



Developments in molecular diagnostics

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Abstract: The present article provides an overview on the rapidly evolving scenario in clinical molecular diagnostics. The concept of clinical validity and utility underpins the use of molecular diagnostic tests in the clinical laboratory, and continuous sharing of expertise between clinical and laboratory partners will enhance appropriateness of molecular testing and interpretation, thus improving personalized medicine. Thanks to the technological advances made in molecular diagnostics, above all in next generation sequencing (NGS) and digital polymerase chain reaction (dPCR) platforms, clinical laboratories benefit from rapid translation of established and emerging molecular tests. The examples of their application which will be discussed encompass cystic fibrosis (CF) (qualitative molecular) and BCR/ABL1 (quantitative molecular) tests. A final consideration on total quality assurance in molecular testing is also made and the role of accreditation is underlined as one of the main factors in guaranteeing the overall quality of molecular test results.

Keywords: Next generation sequencing (NGS); digital polymerase chain reaction (dPCR); quality assurance; real-time PCR (RT-PCR)

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Introduction

Molecular diagnostics has revolutionized modern medicine, genomic data now being increasingly used to guide tailored patient care, thanks to the rapid evolution of technology on the one hand, and medical knowledge on the other. Since 2001, when the first complete human genome sequence was described by Lander *et al.* (1) and Venter *et al.* (2), technology has made enormous progress, with platforms for “next generation sequencing” (NGS) available on the market now allowing complete sequencing of the human genome to be obtained rapidly (within a couple of days) at a relatively low cost (about 1,000 Euros per test). This technological breakthrough has prompted the clinical use of studies identifying the molecular basis of both diseases and treatment, thus resulting in the currently emerging concept of personalized genomics (3).

The parallel progression of technology and discovery in molecular diagnostics is underlined also by the history of Nobel prizes awarded in this field, with three in the sixties for identification of biological basis of molecular diagnostics: Crick, Watson and Wilkins for their discovery of the mechanisms in the biological synthesis of ribonucleic and deoxyribonucleic acid; Jacob, Lwoff and Monod for their discoveries concerning the genetic control of enzyme and virus synthesis, and Holley, and Khorana and Nirenberg for their interpretation of the genetic code and its function in protein synthesis. From the late seventies to the nineties the several technological breakthroughs made included the identification of restriction enzymes, recombinant DNA and the polymerase chain reaction (PCR) method (<https://www.nobelprize.org/prizes/uncategorized/all-nobel-laureates-in-physiology-or-medicine/>).

Within this rapidly evolving scenario, the role of clinical

laboratories has become increasingly relevant, since their mission is rooted at the crossroad between technological improvement and personalized care for patients. The first challenge that clinical laboratories must address is clinical validity and utility (4-7). A molecular laboratory can perform molecular testing to identify alterations in nucleic acids, whereas a clinical molecular laboratory performs clinically useful molecular testing. The analysis of any individual human genome should reveal 4 to 5 million variants that collectively differentiate one individual from another, although a limited number of variants might impact on the health of a subject. While the pathological effects of some disease-associated variants are now known, those of others may thus far be unknown. The term 'molecular diagnostics' should therefore be used for any procedure allowing the identification of alterations in germline or somatic nucleic acids, while 'clinical molecular diagnostics' refers to any procedure allowing the identification of clinically valid and useful alterations in germline or somatic nucleic acids. A clinical validity test enables to correctly classify a patient for any clinical purpose, from diagnosis to treatment.

