February 2016 ISSN 1650-3414 Volume 27 Number 1

eJIFCC

Communications and Publications Division (CPD) of the IFCC

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The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

In this issue

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eJIFCC

Foreword of the editor

Editor in Chief: Gábor L. Kovács, MD, PhD, DSc

The current issue of the eJIFCC is devoted to laboratory harmonization. Harmonization is a fundamental aspect of quality in laboratory medicine and its ultimate goal is to improve patient outcomes through the provision of accurate and actionable laboratory information. Two excellent and renowned laboratory scientists (Ms. Jillian Tate from Australia and Dr. Gary L. Myers from the US) were asked to invite specialists on harmonization and guest-edit the issue.

Jill Tate is a Senior Scientist working in the Department of Chemical Pathology at the Pathology Queensland Central Laboratory in Brisbane, Australia and currently co-ordinates the laboratory's Research and Development Unit which collaborates closely with local, national and international clinical and laboratory groups. She has been involved with harmonization activities since the 1990's through work with lipoprotein(a) standardization and the IFCC Working Group on the Standardization of Lp(a) Assays, then with cardiac troponin and the IFCC Committee on the Standardization of Markers of Cardiac Damage. Between 2008 and 2014 Jill chaired the IFCC WG-TNI, which is developing a secondary reference material for the standardization of troponin I assays. In October 2010 in Gaithersburg, USA, the AACC held their inaugural harmonization meeting. Following this meeting, which was attended by Jill on behalf of the Australasian Association of Clinical Biochemists (AACB), the AACB Harmonization Committee was formed in 2011. As chair of the committee since its inception, Jill coordinates many of the AACB's harmonization activities including workshops and the formation of working parties involved with various aspects of harmonization, e.g. AACB Committee on Common Reference Intervals, AACB-RCPA Working Party on Management of Critical Laboratory Test Results. Over this time, she has guest-edited special issues on harmonization for The Clinical Biochemist Reviews and Clinica Chimica Acta.

Jill's main passion in the routine laboratory for over 30 years has been to work in the protein electrophoresis area and she has written widely on serum free light chain measurement. Standardization and harmonization of free light chain measurements remain controversial. Currently she is co-guest editing a special proteins issue on protein electrophoresis and serum free light chain measurement for Clinical Chemistry and Laboratory Medicine, due out in May this year. Above all Jill is enthusiastic about the role of the profession in Laboratory Medicine and believes that harmonization is an important way that the profession can add value to Laboratory Medicine.

Gary Myers, PhD, currently serves as Chair of the Joint Committee for Traceability in Laboratory Medicine. He also serves as Chair of the Council for the International Consortium for Harmonization

of Clinical Laboratory Results (ICHCLR). His most recent position was Vice President, Science and Practice Affairs for the American Association for Clinical Chemistry (AACC). Prior to joining AACC, Dr. Myers served as Chief, Clinical Chemistry Branch at the United States Centers for Disease Control and Prevention (CDC). During his 33+ year career at CDC he directed programs to improve and standardize the laboratory measurement of biomarkers used to assess chronic disease status, particularly for cardiovascular disease and diabetes. Dr. Myers served as Secretary for the Scientific Division of the International Federation of Clinical Chemistry and Laboratory Medicine from 2009-2014. In 2015 Dr. Myers received AACC's Outstanding Lifetime Achievement Award in Clinical Chemistry and Laboratory Medicine. He served as AACC President in 2007.

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

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Harmonization of clinical laboratory test results

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Key words:
harmonization, standardization, laboratory medicine, total testing process

EDITORIAL

Clinical laboratory testing is now a global activity with laboratories no longer working in isolation but as regional and national networks, and often at international levels. We now have all of the electronic gadgetry via internet technology at our fingertips to rapidly and accurately measure and report on laboratory testing but are our test results harmonized?

WHAT IS HARMONIZATION OF LABORATORY TESTING?

In the context of Laboratory Medicine, harmonization of laboratory testing refers to our ability to achieve the same result (within clinically acceptable limits) and the same interpretation irrespective of the measurement procedure used, the unit or reference interval applied, and when and/or where a measurement is made.

Laboratories may use different analytical methods that may not be harmonized, possibly with different units of reporting. We should not assume that the differing numbers can be directly compared especially if the transfer of results from the laboratory to the report recipient does not highlight differences in units of reporting or in assay methods in use. To

the contrary, the assumption made by patients, clinicians and other healthcare professionals is that clinical laboratory tests performed by different laboratories at different times on the same sample and specimen are comparable in their quality and interpretation.

WHY IS HARMONIZATION NEEDED IN LABORATORY MEDICINE?

When laboratory test results differ the potential exists for misinterpretation of results, wrong treatments and adverse patient outcomes. It is our responsibility as laboratory professionals to identify where gaps exist in laboratory testing and endeavour to harmonize these where possible, thereby minimising misinterpretation of test results.

WHO IS HARMONIZATION OF LABORATORY TESTING INTENDED FOR?

The key stakeholders who will benefit from harmonization are the patients, the clinical laboratory community, diagnostic industry, clinicians, professional societies, information technology providers, consumer advocate groups, regulatory and governmental bodies. The clinical laboratory community includes all disciplines of Laboratory Medicine. As potential consumers of laboratory testing ourselves, we expect to receive not only the Right result on the Right patient at the Right time in the Right form, but also the Right test choice with the Right interpretation with the Right advice as to what to do next with the result. This should be irrespective of the laboratory that produced the result and is achievable through harmonization (1).

AN OVERVIEW OF HARMONIZATION

In this harmonization issue Mario Plebani, who has been a proponent of harmonization in Laboratory Medicine for over 20 years provides an overview of the current and future strategies needed to achieve harmonization of clinical laboratory information (1, 2). He emphasises the importance of considering the complete harmonization picture to ensure the comparability of laboratory information in all aspects of the total testing process (TTP) including the request, the sample, the analysis and the report.

As discussed by Plebani and others in this issue, a systematic approach to harmonization is needed that requires the following:

1. Awareness by the Laboratory Medicine community that there is a need for harmonized processes not only for the analytical phase but across all steps of the TTP (3);
2. Awareness that harmonization processes are complex; hence a systematic and evidence-based approach that reflects best laboratory practice is needed;
3. An organizational plan or roadmap for the set-up and implementation of each harmonization activity is a pre-requisite and must identify and describe the problem in detail, identify relevant groups including external bodies when forming a working group, determine a funding source, gather technical information and data from various sources, consider the solutions, produce a discussion paper, seek feedback comments from the relevant stakeholders through discussion and revise recommendations, publish endorsed recommendations, promote and implement them, then monitor and survey their introduction (4-6);
4. Communication with main stakeholders, i.e. pathologists, scientists, clinical groups, regulatory bodies, IT developers, and consumer groups is central to the success of any harmonization project with a consensus outcome arrived at through cooperation and discussion (4,7,8).

What is the status of harmonization activities globally?

In Europe there is a recent initiative to promote harmonization activities among the 40 European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) member societies. The Working Group on the Harmonization of the Total Testing Process (WG-H), chaired by Ferruccio Ceriotti, was formed the aims being to survey national European harmonization initiatives, coordinate the dissemination of promising harmonization initiatives among the EFLM member societies, and specifically to harmonize nomenclature, units and reference intervals where possible at a European level. As described by Ferruccio Ceriotti in this issue (9), based on the results of a survey questionnaire some activities promoting the dissemination of best practice in blood sampling, sample storage and transportation, in collaboration with WG on the Preanalytical Phase (WG-PRE), are already being promoted (10-13). See Table 1.

Table 1 Harmonization of the Total Testing Process (TTP) – global harmonization activities

TTP phase	Harmonization activity	International and national stakeholders
Pre-analytical	1. Test requesting – demand management and reflex testing – harmonized test profiles	1. ACB Clinical Practice Section – National Minimum Retesting Interval Project (UK)
	2. Guidelines/position papers	2. CDC, CLSI, EFLM WG-CM, EFLM WG-G, EFLM WG-PRE, AACC
	3. Patient preparation and sample collection	3. EFLM WG-PRE, RCPAQAP KIMMS
	4. Sample handling and transport	4. EFLM WG-PRE
	5. Quality indicators	5. IOM, IFCC WG-LEPS, EFLM TF-PG
Analytical	1. Traceability – promoting use of traceable assays	1. BIPM, JCTLM, ILAC, EQAS
	2. Development of commutable secondary reference materials (RM)	2. NIST, IRMM, WHO, IFCC WG-Commutability
	3. Harmonization of measurement values for methods where no RM or reference measurement procedure	3. ICHCLR, IFCC
	4. Harmonization of Mass Spectrometry (MS) methodology	4. APFCB WP-MS Harmonization, AACB MS Harmonization SIG, CDC Hormone Standardization program, COST DSDnet –WG-3: Harmonization of Laboratory Assessment

