



**EuroGentest Unit 1 WP1.3
EQA for Molecular Genetic Testing**



The European Molecular Genetics Quality Network

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**ORGANISATION OF MOLECULAR GENETIC EXTERNAL QUALITY ASSESSMENT
SCHEMES:
DRAFT BEST PRACTICE AND TEMPLATE DOCUMENT**

Based on the discussion held at the EMQN assessors' meeting in Cyprus 2004 and on consideration of ISO guide 43-1.

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To guide the assessment of EQA schemes and for subsequent discussion and revision at follow-on EuroGentest Best Practice meetings.

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1.0 Introduction

This document is intended to be a template for the organisation and realisation of external quality assessment (EQA) schemes in molecular genetic testing. It is based on experience gained with the British (UKNEQAS) and German (BVDH) national EQA schemes and within the European Molecular Genetics Quality Network (EMQN). The aims of the document are (1) to serve as a starting point for the development of new EQA schemes and (2) to facilitate harmonisation and mutual recognition of EQA schemes within Europe.

2.0 Organisation of EQA schemes

2.1 Selection of assessors / scheme organisers (staff)

The scheme organiser is responsible for the whole organisation of the scheme which includes:

- Recruitment of and contact with participants
- Sample collection, preparation and validation
- Distribution of the samples
- Evaluation of the reports

Furthermore the scheme organiser is the contact person for all administrative and content questions.

The scheme organiser and assessors have the joint task of:

- Selecting the samples
- Formulating the cases
- Defining the evaluation criteria
- Evaluating the reports and preparing individual reports and the final scheme report.

The assessors and scheme organisers should be enlisted from the following fields, in order to provide specific expertise:

- Molecular diagnosis laboratory experts
Providing input on technical accuracy of the results, the validity of the methods applied for routine analysis, the workflow of samples in the laboratory and the reporting of the data.
- Molecular genetics research experts
Providing knowledge of different methods used for diagnostic analysis and novel technical advances and the scientific state of the art.

- Clinicians
Providing information on the appropriateness of request forms and interpretation of laboratory reports and advice given on laboratory reports from the clinical point of view.

The assessors should have extensive experience of the following:

- diagnostic analysis of the particular disease (technical know how),
- interpretation of the laboratory data and writing of reports,
- quality assurance in a molecular diagnostic laboratory.

2.2 Scheme structure

Before the commencement of an EQA scheme the scheme organiser and the assessors should agree upon a scheme plan, which should be documented and include the following information:

- the name and the address of the organisation that provides the scheme
- the name and the address of the scheme organiser and the assessors
- the nature and the purpose of the EQA scheme
- where appropriate the criteria that need to be met before participation is allowed
- the name and address of the laboratory(ies) performing (parts of) the scheme
- the number of expected participants
- the nature of the test samples and test(s) selected
- a description of the steps by which the test samples are obtained, processed, validated and transported
- a description of the information that is supplied to the participants
- a time schedule for the various phases of the EQA (expected initial and target dates or deadlines)
- (for on-going schemes, the frequency at which test samples are distributed)
- information on methods or procedures that participants may need to perform the tests
- a description of the data or information to be returned by the participants as the results of the EQA scheme
- an outline of the criteria and procedures to be used for the evaluation of the participants' results
- a description of the data or information to be returned to the participants by the scheme organiser and the assessors

- where appropriate a description of the extent to which the test results and the conclusions of the scheme are to be made public

It is recommended that new EQA schemes are introduced over a three-year cycle, as pilot, intermediate and full-schemes. In the first year an EQA scheme may be offered as a pilot with a limited number of participants. This is to test for the suitability of EQA materials, the appropriateness of scheme questions and the competence of participants to handle the EQA situation. In the second year, intermediate schemes are limited (to perhaps thirty centres) and in the third year full schemes are open to all participants, although scheme organisers may choose to limit the number of participants or geographic scope of the scheme.

3.0 EQA materials

Scheme organisers should consider the following issues before preparing EQA samples.

3.1 Collection of EQA samples

Written consent specific for EQA purposes including the option for establishment of cell lines should be obtained from each patient who is approached to donate a sample for EQA purposes (a template consent form is available from EMQN). National regulations concerning patient privacy and consent must be taken into account, in order to protect the scheme organiser from subsequent liability and royalty claims. The wording on patient consent forms should be formulated carefully in order that differences between regulations in different countries do not cause any repercussions if samples are to be sent around the world. The use of pre-existing materials (e.g. cell lines) is acceptable, if they are irreversibly anonymised. It is recommended that established procedures are followed for cell culture as well as storage and documentation of cell lines to establish an audit trail for the materials. Cell lines should only be used in EQA schemes relevant to the disease for which the line was deposited; similarly reference materials generated by third parties can only be used with the consent of the originators and as long as the above policy is followed.

