to-Southern European populations. *CFTR* mutations may be missed by scanning techniques, especially when homozygous, and even direct sequencing cannot identify 100% of mutations. Undetected *CFTR* mutations may lie deep within introns or regulatory regions which are not routinely analysed. For example 3849+10kbC>T (c.3718-2477C>T) and 1811+1.6kbA>G (c.1679+1.6kbA>G), the detection of which require particular methodologies.

It should also be noted that locus heterogeneity has been documented in patients with the classical form of CF, including a positive sweat test; but this probably concerns less than 1% of cases. In addition mutations in the *SCNN1* genes, encoding sodium channel (ENaC) subunits, have recently been found in non-classic CF cases where no *CFTR* mutations could be identified by extensive mutation scanning. However, the diagnostic utility of ENaC testing in routine practice has not been determined.

Table 1 summarises the approaches to different kinds of referral for CF testing and includes the initial test, subsequent or reflex testing, the possible outcomes and the recommended report style and further action required.

6.2 Population frequencies of CF mutations.

There is considerable heterogeneity in CF mutation frequencies throughout the world. Therefore the proportion of mutations identified in any one population using commercial kits will vary. It is recommended that an estimate of this figure be included in individual reports. Table 2 shows an estimate of CF mutation frequencies of individual populations worldwide [see also section 16(i)]

7 Fetal Echogenic Bowel

7.1 Background

Fetal echogenic bowel (FEB) is observed in 0.2 - 1.8% of 2nd trimester pregnancies and appears to have a multifactorial aetiology. Conventionally the bowel hyperechogenicity might be graded 1 to 3 relative to the sonodensity of the iliac crest, grade 3 being considered to be 'as bright as bone'. Bowel hyperechogenicity may be observed as an isolated finding, in association with other scan anomalies and may be transitory. Fetal hyperechogenicity at grade 2 or above is associated with a range of perinatal outcomes: normal (65.5%), severe malformation (7.1%), prematurity (6.2%), intrauterine growth retardation (4.1%), severe chromosomal abnormality (3.5%), placental/maternal problem (3.5%), CF (3%), viral infection (2.9%), *in utero* fetal death (1.9%) (Simon-Bouy *et al*, 2003).

7.2 Strategy

It is recommended that parental samples are tested in the first instance to determine the likely risk of CF and whether the situation is informative for prenatal diagnosis. Prenatal samples, even if available, are not tested in the first instance in order to avoid a carrier test. Analysis involves a mutation screen for a panel of mutations typically using the OLA32 or CF29 kits. Evidence suggests that cases of CF ascertained by hyperechogenicity have pancreatic insufficient (PI) (severe) mutations and most commonly p.Phe508del (F508del). Referral forms should ideally specify the grade of hyperechogenicity, whether it is an isolated finding, results of any other relevant investigations (specifically karyotype and CMV testing), gestational age, ethnic origin, consanguinity and any known family history of CF.

7.3 Risk figures

Due to the subjective nature of the assessment for hyperechogenicity it is recommended that laboratories derive their own risk figures by determination of the overall incidence of CF in their referrals for echogenic bowel and determination of the mutation sensitivity for the relevant ethnic group. If this is not possible, an estimate may be used based on the recent studies (Scotet *et al,* 2002; Patel *et al*, 2004; Jones *et al,* 2006) which suggest an incidence of CF in FEB at grade 2 or above of around 2 - 4% in routine referrals (Ogino *et al* 2004).

7.4 Extended screening

Extended screening is not recommended unless the analysis will significantly increase the mutation detection rate and can be completed in an appropriate timescale for the management of the pregnancy.

7.5 Reporting

7.5.1 Cases in which neither parent is found to carry a mutation

These should be reported as reduced carrier risk for both parents, and that prenatal diagnosis for cystic fibrosis is not indicated. The residual risk may be quoted (see section 7.3). Reports should state that further testing by karyotype analysis, virology screening and fetal ultrasound scan should be considered. If only one parent has been tested, screening should be offered to the other partner.

Referral type	**First test**	**Subsequent tests (in *addition* to reflex testing (see Section 8)**	**Outcomes**	**Report**	**Further Action**
?CF	CF29, CF-HT, OLA		2 mutations	Provides a molecular confirmation of a diagnosis of CF	
			1 mutation Increases likelihood of CF	DNA Sequencing, MLPA if subsequent clinical diagnosis of CF or strong suspicion	Request further clinical information. Further analysis if appropriate.
			0 mutation	CF unlikely	Clinical (re-) evaluation. Further analysis only if subsequent clinical diagnosis of CF or strong suspicion.
Confirmation of CF	CF29, CF-HT, OLA etc	DNA Sequencing MLPA (as requested or indicated)	2 mutations	Provides a molecular confirmation of a diagnosis of CF	
			1 mutation	Increased likelihood, but not confirmatory of CF (increases likelihood)	Request further clinical information. Further analysis if appropriate
			0 mutation	Significantly decreases likelihood of CF	Clinical (re-) evaluation . Further analysis only if subsequent clinical diagnosis of CF or strong suspicion
Relatives	Relevant mutation (if in CF29, CF-HT, OLA etc)	None	Carrier of familial mutation. Negative for familial mutation and for CF29: risk is greatly reduced (less than 1 in 300)	a) Report as carrier of CF b) Report with reduced risk	a) Genetic counselling as appropriate
	If not covered by CF29 etc, specific test required	Risk may be further reduced by testing by CF29 etc	Carrier of familial mutation. Negative for familial mutation and for CF29: risk is greatly reduced (less than 1 in 300)	a) Report as carrier of CF b) Report with reduced risk	a) Genetic counselling as appropriate
Partners	CF29, CF-HT, OLA etc	None	Carrier	Relative carrier : inform of 1 in 4 risk of having an affected child	Genetic counselling
			Negative	Low risk	
Sperm/egg donors	CF29, CF-HT, OLA etc	None	Carrier	Report as carrier of CF	Genetic counselling as appropriate
			Negative	Report with reduced risk	
CF-RD	CF29, CF-HT, OLA etc	DNA Sequencing MLPA (as requested or indicated)	2 mutations	Provides a molecular confirmation of a diagnosis of CF-RD (or cystic fibrosis)	
			1 mutation	Indicative of, but not confirmatory of CF-RD or CF (increases likelihood)	Request further clinical information Further analysis if appropriate
			0 mutation	Probably rules out CF-RD (significantly decreases likelihood that disease is CF related)	Clinical (re-) evaluation
FEB – parents' samples provided	CF29, CF-HT, OLA etc	None	a) 1 mutation b) 2 mutations	a) Report at increased risk b) Recommend testing fetus	Investigation of fetus if appropriate
FEB – fetal sample provided		None	a) 1 mutation b) 2 mutations c) 0 mutations	a) Report at increased risk b) Report as affected c) Report as low risk	
Infertile males	CF29, CF-HT, OLA etc	Intron 8 Polythymidine repeat (5T) if one mutation is found or if CBAVD is confirmed	Carrier of CF mutation Carrier of CF mutation plus variant e.g. 5T	a) Test relatives; test partner for common mutations b) Test relatives; test partner for common mutations; follow-up for lung disease	

