

CCMG MOLECULAR GENETICS GUIDELINES

*Prepared by the CCMG Molecular Genetics Committee, 2002:
Drs. Allingham-Hawkins, Bernard, Carson (Chair), Dawson, Graham, Greer, Ray, Somerville,
Spriggs*

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*Revised, Section A 8 Mutation Nomenclature, Approved by the Board September 2004:
Molecular Genetic Committee: Drs. Spriggs (Co-Chair), Stockley (Co-Chair), Somerville
(BOD), Armstrong, Buchanan, McGowan-Jordan, Parboosingh, Scott, Wei*

*Revised, Section A 7a) Recommended Turn Around Times, Approved by the Board September
2006: Molecular Genetics Committee Drs. Armstrong, Buchanan, McGowan-Jordan,
Parboosingh, Ray, Scott, Stockley (Chair), Somerville (BOD), Wei*

DEFINITION OF A MOLECULAR GENETIC TEST

Analysis of DNA and/or RNA to detect heritable disorders as well as germline mutations that confer an increased susceptibility to a disease. This includes prenatal, diagnostic, presymptomatic, carrier and susceptibility testing. These guidelines do not apply to the detection of acquired genetic changes.

MOLECULAR GENETIC ANALYSIS OF CLINICAL SPECIMENS

These guidelines are minimum recommendations only. They are subject to the discretion of the laboratory director and the requirements and capabilities of the local laboratory. Adherence to these guidelines is voluntary and does not guarantee that the results obtained are accurate. In addition, it is recognised that there are acceptable variations in methodologies not outlined in this document. It should be recognised that these guidelines are based on information available at the present time and will change with advances in knowledge. The CCMG Molecular Genetics Committee will undertake to review and revise these guidelines as necessary.

A. GENERAL LABORATORY GUIDELINES:

1. Staff

- a) A laboratory director/head should have a doctoral degree and at least 2 years of postdoctoral training in molecular genetics and either Canadian College of

Medical Geneticists (CCMG) certification/eligibility in molecular genetics or American Board of Medical Genetics (ABMG) certification/eligibility in molecular genetics.

- b) We strongly recommend that technologists be certified in genetics or molecular genetics in accordance with provincial and/or institutional regulations (e.g. Canadian Society of Medical Laboratory Sciences, College of Medical Laboratory Technologists of Ontario). However, as this is an emerging field we recognise that human resource shortage may make this impossible. Therefore, if provincial/hospital regulations permit, certification is not necessary if the technologist has molecular laboratory experience.
- c) All molecular genetics staff should be provided with regular opportunities to attend local, regional, national or international education sessions.

2. Facilities and equipment

- a) Laboratory space, equipment, facilities and supplies should be sufficient to ensure safe, accurate and acceptable standards of performance (1).
- b) Temperature-dependant equipment must be monitored daily for appropriate temperatures and documentation of monitoring must be kept.
- c) Laboratory equipment should be maintained according to manufacturer recommendations and a record of maintenance should be kept.
- d) All safety precautions related to handling of specimens, chemicals and radioactivity should be followed and a manual outlining these precautions should be available. In addition, Material Safety Data Sheets (MSDS), when available, should be readily accessible for all chemicals used in the laboratory.

3. Specimen receiving and requisitions

- a) Biological samples should be handled according to universal precautions.
- b) A policy outlining criteria for the rejection of samples should be available. When a sample is rejected it should be documented and the referring physician/counsellor should be notified as soon as is feasible.
- c) Specimens should have at least two identifiers which must include patient name, and any of the following; date of birth, hospital number or other unique identifier.
- d) All specimens should be accompanied by a requisition which should include patient name, date of birth, date of collection, gender, specimen identifier, specimen type, reason for referral, information requested, relevant clinical/laboratory/family history information, referring physician and if

applicable, ethnicity. Pedigrees should be required for linkage analysis and are recommended for all analyses (1, 2).

- e) Each specimen should be assigned a unique laboratory identifier.
- f) As DNA samples can be stored indefinitely, each lab should develop a policy indicating how long samples will be stored. All referring physicians/counsellors should be aware of this policy. In general, samples should be kept a minimum of 6 months after the completion of the analysis.

4. Records

- a) Records should be maintained linking patient requisition and report with the patient's laboratory results.
- b) Records must be maintained in a manner that will preserve confidentiality (1, 2).
- c) Records and reports should not be released to any physician/counsellor or other individual not indicated on the requisition without the appropriate authorisation.
- d) Records of results, requisitions and reports should be kept indefinitely.

5. Procedure manuals

- a) Procedure manuals detailing all methods used in the laboratory should be developed. All methods should be reviewed annually and signed by the laboratory head/director.
- b) Procedures should include references, brief outline of the principle of the technique, details of all steps in the method and interpretation of results.
- c) Records of the probes used for hybridization and sequence of PCR primers should be kept.

