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Laboratory Medicine:
Meeting the needs of the Mediterranean nations
Part 2

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Foreword from the editor-in-chief

János Kappelmayer, MD, PhD

This issue of the eJIFCC incorporates the second part of a series of manuscripts that were presented at the conference entitled “**Laboratory medicine: meeting the needs of the Mediterranean nations**”.

The conference was held in Rome, between July 2-4, 2018, with professor Sergio Bernardini as the Conference President.

This issue is part 2 of a two-part series, and contains articles covering the following sections:

- Improving efficiency in laboratory medicine;
- Perinatal and pregnancy laboratory medicine; and
- Mediterranean diet and the area’s specific diseases.

János Kappelmayer
Editor

Traceability in laboratory medicine: what is it and why is it important for patients?

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ABSTRACT

The between method variability of patient results is a source of uncertainty that can have adverse consequences for patient safety and clinical outcomes. Globalisation requires that laboratory medicine results should be transferable between methods. Traceability in laboratory medicine aims to reduce between method variability so that results are independent of time or location. Application of the metrological traceability chain facilitates a universal approach based around the preparation, adoption and use of higher order international commutable reference materials and reference measurement procedures, supported by expert reference laboratories. Global collaboration is required, involving several different stakeholder groups ranging from international experts to laboratory medicine specialists in routine clinical laboratories.

INTRODUCTION

Laboratory medicine results influence a high percentage of all clinical decisions. Patients expect that different laboratories, using different methods, will give the same result for an analyte measured in a clinical sample. Often this is not the case and an inappropriate clinical decision for a patient may be the consequence. Laboratory medicine specialists have a professional responsibility to provide a high-quality service that is optimised to the needs of the patient [1].

Traceability in laboratory medicine aims to reduce between method variability so that results are independent of time or location [2]. Achieving traceability is a global multi-stakeholder cooperative activity involving metrologists; international standards organisations; scientific and clinical experts from international professional bodies; healthcare regulators; and the in-vitro diagnostics (IVD) industry that is responsible for the manufacture and sale of diagnostic testing systems [3]. The Joint Committee for Traceability in Laboratory Medicine (JCTLM) was established to co-ordinate the activity of these stakeholders, to provide educational support for traceability, and to establish and maintain a database of reference materials, reference methods and reference laboratories [4].

THE IMPORTANCE OF REDUCING BETWEEN-METHOD AND BETWEEN-LABORATORY VARIABILITY

There are several reasons why efforts should be made to reduce between-method and between-laboratory variability [5]. These include:

- Improving patient safety
- Facilitating patient empowerment
- Ensuring public confidence
- Enabling consolidation and networking
- Supporting laboratory accreditation

- Implementing evidence-based clinical guidelines
- Guaranteeing clinical governance
- Adopting common informatics
- Introducing the electronic patient record

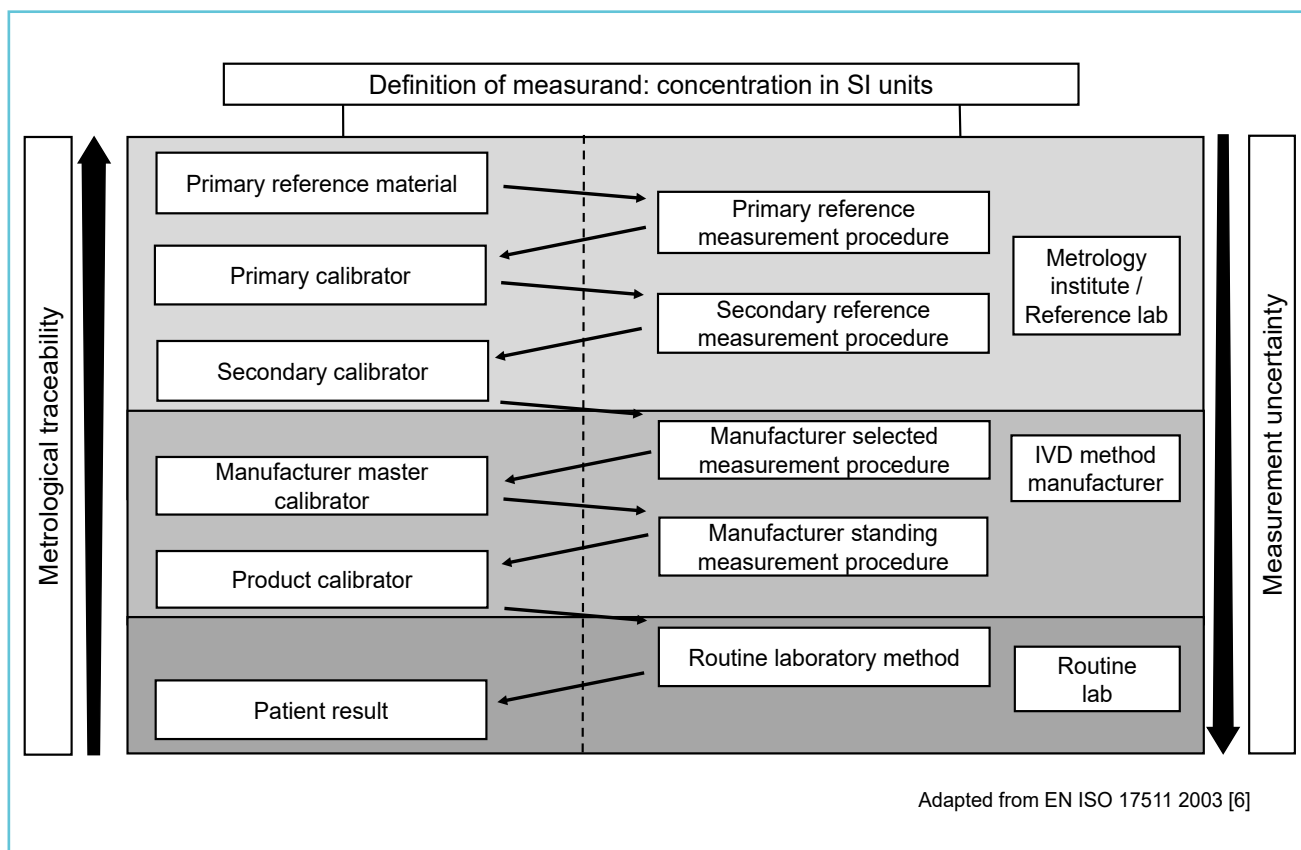
TRACEABILITY IN LABORATORY MEDICINE AND THE METROLOGICAL TRACEABILITY CHAIN

Metrology is the science of measurement. The basics of measurement involve:

- A measurable property, known as a quantity (e.g. concentration)
- Definition of the measurand – the quantity that is intended to be measured. The description of the measurand should include the matrix (e.g. plasma); the component (analyte) of interest, and the amount of substance concentration
- The units in which the measurement will be made. Metrological traceability requires the international system of units (SI) or units with well-established conversions
- The uncertainty with which the measurement can be made

Metrological traceability is the property of a measurement result, which can be related to a reference through a documented unbroken chain of calibrations. The principles of a reference measurement system for establishing metrological traceability are described in the ISO17511:2003 document [6]. The components of a reference measurement system comprise reference materials (calibrators) and measurement procedures (methods), both of which exist at different hierarchical levels.

The inter-relationship between the components of a reference measurement system describes the metrological traceability chain [6]. Figure 1 depicts this traceability chain with higher order

Figure 1 Metrological traceability chain for laboratory medicine

reference materials and measurement procedures at the top and lower order towards the bottom. This hierarchy is depicted by the rising 'metrological traceability' arrow. Descent through the traceability chain is accompanied by increasing measurement uncertainty as depicted by the downward arrow.

The traceability status of an individual measurement result depends on the existence of an unbroken chain to higher order materials and/or measurement procedures. To be effective the unbroken chain requires commutable materials [7] and sufficiently low imprecision at each step. In the case of structurally simple molecules, like many of those measured routinely in clinical chemistry, it is possible to have a complete unbroken chain to primary reference measurement procedures and primary reference materials. Even for some protein molecules it is possible

to achieve full metrological traceability by using a unique, signature peptide as the primary reference material. The measurement of serum cholesterol and blood haemoglobin A1c are examples of full metrological traceability where the agreement between methods is excellent [3]. Serum parathyroid hormone and blood haemoglobin A2 are examples where the between-method variability is unacceptably high, causing clinical risk. In both these cases method standardisation/harmonisation initiatives have commenced [3].

For many biological materials, including complex proteins and viruses it is not possible to prepare secondary calibrators. In these circumstances international conventional calibrators are adopted as being the highest order materials available. The global acceptance of such international conventional calibrators can facilitate reduced between method variability.

SOURCES OF REFERENCE MATERIALS AND REFERENCE MEASUREMENT PROCEDURES

The JCTLM maintains a database of reference materials, reference measurement procedures and reference laboratories [8]. Strict criteria are required for inclusion in the JCTLM database, including evidence of commutability of reference materials and measurement uncertainty.

The World Health Organization Expert Committee for Biological Standardization (WHO-ECBS) maintains a catalogue of international conventional calibrators for blood products and biological standards [9].

CHALLENGES IN IMPLEMENTING TRACEABILITY IN LABORATORY MEDICINE AT A GLOBAL LEVEL

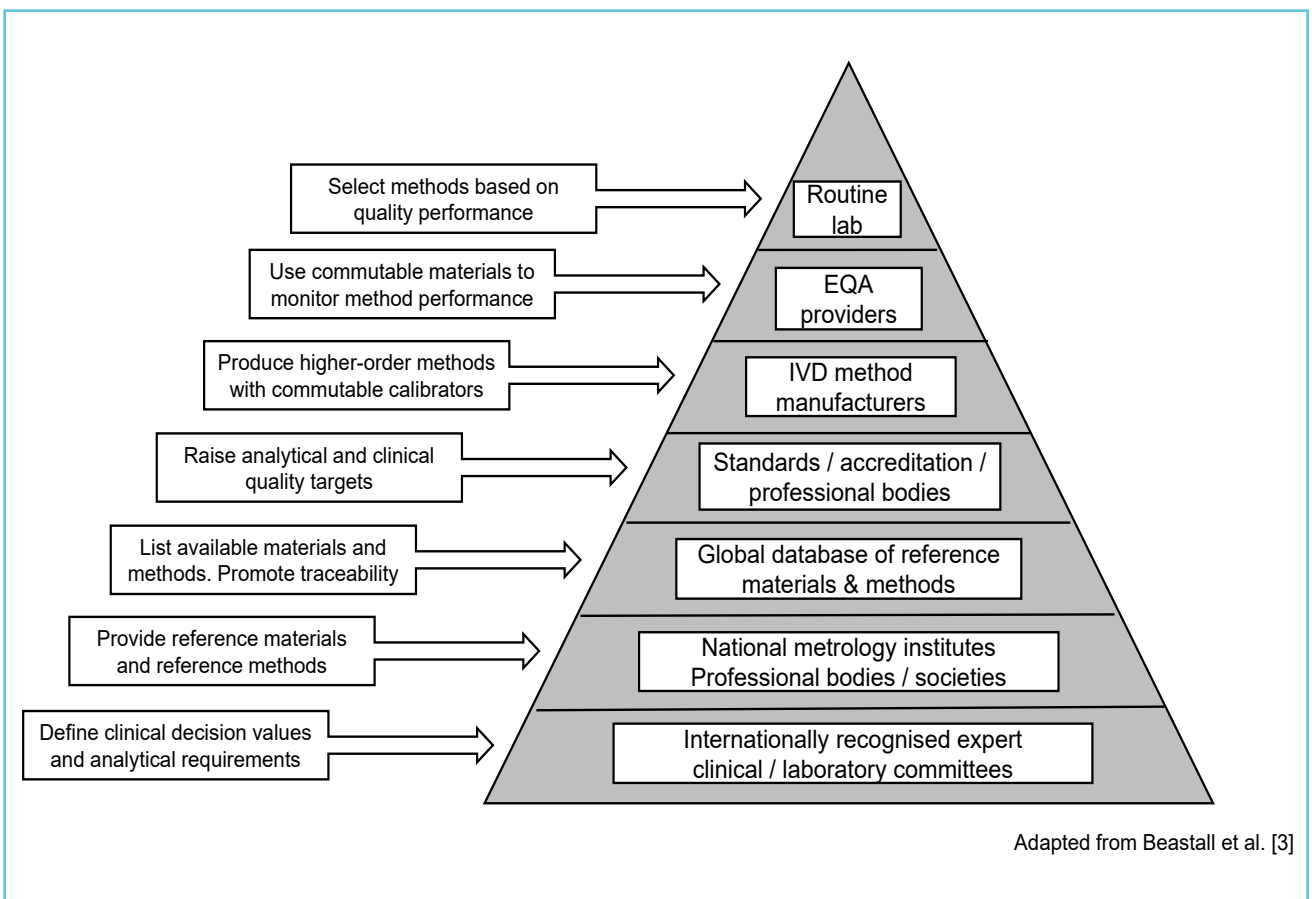
There are several challenges to implementing global traceability [3]. These include:

- Geographical differences
- Lack of uniformity of units
- Complex analytes
- Global coordination

STAKEHOLDERS IN IMPLEMENTING TRACEABILITY IN LABORATORY MEDICINE

The stakeholders involved in delivering traceability in laboratory medicine into routine practice are summarised in Figure 2.

Figure 2 Global stakeholders involved in delivering traceability in laboratory medicine into routine practice



The initiative begins at the bottom of the triangle with international recognition of the need for traceability for a specific analyte. Thereafter, international and national standards organisations and metrology institutes are responsible for producing and listing the available reference materials and measurement procedures. These are used by the IVD method manufacturers to produce the methods made available for routine use with their performance evaluated through external quality assessment (EQA) schemes based on commutable control materials.

ACTION PLAN TO IMPLEMENT TRACEABILITY IN LABORATORY MEDICINE AT A GLOBAL LEVEL

The implementation of traceability in laboratory medicine at a global level requires a coordinated action plan. This can be derived from Figure 2 by assigning actions to each of the seven stakeholder groups [3].

1. Internationally recognised expert clinical/laboratory committees:

- Develop international consortium for communication and sharing information on the need for traceability
- Prioritise and agree methods that require harmonisation and issue invitations to expert groups to undertake method harmonisation projects [10]

2. National metrology institutes/international professional bodies/societies:

- Develop commutable reference materials and measurement procedures for individual analytes to the highest available order of metrological traceability
- Publish the outcome of harmonisation projects in peer-reviewed scientific literature

3. Global database of reference materials and methods:

- Using freely available lists and catalogues publicise available reference materials and methods that meet agreed standards, including information on commutability and measurement uncertainty
- Provide educational support materials to promote the importance of traceability in laboratory medicine

4. Standards/accreditation/professional bodies:

- Include traceability in laboratory medicine in the training of laboratory medicine specialists and in the standards required for laboratory accreditation
- Provide educational support materials to promote the importance of traceability in laboratory medicine

5. IVD method manufacturers:

- Produce IVD methods that conform with the highest available order of metrological traceability
- Provide details of the traceability status of methods in the information for use documentation

6. EQA providers:

- Promote the use of commutable EQA materials
- Provide educational support about traceability for EQA scheme participants

7. Routine laboratory medicine specialists:

- Know the traceability status of the methods used and understand the measurement uncertainty involved
- Educate staff about traceability in laboratory medicine and its importance to healthcare

Resources for educational support are available from JCTLM [4]. Readers of this article are invited to discuss with their peers how they can contribute to the coordinated action plan.

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Laboratory medicine in Palestine

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molecular testing, quality assurance

ABSTRACT

Background

Laboratory Medicine (LM) is one of the cornerstones of healthcare. In Palestine, 3.5% of health expenditure is allocated to clinical laboratories.

Methodology

The Palestinian Ministry of Health (MOH) started to invest in the development and expansion of laboratory services including the introduction of full automation in blood banks and histopathology, molecular testing in microbiology, and testing for autoimmune diseases and metabolic disorders. Improvements have not been limited to new tests but also included external quality assurance (EQA) and accreditation programs.

Results

Latest investments have cut the costs of purchasing tests from outside MOH by more than 3.6 million dollars during the last five years. Al-Quds University established a Center for External Quality Control in LM which was supported by the Palestinian MOH and the National Metrology Institute of Germany. This has led to a significant improvement in the performance of the affiliated laboratories. An accreditation unit was established within the MOH, yet the

number of laboratories that have been accredited with ISO-15189 are still limited but significantly increasing.

The academic institutions have been working in parallel to the MOH in improving LM in Palestine. A new academic curriculum for LM has been developed according to the quality standards of curricula-based competencies determined by the needs of the labor market and the emerging technologies.

Conclusions

Despite the invested efforts, still, the Palestinian LM suffers from shortage of human resources which are qualified in the new emerging diagnostic approaches.



BACKGROUND

The Palestinian population is approximately 4.8 million living in two geographically separate regions; the West Bank and Gaza Strip, ruled by the Palestinian Authority since 1994 [1]. The Palestinian Ministry of Health (MOH) is the primary provider of healthcare services for the Palestinian population. Other healthcare providers include non-governmental organizations (NGOs), United Nations Relief and Works Agency (UNRWA) for Palestine Refugees and Military Medical Services.

The Palestinian population is a young population, although it has been aging over the last two decades. Further, decrease in infant and maternal mortality rates indicate improvements in the healthcare system. Nevertheless, changes in morbidity and mortality patterns which have been shifting from infectious diseases to non-communicable diseases raise new challenges to the healthcare system, especially with the increasing demand for personalized and quality patient care [2].

MEDICAL LABORATORIES IN PALESTINIAN HEALTHCARE SYSTEM

Issues and challenges

The Palestinian MOH has been working on building a comprehensive strategy to address the needs of the healthcare system and LM in particular. The total health expenditure was approximately 430 million dollars in 2016, an increase of 40% since 2010. Of the total health expenditure, 3.5% is allocated for medical laboratories [2, 3]. The role of medical laboratories in medical practices has been highlighted over the last few years, and since then several investments to improve the capacity and quality of LM have taken place. The MOH increased the number of laboratories in their facilities. The statistics of 2016 showed that the number of laboratories increased to 14 medium laboratories (hospital laboratories), 188 peripheral laboratories, three histopathology laboratories and one public health central laboratory. Six-hundred and thirteen laboratory technicians have been employed in the MOH laboratories, representing 12% of the Palestinian laboratory technicians [2]. The improvements have not been limited to only the number of laboratories, but also included investments in new equipment to increase the range of tests performed within the MOH laboratories. The total number of laboratory tests performed within the laboratories of the MOH increased from 4.5 million tests in 2011 to 6.9 million in 2016 [2]. Consequently, the Palestinian MOH significantly reduced the purchased services from the neighboring countries' healthcare institutions.

Education and training

Education in medical laboratory sciences (MLS) was introduced in the West Bank and Gaza Strip in 1979. Currently, ten MLS programs exist; one diploma, three master programs, and the remainder offer bachelor degrees. These programs are

certified by the Palestinian Ministry of Education and Higher Education and by the National Accreditation and Quality Assurance Commission which is the only body responsible for accreditation and quality assurance of Palestinian educational programs.