Table 1: Testing strategies, and outcomes. CF-RD = CF related disease; FEB = fetal echogenic bowel

	Europe, North	Europe, South	America, North	America, South/Central	Australasia	Asia, mainly Middle East	Africa	Relative frequency (%)
Total CF chromosomes screened	21,154	7,281	10,438	758	2,095	608	515	
G85E	30	14	16			7		0.2
R117H	62	3	61		7	0		0.3
621+1G→T	97	37	154		27			0.7
711+1G→T	15	13	21					0.1
1078delT	53	2	1		1			0.1
R334W	18	21	12		2			0.1
R347P	55	24	26		1			0.2
A455E	35	0	27					0.1
ΔI507	57	5	20	2	9	0		0.2
ΔF508	14,866	4,007	6,900	342	2,309	173	351	66.0
17171-G>A	160	65	44		12	3		0.6
G542X	439	259	234	38	56	27	9	2.4
S549N	18	2	5	1	3	0	1	0.1
G551D	356	37	206	1	117	0		1.6
R553X	165	44	96	5	11	0	1	0.7
R560T	40	0	24		3	0		0.1
1898+1G→A	41	10	2					0.1
2184delA	14	7	8					0.1
2789+5G→A	27	10	17					0.1
R1162X	36	68	19		2			0.3
3659delC	39	1	14					0.1
3849+10kbC→T	23	8	57			16		0.2
W1282X	120	43	245		6	120	2	1.2
N1303K	209	179	130	11	23	29	8	1.3
Fraction of mutations detected (%)	80.2	66.7	79.9	52.8	83.7	61.7	72.2	**~70% world wide**

Table 2. Estimates of CF mutation frequencies of individual populations – reproduced from the Toronto 'sickkids' website (http://www.genet.sickkids.on.ca/cftr/app. See Table 4 for HGVS versions of mutation nomenclature.

7.5.2 Cases in which one parent is identified as a carrier

Prenatal diagnosis in this situation is not fully informative. It is recommended that the risk of the fetus being affected assuming it to have inherited the identified mutation is quoted (see above: this is likely to be around 1/7 assuming an incidence of 2% for CF in FEB and a mutation sensitivity of 90%). It should be pointed out that prenatal diagnosis is not fully informative in this case, and some indication of the residual risk included. As in 7.5.1, further testing should include recommendations to determine other possible causes of the anomaly.

The report should include a recommendation that the baby be assessed clinically and biochemically for cystic fibrosis. DNA testing is not advised unless the baby is unwell. As the national newborn screening programme is implemented it is anticipated that such cases should receive appropriate testing. It is recommended that the laboratory report be copied to the local clinical genetics department and to the local Newborn Screening Co-ordinator. The couple should also be advised that the result also has implications for other relatives of the partner identified as a carrier.

7.5.3 Cases in which both parents are identified as carriers

The report should indicate that both partners have been identified as carriers, the risk that the fetus is affected is significantly greater than one in four and that the situation is fully informative for prenatal or postnatal diagnosis. It should be noted that the result has implications for other relatives and the report as above should be copied to the local Clinical Genetics Department and to the local Newborn Screening co-ordinator.

8. Reflex Testing

8.1 Definition:

Reflex testing is additional testing carried out in response to initial analytical results. Most laboratories routinely screen for a panel of 29 (Elucigene CF29), 30 (Elucigene CF-HT), or 32 (Celera OLA v3) mutations as their primary testing strategy. Certain results and clinical situations mean that further testing is required either to:

1) Confirm the initial result or
2) Provide further information to aid interpretation of the initial result.

8.2 Apparent Homozygosity

If there are grounds to doubt a finding of homozygosity e.g. inability to confirm the mutation in both parents where non-paternity has been ruled out, further explanations should be sought. A mutation may appear to be homozygous when the allele on the opposite chromosome 7 is unamplifiable, for example:-

(i) Rare DNA variants can cause failure of amplification or failure of an allele specific oligonucleotide to hybridise.
(ii) A deletion of one or more *CFTR* exons.

8.2.1 DNA Variants

Of particular concern is the presence of apparent homozygosity for the p.Phe508del (F508del) mutation by OLA / ARMS analysis, when true homozygosity has been ruled out.

In the case of OLA, for example, the presence of the p.Phe508del (F508del) mutation on one chromosome (heterozygous) and the c.1523T>G; p.Phe508Cys (F508C) variant on the other chromosome, may result in the failure of the normal probe for p.Phe508del (F508del) to hybridise and the prevention of ligation. As only the probe for p.Phe508del (F508del) (mutant) hybridises, the end result is a false p.Phe508del (F508del) homozygote.

Similarly, there is the potential for c.1519A>G; p.Ile507Val (I507V) to interfere with hybridisation for the normal probe for c.1519; p.Ile507 (I507) leading to a false p.Ile507del (I507del) homozygote.

Elucigene™ CF29 and CF-HT ARMS kits report no cross-reactivity with c.1523T>G; p.Phe508Cys (F508C) or c.1519A>G; p.Ile507Val (I507V) (Instructions for use, P/N CF0HTBY005GB, http://www.elucigene.com/CFHT/pdfs/cfcf0htv2_ifugb005.pdf).