Post-analytical	1. Standardization of reporting units	1. IFCC C-NPU, IUPAC, IFCC WG-HbA1c, Pathology Harmony (UK), RCPA PITUS (Australia)
	2. Standardization of reporting terminology	2. Pathology Harmony (UK), RCPA PITUS (Australia)
	3. Harmonization of calculated parameters	3. ACB Albumin-adjusted calcium, AACB WP-Calculations
	4. Common reference intervals (RIs) across multiple platforms for traceable analytes	4. IFCC C-RIDL, Nordic countries (NORIP), Pathology Harmony (UK), Turkey, Japan, Canada (CALIPER and CHMS), Australia & New Zealand (Common RIs project)
	5. Platform-specific RIs and decision limits for immunoassay analytes where there is method bias	5. AACB Harmonisation Committee (Australia & New Zealand), CALIPER & CHMS (Canada)
	6. Standardization of report formatting	6. RCPA PITUS (Australia)
	7. Critical laboratory results (CLR) – harmonized processes for management and communication of critical results; list of critical tests	7. EFLM, CLSI, AACB-RCPA WP-CLR (Australia)
	8. Interpretative commenting – harmonization of commenting for EQA	8. IFCC WG-Harmonisation of Interpretative Commenting for EQA
	9. Biological variation – harmonized approach to validation of quality of BV data for use with RCV interpretation (EFLM project)	9. EFLM WG-BV
	10. Surveillance of: – pre-analytical and post-analytical processes – common RIs – calculations – test profiles – interpretative commenting – report formatting	10. IFCC WG-LEPS, RCPAQAP KIMMS, EFLM TFG-Harmonisation of performance criteria for EQA program surveillance, RCPAQAP Liquid Serum Chemistry, calculations, RIs and test profiles program (Australia)
	11. Quality indicators	11. EFLM WG-POST, EFLM WG-PSEP

Post-post analytical	1. Promotion of clinical and laboratory relationships	1. IFCC Taskforces, AACC Strategic Clinical and Laboratory partnerships
	2. Lab Tests Online (LTO) – a global educational tool	2. LTO around the globe
	3. Patient focus	3. ACB, EFLM WG-PFLM

AACB: Australasian Association of Clinical Biochemists;

AACC: American Association for Clinical Chemistry and Laboratory Medicine;

ACB: Association for Clinical Biochemistry and Laboratory Medicine (UK);

APFCB: Asia-Pacific Federation for Clinical Biochemistry and Laboratory Medicine;

BIPM: Bureau International des Poids et Mesures;

CALIPER: Canadian Laboratory Initiative on Pediatric Reference Intervals;

CDC: Centers for Disease Control and Prevention;

CHMS: Canadian Health Measures Survey;

CLSI: Clinical and Laboratory Standards Institute;

C-NPU: Committee on Nomenclature: Properties and Units (IFCC and IUPAC);

C-RIDL: Committee on Reference Intervals and Decision Limits (IFCC);

COST-DSDnet: European Cooperation in Science and Technology initiative action BM1303, "A Systematic Elucidation on Differences of Sex Development";

DSDnet; Working group 3; http://www.dsdnet.eu/wg-3.html;

EFLM: European Federation of Clinical Chemistry and Laboratory Medicine;

EQAS: External Quality Assurance Scheme;

ICHCLR: International Consortium for Harmonization of Clinical Laboratory Results (AACC);

IFCC: International Federation of Clinical Chemistry and Laboratory Medicine;

ILAC: International Laboratory Accreditation Cooperation;

IOM: Institute of Medicine;

IRMM: Joint Research Centre Institute for Reference Materials and Measurements;

IUPAC: International Union of Pure and Applied Chemistry;

JCTLM: Joint Committee for Traceability in Laboratory Medicine;

KIMMS: Key Incident Monitoring and Management Systems (RCPAQAP);

LTO: Lab Tests Online;

NACB: National Academy of Clinical Biochemistry (AACC);

NIST: National Institute of Standards and Technology;

NORIP: Nordic Reference Interval Project;

PITUS: Pathology Information Terminology and Units Standardisation (RCPA);

RCPA: Royal College of Pathologists of Australasia;

RCPAQAP: Royal College of Pathologists of Australasia Quality Assurance Programs;

RM: reference material;

SIG: Special Interest Group;

TFG: Task and Finish Group (EFLM);

TF-PG: Task Force on Performance goals in Laboratory Medicine (EFLM);

WG-BV: Working Group on Biological Variation (EFLM);

WG-CM: Working Group on Cardiac Markers (EFLM);

WG-G: Working Group on Guidelines (EFLM);

WG-LEPS: Working Group on Laboratory Errors and Patient Safety (IFCC);

WG-PFLM: Working Group on Patient Focused Laboratory Medicine (EFLM);

WG-POST: Working Group on Postanalytical Phase (EFLM);

WG-PRE: Working Group on Preanalytical Phase (EFLM);

WG-PSEP: Working Group on Performance Specifications for the Extra-analytical Phases (EFLM);

WHO: World Health Organization.

In Table 1 many of the EFLM harmonization activities involving pre-analytical, post-analytical and post-post analytical activities are described. As noted by Ceriotti, a PubMed search for the words “harmonization” or “harmonisation” resulted in 972 items, with a sharp increase in the numbers of publications in the last 5 years. It is apparent that in many countries clinical chemistry societies and other professional groups including External Quality Assurance Schemes (EQAS) are working on harmonization projects (Table 1).

A pathway for global harmonization of assays

While the metrological concepts of standardization, calibration traceability to reference materials and measurements, and measurement uncertainty are described in the International Organization for Standardization (ISO) standards ISO 17511 (14) and 18153 (15) and assure the accuracy and equivalence of clinical laboratory results, harmonization is required to achieve uniform results among different measurement procedures for the same laboratory test where there is no reference measurement procedure available. Gary Myers and Greg Miller describe how an international consortium for harmonization of clinical laboratory results (ICHCLR) has been formed to organize these global harmonization efforts (5, 16).

The role of the ICHCLR infrastructure is to address: 1) prioritizing measurands by medical importance, 2) coordinating the work of different organizations, 3) developing technical processes to achieve harmonization when there is no reference measurement procedure or no reference material and 4) promoting surveillance of the successes of harmonization. A key focus of the ICHCLR is cooperation with other organizations already actively working to improve harmonization of laboratory test results such as the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

The major advantages of harmonized test results include the use of common decision limits specified in clinical guidelines across all methods and uniform interpretation of results. An example of a current IFCC standardization project involving harmonization is that for thyroid function tests with the Committee on the Standardization of Thyroid Function Tests led by Linda Thienpont using a step-up harmonization approach. Other up-to-date information about measurands in need of harmonization is available online at: http://www.harmonization.net, together with a toolkit with information about harmonization protocols.

What is the role of the IVD industry in harmonization?

The In Vitro Diagnostics (IVD) industry is expected to provide traceability information indicating that their routine assays are traceable to reference materials and/or reference methods. However, traceability does not necessarily ensure comparability of patient test results. Rather, both harmonization and metrological traceability of assays are required to provide test results that are clinically equivalent between different manufacturers' analytical systems (5). In their paper on the role of the IVD industry in the harmonization of clinical laboratory test results, Dave Armbruster and James Donnelly describe here the six "pillars" that are needed to achieve traceability and harmonization (17). These are: 1) reference measurement procedures; 2) reference materials; 3) reference measurement laboratories; 4) universal reference intervals; 5) EQA programs using commutable samples with reference method target values to allow accuracy-based grading of manufacturers' assays; and 6) harmonized basic terminology and units.

As both authors state, the new challenge for the IVD industry is to work with the many professional organizations and each other to attain harmonization, and still retain viable businesses. In their view industry support can be best achieved when harmonization initiatives are coordinated and prioritized. Major factors to be considered are:

1. Competing project priorities for companies;
2. Requirements by regulatory agencies for re-registration and associated additional costs and other manufacturing issues;
3. Need for cooperation between companies through contributing to the prioritization of projects, design of experiment, etc.;
4. Device manufacturer's typically register products with the US FDA using a predicate device to demonstrate product acceptance. In such cases proof of substantial equivalence is essential to demonstrate the assay is safe and effective. Ideally companies want to compare their assay with a traceable reference assay that is listed on the JCTLM website (Joint Committee for Traceability in Laboratory Medicine);
5. Does a harmonization effort add value to patient care? The cost of harmonization which includes physician education, patient safety and investment in product redevelopment needs to be assessed against the clinical benefit of harmonization.

How do we derive harmonized Reference Intervals?

In the post-analytical phase laboratory test results are compared to reference intervals (RIs) or decision limits depending on the analyte measured. However, where the same values are interpreted differently due to differences in RIs or decision limits this may lead to inappropriate over- or under-investigation or treatment of the patient. The use of harmonized or common RI across different platforms and/

or assays aims to give the same interpretation irrespective of the pathology provider or the method, provided the same unit and terminology are used. Harmonization of RIs occurs optimally for those analytes where there is sound calibration and traceability in place and evidence from between-method comparisons shows that bias would not prevent the use of a common RI.

Jill Tate, Gus Koerbin and Khosrow Adeli provide an opinion in this issue on how to derive harmonized reference intervals (18). A predetermined checklist approach to acquiring the evidence for common RIs provides an objective means of developing and assessing the strength of the evidence. The selection of the RI will depend on various sources of information including local formal RI studies, published studies from the literature, laboratory surveys, manufacturer's product information, relevant guidelines, and mining of databases.

Several countries and regions including the Nordic countries, United Kingdom, Japan, Turkey, and Australasia are using common RIs that have been determined either by direct studies or by a consensus process. In Canada the Canadian Society of Clinical Chemists Taskforce is assessing the feasibility of establishing common reference values using data from the formal reference interval studies of CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) and CHMS (The Canadian Health Measures Survey) as the basis. Development of platform-specific common reference values for each of the major analytical systems may be a more practical approach especially for the majority of analytes that are not standardized against a primary reference method and are not traceable to a primary or secondary reference material.

The authors encourage laboratories to consider adopting reference intervals consistent with those used by other laboratories in your region where it is possible and appropriate for your local population. Validation of reference intervals by local laboratories is central to the adoption of common RIs nationally as is validation of flagging rates to ensure the expected number of results outside the RI is acceptable.

How do we manage critical risk results?

