



User Manual

East of Scotland Regional Molecular Genetics Service

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Accredited Medical Laboratory

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Also enclosed is a copy of the Human Genetics Request form

General Information

The molecular lab was set up in 1985 as part of the Scottish Molecular Genetics Consortium (the other labs being in Aberdeen, Edinburgh and Glasgow). Each lab offers analysis for a number of common conditions for its local population and a Scottish service for other rarer disorders. Samples received for disorders not offered by the Dundee lab will be forwarded as appropriate. More details of conditions for which analysis is offered in Scotland can be found on pages 7-42.

Analysis for other disorders not listed in this manual may be available from diagnostic labs in other parts of the UK. Please contact the Dundee lab in the first instance as certain sample types, or samples from particular family members may be required for the analysis.

Sample referrals

All samples should be accompanied by a Human Genetics request form (one is included in this manual, please copy as required. More can be supplied on request.)

The following information **must** be supplied **legibly** with **each** sample:

Patient's surname and forename (on sample and form)

Patient's date of birth and gender (on sample and form)

Name and address of requesting consultant/GP (on form)

Analysis requested and reason for request (on form)

Samples received without this information will be rejected.

If you are sending in a sample from someone who has had other family members tested already, please include details of these individuals if known.

Please note that additional forms are required to be completed for some familial cancers eg breast and colon cancer and also for diabetes studies. Contact office for details.

Sample types

For most molecular analysis, a venous blood sample (3ml) in potassium EDTA (purple top vacutainer) is sufficient. There are exceptions however and the requirements for each disorder are included in this manual.

Samples that are clotted will be rejected.

High Risk Specimens

It should be noted that blood samples from patients who are likely to be Hepatitis B antigen or HIV positive, who have infectious hepatitis or who are jaundiced without obvious cause are potentially dangerous to all who handle them. Blood from febrile, undiagnosed patients, especially from abroad, may also be dangerous. Great care should be observed when submitting these samples for laboratory investigations, with strict adherence to the recognised methods of handling, particularly:

- 1. Forms and sample bottles must be clearly marked with a red warning sticker**
- 2. The sample must be sealed in a plastic bag. The accompanying form must not come into contact with the sample.**

For high-risk samples, a mouth wash is preferable to a blood sample although this may not be suitable for all

analyses - please contact the lab for advice, prior to sampling.

Sample transport

Samples sent through the internal mail systems should be placed in sealed plastic bags, with the accompanying form in a separate compartment.

It is no longer permissible to send blood samples through the post in jiffy bags. Proper Royal Mail approved packaging must be used. These are available from the lab on request. All samples should be sent to the address shown on the front cover of this manual.

Reporting guidelines

Written results will only be reported to the consultant or GP whose name is on the request form.

Results may be given out over the phone by senior clinical scientists, but only to the requesting clinician.

Faxing reports

In exceptional circumstances, eg prenatal diagnosis, a report may be faxed to the referring clinician if requested. The surname(s) of the patient(s) will be tippexed out so that there is some degree of anonymity. The fax will only be sent if there is an assurance the clinician can collect it immediately and will inform us of receipt.

e-mail reports

In exceptional circumstances, eg prenatal diagnosis, a report may be e-mailed to the referring clinician if requested. The patient(s) will only be identified by initials and dates of birth for security reasons.

A written report will always be sent as confirmation.

Disease / disorder	Common Trisomy screen
Sample type requested	At least 2ml of amniotic fluid plus 3ml of maternal blood in EDTA
Analysis offered	Microsatellite dosage analysis for trisomy 13, 18 and 21 Sex testing for Turners in appropriate cases
Pick-up rate	
Reporting time	Within 24-48 hours (samples received on Friday will be reported on Monday or Tuesday) If we are unable to get a result, then full karyotype results should be available from cytogenetics within 8-14 days
Notes	Referrals only accepted from Obs+Gynae consultants in Tayside. We may not be able to report fluids which are bloody and show maternal cell contamination (MCC).