Palestinian MLS academic curricula lack international accreditation. Moreover, the shortage of specialized faculty members in many fields of MLS in educational institutions is one of the limitations of education in Palestine, especially in the newly emerging fields. This problem is expected to grow even further as older faculty members retire in addition to the increased need for new technical skills in MLS.

The number of training facilities and supervisors is limited, and annually around 300 students require training in medical laboratories as part of their graduation requirements. This issue created a surplus in laboratory technicians whose skills are not the most optimal. Among the graduates, and according to the records of the Palestinian Medical Technology Association (PMTA), there currently is around a forty percent unemployment rate, primarily among females. Further, the majority of the graduates are bachelor degree holders.

Recently, Al-Quds University launched a joint project with prominent health institutions aiming at improving the education quality of the MLS graduates by developing a new academic curriculum according to national and international standards. The new curriculum will be developed according to the new quality standards of the curricula-based competencies determined by the labor market needs and the provision of the partner institutions. In addition, the emerging technologies in LM will be included within the curriculum which will contribute to enhancing the hands on practice of Palestinian laboratory professionals.

Molecular diagnostics in disease control

Molecular testing is becoming a crucial diagnostic tool in the setting of inherited genetic diseases, neoplastic diseases, and infectious diseases. For the last decade, morbidity and mortality patterns of the Palestinians shifted from communicable to non-communicable diseases. The leading causes of mortality during 2016 were cardiovascular diseases, cancer, cerebrovascular diseases, and diabetes which are responsible for 65.4% of all deaths among Palestinians [2].

Molecular testing has only been introduced into the Palestinian MOH medical laboratories since 2010. Molecular testing requires specialized training and skill set. Currently, ninety-four molecular tests are available at the MOH Central Public Health Laboratory which are used to diagnose viral and bacterial infectious agents. Infectious diseases and respiratory diseases are responsible for less than 10% of all deaths in the West Bank and Gaza Strip [2]. During the period between 2010 and February 2017, a total of 19403 molecular tests had been performed to diagnose seven primary causes of respiratory tract infections including influenza A and B, *Bordetella pertussis*, Adenovirus, enteroviruses, *Streptococcus pneumoniae* and Respiratory Syncytial Virus (RSV). Among these, only 39.3% of the test results were positive and had a confirmed differential diagnosis. Molecular testing of infectious diseases is an essential tool not only for accurate and timely diagnosis and treatment monitoring but also, for disease control and surveillance. Therefore, more molecular tests should be introduced to cover a wider range of infections to enhance the differential diagnosis of communicable diseases. In addition, molecular testing is crucial in the diagnosis of genetic diseases that are common among Palestinians such as thalassemia, hemophilia, inborn errors of metabolism, and cystic fibrosis, but as yet they are not introduced into the Palestinian laboratories.

Moreover, the importance of molecular testing in personalized medicine and in oncology, in particular, has been confirmed, shedding light on its value for accurate diagnosis, targeted therapy, and oncology genomic-based effective treatment. Furthermore, the use of biomarkers is the essence of individualized oncology and has already proven to result in more effective treatment protocols [4]. The use of personalized medicine assays requires a highly qualified team of molecular biologists who are almost absent in the West Bank and Gaza Strip, creating a challenge in introducing the new emerging tests into the Palestinian laboratories.

Accreditation and quality assurance

Efficiency, cost-effectiveness, and quality of healthcare are the top priorities in today's world. Accurate laboratory tests are required for the diagnosis of diseases and monitoring of treatment. Testing errors can be reduced through quality management and accreditation to ensure reliability and quality results. Over the last few years, quality improvement in medical laboratory testing received increased attention from the MOH. In 2014, the Palestinian MOH and the National Metrology Institute of Germany: Physikalisch – Technische Bundesanstalt (PTB), supported the external quality assurance program (EQAS) that was established by Al-Quds University in 1996. The center's activities include preparation of quality control (QC) samples, distribution of samples to the participating laboratories, collection of the QC samples' results, and finally analyzing the results to evaluate the performance of the affiliated laboratories [5]. Subsequently, participation in the EQAS program became mandatory in 2016, resulting in a significant increase in the number of affiliated laboratories from 148 to 435. The program testing panel currently includes the basic laboratory tests in chemistry, hematology and hemostasis. Each laboratory's performance is determined by

calculating the mean-variance index score (VIS). Findings from the EQAS program showed a significant improvement in the performance of the laboratories upon joining the program compared to their performance before joining. As regards to laboratory accreditation, up until 2016 the only laboratory that had already received the ISO 15189 accreditation was the Central Public Health Laboratory (CPHL), which is under the supervision of MOH. It is noteworthy that, recently, a few more laboratories have started working on implementing quality management systems.

Recommendations

The role of medical laboratories in healthcare has been highlighted over the past few years within the Palestinian MOH. Several improvements that facilitated accessibility and added a wide range of new tests were achieved. In addition, the importance of quality assurance and accreditation in providing quality care became well recognized. Regardless, continuous efforts are still required. In this context, there is an urgent need to work on broadening the use of molecular testing in LM for non-communicable diseases especially cancer. Moreover, personalized medicine assays should be implemented to provide the best possible cancer care in terms of treatment and prediction of prognosis of the patient. On the top of that, academic institutions should play an integral part in the continuous improvement of laboratory technicians' qualifications by updating the academic curricula to include the most recent approaches and technologies in LM. In addition, partnership and collaborations with international bodies specialized in laboratory medicine should be initiated to offer opportunities for training and capacity building of human resources which will consequently enforce the transformation process. Finally, a coherent national policy for accreditation and quality assurance should be emphasized and implemented by the Palestinian

MOH to all laboratories and for all types of tests to ensure a high quality of the provided laboratory services.

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Inter-laboratory comparisons and EQA in the Mediterranean area

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Scheme for Clinical Laboratories.

ABSTRACT

The role of Proficiency Testing schemes (PT) or External Quality Control programs (EQA), involves the use of inter-laboratory comparisons for the determination of laboratory performance. EQA-PT schemes are of primordial importance to the analytical quality, standardization of methods and harmonization of the results. Laboratories are familiar with EQA-PT schemes as they are a prerequisite for their accreditation according to the ISO/IEC 15189 standard.

The IFCC Committee on Proficiency Testing (C-PT) conducted a survey among the colleagues of the Mediterranean countries in order to evaluate the status of the EQA-PT providers in the region, their acceptance among laboratories, and possible issues in their implementation. The survey was organized electronically and we received 59 replies from colleagues (IFCC National Representatives and affiliated EQA-PT providers), from 17 of the 23 countries (74%) of the Mediterranean area.

We concluded that there is a broad difference in the application of the rules of External Quality Control programs or Proficiency Testing schemes, among the Laboratories in the Mediterranean countries. Moreover, as the Accreditation of the Laboratories is

not mandatory in the majority of these countries, there is no valid reason for participation in EQA-PT schemes.



The role of Proficiency Testing schemes (PT) or External Quality Control programs (EQA), involves the use of Inter-Laboratory comparisons for the determination of Laboratory Performance (1). The application of comparative analysis adds value to the competency of the laboratories and provides room for improvement (2). Laboratories are familiar with EQA-PT schemes as they are a prerequisite for the accreditation according to the ISO/IEC 15189 standard (3).

EQAs-PT schemes are of primordial importance to the analytical quality, standardization of methods and harmonization of results (4-6).

IFCC has been recognized for its leading role and efforts towards the standardization of Clinical Laboratory methods. Many commercially available IVD tests acknowledge, in their inserts, the use of IFCC methods. The work of the federation also led to novel analytical approaches, which, although recognized by the different regulating bodies are not, yet, unanimously accepted worldwide as in the case of HbA1c.

Meanwhile, the IFCC possesses the resources and knowledge via the involvement of member societies running Proficiency Testing schemes as non-profit organizations, and also the expertise provided by distinguished scientists who have the know-how to design and produce the necessary novel control materials for specialized External Quality Control programs.

The IFCC Committee on Proficiency Testing (C-PT), ex Task Force on Proficiency Testing (TF-PT), helps publicize the existence of specialized and of general-purpose EQA-PT schemes in the field of clinical Chemistry - Laboratory Medicine. The main project of the committee is the creation of

an online database - web application (PTDB) accessible via web browsers and via specific client applications, for the major mobile platforms, offering broader functionality and ease of use. The foundation of this database are the analytes (tests, measurands) and the corresponding analytical methods (assays, instruments, reagents, etc.).

The second part of the database is the PT providers section containing all their contact information, and their programs, their accreditation or certification status, etc. The providers part of the database was completed in mid-February 2017 and June 2018, the PTDB includes 68 providers from all around the globe. The PTDB can be consulted directly at <http://ptdb.ifcc.org/providers>.

On the occasion of the First IFCC, EFLM, AFCB Conference, "Laboratory Medicine: Meeting the needs of Mediterranean Nations", held between 2-4 July 2018 in Rome, Italy, the C-PT conducted a survey among the colleagues from the Mediterranean countries in order to access the status of the EQA-PT schemes in the region, their acceptance, as well as possible issues in their implementation.

The survey was organized electronically, and **59 colleagues replied**, mostly IFCC National Representatives and affiliated EQA-PT providers, from **17 of the 23 countries** of the Mediterranean area (**74%** of the Mediterranean countries).

For the first question

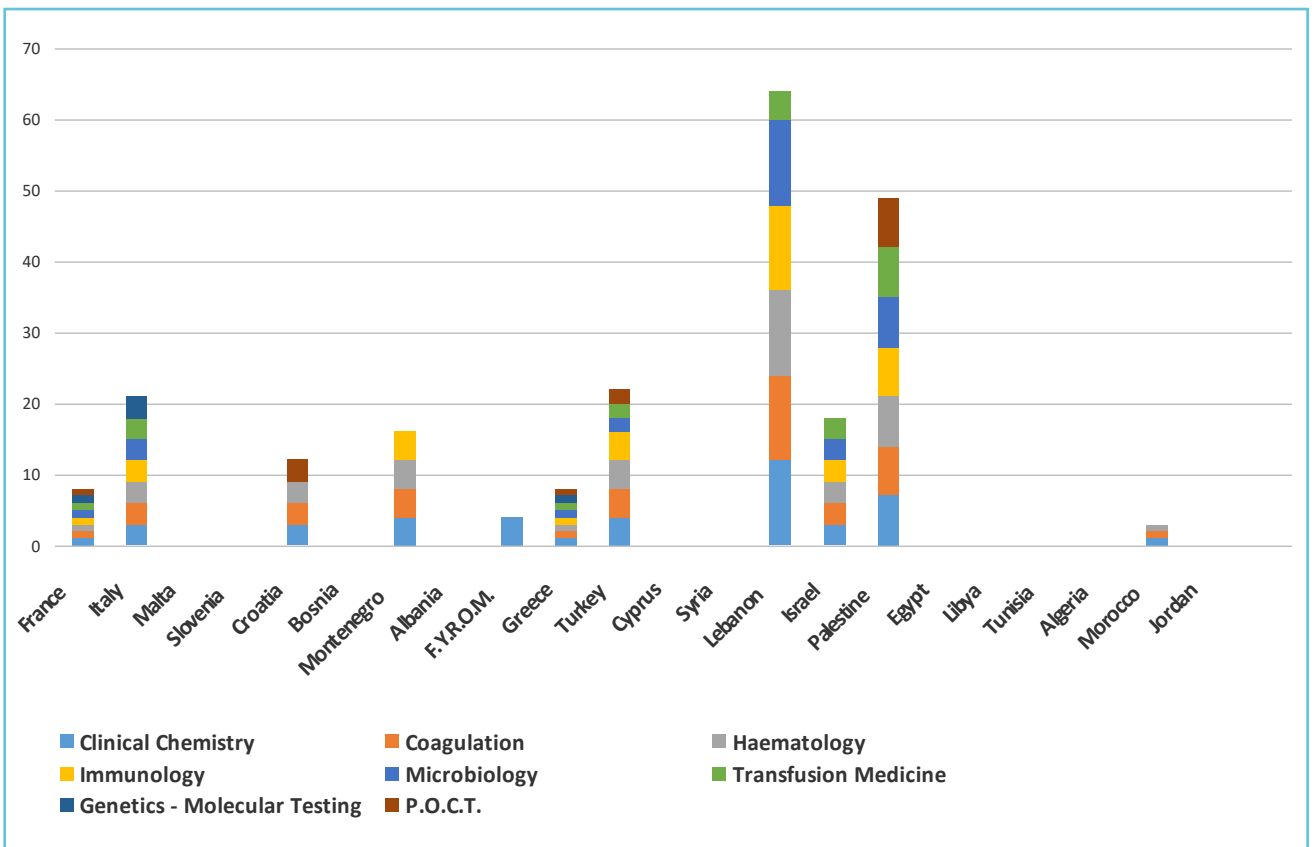
"Is the participation of medical laboratories in External Quality Control - Proficiency Testing schemes in your country mandatory, by:"

53% of the countries replied by **law**, **29%** of the countries replied by **Scientific Society guidelines**, **6%** of the countries replied by **Social Security** organization in order **to reimburse the tests** and finally **47%** of the countries replied

Figure 1 Reasons for participation of medical laboratories in EQA-PT schemes in Mediterranean countries



Figure 2 Frequency of participation by discipline of medical laboratories in EQA-PT schemes in Mediterranean countries



that the participation of Medical Laboratories in EQA-PT schemes **is non mandatory at all** (Figure 1).

For the second question
“If it is mandatory in which sectors and which is the minimum frequency of participation per year”

71% of the countries replied in **Clinical Chemistry** with minimum frequency of participation per year 1 and maximum 12, (*median 3*), **59%** of the countries replied in **Coagulation** with minimum frequency of participation per year 1 and maximum 12, (*median 3*), **59%** of the countries replied in **Hematology** with minimum frequency of participation per year 1 and maximum 12, (*median 3*), **47%** of the countries replied in **Immunology** with minimum frequency of participation per year 1 and maximum 12, (*median 3*), **41%** of the countries replied in **Microbiology** with minimum frequency of participation per year 1 and maximum 12, (*median 2,5*), **41%** of the countries replied in **Transfusion Medicine** with minimum frequency of participation per year 1 and maximum 7, (*median 2,5*), **18%** of the countries replied in **Genetics - Molecular**

Testing with minimum frequency of participation per year 1 and maximum 3, (*median 1*), and **29%** of the countries replied in **Point Of Care Testing (POCT)** with minimum frequency of participation per year 1 and maximum 7, (*median 2*), (Figure 2).

For the third question
“The External Quality Control - Proficiency Testing schemes are organized by the:”

18% of the countries replied by the **State**, **41%** of the countries replied by **a Scientific Society**, **47%** of the countries replied by a **non-profit organization** and **76%** of the countries replied by **commercial companies** (Figure 3).

For the fourth and last question
“Is the accreditation of the laboratories mandatory by:”

30% of the countries replied by **law**, **12%** of the countries replied by **Social Security** organization in order **to reimburse the tests** and finally **58%** of the countries replied that the accreditation of Medical Laboratories **is non mandatory at all** (Figure 4).

Figure 3 Organizers of EQA-PT schemes for medical laboratories in Mediterranean countries



Moreover, we received as remarks that:

In Italy

- The accreditation is mandatory based on Regional requirements.
- Some laboratories are accredited, on voluntary basis, according to ISO 15189:2012.
- The frequency of EQA Schemes participation is not specified in the requirements and depends on test typology. Generally fluctuates, from two surveys (4 control materials) to six surveys (12 control materials) per year.

In Spain

- The establishment of the conditions and technical requirements for clinical laboratories corresponds to the respective Spanish Autonomous Region where the laboratory is located.
- In the Autonomous Regions in which this matter is regulated, the respective regulations require the participation of laboratories in external quality assurance programs, organized by official bodies or by scientific societies of recognized prestige and authority.

- The frequency of participation depends on regional regulation also and, in some of them, it is stated that the frequency should be at least once per month for common tests.

The External Quality Control in Jordan

- Is not mandatory.
- Ministry of Health Law and guidelines encourage the participation in External Quality Assurance programs.
- In fact, the Laboratory Directorate of the Ministry of Health had established an external quality control scheme for HBsAg, HCV, and HIV tests, where they provide two samples twice a year for each of these tests free of charge, and it will be mandatory soon for all laboratories performing these tests to participate in this program.

In Croatia

- The accreditation is not mandatory now, but it will be in the near future.
- The CROQALM is a non-profit organization, one of the CSMBLM activities, where, according to the Croatian Chamber for Medical

Figure 4 Accreditation status of medical laboratories in Mediterranean countries



Biochemistry, each laboratory in Croatia must participate. Laboratories also participate in other type of external quality control according to their practice, accreditation etc.

In conclusion, we can remark that **there is a broad difference in the application of the rules of External Quality Control programs - Proficiency Testing schemes among the Laboratories in the Mediterranean countries**. Moreover, as the **Accreditation of the Laboratories is not mandatory** in the majority of Mediterranean countries, there is not any trend for increased participation in the EQA-PT schemes that helps the harmonization of the quality specifications in Laboratory Medicine.

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An evidence-based laboratory medicine approach to evaluate new laboratory tests

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ABSTRACT

The evidence-based recommendations for the evaluation of new tests to be used in practice are a key issue to improve diagnostic clinical pathway inducing effective care. Emerging precision or personalized medicine requires innovative and pioneering biomarker tests for molecularly targeted therapies, possibly fitted for the individual patient's condition. Beyond the traditional analytical specifications that should guarantee the proper clinical diagnostic performances in response to a specific clinical question, the outcomes of a new test should be clearly defined and evaluated. Analytical and diagnostic performances such as sensitivity, specificity, imprecision, positive and negative predictive values are traditionally established measures but the clinical impact and the healthcare outcomes, to which these accuracy measures are related, are complex to measure. The extent of the improvement of the patients' health due to a diagnostic test remains a "holy grail" notwithstanding it should be the ultimate goal.

HARMONIZATION AND RISK MANAGEMENT POLICIES IN LABORATORY MEDICINE

Harmonization and risk management policies represent the key-issues in laboratory medicine as they directly rely on a patient-centred delivery of laboratory information based on the recognition of the importance of the total testing process for assuring healthcare quality and patient safety.

The term “harmonization” is intended to assure that the results of a test are equivalent, being either traceable to a reference material and based on a consensus approach in agreement with the mean values obtained with different methods (1-3).

Nevertheless, the concepts of commutability, uncertainty and reference intervals to harmonize laboratory results are well known issues, a growing body of evidence demonstrates that clinical benefits can be achieved only by focusing on the total testing process, where the appropriateness of test request and interpretation are the main steps. If the scope of harmonization goes beyond method and analytical results to consider all the other aspects of laboratory testing, including strategies for test demand and criteria for result interpretation (1,2), robust and methodologically high-quality recommendations to evaluate new tests are pivotal tools to promote the cooperation at the clinical-laboratory interface to guarantee a valuable medical decision-making process.

In risk management, the new approaches to quality and patient safety in the healthcare system emphasize that diagnostic improvements are based on the assurance of the desired outcomes rather than on the sole identification of the errors. The outcome-based approach proposed by Epner et al. (4) on testing-related diagnostic errors appeals for a more effective selection and interpretation of useful biomarkers in

order to prevent adverse events, failure to diagnose and to provide the appropriate treatment. Patient safety is compromised by inappropriately requested tests or by misinterpretation of the results so harmonization and risk management policies in the laboratory increasingly recognizes the need to consider patient outcomes in the assessment of tests and test strategies (4,5). The development of high-quality recommendations may be the common framework to promote harmonisation and risk management in diagnostic pathway by evaluation of proposed innovative diagnostic tests translated in clinical practice from research (6).