Que Lam, Eva Ajzner, Craig Campbell and Andrew Young write in this issue about the current situation and existing practices for the management of critical risk results (19). They describe the need for more evidence from outcomes studies of critical risk results management to support laboratory practices and the need for harmonized terminology. New harmonized terminology has recently been proposed, e.g. "high-risk results", results requiring immediate medical attention and action, and "significant-risk results", results which signify a risk to patient well-being and require follow-up action within a clinically justified time limit (20). The authors discuss the recently released Clinical and Laboratory Standards Institute (CLSI) guideline CLSI GP47-Ed1 for the management of laboratory test results that indicate risk for patient safety (21), as well as presenting the Australasian recommendations. In order to promote best laboratory practice, Lam et al. recommend that laboratories consider risk assessment when compiling alert tables and involve laboratory users when setting up protocols. They state: "Harmonization in this area cannot simply be a matter of shared definitions and procedures, but must involve the determination and implementation of best practice. The challenge is to define best practice and to obtain the evidence required to support this".

CONCLUSIONS

It is obvious that harmonization does not happen overnight but is a long term consensus process that ideally is based on hard evidence that has been systematically compiled and has involved close interaction between the laboratory and the clinician to ensure successful implementation. It must be a shared responsibility of all stakeholders interested in patient care. Harmonization aims to add value to Laboratory Medicine measurements and their interpretation. Harmonized test results will ensure that clinical guidelines that call for the use of laboratory tests can be universally implemented. Harmonization still allows for innovation through discussion and the input of new ideas. It should extend beyond clinical chemistry across to all other pathology and Laboratory Medicine disciplines as the problems are not unique to chemistry.

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In this issue: Harmonization of Clinical Laboratory Test Results

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Harmonization of clinical laboratory information – current and future strategies

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Key words:
harmonization, standardization, traceability, interchangeability, quality, patient safety, quality indicators, total testing process

ABSTRACT

According to a patient-centered viewpoint, the meaning of harmonization in the context of laboratory medicine is that the information should be comparable irrespective of the measurement procedure used and where and/or when a measurement is made. Harmonization represents a fundamental aspect of quality in laboratory medicine as its ultimate goal is to improve patient outcomes through the provision of an accurate and actionable laboratory information. Although the initial focus has to a large extent been to harmonize and standardize analytical processes and methods, the scope of harmonization goes beyond to include all other aspects of the total testing process (TTP), such as terminology and units, report formats, reference intervals and decision limits, as well as tests and test profiles request and criteria for interpretation. Two major progresses have been made in the area of harmonization in laboratory medicine: first, the awareness that harmonization should take into consideration not only the analytical phase but all steps of the TTP, thus dealing with the request, the sample, the measurement, and the report. Second, as the processes required to achieve harmonization are complicated, a systematic approach is needed. The International Federation of Clinical

Chemistry and Laboratory Medicine (IFCC) has played a fundamental and successful role in the development of standardized and harmonized assays, and now it should continue to work in the field through the collaboration and cooperation with many other stakeholders.

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INTRODUCTION

Patients, clinicians and other healthcare professionals assume that clinical laboratory tests performed by different laboratories at different times on the same sample and specimen can be compared and that results can be reliably and consistently interpreted (1). Unfortunately, these assumptions are not always justified because many laboratory test results are still highly variable, poorly standardized and harmonized. Harmonization represents a fundamental aspect of quality in laboratory medicine as its ultimate goal is to improve patient outcomes through the provision of an accurate and actionable laboratory information (2). Although the initial focus has to a large extent been to harmonize and standardize analytical processes and methods, the scope of harmonization goes beyond to include all other aspects of the total testing process (TTP), such as terminology and units, report formats, reference intervals and decision limits, as well as tests and test profiles request and criteria for interpretation (3, 4).

Major reasons to focus on a global picture of harmonization are represented by: a) the nature of errors in laboratory medicine and the evidence of the high rates of errors in the pre-and post-analytical phases (5, 6), b) the evidence of large variations in terminology, units and reference ranges (7), c) the increasing demand for improving appropriateness in test request and result interpretation (8), and, finally, d) the risks for patient safety related to previous issues (9).

HARMONIZATION: CURRENT PROJECTS

As recently highlighted by Tate and Coll ”clinical laboratory testing is now a global activity, and laboratories no longer work in isolation” (10). Therefore, there is an increasing awareness of the importance and urgency to achieve harmonization in all steps of the total testing process (TTP) for ensuring comparability and interchangeability of laboratory information.

Harmonizing the pre-analytical phase

Several initiatives and projects are in progress for harmonizing both the pre-pre-analytical as well as the pre-analytical processes. In the initial steps of the cycle, the issue of demand management which focuses on ensuring appropriate requesting is receiving an increasing importance. A step forward in this area has been achieved through the acceptance of the definition of “inappropriate test demand” that appears to be “a request that is made outside some form of agreed guidance” (11). The type of guidance may vary from national and international guidelines to locally agreed behaviours but the basic concept is the application of scientific evidence rather than anecdote to clinical practice (8). Among the several progress, a special attention should be deserved to the National Minimum Retesting Interval Project promoted by the Clinical Practice Section of the Association for Clinical Biochemistry (ACB) in the UK uses a “state of the art” approach to set consensus/evidence based recommendations on when a test should be repeated. (12).

The importance to standardize patient preparation and sample collection requirements to minimize the uncertainty from the pre-analytical phase has already activated efforts to provide better evidence and recommendations. (13, 14). Further work to optimize sample transportation procedures as well as the identification of indicators for their monitoring has been done,

and this is a premise for future harmonization initiatives in this field (15-17). In addition, the harmonization of procedures for evaluating the quality of biological samples, the criteria for their acceptance and rejection even through the use of automated workstations and serum indexes has been largely reported and promoted (18-21).

Harmonizing analytical results

Although the terms "standardization" and "harmonization" define two distinct, albeit closely linked, concepts in laboratory medicine, the final goal is the same: the equivalence of measurement results among different routine measurement procedures over time and space according to defined analytical and clinical quality specifications (22).

While standardization, which allows the establishment of metrological traceability to the System of Units (SI), represents the recommended approach, for a multitude of measurands the SI does not yet apply, in particular when the components in the measurand comprise a heterogeneous mixture. Over the past two decades, several clinical laboratory tests have been standardized through the development of reference measurement procedures, the IFCC playing a major role in this project. In particular, the standardization of glycated haemoglobin contributed to significant improvements in diabetes (23). Other important projects are in progress in order to standardize measurands of high clinical value such as cardiac troponin (24) and carbohydrate-deficient transferrin (25). However, as a matter of fact, for a huge number of measurands neither a reference method nor reference material are available (26). For all these measurands, harmonization of available methods and diagnostic systems should be promoted. In the last few years, significant progress has been done establishing an overarching control system of the harmonization process in all its aspects through improvements in: a) defining the quality and quantity of human samples to be used for standardization and harmonization studies (27, 28), b) identifying new and more robust mathematical models and statistical treatments of the data (29, 30). A major lesson we learnt, is that standardization and harmonization should not be applied only to clinical chemistry measurands, but to the whole field of laboratory medicine, including molecular diagnostics (31). It should be highlighted that one of the most impressive and effective examples of harmonization in laboratory medicine is the expression of prothrombin results as international normalized ratio (INR). PT results are corrected mathematically into INR by raising the PT-ratio to a power equal to the international sensitivity index (ISI) thus harmonizing results stemming from different thromboplastins from patients on treatment with vitamin K antagonists (32). Therefore, the debate on harmonization should not be limited to clinical chemistry scientists but should involve all fields of laboratory medicine to provide comparability and interchangeability of all tests usually performed in clinical laboratories, including "omics".

Under the patient-centered viewpoint, the supposed diatribe between standardization and harmonization should concentrate on more joint efforts to provide equivalence of measurement results among different routine measurement procedures and different clinical laboratories over time and space.

Harmonizing the post-analytical phase

Several issues in the post-analytical phase are increasingly acknowledged as fundamental steps for achieving higher harmonization and effectiveness of laboratory information.

Current evidence collected in the UK and in Australia demonstrates a significant variation in the units used for some tests and even more

widespread variation in the way they are represented on screens and paper, as well as the way they appear in electronic messages (33). This, in turn, creates a potential for misinterpretation of laboratory results and risk for patient safety (7). As test results are increasingly transferred electronically, the argument for adopting a single standardized set of units needs immediate uptake (34).

Reference intervals are the most widely used decision-making tool in laboratory medicine and serve as the basis for many of the interpretations of laboratory results. Numerous studies have shown large variation of reference intervals, even when laboratories use the same assay thus contributing to different clinical interpretation, risk for patients and unnecessary test repetition (35, 36). The importance of obtaining reference intervals traceable to referent measurement systems has been reported (37) and evidence-based approaches to harmonize reference intervals have been promoted (38). The Nordic Reference Interval Project (NORIP) was one of the earliest reference interval harmonization initiatives and established common reference intervals in apparently healthy adult populations from five Nordic countries for 25 of the most common clinical chemistry analytes (39) Several more recent initiatives have already provided data for adopting common reference intervals in huge geographical areas such as Asia (40), Canada (41-43) and Australasia (44). In the Australasian approach, selection of a common reference interval requires a checklist assessment process be adopted to assess the evidence for their use and is based on the criteria summarized in Table 1.

The final decision on the common reference interval to be used involves weighing up each piece of evidence. Importantly, the proposed reference limits should also be supported by flagging rates which provide an indication of the clinical considerations of a reference interval (46). However, the use of asterisks should require further considerations because patients and people who have no training in laboratory medicine now have direct access to their laboratory test results.

