European Consensus Conference for external quality assessment in molecular pathology

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Molecular testing of tumor samples to guide treatment decisions is of increasing importance. Several drugs have been approved for treatment of molecularly defined subgroups of patients, and the number of agents requiring companion diagnostics for their prescription is expected to rapidly increase. The results of such testing directly influence the management of individual patients, with both false-negative and false-positive results being harmful for patients. In this respect, external quality assurance (EQA) programs are essential to guarantee optimal quality of testing. There are several EQA schemes available in Europe, but they vary in scope, size and execution. During a conference held in early 2012, medical oncologists, pathologists, geneticists, molecular biologists, EQA providers and representatives from pharmaceutical industries developed a guideline to harmonize the standards applied by EQA schemes in molecular pathology. The guideline comprises recommendations on the organization of an EQA scheme, defining the criteria for reference laboratories, requirements for EQA test samples and the number of samples that are needed for an EQA scheme. Furthermore, a scoring system is proposed and consequences of poor performance are formulated. Lastly, the contents of an EQA report, communication of the EQA results, EQA databases and participant manual are given.

Key words: external quality assessment, guideline, molecular pathology, oncology

introduction

A novel therapeutic approach based on drugs directed against specific molecular targets that are involved in the proliferation, survival and metastatic spread of cancer cells has been developed in the past two decades. Clinical trials have clearly demonstrated that these agents are active only in molecularly selected populations of patients. Therefore, identification of predictive biomarkers (i.e. markers that assess the effectiveness of a specific treatment) has become mandatory in order to improve this therapeutic approach.

The approval of anti-epidermal growth factor receptor (EGFR) monoclonal antibodies for colon carcinoma patients with wild-type KRAS has represented an important innovation in medical oncology, since this was the first approval of a drug for a frequent solid neoplasm such as colon carcinoma on the basis of a mutational analysis. More recently, kinase inhibitors have been approved for treatment of EGFR mutant non-small-cell lung carcinoma (NSCLC) patients, for BRAF mutant melanoma and for NSCLC with rearrangements of the ALK gene. However, approval of drugs based on molecular testing has also represented a major challenge for medical oncologists, pathologists and molecular biologists who are facing technical and organizational problems in order to ensure an adequate molecular characterization of tumors from their patients.

Testing for gene alterations in tumors is a rapidly emerging field in pathology. The results of such testing directly influence the management of individual patients referred to as personalized therapy [1]. Indeed, both false-negative and false-positive results are harmful for patients. In fact, depending on whether the biomarkers are positive or negative predictors, an error in the molecular characterization might lead to treatment of patients with drugs that are not active or might prevent patients from therapies that are able to significantly affect the course of their disease.

To ensure that cancer patients receive a reliable molecular test result to determine their eligibility for treatment with specific and often expensive drugs that target altered molecular pathways, EQA of laboratories carrying out these tests is of utmost importance. EQA is defined by the World Health Organization (WHO) as ‘a system of objectively checking laboratory results by an external agency’. The main objective is to establish inter-laboratory consistency. The process establishes, harmonizes and standardizes best practice in
correctly identifying mutation(s), interpretation of the results and clerical accuracy. An EQA process can indeed identify systematic errors in methodology that may not be revealed by internal QA processes.

Presently, a number of EQA schemes for gene aberration testing are available in Europe and their results clearly indicate the need for EQA since 10%–15% of laboratories do not carry out according to the standard set by the EQA provider [2–5]. These EQA schemes, however, vary largely in scope, size and execution, and the need for harmonization is urgently felt.

An expert group of clinical oncologists, pathologists, geneticists, molecular biologists, EQA providers and representatives from pharmaceutical industries agreed to develop guidelines for EQA in molecular pathology. A meeting was hosted by the Italian Association of Medical Oncology (AIOM), the European Society of Pathology (ESP), the European Society of Medical Oncology (ESMO) and the Italian Society of Pathology and Cytopathology (SIAPEC) in Naples, Italy, March 2012. Participants were invited who represent stakeholders in Quality Assurance for molecular pathology, namely representatives from European quality assurance programs in pathology, scientific societies (ESP, ESMO, AIOM, SIAPEC), pharmaceutical companies and companies providing samples for quality assurance. Excluded were companies that produce tests or platforms for testing. This guideline aims to harmonize EQA in molecular pathology by describing preferred organization of EQA schemes, criteria for reference laboratories, requirements for EQA test samples, the number of samples needed for a scheme, the scoring system, consequences of poor performance, the content of the EQA report and the communication of the results, the development of EQA databases and participants manuals. A comprehensive version of this guideline will be published shortly elsewhere [6].