THE TEST EVALUATION WORKING GROUP (WG-TE) OF THE EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE (EFLM): IDENTIFYING UNMET CLINICAL NEEDS FOR NEW BIOMARKERS

The Test Evaluation Working Group (WG-TE) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), a working group composed of laboratorians, epidemiologists, evidence-based medicine (EBM) methodologists, health technology assessment and policy experts and the IVD industry, delivered practical tools to improve the clinical and cost-effectiveness evaluation of new biomarkers to facilitate their implementation as medical tests within the clinical pathway (7-8). It proposed an outcome-focused approach that can be used by stakeholders for any medical test, irrespective of the purpose and role of testing to identify clinical management decisions, linking biomarker testing to health outcomes. This method including worked examples are suggested to assist researchers, clinical scientists, and the IVD industry working with clinicians, to identify unmet clinical needs to improve the development of IVD medical tests to improved health outcomes.

The 14-item checklist is organized into 4 domains: 1/ identifying the clinical management problem and desired outcome and 2/ verifying the unmet need and an existing solution; 3/ validating the intended use, how the biomarker contributes to the solution; and 4/ assessing the feasibility of the new biomarker to influence clinical practice and health outcome. A more efficient biomarker development and translation into practice are the purpose of the proposed checklist as it was field tested to promote the role of the clinical laboratory specialist to forward interdisciplinary and multi-professional collaboration. A complete picture of the guide to identify unmet clinical needs for new biomarkers is described in a recent paper (9), this checklist proposed by EFLM TE-WG is aimed to assist all stakeholders engaged in the discovery or implementation of new biomarkers or new diagnostic pathway.

THE GRADE APPROACH: THE GRADING OF RECOMMENDATIONS ASSESSMENT, DEVELOPMENT AND EVALUATION

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to assess the certainty in evidence and to develop recommendations is a widely, patient-centred method (10). This approach is presently used by over 100 organizations worldwide and has become one of the reference standard for providing health care recommendations (11).

The GRADE methodology is increasingly used in the area of medical testing where the GRADE framework is turning away from simple test accuracy to incorporate main health outcomes in light of the resulting downstream clinical actions. Since direct studies assessing the impact of diagnostic tests or strategies on patient important outcomes are rarely available, the GRADE process requires two main steps. The

first is the judgments about directness in assessing the link between test accuracy and the evaluated health outcomes and the second aims to the criteria used in moving from evidence to a recommendation (12).

THE GRADE EVIDENCE TO DECISION (ETD) FRAMEWORKS

EtD frameworks may be utilized to assess the certainty of the evidence and to model the consequences of a decision about a test. The frameworks include not only the traditional criteria to assess test analytical and diagnostic performances but also the assessment of the certainty of evidence to estimate if the test effects match patient outcomes. At first a clear clinical question and related outcomes (important to the patient) are defined and then a structured systematic review of the available evidence is performed. Diagnostic test performances are then judged by taking eight criteria into consideration, of which five are to downgrade the quality of evidence, such as risk of bias, indirectness, inconsistency, imprecision, and publication bias. The last three criteria are to upgrade evidence quality, such as the magnitude of the effect, dose response in relation to the effect, and opposing plausible residual bias or confounders.

The GRADE evidence to decision (EtD) frameworks (6) for tests offer a structured approach as described as follow.

Formulating the question

Formulating a question needs a clear problem draw and the definition of purpose, type and role of a test and alternative intervention(s), the main outcomes focused and the expected setting. PICO, the population intervention comparison outcome format is a suitable method for formulation of the question (13).

Making an assessment

The problem

A definition of the magnitude and the priority of the problem should be established depending on the setting in which the test will be used and the influence on current or future practices.

Test accuracy

A summary of findings from systematic reviews is the means to interpret the accuracy of a test. An acceptable overall accuracy is the starting point for entering a laboratory test into an EtD framework evaluation.

Benefits & harms

The judgment about the benefits and harms to introduce a new test is based on findings about desirable and undesirable effects. Evidence should be derived from up-to-date systematic reviews and summarized in a table of findings (14).

Certainty of the evidence

The GRADE overall rating the certainty of the evidence about the effects of a new test and the subsequent management decisions on patient-important outcomes is now extensively used by guideline developers (15) and a complete report of this approach can be found elsewhere (16).

EtD tests framework includes five criteria for reaching judgments and making assessment of the evidence certainty: 1) test accuracy, 2) any critical or important direct benefits, adverse effects or burden of the test, 3) effects of natural history or the management that is guided by the test results, 4) the link between the test results and the management decisions and 5) the evidence about the effects of the test.

Values

The perceived value of the main outcomes includes test downstream outcomes. For example, a blood test may replace more dangerous

interventions, such as bowel biopsy in coeliac disease diagnosis, tumor prostate biopsy, or fetal cell genotyping through maternal blood sampling instead of amniocentesis.

Balance between the desirable and undesirable effects

Desirable and undesirable effects following the introduction of a new test need to be judged in comparison to the old or traditional test through either formal or informal modeling evaluating the actions due to a new test.

Resource use

In the case of the selection of the proposed diagnostic test, judgments about the magnitude of costs, certainty of evidence of resource requirements and the cost-effectiveness of interventions should include the evaluation of the impact both within the laboratory and the downstream consequence. The great challenge is to identify the overall health care cost and not only the plan cost of the test itself (17).

Equity, acceptability and feasibility

Assessments of equity, acceptability and feasibility comprise both the test and the consequent interventions. The use or misuse of tests for a specific clinical presentation in different professional settings affects equity of access to clinical care. For example, in the same healthcare setting, the introduction of a new test may vary from one hospital to another.

CONCLUSION

High quality and evidence-based recommendations may support a transparent clinical governance policy where the introduction of new laboratory test based on assessed outcome for patients is a value allowing the laboratory people to contract with the management the allocation of resources in terms of health priorities (18).

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Which skills are needed and how they should be gained by laboratory medicine professionals for successful ISO 15189 accreditation

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ABSTRACT

Clinical laboratories worldwide are accredited according to the “ISO standard, 15189:2012: Medical Laboratories—Requirements for Quality and Competence.”

Seeking accreditation has many challenges. Success requires the right competencies and knowledge and the right technical expert and trainer to lead the laboratory through the process. The right competencies and knowledge typically are beyond the core knowledge, skills and attitudes gained during education of laboratory professionals. The main objective of this paper is to discuss what competencies, knowledge and expertise are essential for laboratories to meet accreditation challenges and gain ISO 15189:2012 accreditation.

INTRODUCTION

The “ISO 15189 Standard: Medical Laboratories–Requirements for Quality and Competence” is an internationally accepted accreditation standard based on a series of requirements (1). Like other ISO Standards, ISO 15189 identifies what laboratories need to do, but not how to do. Each laboratory specifies the “how” for its situation using knowledge thought to be acquired during the training of medical laboratory specialists and included in the core curricula/syllabi published by scientific, professional and government authorities (2,3). However, ISO 15189 accreditation typically requires knowledge and competencies beyond the educational scope. A key to ISO 15189 accreditation is the quality management system. In this context, it is useful to understand and apply concepts from “ISO 9001:2015 Quality Management Systems–Requirements,”

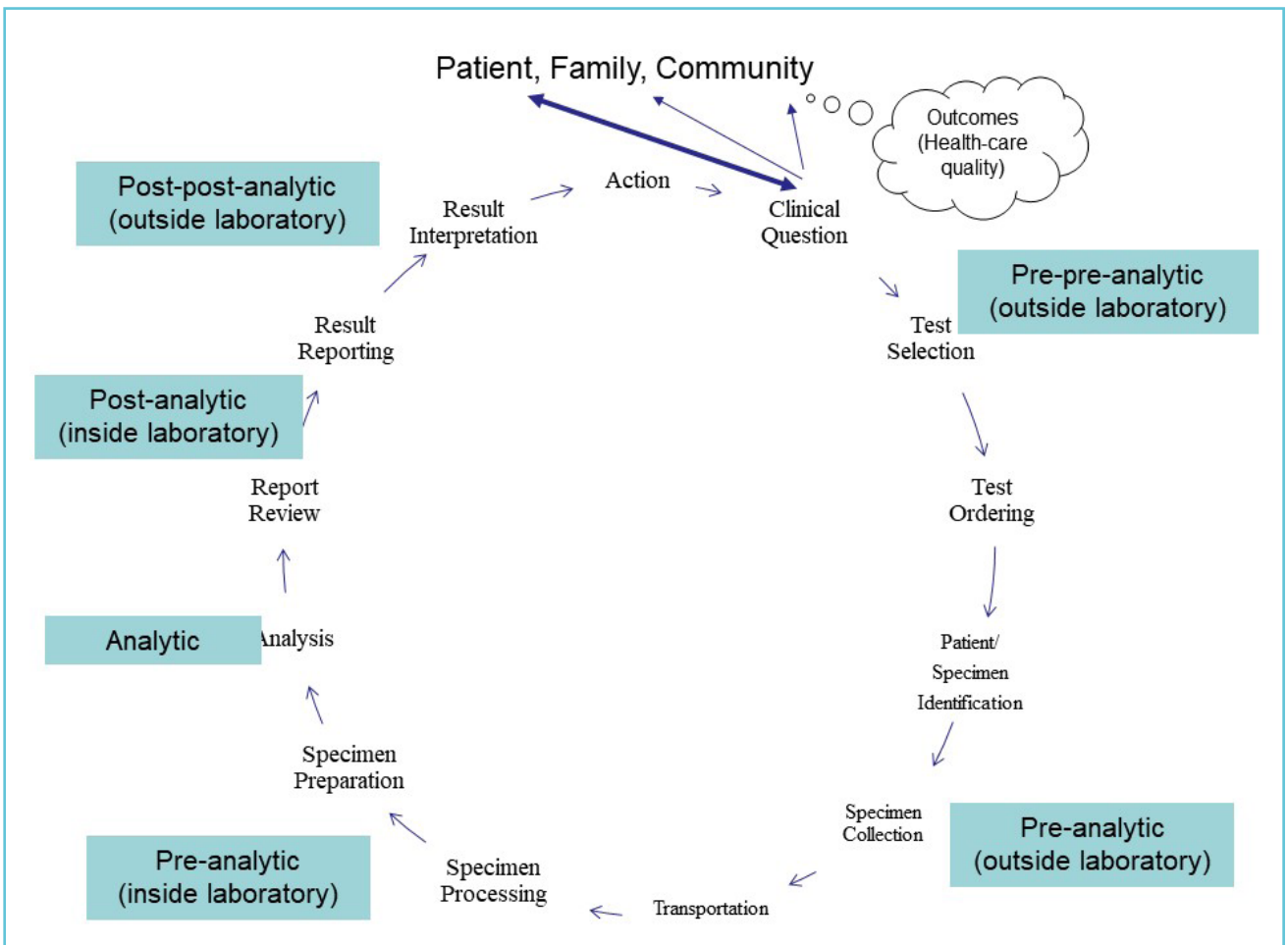
and the CLSI Guideline, “GP26-A3: Application of a Quality Management System Model for Laboratory Services (4,5). This paper presents the main areas and basic tools for gaining the competencies required by ISO 15189:2012 and to share my experiences of the ISO 15189 technical expert and trainer.

MAIN AREAS AND BASIC TOOLS FOR SUCCESSFUL ACCREDITATION WITH ISO 15189:2012

The medical laboratory environment is rapidly changing. In order for effective management, the laboratory directors must be technically and clinically competent in their defined specialty areas, and also have relevant and common managerial, statistical and computer knowledge and skills. These attributes can be collected under the main topics such as “Quality Management”,

Figure 1 Areas and topics for competencies required for the ISO 15189:2012

Areas and Topics for Competencies Required for ISO 15189:2012					
Process Management	Quality Management	Risk-Based Quality Control	Laboratory Mathematics and Statistics	Evidence-Based Laboratory Medicine	Continuous Improvement
<ul style="list-style-type: none"> •Process definition •Process core components •Process elements •Process improvement <p>(Related ISO 15189:2012 Clauses: 5.4, 5.5, 5.7, 4.12, 4.14.7, 5.1, 5.2, 5.3, 5.10)</p>	<ul style="list-style-type: none"> •Quality planning •Quality assurance •Quality control <p>(Related ISO 15189:2012 Clauses: 4.1, 4.2, 4.10, 4.11, 4.14.7, 5.6)</p>	<ul style="list-style-type: none"> •PDCA Cycle •Risk identification •Risk evaluation •Risk ranking •Risk treatment •Risk monitoring •Six Sigma Methodology (Process Sigma Levels) •Failure Mode Effects Analysis (FMEA) <p>(Related ISO 15189:2012 Clauses: 4.11, 4.14.6, 4.14.7)</p>	<ul style="list-style-type: none"> •Laboratory Mathematics •Normal distribution •Descriptive statistics •Method comparison statistics •Six Sigma Methodology (Process Sigma level) <p>(Related ISO 15189:2012 Clauses: 4.12, 5.5.1.2, 5.5.1.3, 5.5.1.4, 5.5.2, 5.6)</p>	<ul style="list-style-type: none"> •Diagnostic accuracy •Diagnostic specificity •Positive predictive value •Negative predictive value •Diagnostic effectiveness •Diagnostic efficiency •ROC Curves •Forest Plots •Cost-effectiveness <p>(Related ISO 15189:2012 Clauses: 4.1.1.4-f,g,k, 4.4.1, 4.7, 5.6.3.4)</p>	<ul style="list-style-type: none"> •Project Management •PDCA Cycle •Related quality tools such as cause-effects analysis <p>(Related ISO 15189:2012 Clauses: 5.4, 5.5, 5.7, 4.12, 5.6.3.4)</p>

Figure 2 The medical laboratory total testing process

Adapted from:

https://ftp.cdc.gov/pub/cliac_meeting_presentations/pdf/addenda/cliac0314/16a_west_cdclabtoolkit2013_handout.pdf

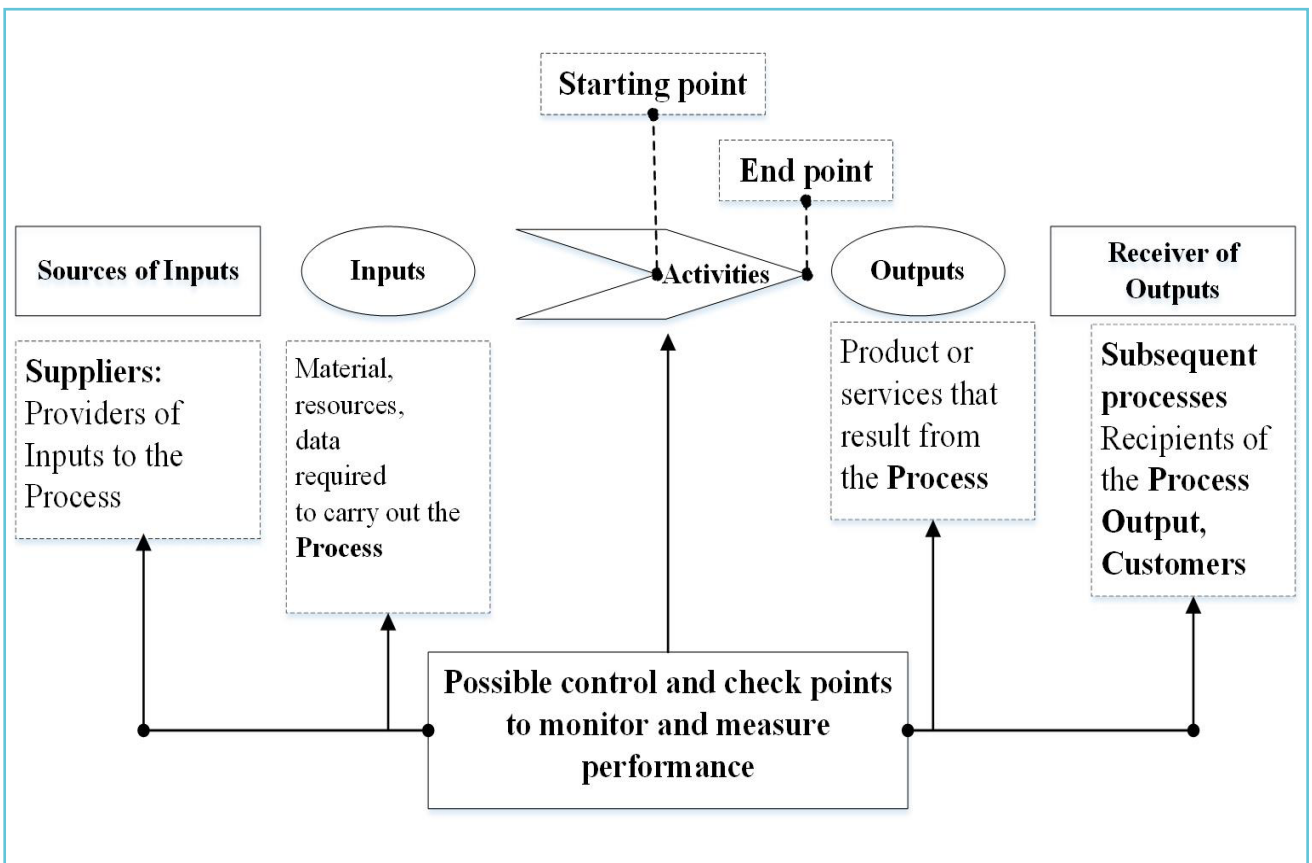
“Process Management”, “Risk– Based Thinking”, “Laboratory Mathematics and Statistics”, “Evidence-Based Laboratory Medicine” and “Project Management” (Figure 1) (6–10).

The clauses’ numbers of the ISO 15189: 2012 Standard that are related to the competencies that should be gained by laboratory professionals are listed under the competency topics in Figure 1.

To establish a quality management system requires a systematic, process-oriented approach so that quality objectives/requirements are achieved. The sub-processes of the total testing process of a medical laboratory seen in Figure 2

can be managed according to the simple work flow shown in Figure 3 in regard to the process core components (e.g., inputs, outputs, resources, activities and controls), and its elements (e.g., personnel, methods, materials, equipment, environment and measures).

Since continuous improvement is one of the key requirements of the ISO 15189, the methodologies and standards such as the “Plan, Do, Check, Act (PDCA)” Cycle, Six Sigma, Lean, Lean Six Sigma, total quality management, statistical process control and cause-effect analysis are the improvement tools used widely as listed in Figure 1 (6,8,11,12).

Figure 3 A process approach

Adapted from the ISO 9001:2015.

In addition to the topics mentioned in the previous paragraph, knowledge of quality management sub-processes is important to provide a systematic initiation. Such that establishment the QMS is composed of three processes; quality planning (QP), quality assurance (QA), and quality control (QC) (Figure 4) (6).

In the QP process, the managerial, operational and functional (supportive) processes of total testing process of a medical laboratory as required by ISO 15189 are defined, the resources according to each test system are allocated, and quality objectives and quality indicators of each subprocess are defined considering each test (Figure 5).

