



Draft Best Practice Guidelines for Laboratory Internal Quality Control

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Disclaimer

These Guidelines are based, in most cases, on the reports drawn up by the chairs of the disease-based workshops run by EMQN. These workshops are generally convened to address specific technical or interpretative problems identified by a QA scheme. In many cases, the authors have gone to considerable trouble to collate useful data and references to supplement their reports. However, the Guidelines are not, and were never intended to be, a complete primer or "how-to" guide for molecular genetic diagnosis of these disorders. The information provided on these pages is intended for chapter authors, QA committee members and other interested persons. Neither the Editor, the European Molecular Genetics Quality Network, the Clinical Molecular Genetics Society, the UK Molecular Genetics EQA Steering Committee nor the British Society for Human Genetics assumes any responsibility for the accuracy of, or for errors or omissions in, these Guidelines.

Definition of IQC

The World Health Organisation (WHO) External Assessment of Health Laboratories (1981) usefully defines Internal Quality Control (IQC) as "the set of procedures undertaken by the staff of a laboratory for continuously assessing laboratory work and the emergent results, in order to decide whether they are reliable enough to be released (either in support of clinical decision making or for epidemiological or research purposes). Thus quality control procedures have an immediate effect on the laboratory's activities and should actually control, as opposed to merely examining the laboratory's output."

Sample reception

- a. All patient information should be stored on a secure computer database system protected by restricted access and passwords. Computer systems should ideally be networked and backed-up on a daily basis to an external medium such as a tape drive, ZIP disk, or external computer server etc. Back-up copies should be stored in a fireproof safe with a second back-up copy located off-site. The use of staff dedicated to the maintenance and back-up of the database is strongly advised.

- b. Laboratories should take measures to ensure that the correct patient and sample referral details are transferred on to the computer. A checking procedure should be used and the transferred information verified by a suitably qualified staff member¹.
- c. Laboratories should have a procedure for dealing with samples with incomplete or incorrect referral data. The laboratory should not analyse this type of sample. The responsibility lies with the referring clinician to provide the correct information. It is advisable that the laboratory keeps a copy of the referral information as documented proof.
- d. Sample tubes accompanying a referral should have at least two pieces of unique identification to link them together such as a barcode, name, date of birth (DOB), national insurance or hospital number.
- e. Manual transfer of information is always liable to error. The less manual transfer of information the better. Printed sample and/or DNA numbers should be used where possible. Ideally, printed labels with all the patient details should be generated by computer at the time of booking a sample in.
- f. If information in permanent records is altered for any reason then an explanation for the change should be inserted and initialled by the person making the changes to it.

Sample storage

- a. Some laboratories store DNA samples long term at 4°C and others at -20° or -40°C. Whatever storage temperature is chosen it should be suitable for maintaining the integrity of the DNA over time.

¹ A suitably qualified member of staff may be a clinical scientist, duty scientist or higher grade technical officer.



- b. Care should be taken to minimise the number of freeze thaws a sample undergoes as this may compromise DNA quality. Laboratories may find it convenient to take a working aliquot to store at 4°C while testing is undertaken.
- c. A duplicate back-up sample of blood or DNA should be stored before and/or after DNA extraction. Duplicates should be stored for a minimum period of 1 year in a second freezer, preferably in another department or building. This is beyond the means of many laboratories but the storage of blood spots on 3MM paper as an emergency back-up may be a viable alternative.
- d. The labels on stored DNA samples and blood spot papers should include at least two pieces of identifying information; e.g., name and DOB or name and DNA number. It is always worth remembering that numbers are more susceptible to transposition errors than words.

DNA extraction

- a. Laboratories should take steps to minimise the risks of contamination during the DNA extraction process. The use of dedicated DNA extraction areas, safety cabinets and filter tips for pipettes are recommended although space and cost may be an issue.
- b. The number of tube-to-tube transfers should be kept to a minimum although this is dependant on the technique used for DNA extraction.
- c. Greater automation of the DNA extraction process would help to avoid some of the problems of human error. Details of automated DNA extraction systems which are in use in at least two European diagnostic lab can be found at <http://www.genovision.com/GenoM48.htm> and http://www.gentra.com/purification_instruments/autopure_home.asp although the authors recognise that for many labs the cost would be prohibitive.

Sample handling

- a. All tube transfers during DNA extraction and setting up of PCR's and digests should be independently checked either by the operator or another suitably qualified member of staff to minimise the risk of sample mix up. Experiment sheets should be initialled by the checker to provide evidence that the check has taken place. Alternatively, a duplicate sample should be set up in parallel and analysed along with the original sample.
- b. Records should be kept of the batch numbers of all laboratory solutions for traceability and

troubleshooting. Ready-made solutions from commercial manufacturers are recommended although the costs involved may mean that some laboratories prefer to use "in-house" reagents.

