

11. EUROPEAN QUALITY ASSESSMENT NETWORKS IN MOLECULAR DIAGNOSTICS

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Molecular Diagnostics has been growing steadily over the last decade and has now conquered a firm place in diagnostic testing in the surrounding of health care and scientific biomedical applications. Aside from testing for xenogenic nucleic acids in infectious diseases, molecular tests are mostly being carried out on human genetic material for the characterization of underlying defects in illness, a presymptomatic diagnosis or a predisposition testing. With the exception of somatic genetic defects e.g. observed in malignant diseases that characterize the phenotype of cancer cells and thus may be helpful for prognosis and therapy, many molecular tests describe the germline genetic make-up of a patient. It must be clear that the increase of potential genetic biomarkers will be enormous within very few years, Specifically, while the complete sequence of the human genome as determined by the Human Genome Project [www.sanger.ac.uk] forms the basis of our knowledge of the human genome, several large-scale international studies have started to improve our knowledge of the extent of genetic variation and its importance as a marker for disease and disease risk. Such projects include the international SNP-consortium [www.snp.cshl.org], the HapMap-Project [www.hapmap.org] and recently started Medical Sequencing Program [www.genome.gov/17516031]. This situation poses important questions pertinent to the issue discussed below in more detail.

Molecular genetic testing results may have important consequences for health and the well-being of an individual and his relatives. Indeed, constituting a permanent individual feature, they possess a potential to stigmatize a carrier. This has led to the perception that genetic test results are exceptional and should be considered separately from other medical information, a perspective termed “genetic exceptionalism”. Similarly, the notion to restrict genetic testing to the medical specialist in Human Genetics has been explained by the necessity for genetic counselling before and after the performance of the test. While such restrictions certainly apply to the classical Human Genetics approach to rare monogenic disorders, they will become less and less feasible in the clinical routine molecular testing in the context of multifactorial or polygenic diseases, e.g. diabetes, obesity, psychiatric conditions, cardiovascular diseases or cancer. In these highly prevalent diseases, a multitude of genetic markers has been associated with low or moderate increases of risks of morbidity and additional ones are being added to such panels as our understanding of metabolic networks increases. In contrast, the contributions of single such markers for the clinical presentation of disease conditions with multifactorial background are unclear at best due to the low diagnostic power of the single marker. It is therefore safe to expect the extended usage of multiparametric testing strategies most probably by technologies like DNA microarrays or high-throughput DNA sequencing in the near future. Indeed, first DNA arrays have already been introduced into clinical research and clinical diagnostics. Examples are the ROCHE AMPLICHIP for pharmacogenetic testing, the ROCHE SEPTIFAST system for microbe characterization. Also, small and medium enterprises in the biotechnology/biomedical field offer highly sophisticated tests like the extensive array for the multiplex NAT testing of 34 bacterial and 6 fungi species together with typing of 5 resistance genes as introduced by

SIRSLAB, a company situated in Jena, Germany. Moreover, developmental studies propose the complete sequencing of genomes of infectious agents.

Taken together, the clinical laboratories face a rapid increase in different nucleic acids tests for monogenic and multifactorial diseases and predisposition conditions not only from regional senders. Indeed, it has been pointed out by the OECD that a high increase of cross-border traffic of human genetic material is currently being observed, thus providing a challenging issue for new international regulations in biolegal and bioethical fields. It should be sufficiently clear from the above introductory remarks that quality management in molecular testing must be a highly prioritized activity. Indeed, the importance of quality assurance in molecular diagnostics is now increasingly being emphasized. While there are few active legislative regulations at present, some recommendations and guidelines have recently been published.

Currently, few European countries i.e. Norway, Austria and Switzerland have active national legislations on molecular genetic diagnostics. In others like in Germany, specialist committees have been installed to draft bills that will also specifically address the quality issues that need to be followed when performing molecular diagnostics in a health care setting. For example, in Austria the compliance of molecular genetics laboratories with quality standards may be controlled directly through the State Department for Health. The “molecular diagnostics portfolios” offered by genetic testing laboratories and the respective external quality programs, in which they participate are to be communicated to the department, which will support the availability and access to additional schemes to the labs, as appropriate. In this respect, the Austrian legislation closely follows guidelines issued by the OECD in 2007 (see below).