Table 1 Selection of common reference interval (RI): criteria to be adopted

1.	Define analyte (measurand)
2.	Define assays used, accuracy base, analytical specificity, method-based bias
3.	Consider important pre-analytical differences, actions in response to interference
4.	Define the principle behind the RI (e.g. central 95%)
5.	Describe evidence for selection of common RIs data sources (literature, lab surveys, manufacturers, data mining and the allowable bias goal as quality criterion for acceptance)
6.	Consider partitioning based on age, sex, etc
7.	Define degree of rounding
8.	Assess clinical considerations of the RI
9.	Consider use of common RI
10.	Document and implement

Adapted from ref 45, modified.

Various practices, a number of different terminologies and extremely different values have been described in the literature affecting the quality of critical results management. Large variability in critical results practices have been reported not only when comparing different geographical areas but even in the same country (47). Very recently, a study on the outcomes of critical values notification, demonstrated that in more than 40.0% of cases, they were unexpected findings, and that notification led to a change of treatment in 98.0% of patients admitted to surgical and in 90.6% of those admitted to medical wards, thus confirming their importance for an effective clinical decision-making (48). Several initiatives and recommendations on the harmonization of critical result management have been released (49-52) and, finally, a better awareness of the importance of this issue for improving the quality of laboratory services and patient safety has been achieved.

Quality indicators

The definition, implementation and monitoring of valuable analytical quality specifications have played a fundamental role in improving the quality of laboratory services and reducing the rates of analytical errors. However, a body of evidence has been accumulated on the relevance of the extra-analytical phases, namely the pre-analytical steps, their vulnerability and impact on the overall quality of the laboratory information. The identification and establishment of valuable quality indicators (QIs) represents a promising strategy for collecting data on quality in the total testing process (TTP) and, particularly, for detecting any mistakes made in the individual steps of the TTP, thus providing useful information for quality improvement projects (53). In addition, QIs represent a fundamental requirement for the accreditation of clinical laboratories according to the International Standard ISO 15189 (54). While some interesting programs on indicators in the TTP have been developed in some countries, there was no consensus for the production of joint recommendations focusing on the adoption of universal QIs and common terminology in the total testing process. A preliminary agreement has been achieved in a Consensus Conference organized in Padua in 2013, after revising the model of quality indicators (MQI) developed by the Working Group on "Laboratory Errors and Patient Safety" of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The consensually accepted list of QIs, which takes into consideration both their importance and applicability, could be actually tested by all potentially interested clinical laboratories to identify further steps in the harmonization project (55). Preliminary performance criteria based on data collected have been proposed to allow a benchmark between different laboratories and to support improvement initiatives (56).

FUTURE STRATEGIES

Although standardization and harmonization in laboratory medicine have been recognized as essential requirements for improving quality and value for patients for a long time, some major barriers have affected the success of such projects. In fact, the processes required to achieve harmonization are complicated, costly, and time consuming: a systematic approach, therefore, is needed. This should be based on an infrastructure with "well-defined procedures, transparent operations, effective communication with all stakeholders, and a consensus approach to cooperation" (57). This systematic approach and roadmap represent essential steps for more successful harmonization initiatives. The increasing demand for standardization and harmonization in laboratory medicine requires incremental progress in addressing these issues through the cooperation between many stakeholders: laboratory professionals and their

scientific societies and federations, clinicians, in vitro manufacturing industry, accreditation and regulatory bodies, and patients' representatives (2). Several organizations, such as the IFCC, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), the American Association for Clinical Chemistry (AACC), the World Health Organization, the recently formed International Consortium for Harmonization of Clinical Laboratory Results (ICHCLR) that are working in the field should cooperate and integrate their efforts to avoid duplication of initiatives and to provide joint programs. Other scientific organizations such as the Clinical and Laboratory Standards Institute (CLSI) and the Joint Committee for Traceability in Laboratory Medicine (JCTLM), are recognized to play a major role in providing guidelines and lists of reference materials and reference procedures. But, first and foremost, laboratory professionals have to better understand the urgent need to improve harmonization in everyday clinical practice and to take a proactive role in efforts to assure comparability and interchangeability of laboratory information.

CONCLUSIONS

According to a patient-centered viewpoint, the meaning of harmonization in the context of laboratory medicine is that the information should be comparable irrespective of the measurement procedure used and where and/or when a measurement is made: this represents the major driver for implementing harmonization initiatives. In recent years, further demanding drivers have increased the need for, and relevance of, efforts for harmonizing laboratory information, first and foremost the evidence that variations in laboratory information not only cause confusion but are potentially dangerous. There is convincing evidence that errors in laboratory medicine affect patient outcomes and affect patient safety (6). Two major progresses have been made in the area of harmonization in laboratory medicine: first, the awareness that harmonization should take into consideration not only the analytical phase but all steps of the TTP, thus dealing with "the request, the sample, the measurement, and the report". Second, as the processes required to achieve harmonization are complicated, a systematic approach is needed. A further achievement is the recognition of the need to also apply the concepts of harmonization and standardization in clinical research and in projects of translational medicine (58). The cooperation between laboratory professionals, clinicians, IVD manufacturers, accreditation and regulatory bodies is essential.

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In this issue: Harmonization of Clinical Laboratory Test Results

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Harmonization initiatives in Europe

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Key words:
harmonization, total testing process

ABSTRACT

Introduction: Modern medicine is more and more based on protocols and guidelines; clinical laboratory data play very often a relevant role in these documents and for this reason the need for their harmonization is increasing. To achieve harmonized results the harmonization process must not be limited to only the analytical part, but has to include the pre- and the post-analytical phases.

Results: To fulfill this need the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) has started several initiatives. A Working Group on harmonization of the total testing process (WG-H) has been created with the aims of: 1) surveying and summarizing national European and pan European harmonization initiatives; 2) promoting and coordinating the dissemination of especially promising harmonization initiatives among the EFLM member societies; and 3) taking initiatives to harmonize nomenclature, units and reference intervals at a European level. The activity of the WG started this year with a questionnaire targeted at surveying the status of various harmonization activities, especially those in the pre- and post-analytical phase categories, among the European laboratory medicine societies.

Conclusions: Based on the results of the questionnaire, some activities promoting the dissemination of best practice in blood sampling, sample storage and transportation, in collaboration with WG on the pre-analytical phase, will be promoted, and initiatives to spread to all the European countries the use of SI units in reporting, will be undertaken. Moreover, EFLM has created a Task and Finish Group on standardization of the color coding for blood collection tube closures that is actively working to accomplish this difficult task through collaboration with manufacturers.

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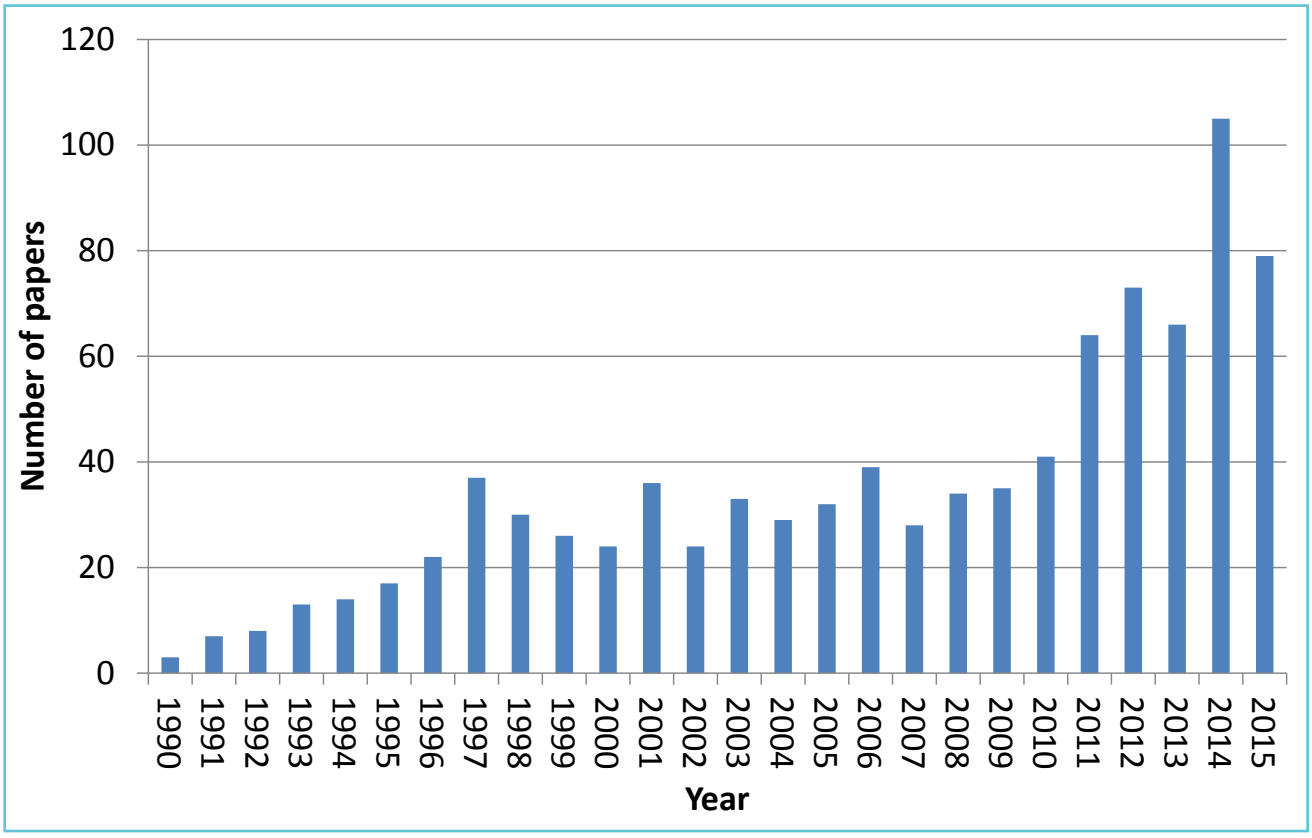
INTRODUCTION

In the last few years there has been a continuous growth in the awareness of the importance of harmonization in all medical fields. A PubMed search for the words "harmonization" or "harmonisation" in the title field resulted in 972 items, with a sharp increase in the numbers of publications in the last 5 years (fig. 1).