3.2 Selection of genotypes

EQA is primarily an educational activity, therefore, it is recommended that EQA samples are chosen to represent the normal range of genotypes observed during routine diagnostics in an average laboratory. Population and group-specific genotypes should be adequately represented. Cases requiring the use of technologies, knowledge and effort

beyond normal practice of most laboratories should be avoided. Scheme organisers should be cautious in choosing materials with genotypes that are known to pose analytical problems. However, in well established schemes, one 'difficult' case may be included to test the limits of the laboratories' analytical procedures. If a previous EQA scheme has high-lighted a specific analytical problem, the following scheme should contain a case suitable to follow up on laboratories' management of the preceding problem.

3.3 Timescale for collection of material

Due to the long preparatory phase needed to produce sufficient sample material it is advisable to collect cases which may include normal genotypes, those encountered in routine practice and interesting cases during the year and to work in parallel on two sets of EQA-materials i.e. one for the upcoming scheme and one for the following year. This could also save time and money in terms of preparation costs. Additionally, it can be beneficial to liaise with colleagues who may be able to provide other useful samples.

3.4 Documentation of samples

The documentation should comprise:

- a copy of the patient's consent form
- the critical steps in processing the sample and any problems or departures from procedure during the preparation and validation of EQA materials
- the original genotype in detail including the analytical method used for the initial analysis and its validation
- the labelling, storing and handling of the samples.

3.5 Sample validation

Prior to the distribution of an EQA scheme, all samples must be validated i.e. the genotype initially established must be confirmed independently. The scheme organiser takes responsibility for the validation of the EQA materials. The analytical methods applied by the participating laboratories will vary greatly, therefore, scheme organisers should ensure that the validating centres use complementary methods and cover at least the standard technologies.

The centres chosen for validation should have a wide experience of the analytical methods applied and the gene under examination. It is recommended that the validation centres are accredited according to recognised international standards for example those based on ISO 15189. Usually two validating centres will be sufficient.

The documentation of the sample validation should include the analytical methods in detail (Standard Operating Procedure or an equivalent document). The validation file should be as detailed as is reasonably possible (for example originals of sequence traces, autoradiographs from Southern blots, gel photographs etc.).

If any inconsistencies are found the validation centres should establish the source of error. As soon as the error is identified the validation must be repeated in both validation centres. If the inconsistency persists the scheme organiser should refer back to the primary stocks of the EQA materials used and should repeat the validation in another independent laboratory. In cases where the inconsistency still persists alternative materials should be recruited for EQA purposes.

The scheme organiser should store all validation documentation for an indefinite period.

4.0 Formulation of EQA questions

EQA schemes should address all phases of the analytical process. Therefore, each sample should be given a mock name, first name and date of birth to be entered in the laboratory's patient database. Likewise, a mock clinical referral should accompany each sample. The information given in the referral should be adequate to the disorder in question and should contain details of any family history and of any previous testing and results. The cases should generally be fully explained and be as straightforward as possible. As for the selection of samples, unusual clinical scenarios should generally be avoided.

The choice of cases over a number of EQA cycles should in general mirror the range of referral type that a laboratory might expect to receive, including:

- diagnostic tests,
- scanning for unknown mutations,
- carrier detection,
- pre-symptomatic predictive tests,
- pre-natal diagnosis (where relevant).

For most disorders not every sample will show a mutation and this should be reflected by the design of cases and choice of materials for EQA. EQA cases requiring mutation scanning should not overburden the laboratory. It is acceptable to limit the search required. Examples include naming a small number of exons to be screened or giving the laboratory the information that whole exon deletions have already been excluded from the case.

The participants should be provided with detailed information and instructions on the scheme (e. g. formal scheme protocol) including

- known factors which could influence the testing of the sample,
- treatment of the sample (normally like routine samples),
- specific instructions on the recording and reporting of the test result (for example making clear if allele sizes are required in addition to a standard clinical report).

In cases where samples are not available for a particular scenario that is part of the routine work, theoretical cases can be offered. Experimental data or results are supplied and laboratories are asked to interpret these in writing.