As for recommendations and guidelines, a number of initiatives have addressed different aspects of molecular diagnostics on national levels. In 2005, a working group for the EU commission has laid down very concise recommendations on genetic testing entitled “25 Recommendations on the ethical, legal and social Implications of genetic Testing”. These recommendations put genetic test results - together with other medical (and for that matter confidential) information - into the general realms of medical laboratory diagnostics and are specifically opposed to the concept of genetic exceptionalism.

[http://ec.europa.eu/research/conferences/2004/genetic/recommendations_en.htm]!

Somewhat in contrast, the 2007 OECD “Guidelines for Quality Assurance in Molecular Genetic Testing” address the special nature of genetic test results (possibly due to the different national composition of the OECD consortium). Both documents give broad room to the various issues related to quality assessment. The OECD paper is highly recommended for their detailing aspects. For example, all molecular genetic testing should be provided and practised under a quality assurance framework, which is subject to adaptation and interpretation by regulatory and professional bodies. Accreditation or equivalent recognition has been recognized as effective procedure to assure the analytical and diagnostic quality. The instalment of monitoring systems is proposed to address instances where laboratories do not meet the standards. It is clearly emphasized in the OECD guidelines that proficiency testing should be implemented to monitor the quality of laboratory performances. Accreditation or equivalent measures should be the basis for the international recognition of providers of external quality assessment programs. Proficiency testing providers need to develop their schemes to keep up with technological advances. Importantly, where proficiency testing is not available for a molecular test performed, the lab should strive to use alternative methods to assure the quality of the test result. Best practise includes the identification of persistent poor performance in each and all single steps including the result reporting. Quality of reporting

includes the definition of requirements for adequate reporting by medical care professionals. Finally, the OECD guidelines address procedures to ensure proper education and training of laboratory staff and medical professionals involved in molecular genetic testing.

While internal quality procedures can be expected to be implemented in every laboratory, External Quality Assessment Programs (EQAP) are important tools to increase the analytical or diagnostic proficiency of the diagnostic lab. Moreover, EQAP allow comparing the performance between laboratories with agreed independent standards. Although there is a general recognition of the necessity of EQAPs, they are usually not mandatory in EU countries so far. We speculate that the minority of labs actually participate in EQAPs on a regular basis. While legislation aims to define genetic testing or the protection of individual personality rights, or regulates the legitimation of professionals to perform genetic analyses, EQAPs are less strictly fixed. Indeed, it may be argued that a proficiency framework will contribute to this patient protection in a number of ways. In this context it is interesting to note that the 25 EU recommendations have defined quality management and assurance in molecular testing as an ethical task in its own right.

In Europe there exist a number of EQAPs for molecular genetic testing that operate on national and in few cases on supranational levels (they can be addressed via the directory of the Eurogentest network, see below). Molecular Diagnostics EQAPs have been initiated in the 1990s in different fields of Laboratory Diagnostics. For example, the European Molecular Genetics Quality Network (EMQN) started in October 1998. EMQN has been funded by the EU during the Standards Measurement and Testing Programme until 2002. It has since then supported itself by subscriptions from its users. EMQN concentrates on monogenic disorders and offers a range of services including methodological EQAPs and proficiency assessments for reporting of finding of inherited diseases. Currently, EMQN currently offers 18 schemes mostly for rare monogenic disorders in the context of Human Genetics (http://www.emqn.org/emqn/EQA/mainColumnParagraphs/04/document/EMQN_scheme_catalogue_2007v2.pdf). Similarly, the European Union Quality Control Concerted Action (EU-QCCA) in diagnostic virology has been established in 1998 and has been replaced in 2001 by QCMD [<http://www.qcmd.org/Index2.htm>], a not-profit organisation dedicated to molecular diagnostics EQAPs in microbiology and virology.