In these and other PCR-based assays rare Single Nucleotide Polymorphisms (SNPs) in primer binding sites can also lead to non-amplification of one allele leading to a false homozygous result.

Reflex testing.

All unexpected homozygous results should be confirmed by a second type of analysis. If p.Phe508del (F508del) homozygosity cannot be supported by alternative confirmatory evidence e.g. heterozygosity in both parents, then further analysis is indicated.

The OLA v3 kit includes a separate reflex assay to genotype p.Ile506Val (I506V), p.Ile507Val (I507V) and p.Phe508Cys (F508C) should unexpected homozygotes for p.Phe508del (F508del) or p.Ile508del (I507del) need confirmation. Laboratories using other methodologies should confirm the presence / absence of rare variants by sequence analysis or by using alternative primers (e.g. simple exon 10 sizing assay for p.Phe508del (F508del).

8.2.2 UPD 7

Homozygosity for a CF mutation in a child of unrelated parents may be due to unmasking of the recessive allele by uniparental isodisomy of chromosome 7 (UPD7). In particular, this scenario should be considered when a rare mutation is seen in an apparent homozygous state.

The referral may indicate symptoms, such as IUGR, in addition to those associated with CF, as maternal UPD7 is also a cause of Russell Silver syndrome (RSS, MIM 180860).

In these rare cases, where one parent has been demonstrated not to be a carrier and UPD has been confirmed, the recurrence risk for CF is low, and prenatal diagnosis for cystic fibrosis is not indicated.

Reflex testing

Parental samples should always be requested to confirm their CF carrier status prior to offering prenatal diagnosis or extended family carrier screening.

Reflex testing using intragenic microsatellite markers for the *CFTR* gene and also spanning chromosome 7 should be used to confirm UPD7 and exclude the presence of a *CFTR* deletion in these rare cases.

8.2.3 *CFTR* deletion

Apparent homozygosity for a rare *CFTR* mutation in a child of unrelated parents may also be caused by the deletion of one or more exons of *CFTR* on the opposite allele.

Reflex testing

Parental samples should always be requested to confirm their CF carrier status prior to offering prenatal diagnosis or extended family carrier screening. MLPA analysis may be considered as a reflex test in these rare cases.

8.3 The Intron 8 Polythymidine Repeat in *CFTR*-related disease: Congenital Bilateral Absence of the Vas Deferens (CBAVD), Chronic Idiopathic Pancreatitis and Disseminated Bronchiectasis.

The intron 8 poly T tract, [c.1210-12T(5_9)], adjacent to the *CFTR* exon 9 splice acceptor site can contain 5, 7 or 9 thymidine bases. This variation affects the efficiency of the splice site, with the 5T (c.1210-12T(5) variant being associated with the least efficient splicing of exon 9 and subsequently the highest levels of mRNA lacking exon 9, leading to reduced production of functional CFTR protein. It is estimated that the 5T allele is found in approximately 5% of alleles in the general population.

The 5T allele is known to modify the expression of the p.Arg117His (R117H) mutation when it is present on the same chromosome (in *cis*). Patients who are compound heterozygous for p.Arg117His and 5T (R117H/5T) and a typical severe mutation have been reported with classical CF of variable severity. p.Arg117His and 7T (R117H/7T) has a much more variable phenotype when in compound heterozygosity with a CF mutation and can even be benign (Girodon 1997). The 9T allele, c.1210-12T(9) is only very rarely found in cis with p.Arg117His (R117H) and is thought to be benign.

In this situation DNA from parents should always be requested to establish phase. However as p.Phe508del (F508del) is in strong linkage disequilibrium with the 9T allele, phase can be inferred without parental samples in compound heterozygotes for p.Arg117His (R117H) and p.Phe508del (F508del).

When the 5T allele is found on the chromosome opposite another CFTR mutation (in *trans*), or when a person inherits two copies of the 5T allele, the phenotype is highly variable; The 5T variant can be associated with *CFTR* related diseases such as congenital bilateral absence of the vas deferens (CBAVD), disseminated bronchiectasis and chronic pancreatitis.

Reflex testing

It is recommended that laboratories carry out reflex testing for 5T in the following situations:

1) All males referred with infertility caused by obstructive azoospermia irrespective of mutation screen results since 5T homozygotes have been reported.

2) Patients referred with bronchiectasis / pancreatitis in whom one pathogenic mutation has been identified.

3) Patients in whom p.Arg117His (R117H) has been detected. c.1210-12T(5_9) (Poly T) analysis should be carried out for both diagnostic and carrier referrals. Isolated carriers of p.Arg117His and 7T (R117H/7T) are far more frequent than predicted from the frequency of R117H derived from CF patients. This suggests that p.Arg117His with 7T (R117H/7T) is frequently benign.

4) Poly T reflex testing modules are included with both OLAv3 and Elucigene™ CF-HT kits. Elucigene™ also produce an independent gel based ARMS kit for the analysis of the CFTR intron 8 polyT tract [c.1210-12T(5_9)].

8.4 Poly T reporting guidelines for diagnostic referrals

Care should be taken when reporting the results of PolyT testing, and if possible full clinical information should be obtained before a report is written. What may be significant in one context may be less important in another. For example, if a p.Arg117His and 7T, (R117H/7T) compound heterozygote is picked up in a population screen, the likelihood is that it will be relatively benign, since most 7T alleles are associated with a HGVS TG11 (TG11) repeat (see table 3).

Allele 1	***Allele 2***	***Report***
R117H (5T)	Known mutation	Consistent with diagnosis of cystic fibrosis of variable severity.
R117H (7T)	Known mutation	Consistent with a *CFTR* related disease, classical CF unlikely. May be benign.
R117H (7T)	5T only	May be associated with *CFTR* related disease; may be benign.
5T only	Known mutation	May be associated with *CFTR* related disease; may be benign.
5T only	5T only	May be associated with *CFTR* related disease; more likely benign
5T only	No mutation	Unlikely to be associated with *CFTR* related disease

Table 3: Reporting recommendations for Intron 8 Polythymidine Repeat/p.Arg117His (R117H) genotypes.