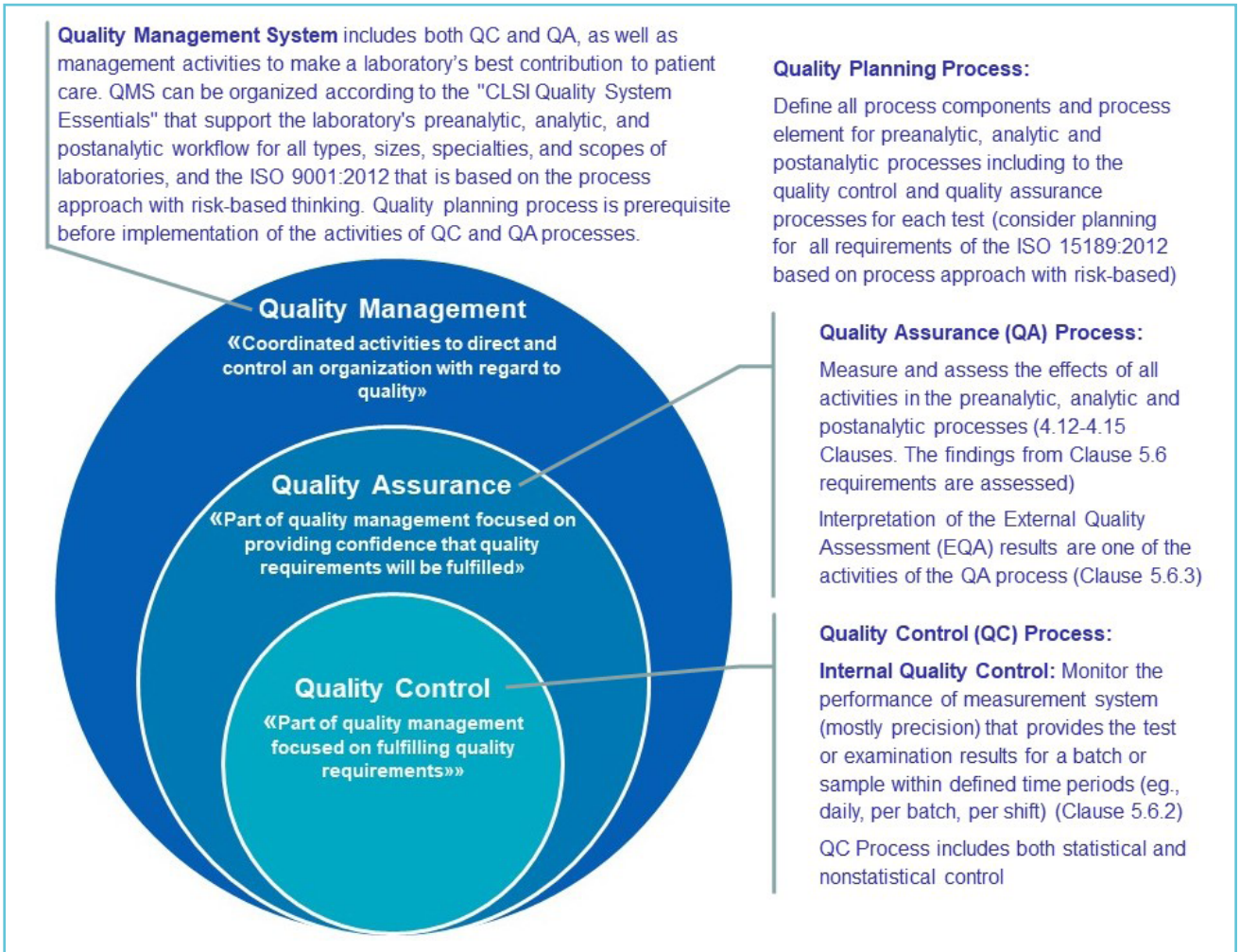
QC process covers the operational process control techniques to fulfill quality requirements

defined for each test in order to achieve each subprocess (analytical and non-analytical processes) performance.

QA process is composed of planned and systematic activities to provide evidence-based information that a medical laboratory fulfills quality requirements defined as quality objectives and quality indicators for each sub-processes in the QP stage, and provides the report for continuous improvement activities (Clause 4.12). Learning resources, required knowledge, skills and competencies for each activities in the sub-processes are summarized in Figure 5.

The important common issue in preparation for accreditation is confusion about the differences between quality system certification and accreditation.

Figure 4 Quality levels that define the quality management processes required to establish a quality management system



DIFFERENCE BETWEEN ACCREDITATION AND QUALITY SYSTEM CERTIFICATION

It is important to realize the difference between the ISO 9001 quality system certification and the ISO 15189 accreditation. The ISO 9001 certifies the consisted business processes are being applied, but it is not guarantee the quality of the end products and services such as the ISO 15189 that focuses on the technical and clinical competencies for reliable and cost-effective test results (1,4). The first part of the ISO 15189 (Clause 4 Management Requirements) is based on the ISO 9001. However, there are

correlations of the second section of the ISO 15189 (Clause 5 Technical Requirements) with the ISO 9001:2015 that uses process approach and risk-based thinking (1,4). In this context, the ISO 9001:2015, especially risk-based process approach, should be well understood before accreditation process. Although there are correlations between two standards, it is crucial to keep in mind the differences because accreditation is deserved for specific activities such as to provide effective and efficient test results whereas certification relates to the whole organization.

Figure 5 The decision, design and implementation processes for a new analyte, and required knowledge, skills and competencies

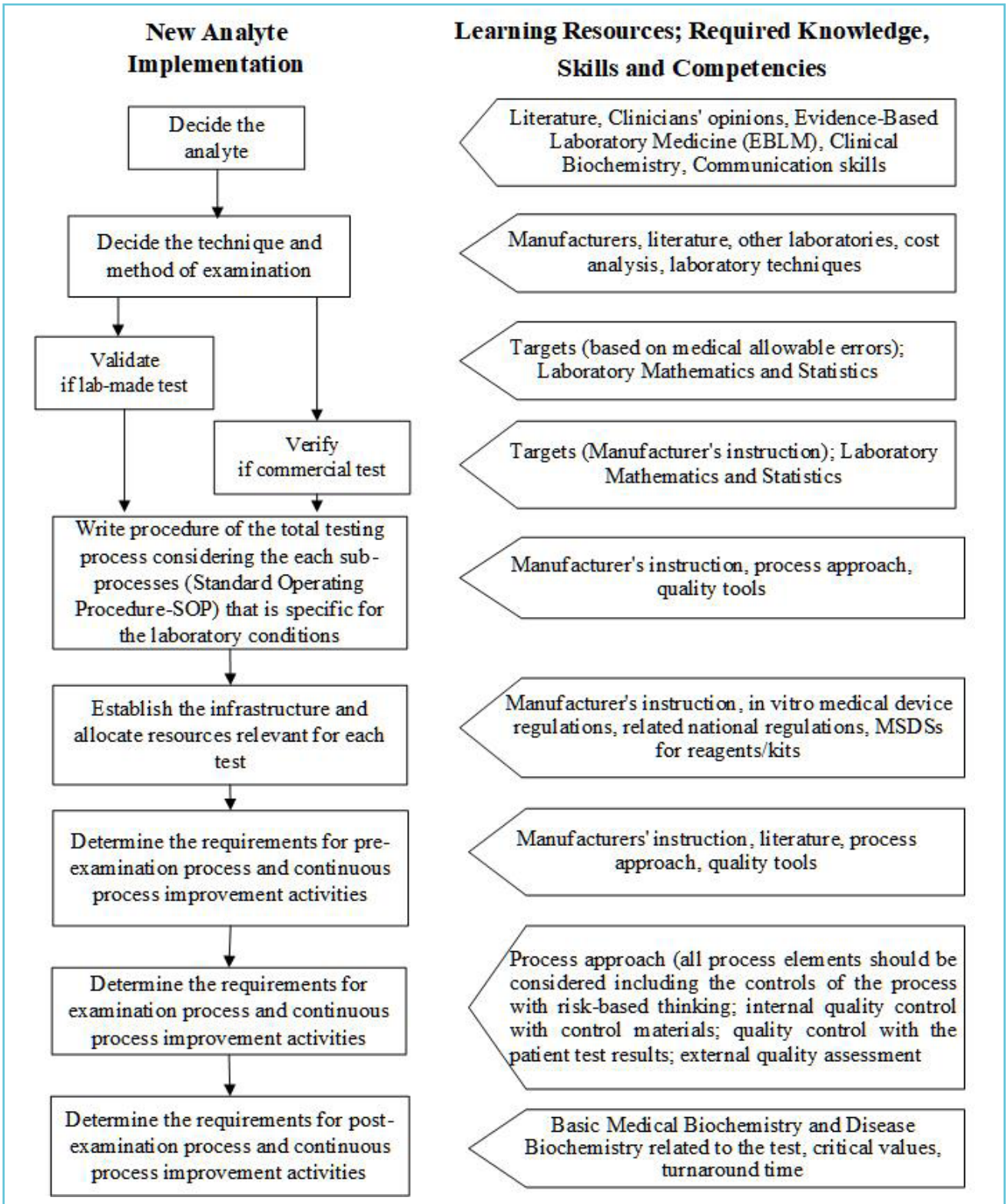


Table 1 Correlations between “NCCLS (CLSI) GP26-A3 Application of a Quality Management System Model for Laboratory Services; Approved Guideline — Third Edition (2004)” with the ISO 15189:2012

GP26-A3	ISO 15189:2012
Documents and Records	4.3 Document control
	4.13 Control of records (quality and technical records)
Organization	4.1 Organization and management responsibility
	4.1.1.3 Ethical conduct
	4.2 Quality management system
	4.15 Management review
Personnel	5.1 Personnel
Equipment	5.3.1 Laboratory equipment
	5.10 Laboratory information management
Purchasing and Inventory	4.4 Service agreements
	4.5 Examination by referral laboratories
	4.6 External services and supplies
Process Control	5.4 Pre-examination processes
	5.5 Examination processes
	5.6 Ensuring quality of examination results
	5.7 Post-examination processes
	5.8 Reporting of results
Information Management	5.10 Laboratory information management
Occurrence Management	4.11 Preventive action
	4.10 Corrective action
	4.14.6 Risk management

Assessment: External and Internal	5.6.2 Quality control (IQC)
	5.6.3 Interlaboratory comparisons
	5.6.3.2 Alternative approaches
	5.6.4 Comparability of examination results
Process Improvement	4.12 Continual improvement
	4.14 Evaluation and audits
	4.14.7 Quality indicators
	4.15 Management review
Customer Service	4.1.2.2 Needs of users
	4.7 Advisory services
	4.8 Resolution of complaints
	4.14.3 Assessment of user feedback
	4.14.4 Staff suggestions
	4.14.8 Reviews by external organizations
Facilities and Safety	5.2 Accommodation and environmental conditions

PREPARATION FOR ISO 15189 ACCREDITATION

The quality system essentials in the GP26-A3 can be used as framework with the process approach (5). Table 1 depicts the correlations between the QSEs and the ISO 15189. The QSEs are matching to the process core components and elements in Figure 3 of total testing process in Figure 1. As seen in Figure 5, all activities that are performed for decision of a new analyte and its application are also in between the requirements of the ISO 15189.

Preparation for the ISO 15189 accreditation is challenging, and requires comprehensive knowledge and competency (13). Learning the topics

listed under the main areas related to the competencies required by the ISO 15189 accreditation summarized in Figure 1 may be helpful for successful accreditation.

The steps may be organized as:

- 1) Defining activities according to the quality levels (Figure 4);
- 2) Defining the process core components and process elements of the total testing process and its sub-processes for each test according to the process approach (Figure 3);
- 3) Defining all key process controls (key performance specifications and quality indicators) for each test process that is in the accreditation scope.

TRAINING PROGRAMS, PROFESSIONAL DEVELOPMENT AND CAREER PLANS

The main duty of a laboratory professional is to provide the reliable and accurate laboratory test results cost-effectively and timely. Besides this, there are responsibilities of laboratory in respect to the value-based health care (14,15).

Accreditation against the ISO 15189, if it is performed ideally, adds value to health care quality in respect to both human health, and the tracking and evaluation of in vitro diagnostic medical devices at the national level if the government establishes infrastructure for collecting the data. However, acquiring diverse knowledge and competencies that are growing exponentially is a challenging issue for a laboratory director.

The other challenging issue is that it is not possible to find a university or teaching hospital that has sufficient trainers who have all required knowledge and skills. One of the solutions may be the training courses organized by experts both in classroom and in distance-learning formats. The training programs should be competency-based in the concepts of “case-based”, “entrustable professional activities” and task-based (16,17).

Distance-learning courses (DLCs) can provide the rapidly increasing knowledge that should be learned and replace live on-site courses, which have high participation costs. However, the DLCs on the “Medical Laboratory Mathematics and Statistics” organized for the last 2 years encountered some challenges (18). These courses are 1.5 - 2 months long and require 3-hours of study per week. They contain texts, presentations, virtual classrooms and videos that enable the participants to practice with Microsoft Excel and SPSS calculations of problems and the assessment quizzes. However, it was observed that the laboratory specialists usually do not have enough time to work on the material.

Standardization of core knowledge of laboratory professionals required for the ISO 15189 accreditation can be established and online central examinations which would assess these requirements can be organized by the IFCC or the Regional Federations. Such initiatives will be helpful for laboratory professionals to decide what they need to learn and self-evaluate their knowledge.

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Prenatal screening for chromosomal abnormalities: where do we stand today in Mediterranean countries?

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ABSTRACT

Over the last 4 decades the practice of prenatal screening has evolved from the second-trimester triple test to complex combinations of biophysical and biochemical testing for aneuploidy, testing of fetal DNA in the maternal circulation and development of screening tests for adverse pregnancy outcomes. Presently, combined test in the 1st trimester is the preferred multimer screening protocol in most countries. Since 2010, cell-free fetal DNA (cffDNA) in maternal plasma, in combination with the next generation sequencing techniques, made a big breakthrough step in screening for Down Syndrome (DS) and other aneuploidies. It seems that the position of cffDNA in the current screening strategies is a secondary contingent use to combined test, at least as long as its price is still high and its use as a primary test is not cost effective. Concerning the situation in Mediterranean countries, at least with those who answered the questionnaire, screening in the 1st trimester is an established practice, reimbursed from social security organizations, and not compulsory. cffDNA is used in all countries and its average cost is about 500 €.

INTRODUCTION

Screening is the process of surveying a population with specific markers in order to identify those individuals with a higher risk for a particular disorder. For high risk individuals, a diagnostic test is applied to definitely diagnose the disorder. A successful screening program should be complemented with an accurate diagnostic test to identify those who are truly affected and also with a clear strategy of how to treat the affected individuals.

Over the last four decades, the practice of prenatal screening has evolved from the simple second-trimester maternal serum α -fetoprotein (AFP) test for open neural tube defects (NTDs) to complex combinations of biophysical and biochemical testing for aneuploidy. It continues to evolve with the testing of fetal DNA in the maternal circulation and the development of screening tests for adverse pregnancy outcomes such as pre-eclampsia. Diagnosis of major fetal chromosomal aneuploidies is done by karyotype of fetal cells, obtained through amniocentesis after the 15th week of pregnancy, or chorionic villus sampling (CVS) between the 12th and 14th weeks. There are several financial and ethical implications of how pregnancies with affected fetuses are treated in different countries and these differences are even bigger between Mediterranean countries with different economical, social and cultural status.

BRIEF HISTORICAL OVERVIEW

Screening for fetal aneuploidy in pregnancy began in the 1960-1970s with maternal age as the only available marker. As maternal age increases, the chance of delivering a child with Down syndrome (DS) or other major autosomal trisomies like trisomy 18 (T18; Edwards syndrome) or 13 (T13; Patau syndrome) increases. However, screening with maternal age alone (cut-off >35years), could detect about 30% of trisomies.

The majority of babies with DS are born by women less than 35 years of age.

The first breakthrough in screening for fetal chromosomal abnormalities was done in 1988 with the introduction of a multiple marker screening test, based on a "risk" calculation for each pregnant woman using her age and the concentrations of 3 biochemical markers: human Chorionic Gonadotropin (hCG), AFP, and unconjugated Estriol (uE3) (Triple test) from blood samples in the 2nd trimester of pregnancy [1]. Such screening has led also to the diagnosis of a large proportion of the other common trisomies, like T18 and T13. In 1992, ultrasound fetal nuchal translucency (NT), by far the single best individual marker, was introduced [2] and in 1997, a new multiple marker screening test the Combined test, using NT, Fb-hCG and Pregnancy Associated Plasma Protein –A (PAPP-A) was started [3]. The following years, several complex screening protocols were introduced using both first- and second-trimester markers. Table 1 [4] shows the model predicted detection rate (DR) and positive predictive value (PPV) for a 1% or 5% false positive rate (FPR) for the traditional screening strategies described above.

Comparing the first and second trimester screening protocols, 1st trimester's Combined test has better DR than the Triple or Quadruple (Triple+inhibin) test in the 2nd trimester, for 5% FPR. Presently, the Combined test is the preferred multimarker screening protocol in most countries. Protocols combining 1st and 2nd trimester markers, in one step or contingently or using more biochemical or ultrasound markers gave better performance but made the screening more cumbersome, expensive and time consuming.

DNA SCREENING FOR ANEUPLOIDIES

The discovery that there is sufficient cfDNA in maternal plasma, in combination with the next generation sequencing techniques, made the

next breakthrough step in screening for DS and other aneuploidies [5]. In principle, the screening is based on counting a large number of DNA fragments (both maternal and fetal) using massive sequencing, assigning them to a chromosome and quantifying the proportion assigned e.g. to chromosome 21. The results are expressed as a z score computed by comparison to an expected proportion for a sample from a euploid fetus.

In a recent review and metanalysis [6], the DR of cffDNA for DS was found 99.4% for 0.1% FPR (results from 148344 tests); for T18, 97.7% for 0,1% (results from 146940 tests); and for T13, 90,6% for 0,1% (results from 134691 tests). The authors concluded that “cffDNA based non-invasive prenatal testing (NIPT) can be diagnostic for fetal sex and rhesus D, but only screening test in aneuploidy”.