Disease / disorder	Cystic Fibrosis
Sample type requested	3ml of venous blood in EDTA (>1ml from infants)
Analysis offered	<p>CF29 kit will detect 29 of the most common mutations in the Northern European population</p> <p>(p.Phe508del; p.Gly542X; p.Gly551Asp; c.489+1G>T; p.Arg553X; p.Arg117His; p.Arg1162X; p.Arg334Trp; c.1585-1G>A; p.Trp1282X; p.Asn1303Lys; c.3727+10kbC>T; p.Ala445Glu; c.2051_2052delAAinsG; c.3528delC; c.948delT;p.Ile507del; p.Arg347Pro; p.Ser1251Asn, p.Glu60X, p.Asp1152His, c.2988+1G>A, c.2657+5G>A, c.1766+1G>A, c.579+1G>T, p.Gly85Glu, c.2052delA, c.262delTT, p.Arg560Thr).</p>
Pick-up rate	Detects >80% of CF mutations in Scottish Caucasian population
Reporting time	<p>Within 1 month</p> <p>Prenatal or urgent referrals will be reported within 7 days</p>
Notes	If only 1 mutation is detected, further mutation analysis can be arranged in a specialist laboratory

Disease / disorder	Haemochromatosis
Sample type requested	3ml of venous blood in EDTA (mouthwash acceptable)
Analysis offered	PCR analysis to detect p.C282Y and p.H63D mutations in HFE gene
Pick-up rate	Approximately 90% of affected individuals are homozygous (carry 2 copies) for the p.C282Y mutation
Reporting time	Within 1 month
Notes	Mouthwash samples should be provided from suspected high- risk individuals.

Disease / disorder	Gilbert Syndrome
Sample type requested	3ml of venous blood in EDTA
Analysis offered	PCR analysis of TA repeat in promoter region of the UGT1 gene
Pick-up rate	The majority of Caucasian Gilbert patients carry the 7/7 genotype.
Reporting time	Within 1 month
Notes	In severe cases of jaundice where the Gilbert screen is negative, screening for Crigler-Najjar may be offered.

Disease / disorder	Thrombophilia
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Factor V Leiden mutation (p.Arg506Gln) Prothrombin g.20210G>A mutation
Pick-up rate	Both of these mutations are relatively common in the population.
Reporting time	Within 1 month
Notes	

Disease / disorder	Medium chain acyl-CoA dehydrogenase (MCAD) deficiency
Sample type requested	3ml of venous blood in EDTA (>1ml from infants)
Analysis offered	Sequence analysis of entire coding region of ACADM gene
Pick-up rate	Approx. 80% of patients with MCAD deficiency are homozygous for the mutation at position 985
Reporting time	Within 1 month Urgent referrals will be reported within 14 days
Notes	

Disease / disorder	Myotonic Dystrophy
Sample type requested	3ml of venous blood in EDTA
Analysis offered	PCR analysis of CTG repeat in DMPK gene
Pick-up rate	The PCR analysis will detect normal alleles and small expansions
Reporting time	Within 1 month
Notes	

Disease / disorder	Neurofibromatosis Type 1
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Linkage only - polymorphic intragenic markers in families with at least 2 clinically affected members. Mutation analysis is not available for NF1 in Dundee
Pick-up rate	At least one intragenic marker will be informative in >90% of families tested.
Reporting time	Within 2 months
Notes	Mutation analysis for NF1 can be arranged elsewhere in the UK but there would be a considerable cost implication.

Disease / disorder	Polycystic kidney disease (APKD or ADPKD)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Linkage analysis in families with at least 2 clinically affected members. Markers available for both the chromosome 4 + 16 loci (PKD2 and PKD1)
Pick-up rate	
Reporting time	Within 2 months
Notes	Mutation analysis is available outwith Scotland and has cost implications.

Disease / disorder	Hereditary non-polyposis colorectal cancer (HNPCC)
Sample type requested	All patients suspected of having HNPCC should be referred to Dr David Goudie in Clinical Genetics for an assessment of the significance of their family history
Analysis offered	Samples accepted for screening will be analysed for MLH1 mutations or examined for MSI instability.
Pick-up rate	Around 60% of classical HNPCC families will have a detectable MLH1 mutation
Reporting time	Within 2 months
Notes	Part of any sample accepted for screening will also be sent to Edinburgh for MSH2 and MSH6 mutation screening