8.5 Intron 8 Poly TG:

A poly TG tract (intron 8 polyTG) consisting of between 9 and 13 repeat units lies directly upstream of the poly T tract in intron 8. Increasing length of the intron 8 poly TG is reported to have an additional impact on the efficiency of exon 9 splicing and therefore levels of functional CFTR protein (Groman *et al*, 2004). This tract commonly has 11 repeat units - less commonly 12 or 13 TG repeats. When a TG12 or TG13 is inherited in *cis* with 5T it leads to increased skipping of exon 9. Individuals who inherit this combination with a severe mutation on the opposite allele are more likely to exhibit *CFTR* related disease than those with 5T and TG11.

In the light of the association of TG13 T5 with atypical CF and TG12 T5 with CBAVD, reflex testing for the TG repeat may be indicated when a 5T is identified. The method of choice is DNA sequencing which has the benefit of simultaneously typing the intron 8 Poly T and Poly TG tracts.

8.6 c.443T>C; p.Ile148Thr (I148T)

The c.443T>C; p.Ile148Thr (I148T) variant was first described as a pathogenic mutation. It has now been established as non-pathogenic. (Claustres *et al,* 2004; Rohlfs *et al,* 2002). Consequent to its original inclusion in the ACOG / ACMG panel of recommended screened mutations, p.Ile148Thr (I148T) was included in commonly used mutation detection kits (OLA v3 and Elucigene™ CF-HT and CF29) but has been removed from later versions. Carriers of this variant were therefore detected as part of routine screening. Where p.Ile148Thr (I148T) is detected, it should not be referred to in reports as a pathogenic mutation.

The reason for its earlier inclusion was the finding that a small proportion of p.Ile148Thr (I148T) alleles (<1%) (Monaghan *et al*, 2004) were in *cis* with a 6 base pair deletion c.3067_3072del6 (3199del6). This deletion is a pathogenic mutation in its own right and it was common practice for the detection of p.Ile148Thr (I148T) to trigger a reflex test for c.3067_3072del6 (3199del6). This practice is no longer warranted.

Reflex testing

In diagnostic testing, the detection of p.Ile148Thr (I148T) and a known mutation is not an indicator for reflex testing of c.3067_3072del6 (3199del6) in isolation. Should the patient meet local criteria for extended screening of *CFTR*, this mutation would be detected by exon sequencing. Reflex testing for c.3067_3072del6 (3199del6) in patients who carry p.Ile148Thr (I148T) alone is not indicated.

In carrier queries, reflex testing for c.3067_3072del6 (3199del6) in patients heterozygous for p.Ile148Thr (I148T) is generally not indicated; however, exceptional cases may merit this, for example if an individual with I148T is the partner of a known carrier.

8.7 Compound heterozygosity

In cases of apparent compound heterozygosity parents or other family members should be examined to ascertain that both mutations are in *trans*.

9. Prenatal Diagnosis (PND)

Accurate Prenatal Diagnosis (PND) is feasible for CF and should always be performed in conjunction with appropriate genetic counselling. PND can be offered to the parents of a child affected with CF, where both parental mutations have been identified and to couples identified through cascade carrier testing.

In the case of an affected child in whom both mutations have not been identified, family studies using linked markers may be possible.

9.1 Recommendations for PND practice

Test(s) for maternal cell contamination (MCC) of the fetal sample must be carried out, for example by studying a panel of microsatellite markers. Care should be taken to consider whether the MCC analysis is quantitative or qualitative: the comparative sensitivity of the CF and MCC analyses and the threshold level for rejecting a sample as contaminated/maternal in origin. Analysis of multiple samples may be helpful and any chorionic villus sample should be dissected by an experienced analyst. Best practice guidelines for MCC can be found on the CMGS website; http://cmgsweb.shared.hosting.zen.co.uk/

In some genetics departments, preimplantation genetic diagnosis (PGD) can be offered for at-risk couples as an alternative to PND (see 9.2 below).

9.2 Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis (PGD) is considered a very early form of prenatal diagnosis where, following IVF procedures, an embryo is usually tested for particular *CFTR* mutations at day 3 post fertilisation. Only embryos predicted to be free of cystic fibrosis are considered suitable

for transfer to attempt a successful pregnancy. Testing is usually undertaken along with a linked marker to identify allele drop out and possible contamination by extraneous DNA (Goossens *et al,* 2003). An indirect haplotype approach allows PGD to be offered for any CFTR mutation. Haplotypes can be identified in the couple using informative markers chosen from a selection of available multiplexed marker combinations (Moutou *et al,* 2004). More recently, this indirect approach has been combined with whole genome amplification using a universal panel of linked markers, termed Preimplantation Genetic Haplotyping (PGH) (Renwick *et al,* 2006). Currently PGD is a very specialised procedure for which only a small number of laboratories are licensed. Full details are outside the scope of these guidelines. (Best practice guidelines for PGD (Thornhill *et al,* 2005) and information on ESHRE PGD consortium data collections can be found on the ESHRE website : http://www.eshre.com)

10. Congenital Absence of the Vas Deferens (CBAVD/CUAVD) / Infertility

10.1 Introduction

Infertile males with CBAVD or congenital unilateral absence of the vas deferens CUAVD have an increased risk of carrying CFTR mutations. Approximately 2% of male infertility is caused by CBAVD, which is present in about 25% of males with excretory azoospermia (Dubin and Amelar, 1971). CUAVD is a rarer condition occurring in less than 1/1000 males (Martin *et al,* 1992).

Testing for *CFTR* mutations in infertile males, who have an increased risk of carrying non-classical *CFTR* mutations, produces difficulties for the diagnostic laboratory in both appropriate testing and reporting. The extent of genetic testing for male infertility varies between laboratories; *CFTR* testing is usually applied in cases of obstructive azoospermia ; however Y microdeletion testing is provided by fewer laboratories and is associated with cases of non-obstructive azoospermia/oligozoospermia.

CFTR testing is usually carried out for one of two reasons:

(i) To support a diagnosis of infertility/CBAVD arising from the finding of *CFTR* mutations.