Table 1 Model predicted DR and PPV for different policies for Down syndrome screening according to FPR

Policy	FPR = 1%		FPR = 5%	
	DR (%)	PPV ^a	DR (%)	PPV ^a
Second trimester				
Quad	50	1 in 16	71	1 in 54
First trimester				
Combined	72	1 in 12	85	1 in 46
Both trimesters				
Serum integrated	58	1 in 14	76	1 in 51
Integrated	83	1 in 10	92	1 in 42
Contingent ^b	83	1 in 10	91	1 in 42
Improved				
Combined plus NB	88	1 in 10	94	1 in 41
First trimester contingent ^b	86	1 in 10	90	1 in 43
Quad plus facial profile	83	1 in 10	93	1 in 42
Combined plus PIGF and AFP	77	1 in 11	89	1 in 44

^a At term

^b First stage cutoff risks 1 in 50 and 1 in 2000 at term

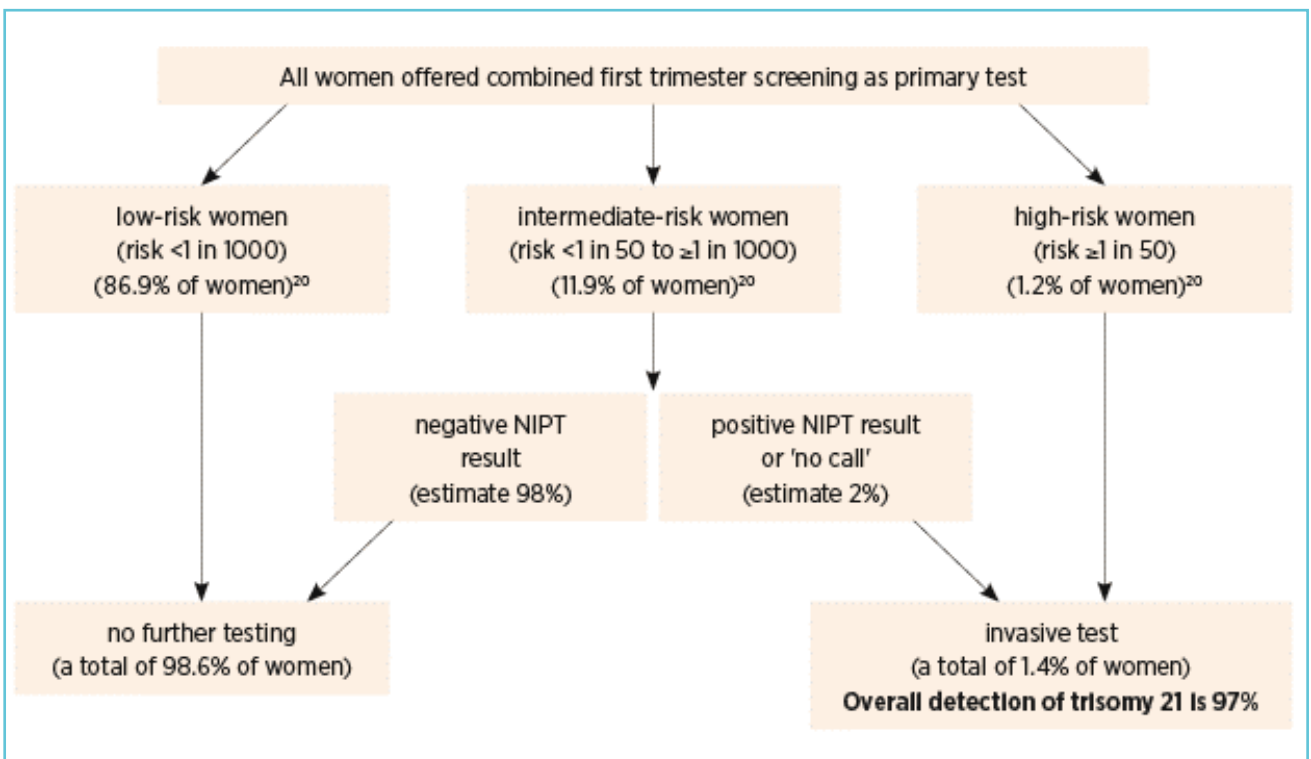
Concerning the position of cffDNA based NIPT in the established screening strategies, two main approaches have been suggested:

- as “primary” testing replacing the conventional screening; and
- as “secondary” testing offered after the 1st trimester’s Combined test.

In both approaches, a confirmatory invasive prenatal diagnosis by CVS or amniocentesis is necessary for positive results. As a primary test, cffDNA has a much higher screening performance, at least for Down syndrome, than any of the conventional policies summarized in Table 1. However, the major concern of this approach is the cost. With the cost for a Down syndrome birth avoided to be almost 10

times higher for cffDNA than the conventional screening [7], this screening approach could be an unaffordable burden for every health care system. Another consideration is the test failure rate of cffDNA testing. The reported rates for “no-call” results from the commercial companies vary between 2-6%. The main reason for the test failures is the low fraction of fetal DNA in the total amount of free DNA in the maternal circulation. The fetal fraction has to be higher than 10% optimally, and today all the main commercial companies include the fetal fraction in their results’ report. As a secondary test, a contingent use of cffDNA test is more cost effective than the use as a primary test. With this approach, conventional 1st trimester

Figure 1 Example model for contingent screening for Down syndrome



NIPT: non-invasive prenatal tests, “no call”: test could not be reported

Non-invasive prenatal testing may be best used in a contingent approach. In the example, combined first trimester screening is offered to all women as an initial screening tool. From this, women are stratified by risk to determine further management. Women with a high risk are offered an invasive test (chorionic villus sampling or amniocentesis). Women with a low risk are reassured and advised that no further testing is needed. Women with an intermediate level of risk are offered a non-invasive prenatal test. Contingent screening allows highest detection rate (in the example 97%) while reducing the false positive rate (in the example 1.4%).

Table 2 Brief summary of the responses received from some Mediterranean countries

Question	Slovenia	France	Greece	Turkey	Israel	Albania
Is screening regulated by law?	Yes	Yes	No	Yes	Yes	No
Is screening compulsory?	No	No	No	No	No	No
Is screening reimbursed?	Yes	Yes	Yes	Yes	Yes	No
Screening strategies						
1 st trimester only	No	Yes	No	No	No	No
1 st or 2 nd trimester	Yes	-	Yes	Yes	Yes	Yes
cfDNA testing	Yes	Yes	Yes	Yes	Yes	Yes
cfDNA testing reimbursed?	No	No	No	No	No	No
Primary or secondary	Sec		Sec	Sec		
Cost of cfDNA testing (€)	~ 450		350-650	400-600	-	600-800
Invasive cytogenetic diagnosis						
Woman's age only	Yes	No	Yes	Yes	Yes	Yes
Screening results	Yes	Yes	Yes	Yes	Yes	Yes
Other indications (US, family)	Yes	Yes	Yes	Yes	Yes	Yes
US monitoring	Yes	Yes (3)	Yes (3)	Yes	Yes (3)	Yes

combined screening is offered to all women. To those women with a very high risk for all type of aneuploidy (e.g. >1:50), invasive prenatal diagnosis is offered.

To all other women, the result of the combined test could be used for counseling, giving them the choice of selecting either:

- a) no further action with a result lower than e.g. 1:1000;
- b) proceed with cffDNA testing or
- c) having invasive diagnosis.

An option for this approach is depicted in Figure 1. (<https://www.nps.org.au/australian-prescriber/articles/non-invasive-prenatal-testing-for-down-syndrome>)

RECENT GUIDELINES FOR SCREENING

In recently published recommendations of the American College of Obstetrician and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine for screening for fetal aneuploidy (<https://www.acog.org/Clinical-Guidance-and-Publications/Committee-Opinions/Committee-on-Genetics/Cell-free-DNA-Screening-for-Fetal-Aneuploidy>), among others it is mentioned that:

- A discussion of the risks, benefits, and alternatives of various methods of prenatal screening and diagnostic testing, including the option of no testing, should occur with all patients.
- *Given the performance of conventional screening methods, the limitations of cell-free DNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.*
- The cell-free DNA test will screen for only the common trisomies and, if requested, sex chromosome composition.
- Given the potential for inaccurate results and to understand the type of trisomy for recurrence-risk counseling, a diagnostic test should be recommended for a patient who has a positive cell-free DNA test result.
- Cell-free DNA screening is not recommended for women with multiple gestations.
- If a fetal structural anomaly is identified on ultrasound examination, diagnostic testing should be offered rather than cell-free DNA screening.
- Patients should be counseled that a negative cell-free DNA test result does not ensure an unaffected pregnancy.

THE SITUATION IN MEDITERRANEAN COUNTRIES

Trying to imprint the situation of prenatal screening for chromosomal abnormalities in the different Mediterranean countries, a questionnaire in cooperation with MZ Congressi, was sent to the members of Scientific Committee and was uploaded as a survey (https://docs.google.com/forms/d/e/1FAIpQLSdu6i2dvWbeCbObkGToJV51V8C0A6vA7LYTu0JBPtr4UrL_TQ/viewform). Unfortunately, a limited number of responses was received (Slovenia, France, Greece, Turkey, Israel and Albania) (Table 2).

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The role of laboratory medicine for health during pregnancy

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ABSTRACT

Pregnancy produces profound physiological changes that increase in significance as it progresses. These changes include hormonal changes, metabolic changes, increases of plasma volume up to 50%, alterations to the balance of the coagulation system in favour of clotting, and GFR increases to a peak 50% above pre-pregnancy levels. Since healthy physiological changes occur during pregnancy, different reference intervals may be needed. First antenatal screens usually include Complete blood count, Blood group and antibody screen, rubella antibody status, syphilis serology, Hepatitis B serology and HIV abs testing. Additional testing in early pregnancy may be added to the first antenatal screen such as varicella, Chlamydia and vitamin D tests. The most important test in the second antenatal testing screen is gestational diabetes screening and protein detection in urine to rule out preeclampsia. Screening for Down syndrome, other chromosomal abnormalities and neural tube defects is recommended for all pregnant women above the age of 35 years. Additionally, 37 weeks into pregnancy, a swab to detect Group B streptococcal (GBS) infection is recommended.

INTRODUCTION

Human Pregnancy is not a disease, it is a physiological condition; pregnancy produces profound physiological changes that become more significant as pregnancy progresses.

The hormonal changes starts from the **ovaries**, and then later the **placenta**. The first hormone to make its appearance after conception is human chorionic gonadotropin (hCG) then followed by hormones include estrogen, progesterone, prolactin, renin and human placental lactogen. It is also worth mentioning that adequate levels of circulating thyroid hormones are of primary importance for normal reproductive function, all these changes are accompanied by growing uterus with gradual mechanical effect.

Metabolic changes are also due to an increased insulin production, and pregnancy is associated with insulin resistance caused predominantly by human placental lactogen. This facilitates placental glucose transfer and any carbohydrate load will cause a greater than normal increase in plasma glucose.

Plasma volume increases progressively throughout normal pregnancy; most of this 50% increase occurs by 34 weeks' gestation and is proportional to the birth weight of the baby. Because the expansion in plasma volume is greater than the increase in red blood cell mass, there is a fall in haemoglobin concentration.

Changes in the coagulation system during pregnancy produce a physiological hypercoagulable state. The concentrations of certain clotting factors, particularly VIII, IX and X are increased. Fibrinogen levels rise significantly by up to 50% and fibrinolytic activity is decreased. Concentrations of endogenous anticoagulants such as antithrombin and protein S decrease. Thus, pregnancy alters the balance within the coagulation system in favour of clotting.

During pregnancy renal vasodilatation increases renal blood flow early during pregnancy, this increase cardiac output (CO), GFR and renal plasma flow (RPF) by 50%, also an increase in Renin & Aldosterone level promotes Na⁺ retention leading to volume overload.

The kidneys face remarkable demands during pregnancy, GFR rises early to a peak of 40% to 50% that of pre-pregnancy levels, resulting in lower levels of serum creatinine, urea, and uric acid. There is a net gain of sodium and potassium, but a greater retention of water, with gains of up to 1.6 L.

Clinicians should be aware of the role of pregnancy specific reference ranges and how these can assist correct diagnosis and management in pregnancy. Appropriate pregnancy reference intervals help clinicians to avoid interpreting normal results as pathological and help them to identify when results are truly abnormal.

REVIEW

Tests included in the first antenatal screen

- Complete blood count
- Blood group and antibody screen
- Rubella antibody status
- Syphilis serology
- Hepatitis B serology
- HIV

Although the first antenatal screen usually occurs early in pregnancy, it may be requested at any stage of pregnancy, i.e., if a woman presents for the first time late in pregnancy, she should still receive a first antenatal screen.

Complete blood count

Anaemia is most common medical disorder especially in underdeveloped countries which

increases maternal morbidity and mortality; it is well accepted that there are three main causes:

- Decreased erythrocyte production as in iron, vitamin B₁₂ and folate deficiency.
- RBCs destruction as in hemoglobinopathies.
- RBCs loss as in any haemorrhage.

Gestational age should be considered when assessing haemoglobin, as levels decrease during pregnancy due to haemodilution caused by increased plasma volume. The lower limit for haemoglobin is usually 12 g/dL, but for pregnant women the lower limit is usually reported as 10 g/dL.

Pregnancy causes a two- to three-fold increase in the requirement for iron, not only for haemoglobin synthesis but also for the foetus and the production of certain enzymes. There is a 10- to 20-fold increase in folate requirements and a two-fold increase in the requirement for vitamin B₁₂.

The platelet count tends to fall progressively during normal pregnancy, although it usually remains within normal limits. In a proportion of women (5–10%), the count will reach levels of 100–150 × 10⁹ cells/L by term and this occurs in the absence of any pathological process

Blood group and antibody screen

Identifying ABO blood group, rhesus D status and red cell antibodies in pregnant women is important to prevent “haemolytic disease of the new-born” in subsequent pregnancies. If the foetus is rhesus D-positive (and the mother is negative), the mother may form anti-D antibodies, which may affect a subsequent rhesus D-positive foetus. Haemolytic disease of the new-born in subsequent pregnancies.

Recently Non-invasive prenatal genetic testing (NIPT) is used to determine foetal rhesus D status and prevent rhesus D negative mothers from undergoing unnecessary prophylactic treatment, this allows to prevent the risk of

foetal anaemia and haemolysis when the mother is serologically RhD negative and the foetus is RHD positive.

Rubella antibody status

All pregnant women should be screened for rubella antibodies. Congenital Rubella Syndrome occurs when the rubella virus infects the developing foetus, especially during the first trimester when up to 90% of affected infants will be born with a birth defect, e.g. deafness, eye defects, heart defects, mental retardation. The risk of birth defects is decreased when infection occurs after 20 weeks' gestation.

The aim of screening is to identify women who have not been immunized or have diminished immunity and are susceptible to contracting rubella, so they can be immunized in the post-natal period to protect future pregnancies. Rubella antibody titers should be measured in each pregnancy as levels may decline and fall below protection levels.

Syphilis serology

All pregnant women should be screened for syphilis, mothers infected with syphilis can experience long-term morbidity and the complications for pregnancy are significant; 70 to 100% of infants will be infected and one-third will be stillborn.

Treponema Elisa Screen assay is used to screen for syphilis as this can detect primary or secondary infection.

Hepatitis B serology

Up to 85% of infants born to mothers infected with hepatitis B (particularly mothers who are HBeAg positive, i.e. with active infection), will become carriers and will be more likely to develop chronic liver disease, including cirrhosis, liver failure or liver cancer. Transmission of the hepatitis B virus from mother to infant can be

prevented by administration of the hepatitis B vaccine and immunoglobulin to the infant at birth, therefore screening is important.

HIV screening

All pregnant women should be screened for HIV. Women who are HIV positive can be given treatment to reduce the risk of HIV being transmitted to their infant (risk reduced from 32% to less than 1%). Interventions to reduce mother-to-child transmission of HIV infection include antiretroviral therapy, elective caesarean section delivery and the avoidance of breastfeeding.

If a patient is considered at risk for HIV, hepatitis C screening should also be considered.

Additional testing in early pregnancy

Consider checking varicella antibody status in pregnant women with no (or uncertain) history of illness (i.e. chicken pox or shingles) or vaccination. Contracting varicella during pregnancy is associated with a significant risk of harm to both mother and infant.

Testing for chlamydia and gonorrhoea should be considered for those who may be at increased risk based on age (e.g. less than 25 years) and sexual history.

Vitamin D is required for normal bone growth development in the foetus. Mothers with known vitamin D deficiency or at risk for deficiency (e.g. dark-skinned women, women who wear a veil) should receive vitamin D supplementation.

Blood tests included in the second antenatal screen

At 26–28 weeks' gestation, a second round of blood tests, commonly referred to as the second antenatal screen includes:

- 50 g glucose tolerance test (the "polycose" test)
- CBC
- Blood group antibodies

Screening for gestational diabetes

Gestational diabetes affects 5–8% of pregnant, it is recommended that testing for gestational diabetes occurs for all women between 26 and 28 weeks of gestation.

A 50 g glucose tolerance test (the polycose test) is used to screen for gestational diabetes. A 50 g glucose load is given to the non-fasting patient, and a glucose level is determined after one hour. Women with an elevated result should be followed up with a 100 g oral glucose tolerance test (OGTT).

Repeat CBC and antibody screening

The CBC should be repeated at 28 weeks' gestation, to check haemoglobin and platelet levels (see commentary in previous section on how to interpret and manage these levels in pregnancy). Antibody screening should also be repeated at 28 weeks' gestation.

Proteinuria in pregnancy can also be a sign of preeclampsia if it's accompanied by high blood pressure. Preeclampsia is a condition that only occurs in pregnancy and causes high blood pressure. It usually occurs after week 20 of pregnancy and can happen in women who didn't have high blood pressure before pregnancy. It can lead to serious complications with mother and baby that can sometimes be fatal.

Additional tests during pregnancy

Sub-Clinical urine infection

It is recommended that all women have a mid-stream urine culture at the time of the first antenatal screen, again at the second antenatal screen and then at 36 weeks' gestation, to exclude a sub-clinical urine infection (asymptomatic bacteriuria).

Screening for Group B streptococcus

Group B streptococcal (GBS) infection is a significant cause of serious neonatal infection.

Approximately 15–25% of women will be carriers, and one in 200 of these women will have infants who develop neonatal sepsis.

Women may have a vaginorectal culture collected at 35 to 37 weeks' gestation.

Testing for Down syndrome and other genetic conditions

Screening for Down syndrome, other chromosomal abnormalities and neural tube defects is recommended to all pregnant women above the age of 35 years.

First trimester screening is based on the combination of results of the following:

The PAPP-A and β hCG tests must be taken between nine and 13 weeks' gestation (ideally between 10 and 12 weeks), and the NT scan carried out after 11 and before 14 weeks' gestation.

Second trimester screening can be offered to all women who present after 14 weeks' gestation but before 20 weeks, who have not completed first trimester screening (bloods are ideally taken between 14 to 18 weeks gestation). This serum screen measures β hCG, alpha-fetoprotein (AFP), unconjugated estriol (μ E3), and inhibin A.

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Alcohol abuse

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ABSTRACT

Chronic alcohol consumption is a world-wide socio-economic problem. Three metabolic pathways of ethanol were describe in human - alcohol dehydrogenase (ADH), microsomal ethanol oxidizing system (MEOS, CYP2E1) and catalase. Ethanol directly bounds to different molecules (e.g. etylglucuronid) and ethanol per se and its metabolites have toxic effect on biological stuctures.

Alcohol abuse is well known for its liver diseases e.g. cirrhosis (the most frequent cause in Europe and US) and hepatocellular carcinoma.

Chronic alcohol consumption leads to cardiovascular diseases (e.g. hypertension, cardiomyopathy), pancreas damage, myopathies, osteoporosis, neurological and psychiatry diseases including fetal alcohol syndrome and addiction.

Alcohol comsumption may lead to cancer via several mechanisms, per se (solvent for carcinogens) and its metabolites. Acetaldehyde, a cancerogen, has mutagenic effect on DNA, oxidation of ethanol produces the reactive oxygen and nitrogen species with different effects e.g. cell transformation, DNA, protein and lipid damage. The changes of folate metabolism, altered methylation of DNA, reduction of retionic acid influences on cancer development.

