MOLECULAR DIAGNOSTICS: QUALITY ASSURANCE PROGRAM IN GENOTYPING. A CROATIAN EXPERIENCE

Jadranka Sertić
Clinical Institute of Laboratory Diagnosis, Zagreb University School of Medicine and Clinical Hospital Center, Zagreb, Croatia

ABSTRACT - A program of molecular diagnostics has developed into a principal part of laboratory medicine over the past 20 years. Although much diagnostic testing for rare disorders is still carried out in research laboratories, testing for more common genetic disorders, such as cystic fibrosis, muscular dystrophies, fragile X syndrome and hemochromatosis is now carried out in specialist diagnostic laboratories. Molecular genetic tests are extremely specific and usually detect presence or absence of a mutation, the number of repeat units, or the number of copies of a gene. Molecular testing laboratories use a wide variety of technologies, equipment and, according to best practice recommendations, participate in external quality assessment. Zagreb University Hospital Center has participated in the scheme run by the European Molecular Genetics Quality Network and Deutsche Vereinte Gesellschaft für Klinische Chemie and Laboratoriumsmedizin e.V. since 1998. The external quality assessment scheme for cytogenetics, molecular genetics, biochemistry and immunogenetics is an important step in the new millennium towards harmonization of laboratory and clinical services at European, regional and national level.

Key words: Genotype+analysis; Quality Control+standards; Health Planning Guidelines

Objectives of the strategy of molecular diagnostics are diagnosis, its confirmation, and its predictive dimension in health care and health promotion. The prevalence of genetic diseases varies significantly among ethnic groups and geographic regions. Molecular genetics testing laboratories must be responsible to individuals and families who seek their service for the quality and validity of services according to recommendations they provide in the course of establishing molecular diagnosis. Therefore, it is essential that all public and private laboratories apply standards for correct genotyping. The European Molecular Quality Network (EMQN), set up in 1998, has designed External Quality Assessment (EQA) scheme for a number of genetic diseases (1,2). The aim was to upgrade and maintain standards of diagnostic clinical molecular genetic testing in the European Union (EU) and non-EU countries through provision of EQA schemes. The recommendations are based in most cases on reports drawn up by chairs of disease-based workshops run by EMQN and molecular genetic societies published on the website www.emqn.org. In 2004, 14 EQA schemes for the following diseases were released: retinoblastoma (RB), fragile X syndrome (FRAX), Duchenne muscular dystrophy (DMD), breast cancer (BRCA1, BRCA2), hereditary haemochromatosis (HFE), Prader Willi / Angelman syndromes (PW/AS), Friedreich ataxia (FRDA), Charcot-Marie-Tooth disease (CMT), Huntington’s disease (HD), hereditary non-polyposis colon cancer, spinocerebellar ataxias (SCA), phenylketonuria, Y-chromosome microdeletions (Y-chrom), and cystic fibrosis (CF). Also, there is a scheme for DNA sequencing. EMQN was founded by and affiliated with the European Commission until 2001. Presently it is linked with European expert centers for different diseases and supported by subscriptions of laboratories for which it provides EQA services.
nation and training (www.ec-4.org/equal). This
tation of PCR assay methods including dissemi-
diagnostics based on performance and interpre-
EQA programme in clinical molecular
in the world participate in the DGKL EQA scheme
(MTHFR), glycoprotein IIb, IIIa (GPIIbIIIa) (8
factor XIII, methylenetetrahydrofolate reductase
(UGT-1A), factor V , factor II (prothrombin),
uridin diphospho-glucuronosyltransferase A1
S-methyltransferase (TPMT), cytochrom p450,
giotensin-converting enzyme (ACE), thiopurin-
apoprotein E (apo E), apoprotein B (apo B),  an
hemochromatosis, antitrypsin deficiency (AAT),
provides EQA schemes for the following genes:
Chemie and Laboratoriumsmedizin e.V . (DGKL)
deutsche Vereinte Gesellschaft für Klinische
confidentiality of results, of timely information
and emphasize the importance of storage and
concerning genotype and interpretation results,
and specific technical or interpretation problems
concerning genotype and interpretation results,
and underestimate the importance of storage and
confidentiality of results, of timely information
to clinicians, and of DNA sample storage (5,6,7).
The Reference Institute for Bioanalytics of
Deutsche Vereinte Gesellschaft für Klinische
Chemie and Laboratoriumsmedizin e.V. (DGKL)
provides EQA schemes for the following genes:
hemochromatosis, antitrypsin deficiency (AAT),
apoprotein E (apo E), apoprotein B (apo B), an-
giotensin-converting enzyme (ACE), thiopurin-
S-methyltransferase (TPMT), cytochrom p450,
uridin diphospho-glucuronosyltransferase A1
(UGT-1A), factor V , factor II (prothrombin),
factor XIII, methylenetetrahydrofolate reductase
(MTHFR), glycoprotein IIb, IIIa (GPIIbIIIa) (8
-11). Many diagnostics laboratories throughout
the world participate in the DGKL EQA scheme
(Table 1). In addition, EC4 EQUAL project starts
this year as a multi-national external quality ass-
ay (EQA) programme in clinical molecular
diagnostics based on performance and interpret-
tation of PCR assay methods including dissemi-
nation and training (www.ec-4.org/equal). This