The importance of harmonization in Laboratory Medicine and the reasons for improving it are clearly stated in several papers (1-6). The message that comes from these papers is that the standardization of the analytical phase is crucial, but the harmonization process has to include the total testing process, from the pre-pre-analytical to the post-post-analytical phase (2-6).

Starting from these considerations, the Executive Board of EFLM (European Federation of Clinical Chemistry and Laboratory Medicine) decided to create an ad hoc working group within the Science Committee.

Figure 1 Papers in PubMed with the word "harmonization" or "harmonisation" in the title (last 25 years)



The Working Group on the "Harmonisation of total testing process" (WG-H) has the following terms of reference:

- Survey and summarize national European and pan European harmonization initiatives.
- Promote and coordinate the dissemination of at least two especially promising harmonization initiatives among the EFLM Member Societies.
- Undertake initiatives to harmonize nomenclature, units and reference intervals at a European level.

The plan of action for the first two years is the following:

1. WG-H will act as a collector of the harmonization initiatives arising from other WGs or Task and Finish Groups of EFLM and from National Member Societies active in the field and will disseminate them to all the EFLM Member Societies to monitor their application and effects.
2. WG-H will survey and promote the use of harmonized nomenclature for measurands and promote the use of amount of substance units in the European countries.
3. WG-H will promote the implementation of common reference intervals for the measurands where this approach is feasible.

The European situation regarding harmonization is particularly critical essentially for two reasons: there are many different countries (the members of EFLM equal 40), each one with unique traditions, culture and legislation as well as many different languages. The first initiative taken by the WG-H was a survey aimed at identifying those harmonization initiatives already in place in the different European countries and to obtain a picture of the units of measurement presently in use.

EFLM SURVEY ON HARMONIZATION OF TOTAL TESTING PROCESS

The survey aimed to collect information on the harmonization activities already carried out, or currently on-going, by the different national societies of Europe. It was mainly based on the ideas presented in the references 4 and 5 and covered the 3 main phases of the clinical laboratory process: pre-analytical (8 questions), analytical (5 questions) and post-analytical (8 questions). It was distributed to the Presidents and National Representatives of the 40 EFLM Member Societies in 2 phases. In the first phase held at the end of March 2015 the complete survey consisting of 21 questions was sent out. After an evaluation of the replies received from 22 National Societies, it was decided to send a second reduced version (with only 9 of the original 21 questions) and to focus on the most relevant aspects of the pre- and post-analytical phases. This second questionnaire was sent in July 2015 only to the representatives of the 18 National Societies that did not reply in the first phase. This second phase was successful and we received 14 replies, with only 4 countries not responding, hence allowing us to draw an almost complete picture of the European situation regarding the harmonization activities in the pre- and post-analytical phases.

I will present hereafter only the results relative to the 9 questions that received a reply from 36 out of 40 countries.

Questions on harmonization activities in the pre-analytical phase

1. *Is it common practice in your country to use "profiles" (e.g. liver function, electrolytes, etc.) for test requesting?*
2. *If YES, did/does your society produce some document on harmonization of test requesting profiles?*

The questions aimed at identifying how widespread the practice of requesting tests by profiles instead of test by test was and if the societies gave any indication of their intention to standardize the content of each profile (e.g. Electrolytes as only sodium, potassium and chloride or to include also bicarbonate and anion gap). Twenty countries replied that the use of profiles is common practice, but only 7 of them had undertaken test profile harmonization initiatives and only 3 sent us their practice documents indicating the suggested profile contents (Russia, Kazakhstan, The Netherlands); unfortunately all were in the national language and were not understandable (a translation is in progress).

3. *Did/does your society, alone or in collaboration with clinical societies, elaborate guidelines for diagnostic approaches to specific diseases? (e.g. myocardial infarction, coeliac disease, etc.)*

Eighteen societies gave a positive reply and we received several documents. The topics addressed were the following: Autoimmune diseases, Coeliac disease, Chronic Kidney Disease (CKD), Diabetes and Gestational Diabetes, Dyslipidemia and Lipoprotein reporting, Myocardial infarction (MI), Proteinuria, Thyroid diseases and Thyroid disease in pregnancy, Tumor markers.

Several topics (diabetes, MI, CKD, tumor markers) were covered by guidelines in various countries; the material received was heterogeneous and, as expected, in many different languages. The WG-H has not yet been able to examine all of them in detail, but probably there is a need to promote European or international guidelines from which each country can derive its own document. In this way all 40 countries will be able to propose a harmonized approach to the diagnosis of at least the most common diseases.

4. *Did/does your society publish indications for optimal timing for test repetition or minimal retesting intervals?*

Most of the replies (30) were negative with 6 positive. However, only the UK has officially published a document (7). The minimum retesting interval is an important element for governing the appropriateness of test requesting and initiatives to expand similar documents at the European level are planned.

5. *Did/Does your society produce a document on quality of the diagnostic samples or have some activity currently on this topic?*

This is a very sensitive topic, especially in this period when centralization and laboratory consolidation is occurring throughout Europe. Twenty-two societies replied 'No', 14 'Yes' and two of them (Spanish and German Societies) sent us very detailed documents. The EFLM working group on the pre-analytical phase (WG-PRE) is working on this matter and specific documents are in preparation.

Another important harmonization activity in the pre-analytical phase is the harmonization of blood sampling processes. Several European scientific societies have produced documents on this topic namely: Italy (8, 9), Croatia (10), Slovenia, Norway, Russia, and The Netherlands. Moreover the EFLM WG-PRE has already prepared a specific document (11) after conducting a survey of national guidelines, education and training in phlebotomy (12).

An important initiative for the safety of the operator during blood drawing is the European Directive 2010/32/EU implementing the Framework Agreement on prevention from sharps injuries in the hospital and healthcare sector concluded by HOSPEEM (European Hospital and Healthcare Employers' Association) and EPSU (European Federation of Public Service Unions) (13). This directive has been converted in national law by each member state, but its application is not yet

complete and the use of safety-engineered devices for blood sampling has to be fully implemented.

A comprehensive overview of harmonization activities in the pre-analytical phase was published by the EFLM WG-PRE (14).

A further harmonization initiative of EFLM is the creation of a Task and Finish Group on Standardization of the colour coding for blood collection tube closures. This group is trying to define a road map to arrive at a uniform coloring of the tube caps produced by the different manufacturers with the aim of reducing the possible errors when changing manufacturer or when receiving tubes from different laboratories (15). All stakeholders, including all manufacturers working in the field, have been invited to join a dialogue to establish a universally acceptable colour coding standard for blood collection tube closures.

Table 1 Current use of SI units in Europe

	Nation	Use of SI units	Intention to promote SI		Nation	Use of SI units	Intention to promote SI
1	Albania	<10%	NO	21	Latvia	-	-
2	Austria	-	-	22	Lithuania	>80%	Yes
3	Belgium	50 – 80%	Yes	23	Luxembourg	-	-
4	Bosnia Herzegovina	100%	Yes	24	Macedonia	>80%	Yes
5	Bulgaria	100%	NO	25	Montenegro	>80%	Yes
6	Croatia	>80%	Yes	26	Norway	>80%	Yes
7	Cyprus	<10%	NO	27	Poland	50 - 80%	Yes
8	Czech Republic	>80%	NO	28	Portugal	10 – 25%	NO
9	Denmark	>80%	Yes	29	Romania	10 – 25%	Yes
10	Estonia	50 – 80%	Yes	30	Russia	100%	Yes
11	Finland	>80%	Yes	31	Serbia	100%	Yes
12	France	100%	Yes	32	Slovak Republic	>80%	Yes
13	Germany	25 – 50%	Yes	33	Slovenia	100%	Yes
14	Greece	<10%	Yes	34	Spain	<10%	Yes
15	Hungary	>80%	NO	35	Sweden	>80%	Yes
16	Iceland	>80%	Yes	36	Switzerland	>80%	Yes
17	Ireland	<10%	Yes	37	The Netherlands	>80%	Yes
18	Israel	<10%	Yes	38	Turkey	<10%	Yes
19	Italy	<10%	Yes	39	Ukraine	100%	Yes
20	Kosovo	-	-	40	UK	>80%	Yes

Questions on harmonization in the post-analytical phase

1. *Did/does your society make documents or guidelines on use or definition of autovalidation rules?*

Six societies replied 'Yes', but only Switzerland supplied a document that is now in evaluation for possible promotion at the European level.

2. *Do you have any data on the diffusion of the use of SI unit (amount of substance units, e.g. mmol/L) in your country?*
3. *Did/does your society promote officially the use of SI units?*
4. *Would your society be in favour of initiatives devoted to the introduction of SI units (mmol/L)?*

The replies to these questions are summarized in Table 1 (above).

After the distribution of the survey we posed a further question on the use of katal for the expression of enzyme catalytic activity. Five countries replied that µkat/L is the unit used by all of the clinical laboratories in Slovenia, Slovakia, Sweden, Czech Republic and Ukraine, 22 use U/L and we received no replies from the 13 other countries.