(ii) To alert the clinician to the risk of CF in the offspring, and any carrier risks to the patient's extended family.

10.2 CBAVD

Congenital bilateral absence of the vas deferens (CBAVD) is found in 1-2% of infertile males. CBAVD associated with CFTR mutations was found in 2% of males with obstructive azoospermia, however the incidence varies between populations. *CFTR* mutations have also been found in cases of non-obstructive azoospermia (Jarv *et al,* 1995; Mak *et al,* 1999; Meschede *et al,* 1997). Obstructive azoospermia is present in over 95% of CF males. In general, patients with obstructive azoospermia have about 80% risk of being carriers of at least 1 *CFTR* mutation. (Casals *et al*, 2000; Cuppens *et al*, 2004; Mercier *et al,* 1995; Mickle *et al*, 1995; De Braekeleer *et al*, 1996; Dork *et al*, 1997; Wang *et al*, 2002).

Unlike patients with classic CF, CBAVD patients are less likely to carry two classic severe *CFTR* mutations. Often the patient will be a compound heterozygote, with either one severe and one 'mild' mutation or two 'mild' mutations (Chillon *et al,* 1995; Dork *et al,* 1997). The c.1210-12T(5), (5T), allele of the polythymidine tract in intron 8 is the most frequent variant associated with the CBAVD phenotype. It is seen at a much higher frequency in CBAVD males compared to the general population. In such cases the 5T allele is often seen in *trans* with a *CFTR* mutation and also rarely as a 5T homozygote (Chillon *et al*, 1995).

Assisted reproductive technologies can be offered to CBAVD males. Sperm can be surgically retrieved by Percutaneous Epididymal Sperm Aspiration (PESA) followed by fertilisation using intracytoplasmic sperm injection (ICSI). If the female partner is also found to be a CF carrier then prenatal or preimplantation genetic diagnosis can be offered to the couple; alternatively they can choose donor sperm.

10.3 CUAVD

Congenital unilateral absence of the vas deferens (CUAVD) occurs in <1/1000 males. A proportion of these males may be fertile, but there may be an association with oligospermia; and some may carry *CFTR* mutations. There are 2 distinct subgroups of CUAVD males; (i) those with one anatomically complete vas deferens and associated incidence of renal anomalies, and (ii) those with a single vas deferens ending abruptly. *CFTR* mutations are found in the latter subgroup but at a lower frequency than in men with CBAVD (Casals *et al,* 2000; Mickle *et al,* 1995).

10.4 Testing Guidelines

Tests for CFTR mutations should be undertaken in men who are found to have bilateral absence or bilateral abnormality of the vas deferens or unilateral absence of the vas deferens and/or seminal vesicles with normal kidneys (EAU 2004, ESHRE 2000, NICE 2004, Lissens *et al,* 1996). It is also important to test the partner of a man with CABVD for CF mutations

Genetic counselling should be offered to couples with a genetic abnormality found in clinical or genetic investigation and to patients who carry a (potential) inheritable disease (EAU, 2004, NICE 2004).

A diagnosis of CBAVD can only be made on clinical examination. Therefore genetic testing should be carried out on couples only following a clinical examination (NICE; Claustres, 2005).

10.5 Referrals

Infertility referrals originate mainly from NHS and private fertility clinics, GPs, and occasionally urologists. Information should be sufficient to ensure correct testing, and to enable an appropriate report to be written. 'Infertility investigations' is insufficient for most laboratories; '*CFTR* analysis' should be stated on the referral form if required. A sperm count is useful to indicate whether the patient has azoospermia or oligospermia, and therefore whether CBAVD or CUAVD is to be considered when writing reports. If the patient has definitive CBAVD, this should be stated, as intron 8 poly T testing is then necessary. An additional form with appropriate tick-boxes may be useful in order to prompt the clinician for sufficient information, but this depends on the extent of fertility testing in a particular laboratory.

10.6 Testing

Currently the majority of laboratories initiate a routine first level screen using a kit based panel of *CFTR* mutations for example the Elucigene CF29 ARMS kit (Tepnel), the 33 OLA kit (Applied Biosystems), or an 'in-house' assay with selected *CFTR* mutations relevant to CBAVD.

Some first level screens, e.g. the CF29 kit, do not include intron 8 poly T analysis (i.e. testing for 5T). The 32 OLA *does* include 5T, which can be masked. Currently the

majority of laboratories using 32 OLA report the 5T result in cases of infertility.
Testing for 5T in isolation should be discouraged in cases where the precise reason for the patient's infertility is not known, as it is difficult to distinguish a pathogenic 5T allele from a 5T carried in the population. However for some clinicians this is part of their diagnostic process and it is often impractical for laboratories to exclude 5T in their initial analysis.
If there is definitive CBAVD, 5T testing should be undertaken. If intron 8 poly T analysis is not included in the initial test then it should be performed as a reflex test for CBAVD patients where one or two *CFTR* mutations are found (see also section 'reflex testing'). Where one p.Arg117His (R117H) mutation is found, phase needs to be determined following the finding of a 5T or 7T allele. p.Arg117His+5T (R117H/5T) in *trans* with a second *CFTR* mutation may cause a classical CF-PS phenotype, whereas p.Arg117His+7T (R117H/7T) in *trans* with a second *CFTR* mutation is likely to result in a CBAVD phenotype. P.Arg117His (R117H) with 9T in *cis* is thought not to be disease causing (Claustres, 2005).
In the light of the association of TG13/T5 with atypical CF and of TG12/T5 with CBAVD, reflex testing for the TG repeat may be indicated when a 5T is identified, depending on the context of the test. The method of choice is DNA sequencing which has the benefit of simultaneously typing the intron 8 Poly T and Poly TG tracts. An individual with p.Phe508del (F508del) and 5T/12TG or p.Phe508del and 5T/13TG is more likely, although not certain, to have CAVD or non-classic CF (Groman, 2004). Extended testing (i.e. mutation analysis of the whole CFTR gene) is not usually undertaken in the UK. Claustres recommends a clinical diagnosis of CBAVD certified by a competent physician before this is considered (Claustres, personal communication; Claustres, 2005).