| Table 1. | Participation in DGKL schemes - the number of participants per country in 2002 |

<table>
<thead>
<tr>
<th>Country</th>
<th>A</th>
<th>GB</th>
<th>AUS</th>
<th>B</th>
<th>CAN</th>
<th>CH</th>
<th>CZ</th>
<th>D</th>
<th>DK</th>
<th>E</th>
<th>F</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>67</td>
<td>11</td>
<td>3</td>
<td>6</td>
<td>14</td>
</tr>
</tbody>
</table>

(3,4). Also, there are disease-specific recommend-
factions for the following 18 diseases: breast can-
cer, Facio scapular humeral muscular dystrophy,
fragile-X syndrome, Friedreich ataxia, hereditary
motor and sensory neuropathies (HMSN), Prader
Willi / Angelman syndromes, retinoblastoma,
spinal muscular atrophy (SMA), Y-chromosome
microdeletions (AZF), cystic fibrosis, Duchenne
muscular dystrophy, familial adenomatous poly-
posis coli (FAP), hemophilia A, hemophilia B,
hereditary non-polyposis colorectal cancer (HN-
PCC), Huntington’s disease (HD), myotonic dys-
trophy (MD), and spinocerebellar ataxias (SCA).
These recommendations also tend to solve
specific technical or interpretation problems
concerning genotype and interpretation results,
and emphasize the importance of storage and
confidentiality of results, of timely information
to clinicians, and of DNA sample storage (5,6,7).

The project is for laboratories from EU countries, but
Croatian laboratories can also apply and partici-
pate (www.dgkl-rfb.de). These methodological
EQA programmes will be available to laborato-
ries performing molecular diagnostics to improve
technical and interpretation skills required for
correct results. Molecular genetics based bio-
technologies and genomic studies have opened
new perspective for prognosis and treatment of
many diseases. Molecular diagnostics is now
available for the clinical chemistry and clinical
genetics, together with standardization of these
tests in terms of instrumentation, reagents and
procedures. A mechanism is needed for quality
assurance systems to have a permanent place in
the practice of EU molecular laboratories, includ-
ing informed consent. According to a Swiss law,
any analysis of a person’s “genetic heritage” and, in
particular, every genetic test performed for
diagnostic reasons, can be performed only with
the consent of the person concerned. The person
concerned gives a legally valid consent only if
he/she has previously been completely informed
about the nature of the test, its aim, risks, cost and
its implications (www. ssgm.ch). In addition, this
consent includes patient’s decision about the DNA
sample after the test is completed with the possi-
bilities of DNA banking (storage) for reanalysis
upon request, and use for medical research after
anonymization or destruction. Also, information
should be included about costs and whether they
are covered by health insurance or not. According
to a law in the Netherlands, only DNA diagnostic
laboratories are allowed to perform tests for mo-
nogenic disorders. In European countries, more
and more laboratories from peripheral hospitals
are beginning to perform genetic testing by es-
thablishing networks with referral diagnostic lab-
atory in order to organize responsibilities and
ensure quality and patient well-being. There is a
need for harmonization in the process of quality
assurance system, and endeavors for accredita-
tion according to ISO standards.