Another critical issue of the post-analytical phase that requires harmonization is the communication of critical values. EFLM has established a Task and Finish Group with the aim of surveying the critical result management procedures and policies laboratories currently have and how critical values are established and used in European laboratories.

CONCLUSIONS

There are several harmonization initiatives in place in different European countries, but these initiatives are not coordinated. The problem of the different languages precludes the possibility of sharing easily the documents within Europe. EFLM WG-PRE has produced several documents on which harmonization of several aspects of the pre-analytical phase can be based. Implementing these on a European scale and verifying the effectiveness of their application will be the real challenge for the future. The harmonization and standardization of the analytical phase is already covered at the international level by IFCC and by the American Association for Clinical Chemistry's International Consortium on Harmonization of Clinical Laboratory Results (AACC ICHCLR) (1). EFLM is now working on the definition of quality performance specifications (16) that represent the basis for the harmonization of analytical quality.

The most problematic situation regards the post-analytical phase. The unit of measurement problem is really important. While most of the northern European countries (excluding Ireland) declare an almost total adoption of the amount of substance (mole) unit for expressing the laboratory results, the southern countries (Spain, Italy, Albania, Greece, Turkey, Cyprus) are still using traditional units and in some countries like Italy, clinical laboratories use up to 5 different units for the same test (e.g. Free T3: pg/mL, ng/L, pmol/L, pg/dL and ng/dL). Moreover, many of the countries that adopted the SI units do not use katal for reporting enzymatic activity. It may be easier to ask countries that adopted katal to change back to international units rather than moving all the others to katal. Changing old habits is difficult, and requires coordination and collaboration; however, some countries like Albania, Cyprus and Portugal have declared that they are not in favor of any change. WG-H will promote initiatives in the southern European countries to gradually move toward a larger use of the SI units, starting with electrolytes. Finally the problem of reference intervals remains untouched. Initiatives, similar to the Australasian one (17), are very difficult at the European level.

There is an initiative in the UK (18) and the previous studies of the Nordic Countries (19) but I do not foresee pan European initiatives in the short period except for a few specific analytes.

Most of the work has yet to be done – we are just at the beginning. Communication and collaboration with the National Societies will be the key to achieving some progress in this field which is crucial not only for our profession but for medicine as a whole.

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The International Consortium for Harmonization of Clinical Laboratory Results (ICHCLR) – a pathway for harmonization

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Key words:
harmonization, traceability, harmonization consortium, measurand, comparable test results

ABSTRACT

Results from clinical laboratory measurement procedures must be equivalent to enable effective use of clinical guidelines for disease diagnosis and patient management. Analytical results that are harmonized and independent of the measurement system, time, and location of testing is essential for providing adequate patient care. The key to generating harmonized results is establishing traceability to an accepted reference standard where available. Awareness of the benefits of having traceable measurement results that are harmonized has increased along with efforts to develop approaches to enable and facilitate the implementation of harmonization. Although several organizations are addressing harmonization of test procedures, centralized and cooperative global oversight is needed to ensure that the most important tests are being addressed and resources are optimally used. Working with its domestic and international partners, the American Association for Clinical Chemistry (AACC) has created an International Consortium for Harmonization of Clinical Laboratory Results. Advances in this area will improve the quality of patient care.

THE PROBLEM: INTRODUCTION

Many clinical decisions are based upon clinical guidelines that use a fixed laboratory test result for treatment decisions. A basic problem in laboratory medicine is that different laboratory measurement procedures that intend to measure the same measurand may give different results for the same specimen. If different laboratories get different results, clinical guidelines become compromised and a patient may get the wrong treatment. Many clinical studies may use a central laboratory with a single method; however, guidelines resulting from such a study cannot be effectively implemented until all other methods are harmonized to the central laboratory procedure. Other types of clinical studies may use multiple laboratories that use different methods in which case data cannot be aggregated to develop guidelines until the results from the different methods are harmonized.

Over the past two decades, there have been a number of harmonization successes that have contributed to significant improvements in identifying and managing individuals with chronic diseases, such as diabetes and heart disease (1, 2). But despite these successes, the total number of laboratory tests for which a reference system is available remains very small (approximately 80).

WHAT IS HARMONIZATION

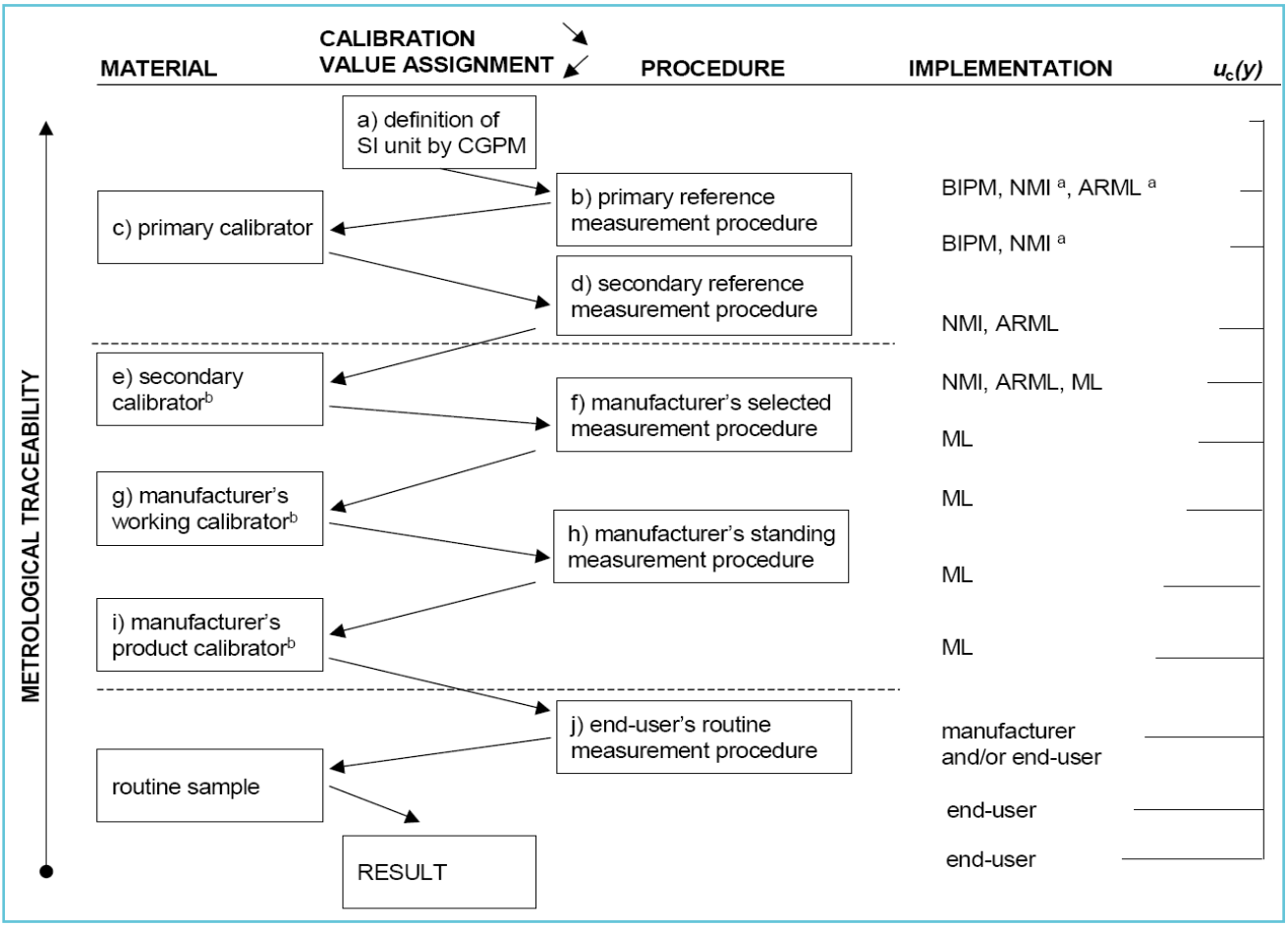
Harmonization is achieving uniform results among different measurement procedures for the same laboratory test. Harmonization usually implies there is no reference measurement procedure available. Harmonization includes consideration of nomenclature, patient preparation, specimen collection and handling, result value, reporting units and interpretative information. The topic for this report focuses on achieving harmonized test results.

ACHIEVING HARMONIZED LABORATORY TEST RESULTS

There are basically two important aspects or requirements to achieve harmonized or equivalent results. The first requirement is that all measurement procedures must measure the same quantity. Secondly all measurement procedures should be traceable to a common reference system. There is an ISO Standard 17511 that describes traceability and puts forth a pathway for establishing a traceable link to a reference system, where one exists (3). Figure 1 shows the traceability scheme based on the ISO Standard. Since it is not practical in the clinical laboratory to use a reference measurement procedure for routine testing, it is important that the patient's result is traceable to the reference measurement procedure. Establishing this "traceability chain" is accomplished through the materials and methods depicted in Figure 1. In many instances it may be necessary to substitute a panel of patient samples when reference materials are not available or deficiencies of the reference materials limit their use. For example, many existing reference materials are not commutable with patient samples and therefore not suitable to be used to calibrate routine clinical laboratory test procedures (4).

It is important to recognize that calibration traceability does not ensure accuracy for an individual patient's sample. The imprecision of the measurement procedure may be too large, the measurement procedure may not be specific for the measurand, interfering substances may influence the result or the measurand itself may not be well defined and the molecular form of clinical interest may not be understood. Consequently different methods may be measuring something a little bit different making it impossible to achieve harmonization of results.