The concept of 'clinical utility' is wide-reaching, encompassing diagnosis, including pre-natal diagnosis, prognosis, as well as prediction of disease and treatment outcome, all of which converge in a test result that improves medical decision making and patient care (8,9). Taken outside the context of clinical laboratory medicine, molecular laboratories, rather than benefitting patients, might put them at risk. Molecular services, now available on line, are bringing genomics to the public at large, and the consumer market is rapidly expanding (3). This might incur a risk, because genomic analyses performed outside a specific clinical reasoning yield reports that are not necessarily interpretable, and may cause unjustified anxiety, induce unnecessary preventive care or false reassurance in case of negative results, as well as cost escalation. In contrast, clinical reasoning and interchange of expertise between clinical and laboratory partners with involvement of patients, described by Lundberg in 1981 (10) and Plebani in 2011 (11) as the 'brain to brain loop', is advocated in laboratory medicine and appears particularly relevant in the context of molecular diagnostics. Physician-laboratory partnership, therefore, appears not only to be of growing importance, since genomic analyses increasingly becoming an integral part of the clinical diagnostic work-up, but it will also improve patient management in new clinical areas and favor rapid translation of new discoveries in clinical practice (12). In this field in particular, the role of laboratory

professionals is not limited to the analytical and clinical validation of laboratory tests, but also encompasses pre-analytical quality assurance and post-analytical interpretation, due to the extensive data generated, especially in the case of next generation genomic platforms (12).

Basic concepts for molecular diagnostics

In the face of the assumption that "DNA makes RNA makes protein", molecular diagnostics comprise the study of all nucleic acids including their structural and functional variations. The human genome comprises about 28,000–35,000 genes, whose expression is strictly regulated in a cell-, tissue- and context-dependent manner. Before final translation into a functional protein, DNA transcription into mRNA undergoes a series of intertwining processes, including alternative splicing, polyadenylation, decay and translation, which all concur in the complex regulation and fine tuning of cellular proteome. mRNA translation is controlled by non-coding RNAs (i.e., microRNA), short sequences of 20–22 base pairs that pair with complementary mRNA sequences, thus potentially regulating protein translation by enhancing mRNA decay and/or by repressing translation. The genome size of genes correlates moderately with the organism complexity, more complex species exhibiting more extensive gene expression regulation than simpler organisms (13). Variation in DNA sequence might cause disease because it determines variations in protein sequence and function, but also because it might induce variations in protein folding or levels of expression. While the former variations mainly involve exons, the latter can be found in the promoter or enhancer regions of the genes (cis regulatory elements) or in genomic regions distant from the target gene that might influence its expression (trans regulatory elements) because they affect transcription factors or other regulatory factors, such as microRNA. This complex regulation of gene expression explains the complex genetic basis of diseases. It includes variants as well as variations in mRNA, microRNA and long non-coding RNA expression levels (14). In the clinical process of disease diagnosis, prognosis and monitoring, including drug monitoring, therefore, the genomic analysis necessarily includes qualitative (i.e., identification of sequence variants), and quantitative (i.e., measurement of expression levels) assessment of nucleic acids.

Qualitative assessment of nucleic acids

Germline testing, traditionally used for qualitative genetic

analyses in the diagnosis of inherited genetic diseases, is also employed to assess risk genotypes for disease and predicting individual response to drugs (pharmacogenetics). In diagnosing inherited diseases, genetic disorders are classically suspected on the basis of particular clinical phenotypes, gene tests being performed one at a time. This approach has its drawbacks. In many genetic diseases the phenotype can vary, with the risk that patients with non-classical phenotypes may not be diagnosed. Moreover, patients with classical phenotypes may harbor unknown mutations, thus remaining without a definitive diagnosis. For family members of patients harboring known germline mutations underlying inherited diseases, the molecular diagnostic approach might be more straightforward, since the laboratory might directly focus on the family-associated mutations to identify the carrier state. The spectrum of mutations potentially causing inherited diseases varies greatly, depending on the population studied. The American College of Medical Genetics (ACMG) and American Society for Human Genetics (ASHG) have published a series of clinical and technical recommendations for molecular testing for single-gene disorders, including carrier testing for cystic fibrosis (CF), biochemical and molecular testing for factor V (Leiden), diagnosis and carrier testing for fragile X syndrome, these practice guidelines being available on the ACMG website (www.acmg.net). A powerful example of clinical molecular genetics is CF, one of the most common life-limiting autosomal recessive Mendelian diseases that affects about 70,000 humans worldwide (15). The classical clinical manifestations of this disease, due to abnormal viscous secretions of epithelial ducts of the lung, sinuses, pancreas, intestine, biliary tract and male reproductive tract, lead to inflammation, tissue damage and destruction. The variety and complexity of clinical manifestations in CF patients depends on various factors, including (I) the CF transmembrane conductance regulator (*CFTR*) genotype, the disease-causing gene; (II) the contribution of genetic modifiers other than *CFTR*; and (III) environmental contributors including infections (e.g., chronic pulmonary colonization by *Pseudomonas aeruginosa*, a feature of advanced lung disease associated with increased mortality). The *CFTR* gene, known to be the main genetic cause of CF for the last 25 years, encodes CFTR protein, a transmembrane chloride channel regulated by cyclic AMP dependent phosphorylation. This channel has three domains: (I) intracellular ATP binding (nucleotide binding domain 1-NBD1); (II) two regions that anchor the protein to the cell membrane, named membrane spanning domains