Disease / disorder	Multiple Endocrine Neoplasia Type 1 (MEN1)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Mutation analysis of exons 2-10 of the Menin gene
Pick-up rate	Over 90% of patients with MEN1 have a detectable mutation in the Menin gene
Reporting time	Within 2 months
Notes	In families with an undetectable mutation, linkage analysis may be carried out to confirm linkage to the Menin locus, and then establish a 'high-risk' haplotype

Disease / disorder	Multiple Endocrine Neoplasia Type 2A/2B + FMTC
Sample type requested	3ml of venous blood in EDTA
Analysis offered	PCR and sequence analysis of exons 10,11,13,14 and 15 (and 918 mutation in exon 16) of RET gene
Pick-up rate	Approximately: 95% of MEN2A cases 98% of MEN2B cases 85% of FMTC cases
Reporting time	Within 2 months
Notes	In families with an undetectable mutation, linkage analysis may be carried out to confirm linkage to the RET locus, and then establish a 'high-risk' haplotype

Disease / disorder	SDHB / SDHD screen (paraganglioma / phaeo)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Mutation analysis of SDHB and SDHD genes
Pick-up rate	
Reporting time	Within 2 months
Notes	Screening of these genes may be performed as part of a wider screening strategy according to the clinical details provided (eg in addition to RET and VHL screening)

Disease / disorder	Von Hippel Lindau (VHL)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Mutation analysis of exons 1-3 of VHL gene plus MLPA kit for large deletions
Pick-up rate	
Reporting time	Within 2 months
Notes	VHL screening may be performed in addition to other genes as considered appropriate according to the clinical details provided

Disease / disorder	Retinoblastoma
Sample type requested	3ml of venous blood in EDTA + tumour material if available
Analysis offered	Linkage using intragenic markers in familial cases. Mutation analysis of RB1 gene. MLPA assay for gene deletions.
Pick-up rate	Up to 70% of familial / bilateral cases Only 10% of unilateral cases unless tumour material is available
Reporting time	Within 2 months
Notes	DNA from tumour tissue is required for any realistic chance of identifying the mutation in unilateral cases.

Disease / disorder	Pelizaeus Merzbacher Disease
Sample type requested	3ml of venous blood in EDTA
Analysis offered	MLPA assay for PLP gene duplication If -ve for duplication, mutation analysis will be carried out on the coding region.
Pick-up rate	50-60% of cases have a duplication of the PLP gene 30% of remaining cases have a point mutation
Reporting time	1 month for duplication test Up to another 2 months for mutation analysis
Notes	To date, no duplications have been observed in clinically affected females. Point mutations have been detected in manifesting carriers. Screening of GJA12 (Cx46.6) gene may also be carried out if appropriate.

Disease / disorder	Leber's Hereditary Optic Neuropathy (LHON)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Mitochondrial mutations (11778, 3460, 14484 and 14459)
Pick-up rate	>90% of LHON mutations in Caucasian population
Reporting time	Within 2 months
Notes	

Disease / disorder	Mitochondrial Disorders (MELAS,MERRF,Leighs)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	PCR for common mutations (3243, 8344 and 8993) plus some rarer mutations SURF1 analysis for Leigh's cases
Pick-up rate	The majority of MELAS and MERRF cases are caused by the 3243 and 8344 mutations respectively
Reporting time	Within 2 months (3months for SURF1)
Notes	Many mitochondrial point mutations (including MELAS 3243) exhibit differences in tissue distribution and may not be detectable in blood samples. The sensitivity of these tests is therefore not 100%. Urine samples or muscle biopsies may be required at a later stage for further analysis.

Disease / disorder	Mitochondrial disorders (PEO,KSS,Pearsons)
Sample type requested	Muscle biopsy (frozen)
Analysis offered	Long-range PCR for large rearrangements of mitochondrial DNA PCR analysis for common point mutations
Pick-up rate	Rearrangements are the most common cause of CPEO and KSS.
Reporting time	Within 2-3 months
Notes	In individuals >20 years of age, deletions are not visible in DNA extracted from blood. Only muscle biopsies will be accepted from these patients for the analysis of suspected PEO or KSS.