In public and private laboratories, molecular
diagnostic program includes molecular genetic
OMIM-diagnostics, paternity testing, microbiol-
y, pharmacogenetics, cytogenetics, HLA typing
and immunogenetics (www.ma.uniheidelberg.de/
inst/ikc/, www.medical.genetic.de). EUROGEN-
TEST is a large collaborative Network of Excel-
ence under the EU Framework, which intends
to develop infrastructure, tools, resources, rec-
ommendations and procedures that should lead
to harmonization and improved overall quality
of cytogenetic, molecular genetic, biochemical,
immunogenetic and clinical genetic services.
This network will bring together experts across Europe on different aspects of genetic testing including researchers, diagnosticians, managers, ethicists, lawyers, sociologists and consumers (www.eurogentest.org).

**MUTATION DETECTION METHODS**

It has been estimated that 60% of all humans will be affected by a disease causing genetic mutations in the lifetime. On the other hand, only a limited number of efficient methods are presently available for detecting mutations in single gene disorders, cancer and common polygenic disorders. Some of them are shown in Table 2. Tests for genetic disorders include different laboratory processes. Some involve direct DNA examination for testing, profile or screening, while others involve conventional biochemical tests for gene product or microscopic examination of stained or fluorescent chromosomes. As commercial kits are only available for a small number of tests, most laboratories use their own in-house (so-called “home brew”) test methodologies and reagents. Automated genetic instruments, such as KingFisher/LabSystems, MagnaPure/Roche, LightCycler/Roche, TaqMan/Applied Biosystems, etc., allow rapid prenatal and postnatal diagnosis of gene abnormalities, whereby genomic DNA is isolated from 10 mg chorionic tissue cells, 1 ml uncultured amniotic fluid or 0.1 ml whole blood, and a particular gene region is amplified and genotyped by use of polymerase chain reaction (PCR) or fluorescent markers (12,13). Rapid pathologic results are referred to the clinician within 1-2 days. Several approaches for rapid detection of many single nucleotide polymorphisms and mutations in a large population by microelectronic microchips have been reviewed. Application microarray and quantitative PCR technologies have improved prediction of therapeutic sensitivity of breast-, colorectal-, head and neck-, and pediatric cancers. Many applications in molecular diagnostics have included quantification of the number of specific targets in a gene, gene dosage. This has generated an increasing need for the development of quantitative PCR techniques for the determination of viral load in blood for diagnosis of HIV or HCV infections, changes in gene dosage of inherited diseases and profiling of cancer cells (14 - 19).

Genotyping tests for molecular mutations associated with clinical syndromes increasingly allow clinicians to identify health risks before clinical problems occur, but can create moral problems for families and serious health policy challenges for communities, and complicate professional ethics. The psycho-social impact of testing, the patient’s privileges with respect to testing, and the potential for effective prevention following testing become important for professional decision-making about genetic testing (20).

The issue of quality management in preanalytics, effects of storage time and temperature on DNA quality is of utmost importance for DNA testing. Also, molecular diagnostic has special aims which are the implementation of external quality assessment, establishment of a network database within societies and implementation of educational program for laboratory experts and physicians (21 -27).