1 and 2 (MSD1 and MSD2); and (III) an area containing several phosphorylation sites, the so-called regulatory domain (R domain). *CFTR* gene variations may be pathogenic, potentially determining amino acid substitutions or deletions, protein misfolding, reduced protein synthesis and/or reduced protein stability (16). To date almost 2,000 *CFTR* gene variations have been described in literature, but only some of them are recognized as causative of disease, their number increasing progressively over time: starting with a panel of 23 diagnostic variants proposed in 2001, currently 312 *CFTR* variants are considered disease-causing and recommended for analysis (CFTR2 database, <https://cftr2.org>, accessed 8 August 2018). On extending the panel of *CFTR* variants recommended for screening, the sensitivity in white Europeans increased from about 85% to about 95%. Causative variants mainly determine single amino acid substitution or alter RNA processing (nonsense, frameshift mis-splicing variants) and, in a minority of cases, affect the promoter region of the gene. Not only does the accurate identification of causative variant in CF have a diagnostic impact, but it also allows guided therapy. Some variants can be efficiently treated by targeted drugs, such as ivacaftor in patients carrying the *CFTR* p.Gly551Asp variant (16). This is an encouraging example of the promise of precision medicine, which might benefit in the future from the exploration of genomic data to identify potentially druggable proteins, in line with the Druggable Genome initiative launched in 2014 by the US National Institutes of Health (17). In view of the heterogeneity and complexity of the *CFTR* gene, and the understanding that *CFTR* testing is indicated not only for diagnosing, but also for identifying carriers in the context of pre-conceptional screening, it is the responsibility of the clinical laboratory to define the best possible strategy for guaranteeing all the analytical requirements for high quality results in large series of samples. In this context, the International Standard for Accreditation of Medical laboratories, the International Organization for Standardization (ISO) 15189:2012, helps laboratory professionals, by including important requirements (18): (I) use of methods validated for their intended use (5.5.1.1), whose performances must be monitored not only through internal quality control procedures (5.6.2) but also by interlaboratory comparison programs [as external quality assessment (EQA) schemes] appropriate to the examination and interpretations of results (5.6.3); and (II) use of quality indicators to identify systemic or random errors (19). Although internal quality controls are relevant for the identification of errors, they

cannot cover all possible erroneous results in each and every analytical setting, and usually allowing us only to ascertain the reproducibility of the diagnostic system for the most frequent gene variants. There are several reasons for this: (I) in each analytical series, one to three internal quality controls can be run in parallel with samples, and therefore one to three genotypes (less than 1% of possible findings) can be verified; (II) since it is often extremely difficult to obtain variants infrequent in the population, they are rarely verified; (III) the construction of an internal quality control representing different genotypes made by pooling different DNA is not recommended because, although the material used is commutable, the effects of dilution on less common variants cause the control to differ from the diploid human genomic DNA; (IV) genetic testing is designed not only to identify known mutations, but also unknown mutations, which are not necessarily covered by internal quality controls; and (V) serious preanalytical issues, especially identification errors or sample/aliquot mismatch during preparation, cannot be identified neither using the most stringent internal quality control protocol. If the laboratory sets up a work flow guaranteeing quality control in each and every phase (i.e., pre-analytical, analytical and post-analytical) of the total testing process, these difficulties may be overcome. The work flow must be organized so as to minimize risk of: patient mis-identification; reagents and samples contamination; reduced detection rate of mutations; errors in the interpretation of data (20). Milestones in achieving these objectives are informatic tools and automated sample processing, choice of the most reliable possible detection method, and adequate personnel training.