The high rate of alcohol consumption has become a great social-health problem. Consumption of alcohol is still increasing in many countries, but in some countries is stable or decreasing (e.g. Mediterranean region). The data across Europe shows that 10% of all cancers in men and 3% of all cancers in women can be attributed to alcohol consumption. Australian data suggests that alcohol intake accounts for 5% of the total cancer burden of disease. Alcohol consumption is one of the leading causes of mortality and morbidity in many developed countries.



Alcohol has been consumed by people since the dawn of mankind. Beer was commonly produced already in ancient Egypt and wine has been known for millennia. However, excessive alcohol consumption has been mainly attributed to the second half of the twentieth century. Alcohol is a very dangerous cytotoxic substance damaging the organism both acutely and chronically; what is even more dangerous is that it causes addiction. Any alcoholic beverage in any quantity is potentially harmful to human health.

Alcohol-induced damage to the organism derives from its direct effect, in particular its metabolism and the substances produced thereof. The direct effect manifests itself mainly in changes to biological membranes and influencing their fluidity, potentially also by intercellular interactions with the possible alcohol-induced malnutrition caused by its effect on the epithelium of the small intestine.

There are four metabolic pathways of ethanol in the human organism:

- 1) alcohol dehydrogenase (ADH);
- 2) microsomal ethanol oxidizing system (MEOS, CYP2E1);
- 3) catalase, and
- 4) non-oxidative metabolism.

These enzyme systems can remove 90–98 percentage of alcohol from the human body; the unchanged residual amount is excreted from the body through breath, sweat and urine.

The main enzyme in ethanol metabolism belongs to the cytosolic dimeric alcohol dehydrogenase (ADH, E.C. 1.1.1.1) which catalyses the conversion of ethanol to acetaldehyde, which is carcinogenic. In addition to oxidation of alcohols, it also contributes to the metabolism of steroids and omega-oxidation of fatty acids. Oxidation of ethanol in the digestive tract can reduce the systemic ethanol concentration by up to 20%. ADH is developmentally and gender conditioned, resulting in lower ADH activity in females. In addition, this enzyme is not inducible and its rate is limited not only by NAD⁺ and oxidises in 92–96% of the alcohol ingested.

The emerging acetaldehyde is further metabolised by aldehyde dehydrogenase (ALDH, E.C.1.2.1.3.) into the end product acetate, which can be involved in a number of metabolic processes within the organism. ALDH can be identified in almost all organs, with high activity in the liver and in tissues that are in direct contact with the ambient environment. In a large portion of the Asian population, the genetic polymorphism ALDH2*2 is described, which results in an increase in acetaldehyde due to the lower activity of ALDH; there is also a higher incidence of tumour growth in this population.

Oxidation of alcohol to acetaldehyde and subsequently to acetate requires reduced nicotinic cofactors, which alter the ratio of reduced and oxidized NAD, thereby altering the redox cell environment. These changes result in increased lactate and ketone body formation and reduced gluconeogenesis and Krebs cycle activity, followed by increasing of acetate for lipid synthesis. Acetaldehyde is a very reactive compound binding itself to nucleic acids, phospholipids and, above all, to proteins, including albumin,

collagen and haemoglobin, thus altering their structure and function. Nucleic acid modifications, the formation of etheno-DNA adducts, are one of the possible mechanisms of carcinogenicity of alcohol, including inhibition of DNA repair.

Microsomal ethanol oxidizing system (MEOS, cytochrome P450IIE1 or CYP2E1), is an inducible ethanol oxidizing system that metabolizes xenobiotics and many other substances including vitamin D. Induced CYP2E1 can activate carcinogens and hepatotoxins by converting them into even more toxic metabolites. Among the by-products of ethanol oxidation, there is the increased formation of reactive oxygen species, changes in human antioxidant protection systems, and the development of oxidative stress, which has many pathobiochemical effects on the organism (1,2,3,4).

Alcohol consumption is associated with more than 200 diseases (5), including a number of tumours, hypertension (6), liver cirrhosis (7), brain damage and diabetes (8). Ethanol abuse also damages the pancreas (causes up to 50% of chronic pancreatitis), the nervous system (psychiatric diseases, addiction treatment) and the muscles; it affects the immune system, the nourishment of the organism; and contributes to the formation of osteoporosis. Drinking alcohol during pregnancy may cause fetal alcohol syndrome with an incidence of 3.7 per 1000 live births in Europe (9). Children and pre-adolescents (people under 18 years of age) who consume alcohol are at an increased risk of alcohol-induced damage to the organism (10), including the risk of alcohol dependence.

Alcohol is most commonly associated with liver damage. Alcohol-induced liver damage includes a variety of nosological units, such as steatosis, alcoholic hepatitis, cirrhosis, and hepatocellular carcinoma. In Europe and the US, alcohol is the most common cause of liver cirrhosis. According to the GDB (Global Burden of Disease) study,

roughly half of the cirrhosis deaths were caused by alcoholic liver cirrhosis. The basic mechanisms of hepatic tissue damage include centrilobular hypoxia, neutrophil infiltration and immune response activation (IL-8 activation, leukotriene B4, inflammatory cell infiltration), cytokine and endotoxin exposure, antigen adduct formation, and oxidative stress damage (7).

The influence of ethanol on the cardiovascular system is very much debated in terms of its cardioprotective effects at very small doses (20 g/day). The protective mechanism is enabled by an increase in HDL-cholesterol, ApoA I, paraoxonase activity and adiponectin through lowering LDL-cholesterol; by an antithrombotic effect; and by an increase in insulin receptor sensitivity (11). In a recent study (12), the authors have processed data from 83 studies on 59,912 participants, deriving interesting conclusions. When evaluating a total of 40,310 deaths, it was found that the risk rose from 100 g of ethanol per week. In the study of deaths for cardiovascular disease (39,018), there was a decrease in the risk of death in people consuming 100–200g of ethanol per week. When analysing these overall data, the decrease was observed only in myocardial infarction. The above-mentioned paper states that a person aged forty who consumes more than 350 g of ethanol per week will shorten his life by four to five years. Consuming higher doses of alcohol means a higher risk of heart attack, atrial fibrillation and also hypertension (approx. 10% of hypertension is estimated to be caused by alcohol consumption) and cardiomyopathy (11).

Chronic alcohol consumption is associated with 10 % of tumours in males and 3% in females. It is considered a risk factor in upper gastrointestinal tract tumours (UADT – oral cavity, pharynx, larynx, and oesophagus), hepatocellular carcinoma, colorectal carcinoma and breast cancer (13, 14). The impact of alcohol abuse on the risk of lung and pancreatic carcinoma is also discussed.

As for the UADT tumours, 25–68% of them are associated with alcohol consumption with the risk increasing significantly in smokers. The risk of developing the tumour is significantly higher – twice to six times – when consuming 50–100 g/day (13). The threshold risk value for colorectal cancer is 20 g of ethanol a day (15). The incidence of hepatocellular carcinoma increases and alcohol is the major risk factor in Europe and the US. There are a number of mechanisms leading to tumour growth – high dose of alcohol, increased oxidative stress, formation of modified DNA bases, DNA repair disorder, increased acetaldehyde, folic acid deficiency (methylation disorder), inflammatory reactions, changes in iron metabolism, increased estrogen, decreased levels of retinoic acid (hyperregeneration and hyperproliferation), risk allelic variants of alcohol metabolising genes (ALDH2*2, ADH1B*2, ADH1C*1) leading to increased acetaldehyde concentration (16,17), coincidence with pre-cancerous condition (gastroesophageal reflux disease, colon polyps, colitis) or other diseases (e.g. hepatitis, NASH, hemochromatosis).

According to WHO data, alcohol consumption declined in Europe between 1990 and 2014, contrary to East Asia, South America and Africa, where there was an increase in consumption. A significant drop in consumption occurred in the Mediterranean countries – Italy, Spain, France, Greece (18, 19). The European Union estimates the damages caused by consumption of alcoholic beverages at 125 billion EUR per year. In the EU countries, 195 000 people on average die of alcohol-related injuries, liver disease, tumours, etc. every year. It is worth pointing out that in the European Union, every seventh death (14 %) in males and every thirteenth (7 %) in females aged 15–64 are related to alcohol consumption, which means about 95 000 males and 25 000 females per year (12 % of all deaths). It is the third most common cause of early deaths and illnesses in the EU, following smoking and high blood

pressure related diseases. Alcohol is linked to the deaths of young people (15–29 years) at 5% globally, 25% in Europe and, unfortunately, 33 % in Eastern Europe. Around 55 million adults in Europe are at risk of alcohol addiction (consuming more than 40 g/day) (20).

Alcohol as a chemical molecule has been accompanying mankind for several millennia. Current data and meta-analysis of studies suggest that consumption of 100–200 g of ethanol per week can be tolerated (not recommended) on condition that there is a two-day gap between consumption. Alcohol-induced damages are important from both the health and the social point of view (psycho-social and economic consequences).

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Urinary proteomics in biomarker discovery of kidney-related disorders: diabetic nephropathy and drug-induced nephrotoxicity in chronic headache

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ABSTRACT

Objective

Urinary proteomics is primarily applied to the study of renal and urogenital tract disorders. Here are reported two distinct successful examples of this approach for the discovery of early urinary biomarkers of kidney-related dysfunctions: diabetic nephropathy (DN), a well-known complication of diabetes frequently leading to dialysis, and drug-induced nephrotoxicity, a possible condition caused by medication-overuse headache (MOH). Early detection of kidney disorders based on selective biomarkers could permit to diagnose patients at the initial stage of the disease, where the therapy may be suspended or prevent disease advancement.

Methods

Urine samples were first concentrated and desalted. Subsequently, they were subjected to two-dimensional gel electrophoresis (2-DE) coupled to mass spectrometry (MS) for protein identification. Furthermore,

some proteins were verified by Western blot and ELISA test.

Results

In diabetes-related study, 11 differentially expressed proteins were detected (8 up-regulated and 3 down-regulated) in type 2 diabetic (T2D) and T2DN patients compared to the healthy control subjects. In the MOH study, a total of 21 over-excreted proteins were revealed in urine of non-steroidal anti-inflammatory drugs (NSAIDs) and mixtures abusers vs controls. Particularly, 4 proteins were positively validated by immunoblotting and ELISA.

Conclusion

Urinary proteomics allows non-invasive assessment of renal diseases at an early stage by the identification of characteristic protein pattern.



INTRODUCTION

Proteomics is the study of protein expression in a definite tissue, cell type or biological body fluid. The comparison of protein patterns between healthy subjects and patients with a given pathological condition can be useful to identify specific diagnostics or prognostics biomarkers of diseases. Particularly, urinary proteomics has rapidly developed and has been extensively applied in the field of early diagnostics and differentiation of renal damage (1).

Urine is a valuable source of proteins and peptides; it has the advantage of being obtained non-invasively, easily and frequently, and in a large quantity. It has been defined as a fluid biopsy of the kidney and urogenital tract, thus providing considerable information about these organs. Consequently, many changes in kidney and urogenital tract function may be detected in the urinary proteome (2).

Urine proteomics studies were conducted in the search for early biomarkers of renal changes in:

1. type 2 diabetic nephropathy (T2DN), a complication linked to diabetes, which leads to end-stage renal disease (3);
2. drug-induced nephrotoxicity in medication-overuse headache (MOH), a chronic disorder associated with overuse of analgesic drugs or compounds for acute headache (4-6).

MATERIALS AND METHODS

Subjects

1) Diabetic nephropathy

Diabetic patients were enrolled from the "Division of Nephrology, Dialysis and Renal Transplantation" of the University-Hospital of Modena and Reggio Emilia, Italy: 10 normoalbuminuric patients with type 2 diabetes (T2D), 12 T2DN patients with microalbuminuria (range 130-280 mg/mL) and/or proteinuria (>10 mg/dL), and a control group of 12 healthy volunteer subjects with a history of regular renal function.

The duration of diabetes was similar in the two patients groups. Moreover, all groups were matched for age and gender (3).

2) Drug-induced nephrotoxicity

A total of 87 MOH patients were recruited from the "Headache and Drug Abuse Center", University-Hospital of Modena and Reggio Emilia, Italy. They were divided into three groups, according to the type of the primary abused drug, as follows: 31 patients who consumed exclusively triptans, 27 non-steroidal anti-inflammatory drugs (NSAIDs) and 29 taking mixtures. Healthy volunteers (n=30) were enrolled as controls. Each group was matched for age and gender; moreover, patients showed similar MOH duration, days with headache/month and about daily drug intake. Kidney diseases and urogenital tract dysfunctions,

together with other important illness, were considered as exclusion criteria (5).

Both studies were in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki. Written informed consent were received from both, patients and healthy subjects.

Urine proteomics analysis

Morning midstream urine samples were collected and immediately centrifuged at 800 g for 10

min at +4°C, to remove cell debris and contaminants. Commonly, human urine has a very diluted protein concentration and, at the same time, a high-salt content, which hampers the proteomic analysis. Sample preparation is therefore a pivotal step in urinary proteomics, especially during two-dimensional polyacrylamide gel electrophoresis (2-DE) (2). In order to concentrate proteins, eliminating the interfering salts, urine samples were treated with filter devices, 3 kDa MW-cut off (Merck Millipore). Subsequently, total protein content was estimated by the

Table 1 Differentially expressed proteins detected in T2D and T2DN patients by MS

Protein name	Acc. number ^(a)	MW (kDa)	Function
Up-regulated proteins			
Transthyretin precursor	P02766	16.0	Hormone-binding
Ig Kappa chain C-region	P01834	11.8	Immune response
Ig Kappa chain V-II region Cum	P01614	12.8	Antigen-binding
Ig Kappa chain V-II region SIE	P01620	11.9	Immune response
Carbonic anhydrase 1	P00915	28.8	Miscellaneous
Plasma retinol-binding protein	P02753	23.3	Transport
Beta-2-microglobulin precursor	P61769	13.8	Immune response
Beta-2-glycoprotein 1	P02749	39.6	Binding protein
Down-regulated proteins			
Prostatic acid phosphatase precursor	P15309	44.9	Dephosphorylation
Ribonuclease 2	P10153	18.9	Miscellaneous
Kallikrein-3	P07288	29.3	Hydrolysis

(a) Primary accession number from the SwissProt database.

spectrophotometric Bradford method, and 100 mg of protein was premixed with a specific lysis buffer. The first-dimension separation (isoelectrofocalization) was performed using IPG strips 17 cm long, wide pH range 3-10 (Bio-Rad), while in the second-dimension separation, 8-16% polyacrylamide gradient gels were used, that finally were staining with a silver-nitrate staining protocol. Afterwards, gels images were acquired with a calibrated densitometer (GS800 model, Bio-Rad) and analyzed by a specific image analysis software (PDQuest, Bio-Rad), to reveal differentially expressed protein spots among the patients and control groups (3, 5). The spots of interest were cut from the gels and subjected to trypsin digestion. Peptides were finally extracted and analyzed by the mass spectrometry using a quadrupole-time of flight-liquid chromatography mass spectrometer (Q-ToF-LC/MS, Agilent-Technologies).

In the second study (drug-induced nephrotoxicity), the results obtained by proteomic analysis

were further confirmed and validated by Western blot and ELISA test (6).

RESULTS AND DISCUSSION

1) Diabetic nephropathy

Comparing the urinary proteomic profiles obtained by 2-DE analysis, 11 differential proteins were identified that progressively changed between controls and T2D and T2DN patients. Precisely, 8 proteins were significantly up-regulated: transthyretin precursor, Ig k chain C region, Ig k chain V-II region Cum, Ig k-chain V-III region SIE, carbonic anhydrase 1, retinol binding protein, beta-2-microglobulin precursor and beta-2-glycoprotein 1.

Except for the last one, all the other proteins were in the low MW range (<30 kDa). Three proteins were found down-regulated: prostatic acid phosphatase precursor, ribonuclease 2 and kallikrein-3 (Table 1).

Proteomic analysis allowed to detect alterations of urinary proteins in both T2DN and T2D

Table 2 Differentially expressed proteins identified by Q-ToF-LC/MS

Protein name	Acc. number ^(a)	MW (kDa)	Over-expression vs controls ^(b)		
			Mixtures	NSAIDs	Triptans
Low - MW proteins					
Prostaglandin-H2-D-isomerase	P41222	18.7	x	x	x
Ig kappa chain C region	P01834	11.8	x	x	NS
Perlecan (fragment)	P98160	479.2	x	x	x
Transthyretin	P02766	15.9	NS	x	NS
Proactivator polypeptide	P07602	9.11	x	x	x
Nuclear transport factor 2	P61970	14.6	x	x	x

Fatty acid-binding protein	Q01469	15.5	NS	x	x
Beta-2-microglobulin	P61769	11.7	NS	x	x
Protein S100-A11	P31949	11.8	NS	x	x
Non-secretory ribonuclease	P10153	18.9	x	x	NS
Cystatin-C	P01034	13.3	x	x	NS
Protein S100-A8	P05109	10.8	x	x	x
Medium - MW proteins					
Alpha-1-antitrypsin	P01009	46.9	x	x	NS
Actin, cytoplasmic1	P60709	42.1	x	x	x
Alpha-1-microglobulin	P02760	39.9	x	x	NS
Apolipoprotein H	P02749	38.3	NS	x	NS
Serpin B3	P29508	44.6	x	x	x
Annexin A1	P04083	38.6	x	x	x
High - MW proteins					
Serum albumin	P02768	66.5	x	x	NS
Uromodulin	P07911	69.7	x	x	x
Inter- α -trypsin inhibitor heavy chain H4	Q14624	70.6	x	x	NS

(a) Primary accession number from the SwissProt database

(b) Expression difference calculated by the PDQuest software: x: significant, NS: not-significant

normoalbuminuric patients. Thus, this protein pattern might be of potential interest to identify diabetic patients prone to develop nephropathy, contributing to a better understanding of diabetic-related renal damage. The strength of proteomics in this research area has been confirmed also by recently published review articles (7, 8).