Figure 1 Traceability scheme



Source: ISO 17511

WHAT TO DO

The AACC convened an international leadership conference in 2010 to address some of the issues that hamper calibration and traceability in laboratory medicine. Professional organizations and in vitro diagnostics (IVD) manufacturers were invited to send representatives to participate in this leadership conference. Ninety individuals from 12 countries representing 62 organizations and IVD manufacturers participated in the conference to review the issues and come up with recommendations for improving calibration traceability in laboratory medicine. The output from this conference was a proposed roadmap that established a pathway to address unmet needs for harmonization of clinical laboratory measurement procedures (5). The key point in the roadmap was a recommendation to develop an infrastructure to coordinate harmonization activities worldwide. The infrastructure needed to address the following key points: 1) prioritizing measurands by medical importance, 2) coordinating the work of different organizations, 3) developing technical processes to achieve harmonization when there is no reference measurement procedure or no reference material and 4) promoting surveillance of the successes of harmonization. These four goals were intended to address the key issues identified by the conference participants.

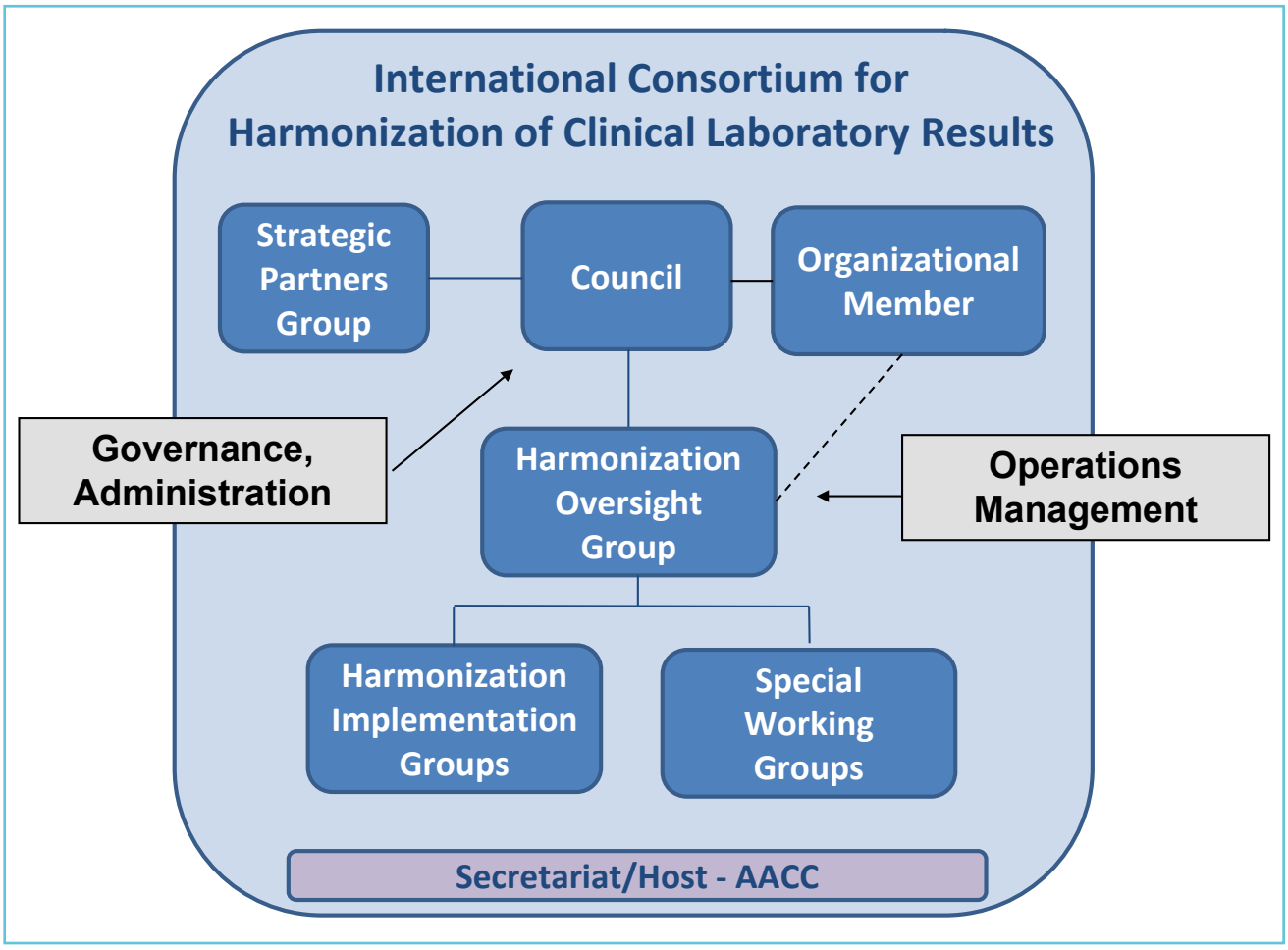
One of the key attributes of this new infrastructure is a focus on cooperation with other organizations already actively working to improve harmonization of laboratory test results. Cooperation is accomplished in part by establishing a communication portal that provides information on what harmonization activities are being conducted by organizations in different countries. A communication portal is essential to minimize duplication of effort and resources.

FORMATION OF A HARMONIZATION CONSORTIUM

Following the international leadership conference, a steering committee was established to fully develop the consortium organization.

Figure 2 shows the organizational infrastructure for the International Consortium for Harmonization of Clinical Laboratory Results (ICHCLR) created to fulfil the roadmap recommendations. The AACC, which supported the development work, agreed to serve as the Secretariat and host organization for this new consortium. The principal components of the ICHCLR include; a Council made up of a small number of professional organizations which is responsible for the governance and administration of the program, a Harmonization Oversight Group (HOG) which is the principle group responsible for the operation and management of harmonization activities, an Organizational Member category which provides an opportunity for organizations (e.g., IVD

Figure 2 An infrastructure for harmonization



manufacturers, professional societies, standard-setting organizations, etc.) to become a member of the ICHCLR and appoint a representative to be a member of the HOG, and a Strategic Partners Group which is open to interested stakeholders to officially join and contribute to the consortium by submitting measurands in need of harmonization and nominating experts for consideration to serve on the HOG.

As the HOG is the central organizing body for managing harmonization activities in the Consortium, a key responsibility is to communicate with strategic partners, which include clinical practice groups, laboratory practice groups, IVD manufacturers, public health organizations, metrology institutes, standards organizations, regulatory organizations and proficiency testing and external quality assessment organizations. It is extremely important that all of these organizations are engaged in the process and know what is going on. Another major responsibility of the HOG is to evaluate measurand proposals submitted by interested stakeholders and determine their priority and technical feasibility for harmonization. To accomplish this, a Special Working Group of experts can be convened to evaluate a submitted proposal and make recommendations back to the HOG. Criteria for prioritization include: medical need, is the test associated with a particular clinical practice guideline, frequency of testing, and performance of routine tests methods in proficiency testing and external quality assurance schemes (EQAS) programs. The HOG will post prioritization information on the Consortium website so that stakeholders around the world will be aware of what measurands are in need of harmonization. The prioritized list will allow standards organizations and IVD companies to decide how to direct limited resources to improve harmonization of clinical laboratory test results.

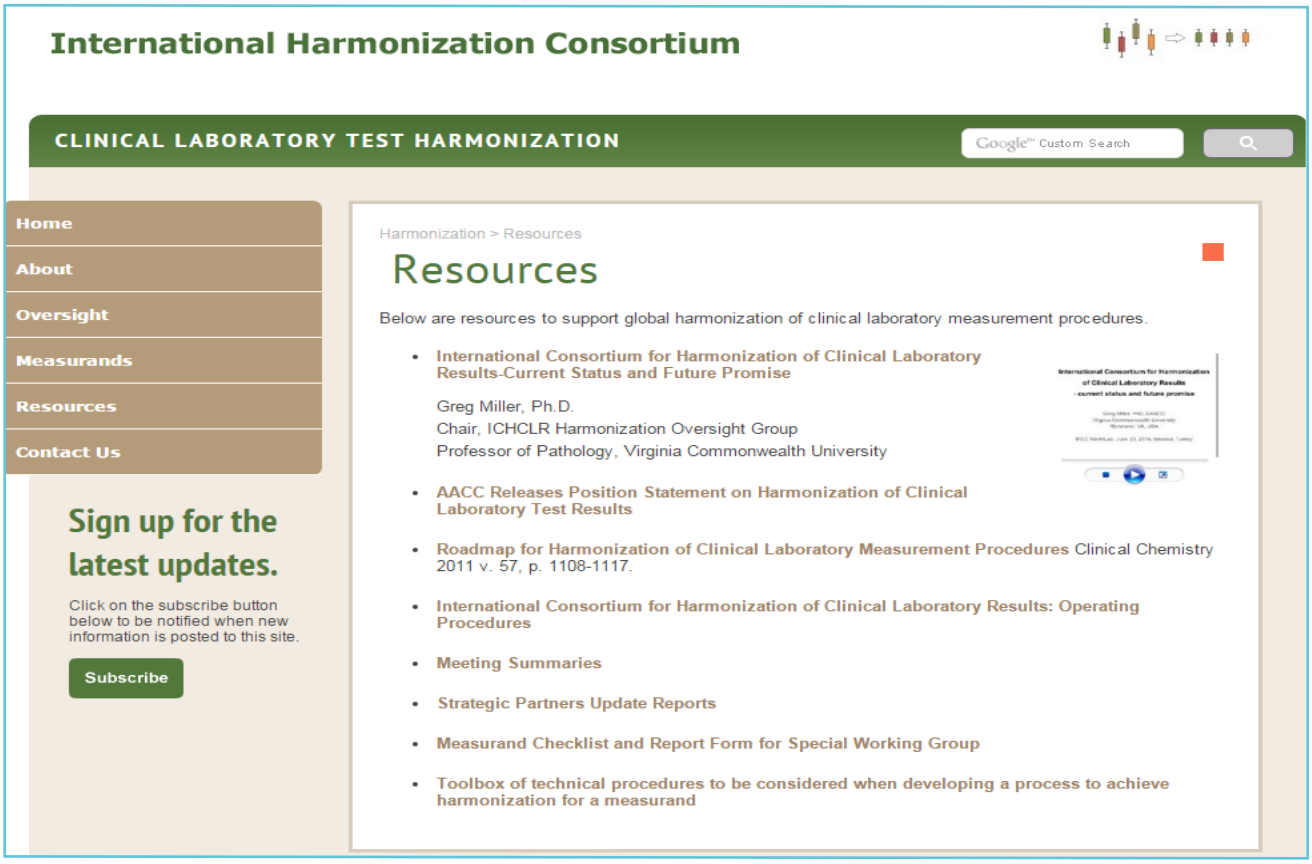
The web site also includes information on what organization is pursuing harmonization of a given measurand. If an organization, such as the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) that is active in harmonization work, is interested to accept a project, then the HOG will refer a project to that organization and provide a link on the web site so progress can be tracked. Alternatively, the HOG may recommend that a project to harmonize the measurand be initiated. The HOG will then identify a champion and appoint a Harmonization Implementation Group (HIG) to develop a technical plan for harmonization with the ultimate goal to achieve listing in the database maintained by the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