Carrier screening for the high mutation-spectrum disorder, CF, is a technically challenging test for the clinical laboratory, also considering that only in 1997 did the US National Institute of Health (NIH) consensus conference recommend carrier screening for all couples planning a pregnancy (4). The methods that can be used for *CFTR* gene mutation detection include a number of scanning techniques, such as heteroduplex analysis, RFLP (Restriction Fragment Length Polymorphism), DGGE (Denaturing Gradient Gel Electrophoresis), SSCP (Single-Strand Chain Polymorphism), and MLPA (Multiplex Ligation-dependent Probe Amplification), all of which have different sensitivities and pose different challenges in setting-up and applying on a large scale. Direct sequencing, on the other hand, is considered a sensitive first line or a confirmatory method after scanning (19). Until a few years ago, the only widely

available direct sequencing approach was first generation automated Sanger sequencing, and currently this approach includes next generation DNA sequencing technologies (NGS) (21). While Sanger sequencing identifies linear sequencing of nucleotides 500 bp to 1 kb in length, NGS monitors the sequential addition of nucleotides to immobilized and spatially arrayed DNA templates, offering shorter (30–400 bp) but redundant and overlapped read lengths, one single analysis being capable of sequencing large numbers of different DNA sequences to include the whole genome. Quantitative sequence coverage or depth of NGS platforms, which refers to the average number of times that a base pair is sequenced in a given analysis, can be considered a metric or index of analytical quality. In practice, however, 100% accuracy is never attained, and coverage is not uniform. The more in-depth sequencing coverage needed to correct sequencing errors can range from 30× to 100× depending on the platform error rate and analytical sensitivity and specificity desired. NGS, with respect to Sanger sequencing, improves throughput of sequencing reactions by several orders of magnitude, also allowing cost reduction. Paradoxically, one of the main strengths of NGS (i.e., the high volume of data generation) may limit its widespread use in the clinical laboratory. Megabases to gigabases sequence data are generated and their informatic management and clinical interpretation calls for resources and skills, necessitating time-consuming in-depth bioinformatic analysis. Data analysis, a critical feature of any NGS analysis, should be well defined on the basis of the clinical goal. In CF, for example, due its inherent complex genetics and genotype-phenotype relationship, an unequivocal mutation search strategy has not yet been defined, and complete *CFTR* sequence analysis by NGS might enable the detection of almost all mutations with a detection rate in the area of 98%. However, this identification includes known disease-causing mutations, and also novel or rare *CFTR* variants of unknown clinical significance or variants associated with a broad phenotypic spectrum. A multistep approach is advocated in order to limit costs and time in CF diagnosis and screening, even in the NGS era (22). The first step in CF diagnosis aims to identify the mutation panels more likely associated with CF in the population under investigation, while the second step, which involves the search for extremely rare variants, copy number variations (macrodeletions and macroduplications) and for new variants, allows an increase of about 2% in the detection rate. The first step analysis is the goal of a Level 1 laboratory, which should define the mutations investigated

in the panel and the detection rate for the panel in the population studied. By using a panel of 188 CF causing mutations, with a frequency in the Italian population ranging from <0.0001 to 0.5, the detection rate of an NGS platform is reportedly in the area of 95% (22). Automated data analysis supported by bioinformatic pipelines that filter NGS output to those mutations defined in the panel of clinical utility, is a relevant tool, making NGS analysis more user friendly, and facilitating its introduction in the clinical laboratory.