### Organization of an EQA scheme

EQA schemes should be developed by an expert group with medical experts (pathologists and oncologists), technical experts and a representing EQA provider. The scheme provider is responsible for all organizational aspects, operation of the scheme according to ISO 17043 standards [7] and should appoint an EQA coordinator for each EQA program. The coordinator is responsible for the selection, distribution and receipt of cases and analysis and reporting of results (Figure 1 and Table 1).

The samples used in an EQA scheme should reflect the diagnostic and clinical reality as close as possible. As the greater part of molecular tests is carried out on formalin-fixed paraffin embedded (FFPE) material, the EQA program should either use FFPE-tumor tissue or close mimics of this such as synthetic control cell lines. Due to the large numbers of participants in an EQA scheme, it is not always possible to provide all participants with the same sample, especially when surgical pathology material is used, making inter-laboratory comparisons more difficult [8]. The distribution of EQA material is then often delegated to reference laboratories, implying that different participants will test different samples. In that case, the EQA coordinator plays a leading role in the

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[Figure 1. Organization of an external quality assurance (EQA) scheme.]
close regulation and control of the scheme and in the central evaluation of the results.

The selection of samples should mirror routine situations with on the one hand the most common aberrations but on the other hand also difficult and borderline cases to uncover potential weaknesses in test performance and interpretation of results. When a laboratory fails to detect aberrations in the latter because the fraction of tumor cells or analyte is below the threshold of their technique, for which they must have provided the analytical sensitivity, this should not be considered as a failure.

The turnaround time, as defined by the EQA provider, should reflect the common clinical situation. Mostly a turnaround time of 10 working days is used for EQA samples, preventing a different approach for these samples when compared with the routine situation. Furthermore, laboratories should report a limited number of samples according to their normal clinical service.

The coordination of the evaluation of the results and raw data will be done by the EQA coordinator in close collaboration with the scheme organizers. At least two members of the assessment team should assess the results independently against validated results and predefined criteria. The medical and technical expert(s) of the EQA scheme should be involved in this process. The final scores and feedback comments should be reviewed by the medical and technical expert(s) and the EQA provider to effectuate consistent assessment across the EQA schemes.

The EQA scheme results should be distributed and made available anonymously among all participants and each laboratory should receive a certificate of performance and individual feedback.

**criteria for a reference laboratory**

Reference laboratories should be selected on their experience with the relevant diagnostic test, access to samples and their ability to coordinate and execute the EQA scheme in collaboration with the scheme provider [3]. The reference laboratory should be a fully equipped ISO 15189 accredited [9] molecular pathology laboratory with well-trained pathologists, clinical molecular biologists and technicians. The reference

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**Table 1. Organization of an external quality assurance (EQA) scheme**

<table>
<thead>
<tr>
<th>Responsibilities</th>
<th>Competence</th>
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<tbody>
<tr>
<td><strong>EQA Scheme Provider</strong></td>
<td>- Initiation, organization and management of EQA schemes</td>
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<td></td>
<td>- Appointing of an EQA team</td>
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<td>- Approval of the final results of the EQA scheme</td>
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<td>- General reporting of the results of the EAQ scheme</td>
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<td>- Provide participants manual</td>
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<td>- Conducting inter-laboratory comparisons</td>
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<td>- Experience in quality management</td>
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<td>- Background in diagnostic domain of the EQA scheme</td>
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<td>- Access to required techniques, samples and facilities to run the scheme</td>
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<td><strong>EQA team</strong></td>
<td>- Development of the EQA scheme</td>
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<td></td>
<td>- Selecting the correct balance of difficult and routine samples</td>
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<td></td>
<td>- Selection of the scheme samples</td>
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<td>- Determine the amount of material supplied</td>
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<td></td>
<td>- Defining the turnaround time</td>
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<td></td>
<td>- Evaluation of results and data in collaboration with the EQA coordinator</td>
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<td></td>
<td>- Review of final scores and scheme feedback in collaboration with medical and technical experts</td>
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<td></td>
<td>- Provide expert commentary on performance of each individual participant</td>
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<tr>
<td></td>
<td>- Medical expert(s) including pathologist and oncologist with knowledge of clinical and pathological background in the specific domain</td>
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<td></td>
<td>- Technical expert(s) with knowledge of the molecular context and technologies used for diagnostic testing</td>
</tr>
<tr>
<td><strong>EQA coordinator</strong></td>
<td>- Selection, distribution, receipt of cases, analysis of results, reporting</td>
</tr>
<tr>
<td></td>
<td>- Regulate, control and evaluation of results in collaboration with the scheme provider and medical and technical expert(s)</td>
</tr>
<tr>
<td><strong>Reference laboratory</strong></td>
<td>- Preparation and distribution of EQA material for a smaller number of laboratories</td>
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<td></td>
<td>- Validation of samples</td>
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<td></td>
<td>- Good experience with diagnostic test(s) of scope on a routine basis</td>
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<tr>
<td></td>
<td>- Access to samples (blocks) for use in the EQA program</td>
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<td></td>
<td>- Ability to co-ordinate and execute EQA schemes in collaboration with the EQA provider</td>
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<tr>
<td></td>
<td>- Fully equipped molecular pathology laboratory with well-trained pathologists, clinical molecular biologists and technicians.</td>
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<td></td>
<td>- Accredited (e.g. ISO 15189) and passed an EQA test</td>
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laboratories are involved in the pre-testing of scheme samples that have been selected by the EQA provider and medical and technical expert(s). Samples should only serve as the standard when at least two reference laboratories pre-tested the samples with the same test result. Only samples that are tested by all reference laboratories with identical results should be used in the EQA scheme [2–4].