**CROATIAN MOLECULAR GENETICS PROGRAM AND EUROPEAN QUALITY ASSURANCE**

Molecular genetic laboratories around the world show growing interest in the systematic application of quality assurance in diagnostic laboratories. A program of molecular diagnosis was established in 1991 by a founding group of laboratory experts and clinicians at the Clinical Institute of Laboratory Diagnosis, Zagreb University Hospital Center, Zagreb, Croatia. The aim was to establish molecular genetic testing in
Croatian molecular genetic program and EMQN/DGKL monitoring of quality control and result interpretation

**Table 3.** Program molekularne genetike u Hrvatskoj i praćenje kvalitete i tumačenja rezultata od EMQN/DGKL

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mutation/Allele</th>
<th>EMQN/DGKL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antitripsin deficiency</td>
<td>PI M, PI Z, PI S</td>
<td>DGKL</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>AF508, N1303K, G542X, G551D, R553X (1/12/17/31)</td>
<td>EMQN</td>
</tr>
<tr>
<td>Duchenne muscle dystrophy</td>
<td>Pm region and exons 4, 8, 12, 17, 19, 44, 45, 48, 51</td>
<td>EMQN</td>
</tr>
<tr>
<td>Hereditary hemochromatosis</td>
<td>C282Y, H63D/S65C</td>
<td>DGKL</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>CGG repeat copies</td>
<td>EMQN</td>
</tr>
<tr>
<td>FMR mutations</td>
<td>CTG repeat copies</td>
<td>EMQN</td>
</tr>
<tr>
<td>Myotonic dystrophy</td>
<td>AZFa/AZFb/AZFc</td>
<td>EMQN</td>
</tr>
<tr>
<td>DMPK mutations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-chromosome microdeletion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial hyperlipidemia</td>
<td>apo E genotype E2/E3/E4</td>
<td>DGKL</td>
</tr>
<tr>
<td></td>
<td>apo B mutation 3500</td>
<td>DGKL</td>
</tr>
<tr>
<td>Pharmacogenetic</td>
<td>ACE genotype I/D</td>
<td>DGKL</td>
</tr>
</tbody>
</table>

Croatia according to recommendations of European experts in a particular clinical entity, with monitoring of quality control in mutation analysis and result interpretation (Table 3). Molecular analyses are performed according to the European recommendations for molecular diagnosis using classic and automated gene systems which in genetic counseling belong to telegenetics; they eliminate the element of distance and include patient database registration. A program comprises several categories: 1) patients and family members at high risk of hereditary disorders, 2) individuals at reproductive age before or during pregnancy, 3) In Vitro Fertilization unit patients, male infertility profile, genetic reproductive profile, 4) individuals included in prevention of polygenic multifactorial disorders, and 5) patients included in pharmacogenetic testing. Most mutations responsible for genetic diseases have a dramatic effect on phenotype. In this regard, molecular medicine offers several procedures including DNA testing, molecular diagnosis, and genetic counseling of inherited disorders. Using the International Referral Network, laboratory experts provide genotype results and interpretation of written reports for clinicians, as well as risk assessment for family members. Prenatal and postnatal diagnosis is available for common severe congenital disorders such as CF, DMD, SMA, MD, FRAGX, etc.

EMQN presently comprises three levels of participants: management group, national partner contacts, and registered members. There is an increase in the number of participants and the number of countries. Most of the registered users are active diagnostic clinical molecular genetic laboratories. Two laboratories participate in this scheme from Croatia: Zagreb Clinical Hospital Center and Rudjer Bošković Institute, along with laboratories from 32 European countries, Australia and the USA. In Croatia, results of DNA testing are monitored through External Quality Assessment scheme for cystic fibrosis, Duchenne muscle dystrophy, and Y-chromosome microdeletions. Cystic fibrosis is a model disease for other genetic diseases. For hereditary hemochromatosis, antitrypsin deficiency, apoprotein E, apoprotein B, and angiotensin-converting enzyme genotypes, genetic quality assurance is supported by Deutsche Vereinte Gesellschaft für Klinische Chemie und Laboratoriumsmedizin.

**MOLECULAR GENETIC TESTING, PROFILES AND SCREENING**

DNA testing is a complex process, and the results depend on reliable laboratory procedures and accurate result interpretation. Interpretation of DNA test results requires information about genetic screening and biochemical and cytogenetic markers, the so-called profile. Some of them are profiles for male genetic reproduction, iron overload, male infertility, mental handicap, etc. (Table 4).