10.7 Reporting
10.7.1 CBAVD
If a diagnosis of CBAVD is not definitively stated on the referral form, there should be a statement on the report e.g. 'The precise reason for this patient's azoospermia was not given on the referral form and may not be known'.
If CBAVD is suspected, there should be a qualifying statement e.g. 'if he has CBAVD there is an increased risk of this man carrying a *CFTR* mutation'.
If the first level screen does not include testing for 5T (e.g. the CF29 kit), this should be stated e.g. 'This analysis does NOT include the CFTR intron 8 variant 5T'
Genetic counselling should be offered, and should be recommended if a mutation is found. It is recommended that the partner of a man with CBAVD is also tested for *CFTR* mutations, and genetic counselling offered (EAU 2004, ESHRE 2000, NICE 2004, Lissens *et al* 1996, Claustres, 2005). This is good practice, even when the CBAVD patient is negative for a *CFTR* mutation screen, as occasionally the partner is found to be a carrier of a *CFTR* mutation, which may require further mutation screening to be undertaken in the CBAVD male to ascertain the genetic risk to offspring (Lewis-Jones 2000).
Most laboratories do not quote the percentage of *CFTR* mutations detected in cases of CBAVD because estimates vary widely, depending on the method and extent of screening (Wang *et al* 2002, Cuppens *et al* 2004, Danziger *et al* 2004, Claustres, 2005). A general statement may therefore be sufficient to the effect that using the laboratory's standard panel of *CFTR* mutations the percentage detection of *CFTR* mutations in CBAVD patients/male infertility will be less than that quoted for the local population, compared to classic CF, and that an increased percentage of non-classic *CFTR* mutations has been reported compared to classic CF, some of which may not be detected by the panel used. Most laboratories do not quote any references for *CFTR* testing in CBAVD patients on their reports. However it is suggested that a good review (such as Claustres, 2005) be included.

10.7.2 CUAVD
If a diagnosis of CUAVD is not definitively stated on the referral form, there should be a statement on the report e.g. 'The precise reason for this patient's oligospermia was not given on the referral for and may not be known'.
If CUAVD is suspected, there should be a qualifying statement e.g. 'if there is a possibility that he may have CUAVD there is an increased risk of this man carrying a *CFTR* mutation'. There should be an indication at the beginning of the report that due to the high carrier frequency in Caucasian populations, there may be a coincidental finding of a *CFTR* mutation e.g. 'Alternatively, he may be at population risk of carrying a *CFTR* mutation'.

10.8 Genotype/Phenotype correlations
If one *CFTR* mutation is found, most laboratories include such phrases as: 'at least a carrier' or 'cannot exclude the possibility of additional mutations'
If one *CFTR* mutation and an intron 8 5T mutation are found in *trans*, there should be an indication that this is 'consistent with the phenotype of infertility'.
In the case of 5T homozygosity, TG testing is indicated and, depending on the TG haplotypes, the report should state that this genotype may be consistent with the phenotype of infertility.
If two *CFTR* mutations are found, there are two alternative scenarios:
(i) Two classic mutations with a more predictable phenotype.
(ii) One of the two mutations is not severe/classic, which may result in a less severe phenotype.
The report should therefore take into account a potential variable phenotype. Referral to clinical genetics is recommended, which may include further assessment for clinical symptoms.

11. Recommendations for nomenclature
It is recommended that mutations are described in accordance with the Human Genome Variation Society (HGVS) recommendations http://www.hgvs.org/ These guidelines recommend use of a coding reference sequence wherever possible and specify that the A of the translation initiation codon ATG is base 1. On reports a reference sequence should be given and a version number (most participants in the UKNEQAS CF scheme for 2006 used the *CFTR* ref seq NM_000492.3).
Because of the established use of previous nomenclature, it is helpful for the common names to be referenced alongside the HGVS version. A list of the HGVS nomenclature for mutations included in the OLA and ARMS kits is given in Table 4.
When a rare mutation has been identified which is not in the commonly used commercial kits, both DNA and predicted amino acid nomenclature, if appropriate, should be given according to HGVS recommendations. Although it can be argued that the amino acid change is almost always a prediction and may not reflect the situation in vivo, it may

help to clarify the position of the mutation at the DNA level. The three letter amino acid code should be used in HGVS versions to avoid confusion with DNA nomenclature.

Table 4 HGVS nomenclature for common CFTR mutations

Traditional	HGVS	
	Nucleotide (DNA level)	Amino Acid (Protein level)
E60X	c.178G>T	p.Glu60X
G85E	c.254G>A	p.Gly85Glu
R117H	c.350G>A	p.Arg117His
Y122X	c.366T>A;	p.Tyr122X
I148T	c.443T>C	p.Ile148Thr
621+1G>T	c.489+1G>T	
711+1G>T	c.579+1G>T	
1078delT[1]	c.948delT	p.Phe316fs
R334W	c.1000C>T	p.Arg334Trp
R347P	c.1040G>C	p.Arg347Pro
R347H	c.1040G>A	p.Arg347His
R352Q	c.1055G>A	p.Arg352Gln
A455E	c.1364C>A	p.Ala455Glu
Q493X	c.1477C>T	p.Gln493X
I507del or DeltaI507	c.1519_1521delATC	p.Ile507del
F508del or DeltaF508	c.1521_1523delCTT	p.Phe508del
V520F	c.1558G>T	p.Val520Phe
1717-1G>A	c.1585-1G>A	
G542X	c.1624G>T	p.Gly542X
S549R(A>C)	c.1645A>C	p.Ser549Arg
S549R(T>G)	c.1647T>G	p.Ser549Arg
S549N	c.1646G>A	p.Ser549Asn
G551D	c.1652G>A	p.Gly551Asp
R553X	c.1657C>T	p.Arg553X
R560T	c.1679G>C	p.Arg560Thr
1898+1G>A	c.1766+1G>A	
2183AA>G	c.2051_2052delAAinsG	
2184delA	c.2052delA	p.Lys684fs
2789+5G>A	c.2657+5G>A	
3120+1G>A	c.2988+1G>A	
D1152H	c.3454G>C	p.Asp1152His
R1162X	c.3484C>T	p.Arg1162X
3659delC	c.3528delC	p.Lys1177fs
3849+4A>G	c.3717+4A>G	
3849+10kbC>T	c.3718-2477C>T	
S1251N	c.3752G>A	p.Ser1251Asn
3905insT	c.3773dupT	p.Leu1258fs
N1303K	c.3909C>G	p.Asn1303Lys
W1282X	c.3846G>A	p.Trp1282X

12. CFTR Variants

Nothing has exercised the European CF community so much in recent years as *CFTR* variants, their significance, their frequency, their role in disease, and even their name.