Disease / disorder	Non-syndromic deafness
Sample type requested	3ml venous blood in EDTA
Analysis offered	Mutation analysis of Connexin 26 gene including 35delG mutation. Also screen for the 309kb and 232kb deletions in Connexin 30. Mitochondrial mutations 1555A>G, 7445A>G and 3243A>G if appropriate
Pick-up rate	Approximately 70% of non-syndromic deafness cases are homozygous for the 35delG mutation.
Reporting time	Within 2 months
Additional notes	Mitochondrial mutations 1555A>G, 7445A>G and 3243A>G may be screened for if evidence of aminoglycoside use or maternally inherited transmission

Disease / disorder	Marfan Syndrome
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Linkage analysis for families with at least 2 clinically affected members.
Pick-up rate	
Reporting time	Within 2 months
Notes	Mutation analysis is available elsewhere in the UK.

Disease / disorder	Hypokalemic Periodic Paralysis (HOKPP1)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	PCR and sequence analysis for the three known mutations in the CACN1AS gene Also analysis of the Arg669 and Arg672 mutations in the SCN4A gene
Pick-up rate	Approx 80% of cases will have one of the above mutations
Reporting time	Within 2 months
Notes	

Disease / disorder	Keratin Disorders
Sample type requested	3ml of venous blood in EDTA
Analysis offered	EBS (K5/K14 analysis) and BCIE (K1/K10) or Prof I McLean's lab for analysis of Keratins 2e, 6a, 6b, 7, 9, 16 and 17
Pick-up rate	
Reporting time	Within 2-4 months
Notes	Where a mutation is identified in the research lab, it will be confirmed by the diagnostic lab then reported

Disease / disorder	CBAVD / Azoospermia
Sample type requested	3ml of venous blood in EDTA
Analysis offered	CF29 kit plus Poly T testing if one mutation identified Y-chromosome microdeletion screening where appropriate
Pick-up rate	
Reporting time	Within 1 month
Notes	NB:- Samples from partners of CBAVD patients should be referred for screening at the same time If only one mutation is identified, sample may be sent to a specialist lab for further screening.

Disease / disorder	Oculopharyngeal muscular dystrophy (OPMD)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Detection of GCG expansion in the PABPN1 gene Sequence for two reported mutations in exon 1
Pick-up rate	
Reporting time	Within 1-2 months
Notes	(GCG ₆) is the normal repeat length. Affected individuals will have an allele with (GCG ₈₋₁₃) repeats. Rarely, affected individuals may be homozygous for (GCG ₇)

Disease / disorder	Primary Torsion Dystonia
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Detection of 3bp deletion in DYT1 gene
Pick-up rate	This mutation is a common cause of torsion dystonia in the Ashkenazi Jewish population
Reporting time	Within 1-2 months
Notes	

Disease / disorder	Crigler Najjar Types I + II
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Mutation analysis of exon 1A1 and exons 2-5 of the UGT1A1 gene.
Pick-up rate	
Reporting time	Within 2 months
Notes	

Disease/Disorder	Cavernous cerebral malformations
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Mutation analysis of CCM2 Krit1 and CCM3 Also MLPA assay for large gene rearrangements
Pick-up rate	
Reporting time	Up to 2 months per gene
Notes	

Disease/Disorder	Peutz Jegher
Sample type requested	3ml of venous blood in EDTA
Analysis offered	Mutation analysis of STK11
Pick-up rate	
Reporting time	2 months
Notes	

Services provided by other Scottish labs

Disease / disorder	Familial breast and/or ovarian cancer
Sample type requested	Patients suspected of familial breast/ovarian cancer should be referred to Clinical Genetics for assessment of the significance of their family history
Analysis offered	Samples accepted for analysis will be sent to Aberdeen and Glasgow for BRCA1 and BRCA2 mutation analysis.
Pick-up rate	Full screening will only detect around 10% of familial cases
Reporting time	Within 2 months
Notes	Samples from individuals in lower-risk families will be extracted and stored.