requirements for EQA test samples
Samples of human tumor tissue are preferable but have limitations. The amount of human tissue is limited, the specimens need to be validated by both pathological review and molecular analysis, differences in the tumor content or tumor heterogeneity may occur as well as possible problems with cross border transportation. Artificial FFPE tissue blocks can be produced by mixing formalin fixed cell lines with and without specific aberrations and embedding them in paraffin with the advantage being the unlimited number of homogenous samples that can be made. However, these samples do not reflect the complex tumor tissue composition and issues related to tumor heterogeneity.

The percentage of tumor cells in the sample is crucial for molecular testing and the laboratory should report this to determine whether the sample is sufficient for analysis in view of the analytical sensitivity of the molecular test that is used. When samples are sent to the participant in an Eppendorf tube, then the percentage of tumor cells needs to be predefined or a separate slide for (virtual) microscopy should be supplied.

The quality and amount of the analyte should reflect routine daily practice where highly heterogeneous material (e.g. FFPE or bronchio-alveolar lavage) is common. This is applied also for artificially made FFPE tissue blocks. Several in vitro diagnostics (IVDs) and CE marked kits need a higher amount of DNA than a single PCR-based analysis. EQA schemes should adhere to the upper limit of available techniques to allow the participation of all laboratories using different techniques.

Informed consent is not mandatory for use of any patient material as long as it is used for validation of testing. Nevertheless, the EQA provider should take highest care that privacy protection is guaranteed.

number of samples needed for an EQA scheme
For a reliable evaluation, at least 10 samples need to be analyzed in one batch or in different smaller batches sent within a year. To offer a statistically substantiated norm for the smallest number of correct cases in EQA, the limit for poor performance should be set at 9 out of 10 cases that should be reported correctly. These do not have to be assessed in one batch. Combining the results of three sets of three to four samples within a year will result in the same statistical confidence with the advantage of a more continuous evaluation of the quality.

scoring system for EQA in molecular pathology
A scoring system for EQA with predefined and peer reviewed criteria should be defined. This scoring system is separated into three categories.

The ‘pre-analytical’ phase assesses the examination by the pathologist, the adequacy of the EQA test sample, percentage of neoplastic cells and the dissection if required. Earlier EQA schemes showed that at the moment there is no gold standard for the estimation of the percentage of neoplastic cells. However, guidelines are being developed concerning this matter. Scoring of the ‘pre-analytical’ phase can only be applied after these guidelines have been introduced. Therefore, we do not recommend scoring of the ‘pre-analytical’ phase at this moment.

The ‘analytical’ phase concerns DNA isolation and genotyping for which a detailed scoring system is mandatory. The proposed system scores a maximum of 2.00 points for each sample with negative points given depending on the type of error. Since it is difficult to predict all types of error in advance, these should be assessed by a board of experts. An example of an evaluation system based on the most common errors encountered in EQA schemes is given in supplementary Table S1–S4, available at Annals of Oncology online. Clearly, the limitations of the technique used for analysis should be taken into account.

The ‘post-analytical’ phase deals with interpretation and reporting of the results and should be applied to all cases for which a report is requested by the EQA scheme. The reports should reflect routine reports provided to physicians. The reports should be scored with respect to patient identification, report look and content and biological and clinical interpretation based on the predefined elements. These key interpretation elements are based on expert consensus and best practice guidelines. Here, the maximum score is also 2.00 per case.

consequences of poor performance
In the United States, the ‘Clinical Laboratory Improvement Act’ of 1988 defines poor performance in EQA either as unsatisfactory or as unsuccessful. In Europe, a standard for EQA and poor performance is lacking. The consequences of unsatisfactory performance and measures for improvement are the responsibility of the laboratory and include internal quality control and validation programs with external samples. The consequences for unsuccessful performance in molecular testing in Europe have not been established but an option is that the laboratory withdraws the test or that either professional organizations or the appropriate government regulatory agency takes measures. It is not the responsibility of the EQA to establish consequences, but the organizer can provide help and support for improvements, such as providing reference material, methodological advice, quality management and help by reviewing corrective and preventive action plans.