CFTR mutations are associated with a broad range of phenotypes which are mainly due to their varied effects on protein synthesis and function. The terms “mutation” and “polymorphism” are loosely and incorrectly used both by clinicians and laboratories for “disease-causing” and “neutral/benign” DNA changes, respectively. It is therefore important to make clear if a mutation or sequence variation is predicted to cause CF, whether its effect is severe or moderate, if it is associated with *CFTR*-related disorders, or if it is phenotypically neutral.

The classification of *CFTR* gene mutations according to their functional effects on CFTR protein production and function, based on functional studies, has been widely used in the scientific literature. However, only a limited number of mutations has been studied, and many *CFTR* mutations have different functional consequences, which cannot be assigned to one particular class. In other respects, it should be noted that categorisation of *CFTR* mutations is not predictive for individual outcomes. *CFTR* genotype/phenotype correlations may be used at a population level to determine associations, but should not be used to indicate definitive prognosis in individual patients.

Variants may be clustered in four groups.

(i) Pathogenic mutations that cause CF disease.

(ii) Pathogenic mutations that result in a *CFTR*-related disorder.

(iii) Variants with no clinical consequences

(iv) Variants of unproven or uncertain clinical relevance.

Some CF mutations may be associated with a wide phenotypic spectrum and discussion should take into account these generally milder phenotypes. As a precaution, variants of unproven or uncertain clinical relevance may be considered as potentially CF-causing. Tables 5 and 6 give a general guide to the phenotype associated with some combinations of CFTR mutations.

13. Linkage analysis using intragenic and flanking polymorphic microsatellite markers.

In most populations, a few mutations account for the majority of CF cases, however many rare mutations may remain uncharacterised. In cases where CF has been clinically confirmed and both mutations have not been found, linkage analysis can be offered using microsatellite markers to follow segregation through a family and determine the haplotype associated with the unidentified *CFTR* mutation. A number of polymorphic markers are summarised in Table 6, including several intragenic markers (Dörk *et al*, 1992; Morral and Estivill, 1992; Renwick *et al*, 2006; Dean *et al*, 1990; Gasparini *et al*, 1991). It should be possible to find informative markers in the majority of cases so that the error rate due to recombination based on linkage with either intragenic and/or flanking markers is «1%. Microsatellite markers can also be used to confirm uniparental isodisomy of chromosome 7 and exclude the presence of a CFTR deletion.

14. QA Schemes

External QA schemes for CFTR molecular analysis.

A scheme for external quality assessment (EQA) is an essential requirement for any professional laboratory seeking to provide a diagnostic service. Furthermore it is a specific requirement of Clinical Pathology Accreditation (CPA) for UK laboratories to take part in an accredited Quality Assessment Scheme (Standard H5.1). Such QA schemes must be accredited either by CPA (EQA) or by another organisation accrediting to standards based upon ILAC G13:2000. For more information on schemes available see: http://www.ukneqas-molgen.org.uk/ukneqas/ and http://www.cfnetwork.be/index.php.

Table 5: Phenotype associated with some combinations of CFTR mutations.

Disorder	Alleles	Genotypes	References
CBAVD	M1I	F508del/M1I	Tz[1]
	R75L	F508del/R75L	DeB
	D110E	F508del/D110E	Tz[1]
	R117C	F508del/R117C	Cl,G
		621+1G>T/R117C	Tz[1]
	R117H-T7	F508del/R117H-T7	Cl,Do,DeB,S,R
	711+3A>G	F508del/711+3A>G	S
	L206W	F508del/L206W	Cl,R
	V232D	F508del/V232D	S,G
	T338I	G542X/T338I	Cl
	R347H	F508del/R347H	Cl,DeB
		3659delC/R347H	Cl
	A349V	F508del/A349V	S
	R352W	F508del/R352W	Tz[1]
	D443Y	F508del/D443Y	Cl
	A455E	A455E/R117H-T7	Do
	T5	F508del/T5	Cl,Do,R
		L932X/T5	Tz[1]
		711+3A>G/T5	Tz[1]
	V562I-TG11-T5	F508del/V562I-TG11-T5	R
	D443Y-G576A-R668C	F508del/D443Y-G576A-R668C	R
	D565G-R668C		Tz[1]
	R668C	F508del/R668C	DeB
	G576A-R668C	F508del/G576A-R668C	S, R
		P67L/G576A-R668C	
	G576A	F508del/G576A	A,DeB
	E831X	F508del/E831X	S
	M952I	F508del/M952I	Tz[1]
	F1052V	F1052V/W496X; F1052V/F1052V	Tz[1]
	A1067V	F508del/A1067V	DeB
	R1070W	F508del/R1070W	Cl,DeB,S
	3272-26A>G	F508del/3272-26A>G	Cl,S,R
	D1152H	F508del/D1152H	Cl,S,R
		D1152H/D1152H	DeB
	D1270N-R74W	F508del/D1270N-R74W	Cl,DeB, R
	Q1352H(G>C)	F508del/Q1352H	R
Bronchiectasis	P67L	F508del/P67L	S
	R117C	F508del/R117C	G
	R117H	F508del/R117H	S
	711+3A>G	F508del/711+3A>G	S
	L206W	F508del/L206W	S
	875+1G>C	F508del/875+1G>C	S
	S307N	F508del/S307N	S
	R347H	F508del/R347H	S,G
	R668C-G576A	F508del/R668C-G576A	G
	R851X	F508del/R851X	S
	3272-26A>G	F508del/3272-26A>G	S,G
	D1152H	F508del/D1152H	S,G
	3600A>G	F508del/3600A>G	S
	R1162L	F508del/R1162L	G
	S1251N	F508del/S1251N	S
	Q1291H	F508del/Q1291H	S
Pancreatitis	R117H-T7	F508del/R117H-T7	Co
	R334W	F508del/R334W; 444delA/R334W	Tz[2]
	R347H	E822X/R347H	Tz[2]
	T5	R1070Q/T5	Tz[2]
	L997F	F508del/L997F	Ca
	3272-26A>G	F508del/3272-26A>G	Tz[2]