2) Drug-induced nephrotoxicity

In this study, both qualitative and quantitative differences in urine of MOH patients were studied and revealed. Interestingly, by 2-DE combined with MS analysis, 21 over-excreted proteins and a significantly higher number of total protein spots were identified in the urine of NSAIDs, mixtures

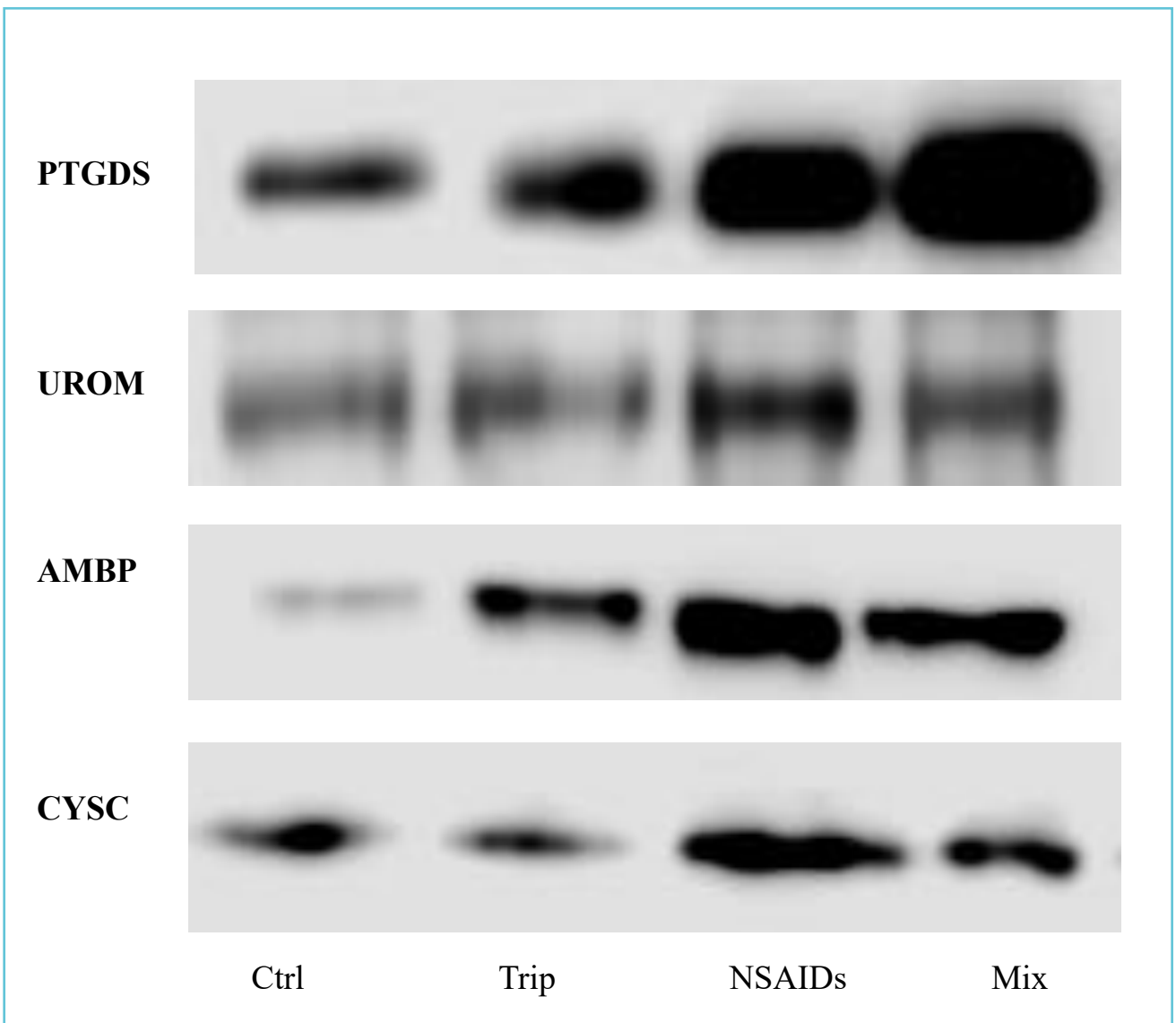
and triptans abusers compared to the controls (Table 2).

Some differentially expressed proteins detected by proteomic analysis were found to be strongly related to renal injury (9), as assessed by an extensive literature review. Particularly, 4 proteins were validated by Western blot: prostaglandin-H2 D-synthase (PTGDS), uromodulin (UROM), alpha-1-microglobulin (AMBP) and cystatin-C (CYSC), as shown in Figure 1.

Immunoblotting allowed to confirm previous data of over-expression of these proteins in urine of MOH patients (especially NSAIDs and mixtures abusers) vs normal controls (6).

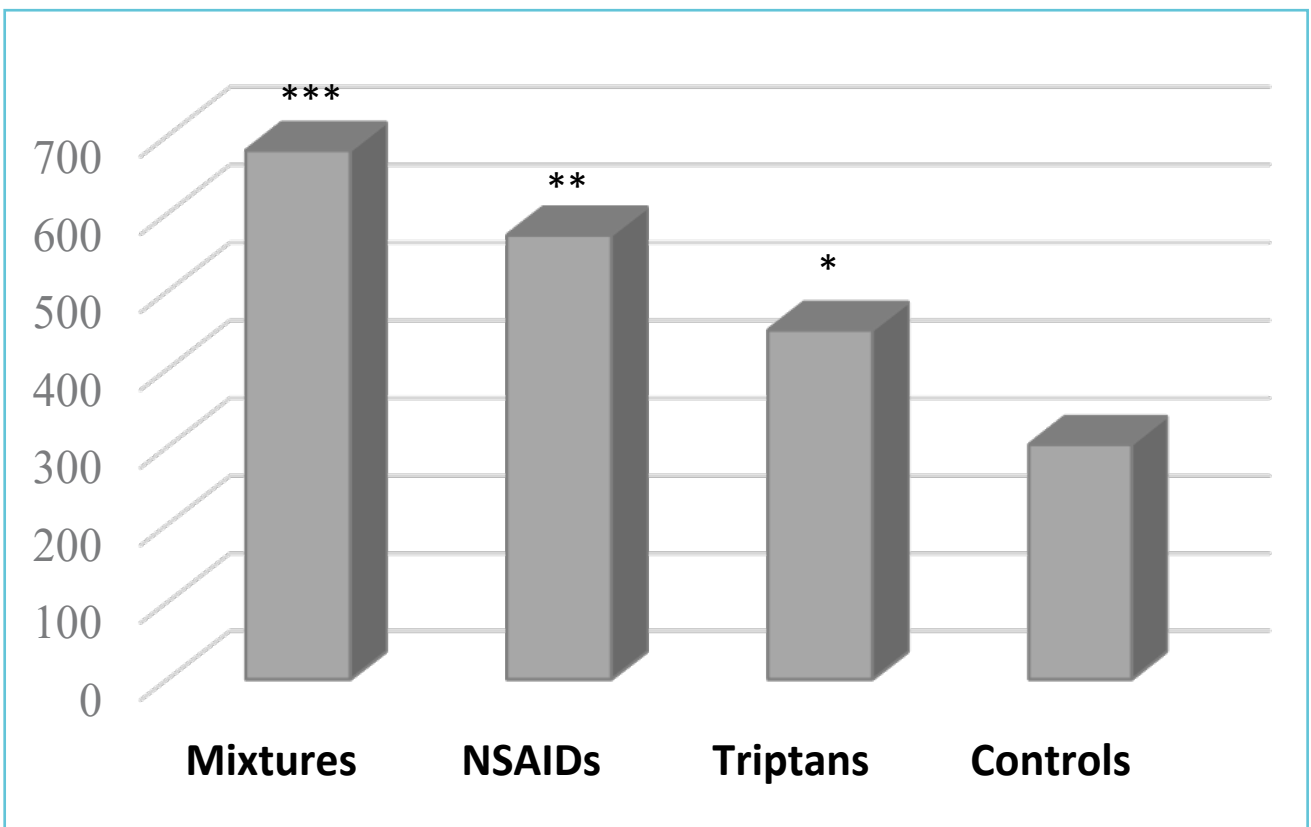
Finally, PTGDS was further quantified by the ELISA test (Figure 2), which proved its significant increase in all MOH groups: mixtures (681 ± 218 ng/mL), NSAIDs (572 ± 135 ng/mL,) and triptans (450 ± 116 ng/mL), compared to the controls (303 ± 130 ng/mL). These data points,

Figure 1 Western blot analysis



The protein expression was evaluated on urine samples in controls (Ctrl), triptans (Trip), NSAIDs and mixtures group (Mix). For each protein tested the signal is higher in NSAIDs and mixtures abusers compared to controls.

Figure 2 ELISA test of PTGDS protein



Significant difference was estimated by the Student's t-test (* $p < 0.01$, ** $p < 0.0001$, *** $p < 1.00^{-06}$ vs controls).

expressed as mean \pm standard deviation, were in strict accordance with MS and Western blot results (6).

The results of this study allowed to define the urinary protein profile of MOH, in relation to the type of drug abused. The use of powerful proteomic methodologies could permit to identify promising candidate biomarkers of kidney dysfunctions, and consequently those chronic headache patients at risk to develop drug-induced nephrotoxicity.

CONCLUSIONS

In conclusion, urinary proteomics proved to be a suitable tool in nephro-toxicological research. Actually, its application may be useful in the search of early biomarkers, providing important diagnostics and prognostics indications.

Additionally, the study of the urinary proteome can offer significant data for a better understanding of renal pathophysiology.

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Standardization of the HbA₂ assay

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Key words:

HbA₂, β -thalassemia,
standardization, traceability

ABSTRACT

Background

A project for the standardization of HbA₂ was launched by the IFCC back in 2004.

Materials and methods

In this work we report on the state-of-the-art of the project on standardization of HbA₂. Data obtained from various EQAS studies, and from previous experimental evaluations, are presented.

Results

We have proven that biases between various commercial methods are still currently significant. We have also shown that calibration by commutable control materials may halve the inter-method variability.

Conclusions

The foundation of the reference system for HbA₂, together with a brief preliminary presentation of the proposed primary reference measurement procedure based on ID-MS are outlined.

WHY TO STANDARDIZE

The promotion of the standardization of routine laboratory methods is one of the mission statement of the International Federation of Clinical Chemistry (IFCC) general policy.

In the specific case of the methods used for the determination of hemoglobin A₂ (HbA₂), their standardization is a real need, not only an academic specific exercise. Indeed, there are several important motivations to meet this:

- most of the routine laboratory methods are poorly aligned;
- important decision limits have been proposed as markers for the diagnosis of thalassemia syndromes;
- the correct diagnosis of carriers in couples at risk is the basic instrument to reduce the burden of thalassemia syndromes;

- β -thalassemia is one of the few examples among genetic disorders in which identification of carriers is performed, mostly, by simple hematological and biochemical tests.

The evidence that the laboratory methods are not aligned may come from the information provided by the external quality assessment schemes (EQAS) or by specific investigations. We have previously reported that the overall inter-laboratory CVs evaluated from a pilot exercise in Italy with 48 Italian laboratories were in the order of 6 to 8% and that the fraction of laboratories reporting unacceptable results ranged from 17 to 32% [1]. We have also shown that data collected in Italy from a mandatory exercise performed in Tuscany and surrounding regions reported CV values between methods similar to those found in another EQAS exercise provided by a manufacturer [2].

Table 1 Performance characteristics of different methods for HbA₂ testing

Method	Between-run reproducibility	Bias versus consensus mean		r
		Slope	Intercept	
Bio-Rad D10	1.7	0.978 to 1.018	0.07 to 0.24	0.9981
Bio-Rad Variant II β -thalassemia	0.9	0.866 to 0.905	0.20 to 0.38	0.9977
Bio-Rad Variant II dual kit	1.7	1.026 to 1.047	-0.23 to -0.14	0.9995
Menarini HA8160	2.3	0.790 to 0.826	0.56 to 0.72	0.9976
Trinity Premier High Resolution	6.6	1.012 to 1.087	-0.08 to 0.24	0.9941
Trinity Premier Quick Scan	1.4	1.115 to 1.156	-0.42 to -0.24	0.9985
Sebia Capillarys 2 FP	1.9	0.898 to 0.946	-0.19 to 0.02	0.9969
Tosoh G8	1.9	1.150 to 1.180	-0.63 to -0.50	0.9992

Data were derived from reference 3. Reproducibility is expressed as CV %. For slope and intercept, the 95% confidence intervals are reported.

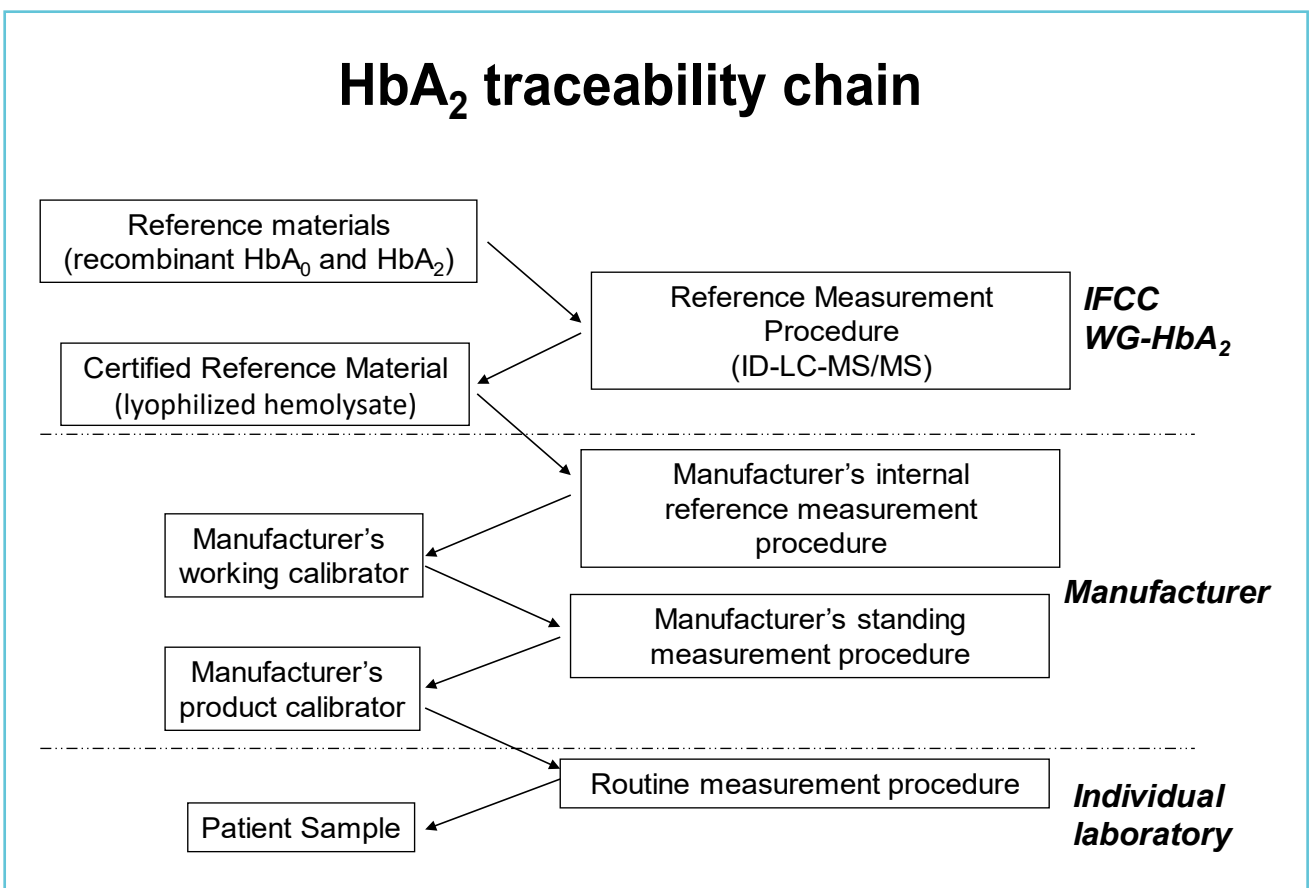
Besides that, we have recently evaluated the performance of current high-performance methods for HbA₂ by measuring 40 blood samples in double over two separate days by the HPLC and one capillary electrophoresis system [3]. We have found a mean imprecision ranging from 0.9 to 6.6 %, and a significant bias between the methods which were however quite well correlated (r between 0.9941 and 0.9995). In Table 1 the main data concerning the performance of the evaluated methods are summarized. In the same work we have tested the commutability of various control materials and we have shown that it is possible to reduce the variability between the methods by using a couple of commutable home-made control materials as calibrators. With regard to the overall variability, we have

seen that after calibration the CVs were reduced from 6.8 to 3.4 %, from 6.6 to 4.6 %, and from 6.7 to 3.0 % for HbA₂ to concentrations lower than 3.0 %, between 3.1 and 4.5 % and above 4.6 %, respectively.

HOW TO BUILD A ROBUST STANDARDIZATION SYSTEM

In 2004, the IFCC approved a project for the development of a complete reference system for HbA₂, as shown in Fig. 1. At the top of the traceability chain, pure recombinant hemoglobins (HbA and HbA₂) are used to calibrate the reference measurement procedure. This reference measurement procedure is based on peptide mapping and isotopic dilution mass spectrometry

Figure 1 IFCC reference system and traceability chain for HbA₂ measurement



(ID-MS), as described previously [2]. Specific tryptic fragments of the δ and α chains (i.e. δ T2 and α T5) are selected as signature peptides representing either HbA₂ or total hemoglobin, and quantified. The principle of the method together with the main performance characteristics are under publication. According to the traceability chain reported in Fig. 1, the reference measurement procedure will then be used to assign the HbA₂ value to certified reference materials which will be produced in collaboration with the Joint Research Centre (JRC).

We have already prepared and characterized one pilot batch of this material consisting of a stabilized hemolysate in the lyophilized form [4]. The material was found to be quite stable with respect to HbA₂ content for at least seven years when stored at -20°C, commutable with the majority of routine methods and to have a total hemoglobin concentration and methemoglobin (MetHb) content similar to that of fresh blood. Manufacturers will then use these certified reference materials to calibrate their methods and to assign values traceable to the primary reference measurement procedure to their calibrators. Finally, the laboratorians using a routine method traceable to a reference measurement procedure will hopefully provide a more robust and accurate result.

TIME-SCALE FOR IMPLEMENTING THE REFERENCE SYSTEM

The first major step is to publish and validate the reference measurement procedure. The principle of this novel method is under publication at the moment of writing this report. The validation of the method according to the various ISO standards will require more work and the involvement of another laboratory because the minimum number of three laboratories is required in order to assign the values to any certified reference material. We estimate at least

one more year to accomplish this second step. Almost in parallel we hope to be able to prepare the certified material to be used as calibrator in collaboration with the JRC. So, it is not unlikely that a couple of years will be necessary in order to have the manufacturers align their methods.

CONCLUSION

Some are of the opinion that the novel molecular techniques such as next generation sequencing (NGS) may substitute the use of HbA₂ in the field of thalassemia screening and diagnosis. We have performed a survey among various opinion leaders and apparently this will not be the case, at least in the next coming years. Indeed, the following points were drawn:

1. Measuring a protein is not the same as sequencing the DNA. Measuring HbA₂ is a functional analysis at protein level, and it will never be replaced by DNA technology.
2. NGS requires a lot of work for the interpretation and, in many cases, the significance of novel SNPs or variants of unknown significance is not known. In addition, it is really critical to know who is going to perform this interpretation.
3. There are still unresolved ethical problems in handling "incidental findings" with DNA technologies.
4. NGS is very expensive and it is unlikely that it could be implemented in poor countries where the burden of thalassemia syndromes is greater than in some richer countries.
5. On the contrary, measuring HbA₂ is simple and fast and it has to be considered that in prenatal screening, as for couples at risk, it is desired to have the result in 1 or 2 days.

As such it can be concluded that HbA₂ assay still remains a gold standard for thalassemia screening and consequently its standardization is a major and topical issue.