However, genomic data produced by clinical laboratories currently offer the unique opportunity to enhance medical knowledge, thanks to data sharing across institutions. The cross matching between the big repositories of clinical records and genetic data will enable the collection of more exhaustive information on rare variants and clarify their potential role in causing predisposition to disease, or causing it, and to assess their potential as outcome predictors and drug-response modifiers, thus facilitating the discovery of new drugs, and innovative clinical approaches. This window of opportunity, highlighted by the American College of Medical Genetics and Genomics in 2017 (23), hinges on the assumption that among the 5,000–7,000 rare genetic diseases, each one harboring considerable clinical and genetic variability cannot be extensively evaluated by a single provider, laboratory, medical center, state, or even individual country. Data sharing will provide key clinical attributes of the phenotype, strength of the association between genetic variants and phenotype, range of benign to pathogenic genetic variants, classification of variants of uncertain significance and, last but not least, will allow the harmonization of variant interpretation among laboratories, and improve standards for variant classification. Moreover, this approach might guarantee a rapid clinical translation, thus allowing a timely diagnosis in an increasing number of cases (3,24). The NIH has prioritized data sharing in its research funding, the aim being to find solutions for the main limitations, linked to privacy protection and data management issues (National Institutes of Health, NIH Genomic Data Sharing Policy, <http://gds.nih.gov/03policy2.html>. Accessed 8 August 2018). Protection and data confidentiality call for a comprehensive approach involving scientists, philosophers, community representatives and bioinformaticians to identify and develop robust rules, whereas important bioinformatic efforts are required for management issues.

Gene sequencing data involve approximately 5 memory terabyte (Tb) for each subject and data from 200 subjects,

1 petabyte (Pt), yet genetic data from 2 million individuals would take up the entire Google repository (about 10,000 Pt), exceeding the 1,000 Pb of Amazon and the 300 Pb of Facebook. Closing the gap between genetic information and clinical action will depend on clinical annotation of data, to be managed by academic medical centers and clinical laboratories using bioinformatic tools that simplify data sharing across institutions and guarantee data protection (25).

Quantitative assessment of nucleic acids

Genomic data in clinical laboratories are both qualitative and, increasingly so, quantitative. These molecular diagnostic tests are mainly applied in the fields of microbiology and oncology, the aim in both fields being to identify and quantify “abnormal” DNA or RNA sequences in different matrixes, including biological fluids and tissues (26). In the microbiology setting, viral, bacterial or fungal genetic sequences sharing low homology with the human genome are targets for identification and quantification. In the oncology setting, the detection and quantification of small amounts of somatic DNA mutations in a background of highly homologous non-mutated genomic DNA is the main target, but often poses analytical challenge. In the last twenty years, clinical laboratories have implemented several systems for nucleic acids quantification based on real-time PCR (RT-PCR) technology, using continuous (real time) PCR reaction monitoring by means of fluorescent probes. The exponential kinetic of the PCR is reflected by the exponential increase in fluorescence that indicates the doubling content of the target nucleic acid at any PCR cycle. The higher the concentration of the starting nucleic acid template, the earlier the amplification cycle corresponding to the beginning of the exponential kinetic, which follows the baseline phase, this cycle being known as the threshold cycle C_t or cycle of quantification C_q (i.e., the cycle number at which the amplification plot intersects the threshold line, which is set significantly above the baseline). By using standards with known contents of the target template, it is possible to construct a standard curve linking C_t and the copy number or quantity of nucleic acids for use as a reference for quantifying unknown samples. RT-PCR thus enables the relative quantification of a target to a calibrator, and remains the gold standard for nucleic acid quantification despite limitations that might be due to differences in the amplification efficiency between standards and samples affecting quantification accuracy, to labor-

Table 1 Features that differentiate the qualitative from the quantitative assessment of BCR-ABL1 fusion gene for diagnosing and monitoring chronic myeloid leukemia