content of an EQA report and communication of EQA results
The results of an EQA should be reported in a general scheme report with anonymized results combined with an individual...
performance score with an independent assessment of the performance with comments and feedback. Additionally, the participants receive a certificate of participation and a certificate of performance. It is highly recommended that results of the EQA samples are published in advance before the general report, so that laboratories can review their performance in the right context. Participants should be given the opportunity to appeal against the reports before the final general report is released. The detailed content of the general report and individual comment to the participants has been published elsewhere [6]. Where required by law, the EQA provider may have also report to regulatory agencies.

EQA databases

The data that will be generated by laboratories that participate in EQA schemes should be combined by linking databases to enable a rapid growth of knowledge on the general performance level. Besides, the European Society of Pathology (ESP) intends to develop a database for the specific results of all EQA providers to provide an overview on what is going on with respect to test performance in molecular pathology. EQA providers should be encouraged to make the general reports available to the public domain.

concluding remarks

The development of drugs capable of blocking molecular alterations promoting tumor growth will lead in the next few years to a significant improvement of personalized medicine in oncology. However, the progress in this field is limited by the availability of reliable methods to detect such target molecular alterations. In this respect, we believe that the activity of European and national scientific societies with the release of guidelines and the conduction of EQA programs has significantly contributed to improve the quality of tumor molecular pathology. Indeed, EQA schemes for laboratories carrying out molecular tests within the framework of personalized therapy are definitely needed and can improve laboratory performance.

During the development of this guideline, consensus could be reached on most issues. However, more experience is needed to further improve EQA schemes. On the other hand, we do expect that the present guideline already leads to a better treatment selection through improved EQA in molecular pathology. The creation of a database of the results of the European schemes in molecular pathology will allow monitoring of this process and assessment of the changes of the quality of molecular testing over time.

Although this guideline addressed important issues in molecular pathology, a number of points still need to be addressed. This guideline is clearly directed to mutational analyses; however, some predictive molecular alterations can be detected only by FISH analysis or by assessment of qualitative or quantitative changes in mRNA. In addition, novel diagnostic approaches aimed to detect panels of molecular alterations and based on genotyping or next generation sequencing techniques are being developed. Therefore, the rules described in this report will need to be adapted to these additional techniques of molecular profiling. Nevertheless, this guideline represents a first fundamental step toward the harmonization of the EQA process in Europe.

disclosure

The authors have declared no conflicts of interest.

references


appendix

Quality Assurance for Molecular Pathology group: F. Blackhall, Manchester University and Christie Hospital NHS Foundation Trust, Manchester, UK; E. Boone, Laboratory for Molecular Diagnostics, H. Hartziekenhuis, Roeselare-Menen, Roeselare, Belgium; G. Botti, Dipartimento di Anastomia Patologica e Citopatologia, IRCCS Istituto Nazionale Tumori, Fondazione G. Pascale, Naples, Italy; F. Carneiro, Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP) and Medical Faculty/ Centro Hospitalar de São João, Porto, Portugal; I. Celik, Merck Serono Global Research & Early Development—Oncology Platform, Merck KGaA, Darmstadt, Germany; F. Ciardiello, Dipartimento Medico-Chirurgico di Internistica Clinica e Sperimentale F. Magnaghi and A. Lanzara, Seconda Università di Napoli, Naples, Italy; I.A. Cree, Warwick Medical School, Clinical Sciences Building, University Hospitals Coventry and Warwickshire, Walsgrave, Coventry, UK; Z.C. Deans, UK NEQAS for Molecular Genetics, UK NEQAS, The Royal Infirmary of Edinburgh, Edinburgh, UK; E. Dequeker, University of Leuven,
Current status of screening for colorectal cancer

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Results: Two screening methods have been shown to reduce CRC mortality in randomised trials: repetitive faecal occult blood testing (FOBT) reduces CRC mortality by 16%; once-only flexible sigmoidoscopy (FS) by 28%. FS screening also reduces CRC incidence (by 18%), FOBT does not. Colonoscopy screening has a potentially larger effect on CRC incidence and mortality, but randomised trials are lacking. New screening methods are on the horizon but need to be tested in large clinical trials before implementation in population screening.

Conclusions: FS screening reduces CRC incidence and CRC mortality by removal of adenomas; FOBT reduces CRC mortality by early detection of cancer. Several other tests are available, but none has been evaluated in randomised trials. Screening strategies differ considerably across countries.

Key words: colorectal cancer, screening, prevention, adenoma, colonoscopy

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