5.0 Choice of analytical methods

In the absence of recognised reference methods for molecular genetic analyses, participants should be free to use the method of their choice consistent with their routine procedures. Where they exist, participants are encouraged to follow published guidelines. EQA schemes should test the ability of a laboratory to perform adequately using the tests which it routinely carries out. Therefore, the samples and cases selected for EQA should be suitable for analysis by established and widespread methods.

EQA schemes should also adapt to technical and scientific developments and therefore may include cases designed to assess the performance of new methods or procedures. These cases should be offered for voluntary handling without marking. Any conclusions drawn from the results of such 'experimental' cases should be fed back to the participants for their benefit. Supplementary exercises may be distributed with EQA schemes to test the performance of reference or control materials. The scheme organiser should always ensure that these exercises are voluntary and that it is clear to participants that their purpose and evaluation is different from the EQA scheme.

6.0 Reporting results

Together with the EQA samples and questions, participants should be provided with clear instructions on the level of detail required for the reporting of their analytical results. In line with the aim to monitor all phases of the analytical process, participants should adhere to their usual clinical reporting format as much as possible. Published guidelines for result reporting should be considered. In disease-specific schemes, the reports should contain the following items at a minimum:

- Identification that unequivocally links the report to the sample.
- The name and contact details of the referrer (EQA scheme).
- The ID number of the participating laboratory.

- The indication for testing and specific medical information where it is relevant to test interpretation.
- The test performed and the methodology used (including the scope of the analysis, the limitations of the test and its analytical sensitivity and specificity).
- The primary sample type where necessary for the interpretation.
- The date of receipt of the sample.
- The test result (the genotype) in recognised nomenclature.
- A biological interpretation of the genotype and its known or likely pathogenetic consequences.
- A clinical interpretation of the result in the context of the indication for testing and all other information provided to the laboratory.
- The date of issue of the report.

Where appropriate, the following information should also be included in the test result report:

- A recommendation for genetic counselling by a qualified health care professional.
- Implications for other family members.
- Recommendations for follow up testing.

In technical EQA schemes, or where data that may be additional to a standard clinical report is required (for example allele sizes or additional details of methodology) the scheme organiser may provide an electronic or printed reporting template to facilitate evaluation and analysis of the results.

7.0 Marking EQA schemes

Before marking the reporting/interpretation, all desirable elements of the report, specific to each case, should be identified. For this purpose the assessors should use professional guidelines and refer to similar cases in previous schemes. The assessors should draft a marking scheme before the reports are assessed. If necessary the marking scheme should be adjusted when the reports have been received from the participants. In this regard, it is important that a consensus is reached among the assessors before marking of the reports is begun. The assessment should be divided between essential points, which should receive a numerical score and desirable points, which are worthy of comment.

Criteria that should be marked in the participants' reports include the genotypes and reporting/interpretation when a full scheme is established and when guidelines are available. Usually the marking should be very straightforward. In a number of schemes

every correct genotype scores two and every incorrect genotype scores zero. Deduction of marks in decimal fractions should be applied if essential points are missing from the report. The essential points and the size of deduction per point should be agreed by the assessors before evaluation as part of the marking scheme. Missing desirable points should not lead to deduction of marks but should be commented on.

In principle the same error in a laboratory report should not be penalised twice. For example, if the genotype is incorrect the assessors should not make a second deduction for an incorrect interpretation. However, if the same mistake was made in all cases that an individual laboratory analysed, an appropriate proportion of marks for each case should be deducted. As an example if two EQA samples are transposed during analysis so that in two cases the genotypes are incorrect the assessors should award zero marks in each case. In each case the interpretation should not be marked.

Where appropriate the availability of further tests for example pre-natal diagnosis should be mentioned. Alternatively, the report may suggest referral to a genetic counsellor who would be able to explain the implications clearly and concisely.

Clerical inaccuracies should not usually be marked, but commented upon by the assessors. However, serious errors, e.g. incorrect patient name, incorrect allele name or incorrect mutation nomenclature, which compromise the integrity and value of the report, should lead to loss of marks for either genotype or interpretation.

It is essential that the assessors achieve consistency both within and between schemes. Therefore it is helpful to develop EQA guidelines, keep a detailed history of schemes and to train organisers and assessors regularly. If meetings of scheme organisers and assessors are held to mark reports plenary sessions should be held to discuss cross scheme issues and harmonise marking practice.

If there is poor performance in the genotype category it is essential to determine the source of the error. Assessors and scheme organisers should (depending on the size of the scheme) provide advice or appropriate assistance to address the problem. In small-scale or low error rate schemes a second sample exchange between laboratories could be considered. This may involve the participant sending samples to the scheme organiser who re-checks the genotypes, re-codes the sample and returns it to the originating laboratory. The participant then re-tests the coded samples.