It is essential to know the frequency of mutations and prevalence of disease in a population subjected to prenatal diagnosis and modified carrier risk assessment. The prevalence of genetic diseases varies significantly among ethnic groups and geographic regions. Most laboratories initially screen for the most frequent mutations and, following a cascade model, turn to more specialized laboratories for complete gene studies if necessary. An example is a monogenetic disease, cystic fibrosis, and studies concerning carrier screening, monosymptomatic form, atypical forms, and fetuses with echogenic anomalies. The Croatian laboratory that performs gene analysis is funded by the Ministry of Health, it operates in collaboration with EMQN, and participates annually in EQA assessments. Furthermore, genetic tests
Moćna dijagnostika: program osiguranja kvalitete u genotipizaciji. Hrvatsko iskustvo

Sažetak - Program molekularne dijagnostike razvio se tijekom proteklih 20 godina u najvažniji dio laboratorijske medicine. Iako se mnoge dijagnostičke pretrage za rijetke poremećaje utvrdjuju prisutnost ili odsutnost mutacija, broj ponavljajućih jedinica, ili broj kopija gena, Laboratorij za molekularnu genetiku koristi različite genetike za pravdu u izražavak laboratorijskih centra u programima procjene kvalitete koje vode EMQn i DGKL od 1998. godine. Program vanjske procjene kvalitete za citogenetiku, molekularnu genetiku, biokemiju i immunogenetiku je važan korak u izgradnji kvalitete u genotipizaciji.

Ključne riječi: Genotip+analiza, Kontrola kvalitete+standardi, Smjernice u planiranju zdravstvene zaštite

Referencije


Tabela 4. Genski profili

<table>
<thead>
<tr>
<th>Genetski profil</th>
<th>Male infertility profile</th>
<th>LH, FSH</th>
<th>Prolactin, testosterone</th>
<th>Glucose, iron</th>
<th>Chromosome analysis</th>
<th>Cystic fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-chromosome microdeletion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystic fibrosis carrier</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron overload profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total iron binding capacity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemochromatosis genetic test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombotic risk profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coagulation profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycoprotein receptor gene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor II prothrombin gene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

will also be developed to determine susceptibility to common disorders, such as diabetes, heart disease, cancer and infectious diseases, as well as for pharmacogenetic applications to predict drug responses. Recently, strong candidate genes and mutations have been discovered, presenting common genetic diseases such as CF and HFE as leading candidates for population screening (28-30). Concerning predictive genetic tests, apo E genotyping is performed on patients with Alzheimer’s disease and subjects with dyslipidemia or hypercholesterolemia of unknown origin. Carriers of the Apo E2 allele have a low risk of developing Alzheimer’s disease as protective effect. The E4 isoform is associated with elevated LDL-cholesterol and reduced HDL-cholesterol level. Carriers of Apo E4 show a predisposition for late onset Alzheimer’s disease. Homozygote carriers of Apo E4 also have an increased risk of breast-and colorectal cancers (31,32). Advances in post-genome science create a new potential in the diagnosis and therapy, particularly concerning gene environment interactions, metabolomics, informatics for post-genomic integration, quantitative proteomics, pharmacogenetics and healthcare. The data indicate that Apo E4 allele decreases the efficiency of statin treatment and indication for ACE genotype is therapy optimization using ACE inhibitor medication (33,34).

EQA scheme is essential for any laboratory that provides molecular diagnostics. Although results from individual laboratories in EQA schemes are confidential, the collation and publication are important to improve quality and in the development of guidelines and accreditation. Laboratories can benefit from activities of international organizations by sharing experience and harmonizing methodology.
Enabling large-scale pharmacogenetic studies by Investigators

Quantitative PCR.

DNA profiling: a valuable tool for Experiences

Effect of apoE genotype on the hypolipidemia response to pravastatin in an outpatient setting.

J. Sertić

Molecular Diagnostics: Quality Assurance Program in Genotyping. A Croatian Experience

J. Sertić