6. Quality control/assurance

- a) All reagents and solutions prepared in the lab should have on their label name, date prepared and/or date expired, concentration, preparer's name (1).
- b) All purchased reagents and solutions should have the date received and, if appropriate, the date they were first opened recorded on the label.
- c) It is strongly recommended that all labs participate in an external molecular genetics proficiency program. When this is not available, interlaboratory comparison can also be used (1).

- d) Appropriate controls should be used in all assays. Refer to specific techniques for suggested controls.

7. Reports

a) Recommended Test Turnaround Times

Appropriate test turnaround time depends on indications for testing, management issues, disease frequency and molecular complexity. In general, the following turnaround times are recommended:

- 1) Prenatal samples (CVS, amniocentesis samples) or urgent samples (pregnant or neonatal cases, testing affects patient management): 1-2 weeks after sample received.
- 2) Routine samples: 6 weeks after sample received.

Exceptions to the above turnaround times may occur, and turnaround time for high complexity tests requiring analysis with more than one technique or more than one gene may exceed recommended turnaround time.

b) Reports should include the following information:

- Name of patient
- Date sample received
- Date of Birth
- Unique lab identifier
- Referring physician(s)/counsellor
- Reason for referral/test done
- Methods used for analysis
- Mutations tested if applicable
- Limitations/sensitivity of the test
- Result
- Interpretation
- Appropriate recommendations
- Signature of the Laboratory Director or their designate
- Date report generated and signed by the Laboratory Director

- c) Reports for linkage analysis should also include a pedigree with genotype data and risk estimates based on the recombination frequency of the closest informative marker(s).
- d) Wherever possible, separate reports should be written for each individual in a family rather than one family report. Clinical or molecular diagnostic information on other family members should not be included in the report unless it is relevant to the interpretation.

8. Mutation nomenclature

It is recommended that the mutation nomenclature guidelines proposed by the Human Genome Variation Society (HGVS) be used to describe sequence changes in reports. These guidelines are updated regularly and can be found at: <http://www.genomic.unimelb.edu.au/mdi/mutnomen/>. Regardless of the mutation nomenclature used, it should be clearly documented in the report with either the appropriate reference cited or enough information provided (such as nucleotide, accession number used, predicted outcome at the protein level) so that another molecular genetics laboratory can interpret the data appropriately.

B. METHODOLOGIES:

1. DNA preparation

- a) DNA preparation must be by accepted protocols (e.g. phenol chloroform, salt, commercial kit).
- b) DNA prepared for Southern blot should be evaluated for purity and concentration by using spectrophotometry, gel electrophoresis or fluorometry.
- c) DNA should be stored at 4°C or frozen.

2. RNA preparation

- a) RNA preparation must be by accepted protocols (e.g. phenol chloroform, commercial kit).
- b) Care must be taken to prevent contamination of the sample by RNases by using reagents and supplies that have been treated to destroy or inhibit RNases.
- c) RNA prepared for Northern blot should be evaluated for purity and concentration by using spectrophotometry, gel electrophoresis or other accepted protocol.
- d) RNA should be stored at -20°C or less.

3. Assay validation

All techniques must be validated prior to using them in the clinical lab. This includes the sensitivity, specificity and reproducibility of the assay. Wherever possible, known positive and negative samples should be tested.

4. PCR methodologies

- a) To avoid nucleic acid contamination of the PCR reaction, a combination of a number of the following practises are recommended:
 - i) A separate work area dedicated to PCR set up
 - ii) Dedicated pipetman and reagents
 - iii) Positive displacement pipets
 - iv) Aerosol barrier tips
 - v) Use of dUTP followed by treatment of preamplification reactions with uracil-N-glycosylase
- b) A negative control (no nucleic acid template) should be included in every PCR assay.
- c) Positive controls should be included in every PCR assay. Examples of positive controls are as follows:
 - i) RFLP analysis - sample with known genotype preferably homozygous cut or heterozygous to test for complete digestion.
 - ii) Mutation analysis - sample positive for the mutation in question.
 - iii) VNTR/microsatellites - sample from an individual with both a large and small sized alleles to test for differential amplification and to help determine size of fragments in unknown samples. Wherever possible, an allelic ladder comprising a mix of all available alleles should be used to allow accurate sizing of patient alleles. When assessing repeat expansions, a sample from an individual with an allele corresponding to the maximum repeat detectable by the laboratory should be included.
 - iv) Heteroduplex, SSCP etc - sample from normal individual to compare electrophoretic mobility of the unknown samples.
- d) When performing an assay based on the presence or absence of a PCR product, a second set of primers specific to another locus and amplifying a fragment of equal or greater size must be included in each PCR reaction to check for positive amplification of the sample.
- e) An appropriate molecular weight standard should be included in every gel electrophoresis reaction to aid in sizing PCR fragments.

5. Southern analysis

- a) Restriction enzyme digests should be checked for complete digestion by visualising the gel stained with ethidium bromide. A photograph should be taken

for future reference.