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Surrogate biomarkers for monitoring healthcare quality for chronic diseases such as diabetes care

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ABSTRACT

Some laboratory tests or biomarkers are used as surrogate outcomes for health care effectiveness. HbA1c is defined as a surrogate biomarker since HbA1c values have been approved to be used in predictions of clinically important complications of diabetes mellitus. With the advance of information technology (IT) the real life data are aggregating as electronic health records (EHRs). About 70-85% of individuals admitted to hospitals have laboratory test results. As such, medical laboratories are the data centers in the hospitals. The test results can be used for assessment of health care delivered, especially for chronic diseases. This information provides insights of healthcare services, and can be used to enhance for individual and population well-being, research, and education. This article focuses on the importance of using laboratory tests results as outcome measures for specific population health status that are important in assessing the quality of health care services. The findings from our studies on the diabetic care quality is presented.

INTRODUCTION

Medical laboratories are one of the key players in the provision of healthcare, and responsible for healthcare quality. In order to create value-based health care, population-level outcomes and cost-effectiveness should be measured as well at the patient-level (1). The test results from the electronic health records can be used for getting information about population-level healthcare quality, especially for chronic diseases. In this context, laboratory professionals can present some valuable information about the quality of care to the health policy makers, since 70-85% of individuals admitted to a hospital have laboratory tests (2).

It is suggested that quality of care can be assessed according to the conceptualized frameworks suggested by Donabedian and the World Health Organization, and randomized controlled trials (RCTs) are suggested as the best model for assessment. However, RCTs of diagnostic procedures are not common because of the challenges in design and implementation (3-9). Electronic health records are providing new opportunities with high data collection capacity and can be used for assessment of the population status with specific disease according to the surrogate outcome measures such as HbA1c for diabetes

monitoring. Although the information obtained from the real life data is not enough for determination of the actual status, it can provide insights into further structured outcome studies. The data can be used by policy makers, especially in countries where no outcome assessments have been performed at the patient and/or population level. Laboratory professionals working in hospitals should have sufficient knowledge and skills in data management for extracting meaningful information from patients' test results besides their core professional knowledge. The objectives of this paper are to emphasize roles of medical laboratory professionals in the value-based health care model, present the examples on diabetes care quality, and to point out what competencies should be gained by laboratory professionals.

HEALTH CARE QUALITY MEASURES, OUTCOMES AND VALUE-BASED HEALTH CARE

Quality measures have been used in order to assess and compare the healthcare quality of an organization, quality of health care delivery services, and population health quality (3). The performance of health care is assessed by outcome measures. The "value-based health care-VBHC"

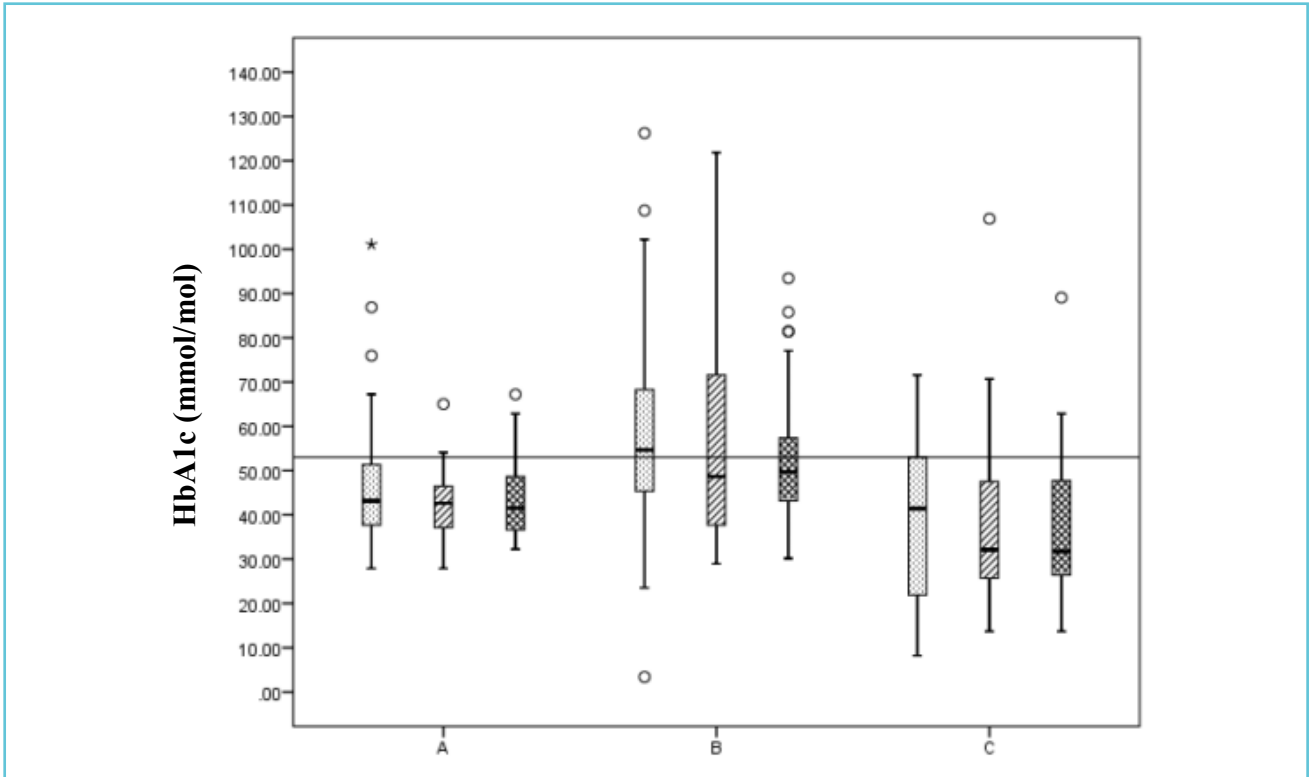
Table 1 Percentages of patients who have test values outside the HbA1c targets at the beginning and after six months

Hospital	Poor controlled diabetics (%)	
	0. month	6. month
Hospital A	19	14
Hospital B	53	39
Hospital C	52	22

Data from our 6-month cohort study of 3 hospitals in different regions in Turkey (11).

Targets: HbA1c <53 mmol/mol (7%) (American Diabetes Association. Standards of medical care in diabetes, 2004).

Figure 1 Six month-cohort study on diabetic populations of three hospitals from three different regions of Turkey (2003-2004)



Hospital A is in the Western Part of Turkey (n=48), Hospital B in the South Eastern Part (n=145), and Hospital C is in the Southern (n=23). A and B are university hospitals, C is a private hospital.

All patient results were collected together with the evidence of quality assurance results of the laboratories.

model that is implemented in the US aims to increase the value that is provided from healthcare services available for a population (1). A report prepared according to the laboratory test results for specific disease population extracted from EHRs can provide an overall look about all the key supporting elements of the VBHC model.

ELECTRONIC HEALTH RECORDS, BIG DATA, POPULATION HEALTHCARE QUALITY

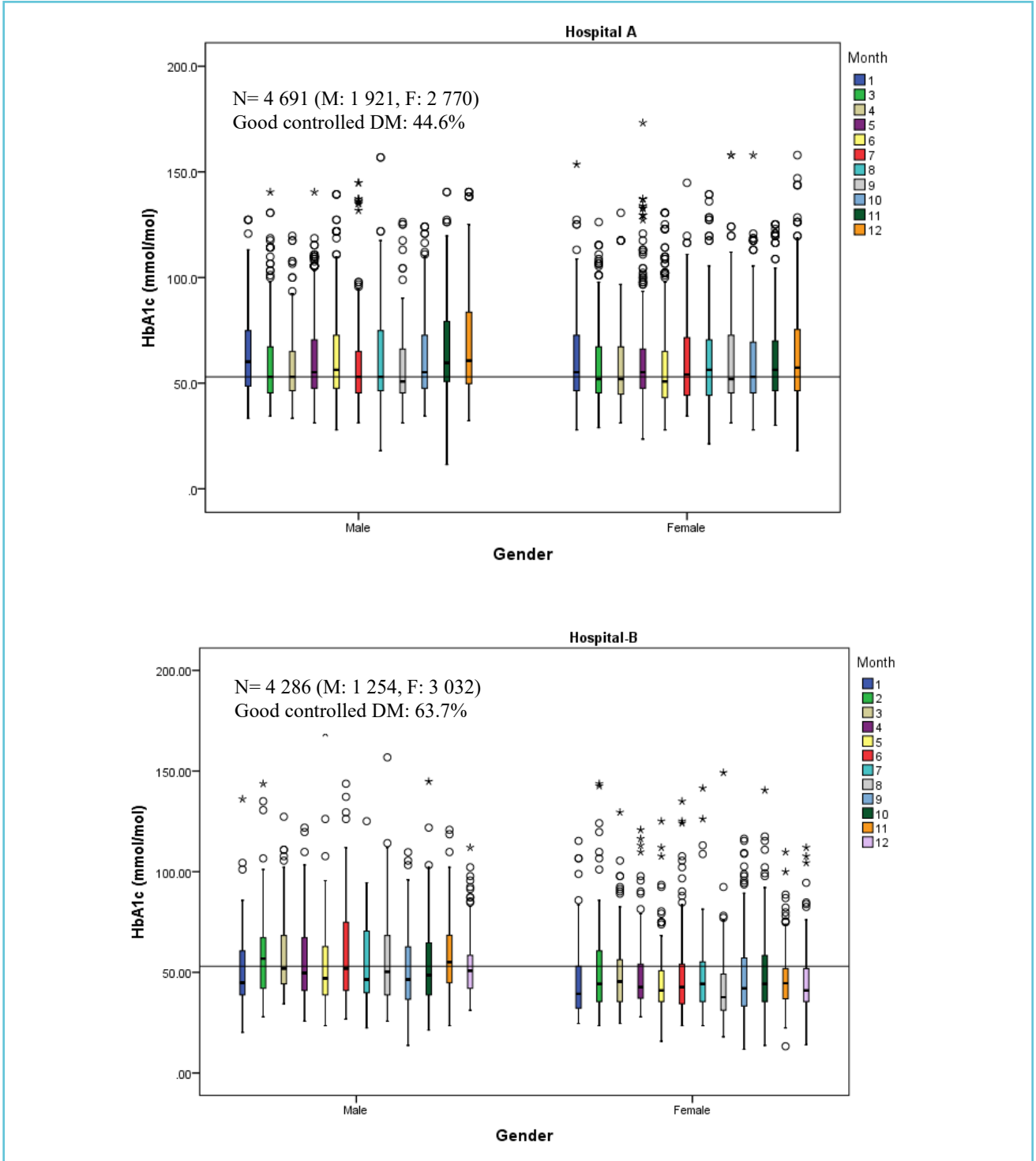
The EHRs are being recognized as an important tool for research as well as clinical care. The main objective should be to learn data mining techniques for extracting meaningful information from database obtained from the EHRs. Some countries have been establishing systems for enhancing the data mining capacities of relevant

organizations (4,5). The laboratory professionals with their data mining knowledge complemented with their core professional knowledge should be part of the data management teams at the hospitals along with epidemiologists and data scientists. They provide meaningful information from test results as a basis for tracking chronic diseases and insights into public health trends, and can aid the management of public healthcare policies (6,7).

DIABETES CARE QUALITY, HbA1c AND BIG DATA

Quality indicators for several diseases, for example, diabetes care quality, are being defined by government organizations and by scientific societies (8,9). Most of quality measurement in

Figure 2 Monthly HbA1c distributions of diabetics in 2017 from data collected in two hospitals in Denizli, Turkey



A is a university hospital, B is a private hospital.

Target for HbA1c: 53 mmol/L (7.0%).

All patient results were collected together with the evidence of quality assurance results of the laboratories.

diabetes mainly includes measures of process and intermediate outcomes, such as HbA1c as surrogate biomarker (10). Laboratory test results can be treated as part of indicative data and the findings from data mining can provide meaningful knowledge to the policy makers at national and individual levels.

DIABETIC CARE QUALITY TRACKING FOCUSING ON THE HbA1c LEVELS OF PATIENTS WITH DIABETES MELLITUS

The HbA1c values of diabetics admitted to the hospitals were collected for 40 years together with the evidence for the analytical quality assurance. In the first 12 years, there were no electronic health records. The test results of diabetics were recorded on their “diabetes test follow-up cards”. Our laboratory collected the results of glucose, HbA1c, and lipids tests and estimated the percentages of poorly controlled diabetics (1982-1994) and 93% of diabetics had HbA1c values higher 7.0% (53 mmol/mol).

Furthermore, HbA1c results of diabetics admitted to the Center of the Turkish Diabetes Society in Denizli between 1999-2003 were also collected and 53% of the patients had HbA1c values higher than 7.0% (53 mmol/mol).

The HbA1c distributions established from our research studies (2003-2005) and the distributions obtained from data extracted from the LISs (2017) are seen in the Figures 1 and 2, respectively (11,12). The percentages of patients that have values outside the targets at the beginning and the 6th month can be seen in the Table 1 extracted from our cohort study (11). All patient results were collected together with the evidence of analytical quality assurance results of the laboratories.

CONCLUSION

Observations and findings from our studies have shown that laboratory professionals should be

part of the data management team in health care organizations along with epidemiologists, statisticians, data scientists, and professionals from relevant disciplines. Laboratory professionals are one of the key players in health care services. They should be aware of the laboratory's value in improving the health of the population, not only the health of a single patient. The key issue is to realize what future challenges will be and what skills should be gained in order to cope with these challenges. Additional skills may be acquired to use relevant information technology and data mining methods in order to be part of the multidisciplinary teams.

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The role of laboratory medicine in addressing migrant health problems

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ABSTRACT

Introduction

A migrant is a person who has relocated to another country for varying reasons. Laboratory medicine is a medical speciality in which body specimens are examined and results interpreted for the appropriate management of patients in healthy and diseased states.

Health challenges of migrants

Migrants have health problems like the general population but may be affected by factors such as their geographic origin, living conditions, and their physical and psychological conditions.

The role of laboratory medicine

Laboratory medicine will play a vital role in the provision of quality healthcare services to migrants. It will be actively involved in the screening, diagnosis, and monitoring of response to treatment. Effective public health surveillance among migrants will require laboratory services. The data gathered from research using laboratory resources will help in the improvement of the quality of migrant health.

INTRODUCTION

Laboratory medicine is a medical speciality involved in the selection, provision, analytical testing and interpretation of tests' results using specimens from patients (1). A medical laboratory is where tests are carried out on specimens in order to obtain information about the health of a patient with regards to the diagnosis, treatment, and prevention of disease.

A migrant can be defined as a person who moves from a place to another in order to find work or better living conditions. Migration may be voluntary (economically motivated) or forced. Each of these forms of migration presents with different health challenges. Some of these challenges are related to where people come from, where they go and how they move. Others are a function of national policies and social attitudes to migrants and their living conditions.

Over 200 million people migrate every year for economic reasons (2). The number of people who are forced to move for reasons of conflict is also growing (3). People flee across borders and become refugees, while at the same time millions of others are forced to flee from their homes but remain within their own borders; the internally displaced.

HEALTH CHALLENGES OF MIGRANTS

The health of displaced populations is mainly affected by infectious diseases, mental health issues and chronic diseases (3). It also depends on the migrant's geographic origin, conditions of migrant camps or urban settings where they live, and the physical and psychological state of the migrant, either pre-existing or acquired (4). Their health problems may be similar to those of the rest of the population, albeit with a higher prevalence. Each migrant should have full, uninterrupted access to high-quality health care, without discrimination on the basis of gender, age, religion, nationality or race. The World

Health Organization (WHO) supports policies to provide health care services irrespective of migrants' legal status. As rapid access to health care can result in cure and reduce the spread of diseases; it is in the interests of both migrants and the receiving country to ensure that the local population is not unnecessarily exposed to the importation of infectious agents (5).

THE ROLE OF LABORATORY MEDICINE

While the roles of clinical laboratories in the management of diseases in a stable population are clearly defined, the same cannot be said for a migrant population. Laboratory testing helps determine the presence, extent or absence of disease and monitor the effectiveness of treatment. An estimated 60 to 70% of all decisions regarding a patient's diagnosis, treatment, hospital admission and discharge are based on laboratory test results (6). A well trained and competent laboratory staff working harmoniously is needed to perform the following roles in addressing migrant health issues.

Screening for specific diseases

Screening is the systematic application of a test to identify subjects at sufficient risk of a specific disorder to benefit from further investigation or direct preventive action, among persons who have not sought medical attention on account of symptoms of that disorder (7). It can detect indicators of active or latent disease that may lead to a cure or diminish the impact medical conditions may have. Some infectious diseases screened for include tuberculosis (TB), hepatitis. Non-communicable diseases can also be screened for e.g., diabetes mellitus (DM), sickle cell disease, malnutrition, etc. Screening for premalignant lesions e.g. cervical cancer (Pap smear), colorectal cancer (faecal occult blood testing) has the potential to reduce mortality from cancer. The WHO does not recommend obligatory screening of migrant populations

because there is no clear evidence of benefits and it may cause anxiety among migrants and the local community (5).

Diagnosis

Laboratory medicine is crucial to the accurate determination of the cause of diseases. Infectious diseases can often be diagnosed using microbiological tests. The Chemical Pathology laboratory is useful in the diagnosis of diseases such as DM, kidney disease, etc. The Haematology and Histopathology laboratories also play crucial roles in the diagnosis of cancers.

Monitoring effectiveness of treatment

Laboratory investigations play a major role in monitoring and evaluating the efficacy of medical treatments. Microbiological tests play an essential part in effective infection control program, and management of antimicrobial resistance. Chemical Pathology and Haematological tests help in monitoring various non-communicable diseases.

Prognostication

Potential adverse outcomes due to disease or drug treatments can be evaluated by laboratory tests. These can minimize the severity of disease and its effect on mortality, morbidity and quality of life.

Quality of patient care

Healthcare delivery systems aim to provide quality care with the patient at the centrepiece via safety, effectiveness, timeliness, efficiency, equity and patient centeredness. Laboratories play crucial role in caring for the patient; over 50% of electronic medical records come from laboratory data (8). Laboratory information enables physicians and other healthcare professionals to make appropriate evidence-based diagnostic or therapeutic decisions for their patients. Clinical laboratory services are the most cost effective,

least invasive source of the objective information used in clinical decision-making.

Public health surveillance

Laboratory medicine plays an important role in the identification and management of public health threats. Accurate laboratory-based information is critical for disease surveillance and control programmes. Before an outbreak, laboratory-supported surveillance allows early detection of cases. During an outbreak a sample of cases should be laboratory confirmed to assess changes in the aetiological agent and to guide decisions about the allocation of resources. Support of varying nature is provided by laboratories of differing capabilities. Field laboratories are useful in providing laboratory services to a migrant population. More complete testing is usually done in nearby regional laboratories. Reference laboratories may identify rare or dangerous pathogens, identify newly described organisms, and provide uncommon diagnostic reagents.

Economic value

When the role laboratory medicine plays in the prevention and useful guidance of treatment is considered, its economic utility as determined by cost-effectiveness analysis is not in doubt. Clinical laboratory tests save time, costs, and lives by enabling early detection and prevention of disease.

Research

Laboratories also play a pivotal role in performing research, especially operational research that supports evidence-based decisions for guiding laboratory practice. Whenever possible, such research should be performed in migrant camps, where the conditions represent the real situation. This is because research performed in academic centres may differ from those in the field and may not always provide reproducible

results under different conditions. Research can also be carried out to improve diagnostic methods and techniques.

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