Disease / disorder	Familial Adenomatous Polyposis Coli (FAP)
Sample type requested	Patients suspected of having FAP should be referred to Clinical Genetics for assessment of the significance of their family history
Analysis offered	Samples accepted for analysis will be sent to Aberdeen for APC mutation analysis
Pick-up rate	
Reporting time	within 2 months
Notes	

Disease / disorder	Duchenne or Becker Muscular Dystrophy
Sample type requested	3ml of venous blood in EDTA
Analysis offered	DNA sent to Glasgow for dosage and intragenic marker analysis of dystrophin gene.
Pick-up rate	
Reporting time	Within 1-2 months
Notes	Mutation analysis of the dystrophin gene is available

Disease / disorder	DRPLA (dentatorubral-pallidoluysian atrophy)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	DNA will be sent to Edinburgh for analysis of the CAG repeat number
Pick-up rate	
Reporting time	Usually within 4-6 weeks
Notes	Patients (or their legal representative) should be advised regarding the implications of DRPLA test results, for themselves and their families, before proceeding with the diagnostic test

Disease / disorder	Fragile X syndrome (FraXA)
Sample type requested	3ml of venous blood in EDTA (>1ml from infants)
Analysis offered	DNA will be sent to Glasgow for analysis of the CGG repeat number
Pick-up rate	Technique will detect full- and pre-mutation carriers. Will not detect rare cases where there is a point mutation in FMR1
Reporting time	Within 1 month
Notes	Although heparinised blood can be used for the PCR method, it is not really suitable for southern blotting, therefore EDTA samples should be obtained wherever possible

Disease / disorder	Friedreich's Ataxia
Sample type requested	3ml of venous blood in EDTA
Analysis offered	DNA sent to Glasgow for GAA repeat analysis of the Frataxin gene
Pick-up rate	
Reporting time	Within 2 months
Notes	Patients (or their legal representative) should be advised regarding the implications of Friedreichs Ataxia test results, for themselves and their families, before proceeding with the diagnostic test

Disease / disorder	Hereditary Motor and Sensory Neuropathy (HMSN) / HNPP
Sample type requested	3ml of venous blood in EDTA
Analysis offered	The DNA will be sent to Aberdeen for dosage analysis of the PMP22 gene
Pick-up rate	
Reporting time	within 2 months
Notes	Non-PMP22 duplication patients with confirmed HMSN1 can be screened for P ₀ , PMP22 and Connexin32 mutations if adequate clinical details and family history are provided.

Disease / disorder	Huntington's Disease
Sample type requested	3ml venous blood in EDTA
Analysis offered	DNA will be sent to Edinburgh for analysis of CAG repeat size.
Pick-up rate	
Reporting time	Usually within 1 month
Notes	Patients (or their legal representative) should be advised regarding the implications of HD test results, for themselves and their families, before proceeding with the diagnostic test

Disease / disorder	Haemophilia A + B
Sample type requested	Patients seeking pre-symptomatic or predictive testing should be referred to the clinical genetics service
Analysis offered	Samples accepted for testing will be sent to Edinburgh for mutation analysis and/or linkage analysis
Pick-up rate	
Reporting time	Usually within 2 months
Notes	

Disease / disorder	Prader-Willi / Angelman's syndromes
Sample type requested	3ml of venous blood in EDTA from the patient <u>and</u> both parents
Analysis offered	DNA will be sent to Glasgow for deletion and UPD analysis
Pick-up rate	
Reporting time	within 2 months
Notes	A small proportion of familial AS cases are caused by mutations in the UBE3A gene. A screening service for these patients is being developed in Glasgow, and should be available soon.

Disease / disorder	Spinocerebellar Ataxias (SCA1,2,3,6 and 7)
Sample type requested	3ml of venous blood in EDTA
Analysis offered	DNA sent to Edinburgh for CAG repeat analysis
Pick-up rate	
Reporting time	Within 1-2 months
Notes	Patients (or their legal representative) should be advised regarding the implications of SCA test results, for themselves and their families, before proceeding with the diagnostic test

Disease / disorder	Spinal Muscular Atrophy
Sample type requested	3ml of venous blood in EDTA
Analysis offered	DNA sent to Edinburgh for deletion analysis of the SMN gene
Pick-up rate	
Reporting time	Usually within 4-6 weeks
Notes	

Referral Labs in Scotland

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Head of lab : Dr Kevin Kelly

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West of Scotland Regional Genetics Service

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Head of lab: Ms Su Stenhouse

Addresses / Contact details of other UK labs which we refer samples to can be obtained by contacting us directly.