Features	Qualitative BCR-ABL1 assessment	Quantitative BCR-ABL1 assessment
Sample	Buffy coat or whole blood EDTA	At least 10 mL whole blood EDTA
Target nucleic acid	mRNA	mRNA
Impact of pre-analytical storage (time and temperature)	Relevant	Mandatory
Reverse transcription into cDNA	Yes	Yes
Primers pairing with the BCR and ABL1 specific sequences that allow PCR amplification of fusion transcript only	Yes	Yes
Internal quality control (IQC) of sample amplification, usually primers pairing ABL1 specific sequences	Yes	Yes
Minimum number of ABL1 copies to be identified	No	10,000
Precise report and evaluation of the BCR-ABL1 Ct	In this case it is sufficient to define the absence (negative samples) or presence (positive samples) of amplification kinetic	Mandatory
Standard curve with calibrators	No	Mandatory
Harmonization across methods/laboratories using a WHO recommended International Standard (IS)	No	Mandatory
Expected amount	BCR-ABL1 transcripts largely exceeding ABL1 transcripts (>50%)	BCR-ABL1 transcripts less than 10% with respect to ABL1 transcripts at three months and less than 0.1% in major molecular response
Third party IQC	cDNA from previous series and/or cell lines (e.g., K562)	The ideal mRNA control is unstable, and this greatly impacts on low amounts, compromising use of any IQC

PCR, polymerase chain reaction.

intensive protocols or to limited reproducibility in some contexts (27). Quantitative molecular diagnostics is currently a relevant component in the diagnostic work up for patients with hematological malignancies. An example of this is the detection of BCR/ABL1 transcripts in chronic myeloid leukemia (CML), a hemopoietic stem cell disease caused by the translocation t(9;22)(q34;q11) with the consequent juxtaposition of the ABL1 gene from chromosome 9 and the BCR gene from chromosome 22, resulting in the BCR-ABL1 fusion gene (28). The transcription of this abnormal gene results in a fusion protein with a high tyrosine kinase (TK) activity that is targeted by tyrosine kinase inhibitors (TKIs), of which three are commercially available for frontline CML treatment: TKIs imatinib, dasatinib and nilotinib. Gene translocation causes shortening of chromosome 22, known as the Philadelphia chromosome

(22q-). Cytogenetic analysis allows the identification of the percentage of Ph+ cells between metaphases in the bone marrow. The identification and quantification of BCR-ABL1 transcripts in blood and bone marrow is possible, thanks to molecular diagnostic techniques. While the qualitative identification of BCR-ABL1 transcripts in blood is recommended at baseline of the diagnostic work up, quantitative assessment is recommended for monitoring response to TKIs (28). Qualitative and quantitative BCR-ABL1 assessment might be successfully performed using RT-PCR systems, although with relevant differences in the analytical set-up, as summarized in *Table 1*. BCR-ABL1 mRNA quantification is the most sensitive available tool for monitoring residual disease. According to guidelines (28), this assay should be performed every 3 months, with results expressed according to the IS (BCR-ABL1IS %) in order

to guarantee comparability of results among laboratories. Following therapy, thresholds differ depending on time points: optimal response is achieved when values are lower than 10% at 3 months, lower than 1% at 6 months, and lower than 0.1% at 12 months, respectively. Lower values (<0.01%) indicate treatment free remission (28).