In Clinical Chemistry, an EQAP for molecular genetic testing has been established in Germany by the German Society for Clinical Chemistry as one area within their extensive quality assessment program that is covering 29 different schemes from all areas of Clinical Chemistry. These first EQAPs were run in 1997 and 1998 (1) and have been offered twice a year since. The program has been extended ever since (2) today encompassing 19 different genetics parameters including metabolic diseases, thrombophilia and pharmacogenetics [http://www.dgkl-rfb.de/index_E.shtml]. Currently, more than 250 laboratories participate regularly, thereby allowing detailed analysis of the results. Additionally, EQAPs on molecular methodology like DNA-sequencing are performed. Finally, this scheme provides so-called “case-based EQAPs” that present clinical cases e.g. in context with biochemical or haematological data, asks the participants to perform molecular diagnostics and communicate back qualified reports.

There is an increasing awareness to improve the quality of molecular genetic testing as recognized by the EU commission ever since the first funding schemes in the 1990s. Indeed, the FP6 has supported a specific support action called EQUAL between 2004 and 2006. In EQUAL, methodological quality assessment schemes have been devised that concentrated on

genotyping (EQUALqual), quantitative rt PCR (EQUALquant) and DNA sequencing (EQUALseq). EQUAL was carried out by a group of laboratory scientists under coordination from researchers of the University of Florence. This program has proved immensely successful and featured offers of training courses for participants with below-the-average performances in all 3 subprograms, also showing demand in this area. A number of reports on the results of EQUAL have been published recently (3-6). Finally and most importantly, the FP6 has funded a Network of Excellence (NoE) called Eurogentest coordinated by Jean-Jaques Cassiman from the University of Leuven. The necessity for such network to support collaboration and the exchange of expertise had been felt after previous reports on high percentages of incorrect test results from Human Genetics testing in rare genetic disorders like cystic fibrosis (7). Since then, Eurogentest has rapidly grown into an extensive network that covers a multitude of information on numerous aspects of molecular diagnostics, proficiency and education, ethics, patient rights etc. It is also a website for active members of the molecular diagnostics community. An updated directory of active EQAPs in genetic testing can be found there [<http://www.eurogentest.org/web/info/unit1/molecular.xhtml>].

The new Framework 7 (FP7) commissioned by the European Commission in 2007 has again encouraged projects that cover areas of quality assessments and control in molecular genetic testing in different programs, and calls have been issued. While this continued EU activities concede that even with today's advances in molecular techniques many areas still need to be optimized, it also is reassuring that the importance of quality on both the national and the international level is now increasingly being acknowledged. This emphasis is certainly well deserved considering the rapid further development and throughput of genetic data in the life sciences and medicine.

Recommended literature:

1. Braun A, Deufel T, Geilenkeuser WJ, Neumaier M, Rohle G, Roscher A, Wagener C. External quality assessment of molecular biology-based methods used in laboratories of clinical chemistry and human genetics. *Clin Chem Lab Med.* 1998;36(4):231-4.
2. Neumaier M, Braun A, Gessner R, Funke H. Experiences with external quality assessment (EQA) in molecular diagnostics in clinical laboratories in Germany. Working Group of the German Societies for Clinical Chemistry (DGKC) and Laboratory Medicine (DGLM). *Clin Chem Lab Med.* 2000;38(2):161-3.
3. Orlando C, Verderio P, Maatman R, Danneberg J, Ramsden S, Neumaier M, *et al.* EQUALqual: A European Program for External Quality Assessment of Genomic DNA Extraction and PCR Amplification. *Clin Chem.* 2007;53(7):1349-57.
4. Dorn-Beineke A, Ahmad-Nejad P, Pfeiffer U, Ramsden S, Pazzagli M, Neumaier M. Improvement of technical and analytical performance in DNA sequencing by external quality assessment-based molecular training. *Clin Chem.* 2006;52(11):2072-8.
5. Ramsden SC, Daly S, Geilenkeuser WJ, Duncan G, Hermitte F, Marubini E, *et al.* EQUALquant: an international external quality assessment scheme for real-time PCR. *Clin Chem.* 2006;52(8):1584-9.
6. Ahmad-Nejad P, Dorn-Beineke A, Pfeiffer U, Brade J, Geilenkeuser WJ, Ramsden S, *et al.* Methodologic European external quality assurance for DNA sequencing: the EQUALseq program. *Clin Chem.* 2006;52(4):716-27.
7. Dequeker E, Cassiman JJ. Genetic testing and quality control in genetic laboratories. *Nat Gen.* 2000;25:259-60.