- b) An appropriate molecular weight marker should be included.
- c) A positive control should be included. See B-4c for appropriate selection of controls.
- d) Southern blotting and hybridisation should be performed using accepted procedures.
- e) All probes used for analysis should be well documented by the Genome Database or the literature. A list of all probes should be kept with the following information:
 - i) Genome location
 - ii) References
 - iii) Cloning vector
 - iv) Cloning site
 - v) Size of insert

6. Sequence analysis

- a) Before offering sequence analysis on a clinical basis the full sequence of the region of interest must be available in Genbank.
- b) The patient sequence must be compared to a known normal control sequence. Whenever possible, a mutation specific control should also be analysed.
- c) Mutations should be verified in both forward and reverse directions.
- d) Care should be taken in interpreting missense mutations unless they have been reported previously as disease causing mutations or are known to affect a functional domain of the protein.
- e) See section A-8 for information on mutation nomenclature.

7. Linkage analysis

- a) An up to date list of all loci used for linkage analysis for a specific disease gene should be kept. It should include location of each marker relative to the disease gene locus, male/female recombination frequency with the disease gene and percent heterozygosity. References used to determine these figures should be included.
- b) If intragenic markers are not available or are not informative, the closest informative flanking markers should be used. If intragenic markers are available and are informative, analysis of one marker is sufficient if the gene is small. If the gene is large and/or there is

a recombination frequency $\geq 1\%$ between the 5' end and 3' end of the gene, analysis of more than one intragenic marker may be necessary.

C. GUIDELINES FOR CARRIER TESTING:

- 1) Carrier testing for asymptomatic individuals for autosomal recessive and X-linked disorders should be limited to individuals of majority age and those adolescents deemed competent to understand the implications of testing. (See the CCMG policy “Carrier testing in Children” for more information).
- 2) All patients should receive adequate genetic counselling prior to carrier testing.
- 3) Appropriate Bayesian calculations should be applied to all individuals who were not identified as a carrier after molecular testing. Such calculations must take into account the patient's *a priori* risk, whether the mutation is known for the patient with a family history of it and the laboratory detection rate for the patient's ethnic population when known.

D. GUIDELINES FOR PRESYMPTOMATIC TESTING:

- 1) Wherever possible, samples for presymptomatic testing should only be accepted from physicians, nurses or counsellors affiliated with a genetics centre to ensure that the individual has received adequate counselling as to the implications and limitations of testing. If this is not possible, then measures should be implemented to ensure that patients being tested have been appropriately counselled as to the implications of the test (e.g. consent forms, information pamphlets).
- 2) Analysis should be limited to individuals of majority age and those adolescents deemed competent to understand the implications of testing unless there is a clinical reason to offer testing prior to this.
- 3) Each centre needs to determine prior to offering presymptomatic testing for a specific disease, how they wish the results communicated to the referring medical professional. Some may prefer to have the results sent in a sealed envelope so that they do not know the results prior to the patient's counselling session.
- 4) Wherever possible, analysis of a sample from an affected family member is desirable to confirm the diagnosis in that family. If no affected family member is available and the test is negative, it should be stated in the report that the results are limited by the lack of a confirmed molecular diagnosis.
- 5) Samples should be accompanied by an up to date pedigree in order to allow the laboratory to determine a) the individual's *a priori* risk and b) if samples from other family members have been analysed previously.

E. GUIDELINES FOR PRENATAL TESTING:

- 1) Wherever possible, samples for prenatal testing should only be accepted from physicians, nurses or counsellors affiliated with a genetics centre to ensure that the individual has received adequate counselling as to the implications and limitations of testing.
- 2) Backup cultures for both CVS and amniocytes should be maintained until the final result has been reported.
- 3) It is strongly recommended that all prenatal samples be checked for the presence of maternal contamination prior to the release of results.
- 4) A sample from an affected individual from the family, when possible, should be analysed in conjunction with the prenatal sample.
- 5) When linkage analysis is being used, samples from the parents and an affected individual from the family should be analysed in conjunction with the prenatal sample.
- 6) If using linkage analysis, appropriate Bayes calculations should be applied utilising known recombination frequencies to determine the risk of the fetus being affected.

F. GUIDELINES FOR DIAGNOSTIC TESTING:

- 1) Under appropriate circumstances, samples for diagnostic testing may be accepted from any physician. For certain molecular genetic tests it may be appropriate to restrict access to the appropriate specialist and/or Geneticist. If a positive result is reported to a non-geneticist, the report should include a recommendation to refer the patient to genetics.
- 2) As diagnostic testing is only offered to patients with clinical symptoms suggestive of a certain genetic disorder, children who are symptomatic can be tested to confirm or exclude the specific disorder.
- 3) It is important to indicate the limitations and sensitivity of the molecular test in the report particularly if the test is negative.

References:

- 1) **American College of Medical Genetics Laboratory Practice Committee (1999). Standards and guidelines for clinical genetics laboratories 2nd edition.**
- 2) **Molecular Methods for Genetic Diseases; Approved Guideline (2000) NCCLS V. 20 #7.**