As in DNA sequencing, technology has also improved for nucleic acids quantification, and digital PCR (dPCR), now undertaken by clinical laboratories allows the absolute quantification of nucleic acid templates and overcomes the shortcomings of RT-PCR (27,29). In dPCR, the sample is initially partitioned in an oil emulsion with a high number of droplets, each containing few or no templates. The PCR reaction, based on specific primers and fluorescent probes as in RT-PCR, takes place in each droplet, yielding a fluorescent signal in droplets containing the template. After PCR, the readout of positive droplets allows the quantification of fraction of positive droplets, the statistically defined accuracy being based on Poisson's statistics. Unlike RT-PCR, template partitioning in single droplets allows the separation of mutated and non-mutated nucleic acids, thus reducing template competition and enabling detection of rare mutations in a background of wild type sequences. Therefore, while RT-PCR quantification is based on continuous fluorescent PCR reaction monitoring, dPCR is an end-point analysis of fluorescence of individual micro-reactors that enumerates a series of positive and negative outcomes, converting continuous or analogue curves into binary or digital signals. dPCR nucleic acid quantification: (I) is independent from a standard curve, being based on binomial statistics that mathematically define its inherent accuracy and performance metrics; (II) allows the reliable detection of very small amounts of mutated, in a background of non-mutated, sequences since it precludes the pitfalls deriving from template competition; and (III) enhances tolerance to enzyme inhibiting substances.

An integrated work-flow incorporating NGS and dPCR with standard RT-PCR and Sanger sequencing platforms in the clinical molecular laboratory, will allow translation into the clinic of several new applications, including the noninvasive pre-natal diagnosis of genetic diseases by detecting fetal DNA in maternal blood, the noninvasive diagnosis of cancer associated somatic DNA mutations, also known as liquid biopsy, and the early identification of graft rejection in transplantation medicine by detecting graft DNA in recipients' plasma. However, there is an urgent need for quality assurance initiatives from established and emerging technologies.

Quality assurance in molecular diagnostics

Qualitative genetic tests have some unique characteristics distinguishing them from other diagnostic tests: (I) since it is inherited, the genetic makeup of an individual does not change throughout a lifetime and, since patients are usually tested once, an erroneous result will stay with them for life; (II) the result of a genetic test can profoundly impact family members; (III) since both the public and medical staff have great confidence in genetic testing, they share the belief that a result is the "state of the art", rarely considering the fact that it might be wrong (30). Yet, of 104 laboratories participating in five EQA schemes for CF for 3 consecutive years, none made no mistakes (30). This 'fallibility' may also apply to quantitative molecular tests, which yield results varying over time, any variation potentially impacting significantly on patient care. Laboratory accreditation is the most effective available approach for reducing the error rate in laboratory testing, molecular testing included, the ISO 15189 being the major standard, as was demonstrated by McGovern *et al.* in their International Survey of Molecular Genetic Testing Laboratories (31). The authors demonstrated that accreditation status was the most important predictor of a quality assurance index, based on general quality and specific molecular diagnostics standards as recommended by the ACMG, the College of American Pathologists (CAP), the UK Clinical Molecular Genetics Society, the National Committee of Clinical Lab Standards (NCCLS) and the Swiss Society of Medical Genetics (SSMG). Yet relatively few laboratories in Europe performing molecular testing have been accredited (23%) or certified (26%), and more than half have been neither accredited or certified (32).

In molecular laboratories, quality assurance must guarantee the standard requirement of each and every laboratory test (i.e., traceability of all pre-analytical, analytical and post-analytical steps) (33), but particular attention must be focused on the validation and/or verification of examination procedures according to the intended use, reference material to assess within-laboratory reproducibility, personnel training and continuous education to guarantee adequate skills for the rapidly evolving scenario, with new tests and technologies, and adherence to EQA and quality indicators. In the molecular biology laboratory, the number and percentage of unexpected results of reference materials (positive controls) and of blanks with all PCR components except for target DNA

(negative controls) could be considered quality indicators of within laboratory reproducibility and of the control of laboratory contamination, respectively. The number and percentage of molecular test undergoing EQA (adherence to proficiency testing) with the number and percentage of failures could be considered quality indicators of laboratory accuracy (34).

In conclusion, in view of the increasing demand for molecular diagnostic tests, hand with the rapid rate of development of new technologies and commercial testing kits, qualitative and quantitative molecular genetic testing will be increasingly carried out in public or private general pathology laboratories, but also in commercial laboratories. In the interests of patients' safety, the highest possible quality standards must be guaranteed by fully accredited molecular genetic testing laboratories.

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