References: [A] Anguiano *et al* 1992; [Ca] Castellani *et al* 2001; [Cl] Claustres *et al* 2000 ; [Co] Cohn *et al* 1998; [Do] Dohle *et al* 1999 ; [DeB] De Braekeleer and Ferec 1996; Girodon E *pers comm*; [R] Ratbi *et al* 2007 ; [S] Schwarz M *pers comm*, [G] [T[1]] Tzetis M *pers comm*, [T[2]] Tzetis *et al* 2007

Table 6: Genotypes occurring in more than one clinical presentation

Genotypes occurring in more than one clinical presentation	Clinical Presentations	References
F508del/R117C	CBAVD, Bronchiectasis	Cl,DeB,G,Tz[1]
F508del/R117H-T7	CBAVD, Bronchiectasis, Pancreatitis	Co,Cl,DeB,Do,R,S
F508del/L206W	CBAVD, Bronchiectasis	Cl,G,S
F508del/R347H	CBAVD, Bronchiectasis, Pancreatitis	Cl,DeB,G,S,Tz[1]
F508del/G576A	CBAVD, Bronchiectasis	A,R,S
F508del/R668C-G576A	CBAVD, Bronchiectasis	G,R,S
F508del/3272-26A>G	CBAVD, Bronchiectasis, Pancreatitis	Cl,G,R,S,Tz[1]
F508del/D1152H	CBAVD, Bronchiectasis	Cl,DeB,G,R,S
F508del/R1162L	CBAVD, Bronchiectasis	G

Table 7: Microsatellite markers flanking and within the *CFTR* gene. Physical distances taken from location along chromosome 7 according to the Santa Cruz genome browser (http://genome.ucsc.edu). Genetic distance is taken from estimated sex averaged genetic location along chromosome 7 according to Mammalian Genotyping Service (http://research.marshfieldclinic.org/genetics/MarkerSearch/buildMap) . NA: marker not placed on Marshfield map; *: Primer sequences as modified by (Renwick *et al*, 2006).

Marker	Type	Het %	Physical distance from *CFTR* (Mb)	Genetic distance from *CFTR* (cM)	Primers	Size range (bp)	Reference
D7S523	(ca)n	80	5.4	1.6	ATGCTTCATAAGCTCTCTATGGC CATTTCCATTACCACTGCTATTATC	304-316	*
D7S2554	(ca)n	76	2.7	NA	ATGGCTTTTTGCATACTAAATGC CAGCATGCTACACCATGTATTG	117-149	*
D7S2502	(ca)n	78	1.7	NA	CTGGAATTGTCTGAGCAGCTAG CATGTATGCTCATGGTTGGA	279-299	*
D7S486	(ca)n	80	1.2	0.5	AGGAGGAAAGGCCAATGGTATATCCC TTGCAATGAGCCGAGATCC	161-184	*
D7S2460	(ca)n	71	0.7	NA	CAGGCTTAGGATCTCCCTGG CTGGACTTTACGCTTCTAATATGCAT	238-248	*
IVS1-CA	(ca)n	73	Intron 1	0	GCTACTCTCGTCAGTACAATGAGT CTTCAGACTCAAACTGGAACATT	277-289	*Moulin, 1997
IVS6-GATT	(gatt)n		Intron 6	0			Dork, 1992 Gasparini, 1991
IVS8-CA	(ca)n	48	Intron 8	0	ATCTATCTCATGTTAATGCTGAAGA ACTAAGATATTTGCCCATTATCAAGT	174-198	*Morral, 1992
IVS17B-TA	(ta)n	87	Intron 17	0	TGCTGCATTCTATAGGTTATCAA GACAATCTGTGTGCATCGG	226-290	*Morral, 1992
IVS17B-CA	(ca)n	39	Intron 17	0			Morral, 1992
CFSTR1	(ttcc)n	93	0.6	NA	TCTTTTTCTGTCTGTGCTGCATTC GAACAATTGCATGCCAGCCT	206-226	*
D7S2847	(tatc)n	84	1.5	0.6	ATTCACCTTCAGAAAGTATTGCC AGGAAACCATTGCGTTTAAATTG	139-171	*
D7S643	(ca)n	75	3.4	NA	GAAATCAAAGCTAATATTGCTCCC GGAGATGGGTGTAGAGTGAATCTG	184-215	*
D7S480	(ca)n	86	3.6	1.4	TTCAGGTAGACAAGTTCCTGTC CTACTCATTTCAACTTTGAGTCTCA	237-257	*
D7S650	(ca)n	83	3.7	2.2	TAGGCTGCTTAGCCCATAATCT AAGACATGTGGAAGCGAACTC	240-261	*
D7S490	(ca)n	77	5.5	3.2	CTTGGGCCAATAAGGTAAGACA TGCAAGCAATTATGTGCTTGT	118-136	*

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16. Further Reading

(i) Worldwide Estimates of Mutation Frequencies:

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The Cystic Fibrosis Genetic Analysis Consortium who published estimates in Human Mutation 4:167-177, 1994. This data is reproduced in Table 2 above and is available on the Toronto 'sickkids' web site as follows:- 'Population variation of 24 common cystic fibrosis mutations' http://www.genet.sickkids.on.ca/cftr/resource/rptTable1Full.html

Registration is required to access the HGMD mutation database at: http://www.hgmd.cf.ac.uk/ac/index.php

(ii) Genotype-phenotype

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(iii) Prenatal diagnosis and Echogenic fetal bowel

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Pam Renwick: Linkage, PGD

Lucy Jenkins, Miranda Durkie: Reflex Testing

Anne Gardner: Infertility