- c. Laboratories should have a procedure in place to ensure that the correct DNA sample is transferred to the correct reaction tube or gel sample lane. Examples would include the labelling of each tube with a unique code written on both the lid and side and the checking of tube codes before, during and after transfers with verification / signing off by a suitably qualified member of staff.
- d. Laboratories should try where possible to maintain separate pre and post PCR areas, preferably in separate rooms within the laboratory, to minimise the risks of contamination. The use of separate pre and post PCR pipettes is strongly encouraged.

Controls

- a. Normal male and female control samples, molecular weight markers, negative controls and specific mutation controls should always be used in diagnostic tests as appropriate.
- b. Laboratories should be aware of the European Commission's directive on the use of In-Vitro Diagnostic Devices (IVD's) and how it will affect them. The exchange of samples between laboratories is not allowed under the directive. Where possible laboratories should use Certified Reference Materials (CRM's) as standards. A copy of the directive can be found on the EMQN website (<http://www.emqn.org/downloads.htm>).

Results

- a. Internal grading of the quality of experimental results e.g., 1 = excellent, clear result, 2 = less than perfect but reportable and 3 = not reportable, is used in some laboratories and may be useful.
- b. Laboratories should store all raw data from experiments for a minimum of 5 years although there may be a statutory requirement in some countries for longer term storage. Transferring data to an electronic format will make for easier storage and access from a computer database.

Reporting

- a. All reports should be checked independently by two suitably qualified members of staff, in conjunction with the original referral, experimental



details, raw data and the report should be signed by both checkers.

- b. The use of standard wording templates for reporting is not recommended as it limits the flexibility required for reporting.
- c. Laboratories may find it useful to draw up local protocols defining procedures to ensure that the results of an experiment are reported to the correct recipient by telephone, fax or email (if appropriate).

Paternity testing

- a. Paternity testing should only be carried out where the results indicate a problem and where correct paternity is vital to the interpretation, for example where genotypes are being inferred. The results of such paternity checking should not normally be included in the report that is sent out to the referring clinician.

Prenatal diagnosis

- a. Testing for maternal contamination of pre-natal samples using a minimum of three highly polymorphic microsatellite loci should be considered, particularly when the genotype is shown to be the same as the mother's.
- b. Some laboratories try to test three independent villi from a Chorionic Villus Sample to try to minimise the risk of an incorrect result due to maternal contamination. This is not always possible but can be useful.
- c. Further guidelines for PND are available on the web. For example, the Society of Obstetricians and Gynaecologists of Canada's guidelines are available from:
http://sogc.medical.org/SOGCnet/sogc_docs/comm/guide/pdfs/ps105b.pdf

CA repeats

- a. Where microsatellite markers are used, blind reading of the results by two independent scientists is recommended.
- b. If using different primers for the same locus, laboratory staff should be aware that the fragment sizes may be different.
- c. The most common convention used for the naming of alleles is to number them within families as 1,2,3 etc with 1 indicating the largest allele. If this convention is adopted it minimises problems when results are transferred from one lab to another.

Documentation

- a. Every laboratory should have a collection of standard laboratory protocols for all the techniques used in diagnostic tests.
- b. Laboratories should have a documented procedure in place to ensure that only the most up to date copies of laboratory documentation are available to staff. The procedure should include a system for periodic review and/or revision of documents, the notification to staff of the withdrawal of old documents, and archiving of all old documentation. Laboratories may wish to consider the use of a computer database to assist document control.

Audit

- a. Laboratories should have a procedure that enables the following of an audit trail on a patient allowing a suitably qualified member of staff to trace all available information on a patient including the original sample(s), referral data, raw data and final report.
- b. Laboratories should consider implementing a procedure that checks and reports on cases/referrals that are outside target turnaround times where appropriate.

Validation

- a. Laboratories should validate all their diagnostic tests to ensure that they meet acceptable performance standards and are fit for the purpose for which they will be used. The use of reference materials and participation in external quality assessment schemes can contribute to this process. Validation can be particularly difficult for genetic testing for rare disorders when it may be difficult to obtain suitable positive mutation controls. There is also very little guidance on the minimum requirements for validation, however the following reference may be of some interest. Prence E.M., 1999, Genetic Testing, Vol 3, pp201-205
- b. Laboratories should have regular independent assessment of the technical performance of their tests and analytical measurements made in one laboratory should be consistent with those made in another laboratory.
- c. Laboratories should have well defined quality control and quality assurance procedures.
- d. PCR: controls of reaction mix without template DNA should be included



e. Southern -blotting: for precise fragment length determination to estimate the number of trinucleotidrepeats (FRAXA) an independent probe should be used together with the specific probe to rule out migration artefacts (as discussed at the FRAXA-BPM in Strasbourg)

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Staff Training.

- a. Staff employed in diagnostic laboratories should be suitably qualified and specifically trained in the methods used for particular disorders. Ongoing safety training is also important.
- b. Staff should be encouraged to continuously update their knowledge by reading the current literature and attending appropriate seminars and conferences. It is useful to keep a record of such continuing education for each member of staff.

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