Poor performance in interpretation of results can be graded at different levels of importance/seriousness. Therefore the assessors should refer to professional guidelines on disease specific and generic reporting.

EQA schemes should develop criteria to identify poor performance and develop policies to assist laboratories that record poor performance. Where an EQA scheme reveals a systematic error amongst a group of laboratories as a result of a failure of methodology

or of analytical materials or instrumentation it should have a mechanism to issue an alert through its aggregate scheme report and if appropriate through other means. For example this may require alerting a commercial manufacturer, government agency or a group of collaborating laboratories.

7.1 Organisation of an assessment meeting

Within six weeks of the closing date of the scheme the scheme organiser should set a date and venue to meet with the assessors. Before the beginning of the assessment the assessors should agree upon case specific marking criteria and after marking the first 5-10 reports these should be evaluated.

In principle, it is also possible that assessment can be conducted remotely e.g. by email or using a web-based reporting system, provided that the assessors are known to each other, experienced with the specific scheme, the cases are standard and the techniques used are uniform. When dealing with schemes with a large number of participating laboratories, it is advisable to make an initial assessment of the reports in advance of the assessors' meeting. This allows the meeting to focus on resolving any problematic cases where the assessors differ. Scheme organisers should be aware of the work-load required of EQA assessors. Where large numbers of reports are required to be marked they could consider dividing the workload such that each report is marked by two (from three) assessors.

7.2 Documentation of assessments

The documentation of an assessment should be standardised e.g. using an EQA reporting template or tool. The aims of the standardisation should be

- to achieve a better consistency of presentation
- to reduce errors
- to speed up the generation of reports
- to reduce the workload for the assessors and scheme organiser
- to have a permanent record for auditing purposes

The reporting tool should cover the following information:

- participant information including the laboratory ID number
- the date the report was received from the laboratory
- the genotyping scores and comments
- the interpretation scores and comments
- the mean score for each case

- the mean scores for all cases for each laboratory (genotyping and interpretation separately)
- up to six defined criteria of the report scored as 'Yes', 'No' or 'Unclear'
- additional comments if necessary

The submission of the EQA data can be achieved using a mail merge tool to generate the assessment reports to laboratories or by direct electronic submission if the website of the EQA provider allows. The scheme reports should be archived by the scheme organiser. All software which is used for the processing of data should be verified, supported and backed up. The storage of the data files should be controlled. Details of a web-based scheme management and reporting tool are available from EMQN and EuroGentest.

7.3 EQA scheme reports

The report for each scheme should include the following information:

- name and address of the organisation conducting or coordinating the scheme
- names of the persons involved in the design and conduct of the scheme
- date of issue of report
- report number and clear identification of the scheme results
- clear description of materials used including details of sample preparation
- laboratory participation codes and test
- statistical data and summaries
- comments on laboratory performance by the scheme organiser
- procedures used to design and implement the scheme
- procedures used to statistically analyse the data
- advice, where appropriate, on the interpretation of the statistical analysis

The participants should at least receive the results of all laboratories in summary (in tabulated or graphical form) and the reports should be made available within a specified time period.

The identity of participating laboratories should only be known to a minimum number of people involved in coordinating the scheme.

At the end of the annual EQA cycle an evaluation of all the schemes offered during the previous period should take place as part of the assessment meeting.

8.0 Definitions and glossary

Case

Mock clinical scenario designed to match the (→) genotype of an (→) EQA material.

External quality assessment (EQA) (→ Proficiency testing)

EQA is a procedure to distribute testing materials (usually DNA) of known (→) genotype to laboratories for blinded analysis. The laboratories are asked to perform a genetic test relevant to the material and to report and interpret their results in writing. The procedure serves to horizontally compare the participants' performance in this test and to identify any shortcomings in their testing procedures, sample handling, result interpretation and reporting.

EQA material (→ Sample)

Biological specimen (usually DNA) with pre-determined (→) genotype prepared for use in an EQA scheme.

Genotype

The combination of sequence variants on the two alleles of a given gene target.

Proficiency testing (→ EQA)

The term proficiency testing (PT) is mainly being used in the US to describe a procedure essentially similar to EQA (sometimes without the interpretation component).

Sample (→ EQA material)

Biological specimen (usually DNA) with pre-determined (→) genotype prepared for use in an EQA scheme.

Validation

A process to objectively describe the characteristics of a material and its appropriateness for the intended use.