The JCTLM uses ISO standards to review reference measurement procedures (ISO 15193), reference materials (ISO 15194) and reference measurement laboratories (ISO 19195) for conformance to ISO criteria. The JCTLM database (www.bipm.org/jctlm/) lists approved reference measurement procedures, reference materials, and reference measurement services that the IVD industry can use as the basis for measurement procedure calibration traceability. What is missing from the ISO standards is a standard that addresses traceability to a harmonization protocol that does not use a reference measurement procedure or certified reference material. To fill this need the HOG developed and submitted to the ISO Technical Committee 212, a preliminary work item proposal on harmonized measurement procedures. This proposal is being addressed by Working Group 2 as a new standard that will allow JCTLM listing of processes to achieve harmonization.

WEBSITE PORTAL

As mentioned previously, a key attribute of the ICHCLR is the establishment of a communication portal to share information on harmonization activities from around the world. A website

Figure 3 Harmonization website resources page



portal for this purpose has been established at www.harmonization.net. Figure 3 is a screen shot of the harmonization website resources page. The site provides information on the Council, the HOG, and the Strategic Partners Group. The site contains resources to support global harmonization of clinical laboratory measurement procedures including: a link to the "Roadmap" paper, an AACC position statement on harmonization, minutes from meetings of the Council and HOG, Strategic Partners Update Reports, operating procedures for the ICHCLR and a copy of the toolbox of technical procedures to be considered when developing a process to achieve harmonization for a measurand. There is a separate section dedicated to measurands which provides information on the status of harmonization and standardization of measurands from organizations around the world. Individuals or organizations can submit a measurand to the Consortium for inclusion on the priority list through the website. A fully electronic process provides an efficient mechanism for submitting measurands for consideration.

TOOLBOX FOR HARMONIZATION

Special attention is drawn to the toolbox of technical procedures to be considered when developing a process to achieve harmonization for a measurand. The toolbox was created by a task force during the formation of the Consortium and contains useful information as a starting point for harmonization. There are two key protocols detailed in the toolbox, 1) the integrated harmonization protocol and 2) a step-up design for harmonization. The integrated protocol is meant to be an assessment study which is a

very carefully designed experiment incorporating clinical samples, pooled clinical samples, admixed clinical samples to assess linearity and any candidate reference materials that may be available. The protocol integrates into one carefully designed experiment the ability to obtain information to enable decisions on feasibility to achieve harmonization given the tools available, the preferred approach to harmonization that is likely to succeed and based on this information a commitment to proceed by interested stakeholders.

The Step-up design is intended for use when there is no reference measurement procedure and no reference material. This particular protocol was developed under the leadership of Professor Linda Thienpont in the context of the IFCC Committee for Standardization of Thyroid Function Tests (6). The step-up design is a sequence of patient sample comparisons between clinical laboratory procedures where success at one phase allows the harmonization process to "step up" to the next phase. The phases are designed to determine whether the methods correlate with each other, which is an essential prerequisite to achieve harmonization, if there is an adequate response over the measuring interval, if there is adequate specificity for the measurand and an adequate value assignment, such as an all methods mean or a trimmed all methods mean that may be agreeable on a consensus basis. After several qualification phases, a panel of patient sera is fit for purpose to harmonize a set of clinical laboratory measurement procedures. Sustainability is assured by a second panel to harmonize new methods entering the market and to be used to transfer values to subsequent panels to maintain consistency of the scheme.

PATH FORWARD

Harmonizing a greater number of clinical laboratory tests will contribute to improved healthcare in many important ways. Harmonized test results will ensure that clinical guidelines that call for the use of laboratory tests can be appropriately implemented. Reliable screening to detect diseases early, when they are easier to treat; appropriate diagnoses of diseases; correct and consistent treatment decisions; and effective monitoring of responses to treatment will be important outcomes of more extensive harmonization of clinical laboratory test results. Furthermore, by reducing incorrect interpretations of laboratory test results, harmonization can help prevent treatment errors and unnecessary — and expensive — follow-up diagnostic procedures and treatments based on inaccurate laboratory test results. The ICHCLR encourages all interested stakeholders to recognize the critical role of clinical laboratory testing in improving health outcomes and to join the ICHCLR in promoting the need for achieving harmonization of laboratory tests results.

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Harmonization of clinical laboratory test results: the role of the IVD industry

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Key words:
metrology, traceability,
standardization, harmonization

Disclosure:
Dave Armbruster is an employee
of Abbott Diagnostics.

ABSTRACT

At the start of the 21st century, a dramatic change occurred in the clinical laboratory community. Concepts from Metrology, the science of measurement, began to be more carefully applied to the *in vitro* diagnostic (IVD) community, that is, manufacturers. A new appreciation of calibrator traceability evolved. Although metrological traceability always existed, it was less detailed and formal. The In Vitro Diagnostics Directive (IVDD) of 2003 required manufacturers to provide traceability information, proving assays were anchored to internationally accepted reference materials and/or reference methods. The intent is to ensure comparability of patient test results, regardless of the analytical system used to generate them. Results of equivalent quality allows for the practical use of electronic health records (EHRs) capture a patient's complete laboratory test history and allow healthcare providers to diagnose and treat patients, confident the test results are suitable for correct interpretation, i.e., are "fit for purpose" and reflect a real change in a patient's condition and not just "analytical noise." The healthcare benefits are obvious but harmonization of test systems poses significant challenges to the IVD Industry. Manufacturers must learn the

theory of metrological traceability and apply it in a practical manner to assay calibration schemes. It's difficult to effect such a practical application because clinical laboratories do not test purified analytes using reference measurement procedures but instead deal with complex patient samples, e.g., whole blood, serum, plasma, urine, etc., using "field methods." Harmonization in the clinical laboratory is worth the effort to achieve optimal patient care.

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INTRODUCTION

The world is experiencing globalization and the clinical laboratory field is no exception. The goal is to provide optimal healthcare to the global population and clinical laboratory practice is inexorably moving towards harmonization. As stated by Greenberg, "An increasingly important objective in laboratory medicine is ensuring the equivalency of test results among different measurement procedures, different laboratories and health care systems, over time (1)." This requires harmonization and metrological traceability of assays to provide equivalence of results derived from different analytical systems (2). This has not been possible historically because assays provided by Industry have not been sufficiently comparable due to a lack of established reference materials and methods to "anchor" tests. As noted by Miller and Myers, "True and precise routine measurements of quantities of clinical interest are essential if results are to be optimally interpreted for patient care. Additionally, results produced by different measurement procedures for the same measurand must be comparable if common diagnostic decision values and clinical research values are to be broadly applied (3)."

A patient's test history would be consistent if a single clinical lab performed all testing (i.e., same methodology, stable analytical performance, etc.) so a significant change in concentration (decrease or increase) would signal a meaningful clinical change. In reality, patients are increasingly mobile and two or more laboratories may test their samples. If the tests performed by different laboratories are sufficiently harmonized so as to produce essentially equivalent results (not necessarily quantitatively equal, but clinically equivalent), changes in concentration can be correctly interpreted by a healthcare provider. As explained by Gantzer and Miller "Clinical laboratory measurement results must be comparable among different measurement procedures, different locations and different times in order to be used appropriately for identifying and managing disease conditions (4)."

Harmonization is needed to use of electronic medical records/electronic health records (EMRs/EHRs) to capture all of a patient's lab results in an electronic file available to patients and healthcare providers. Clinical laboratory results typically account for much of the information in EMRS but the benefit is negated if the cumulative values in EMR for the same analyte are not comparable. Perhaps not a problem for traceable analytes, e.g., electrolytes and glucose, but very much an issue for immunoassays such as thyroid and fertility hormones and cancer markers. Interpretation of sequential values using common reference intervals and medical decision levels (MDLs) is difficult, if not impossible. It's been suggested laboratory data accounts for about 70% of clinical decisions. Hallworth has challenged that blanket statement but allows "The value of laboratory medicine in patient care is unquestioned (5). That value is greatly diminished without comparability of test results.

Cholesterol is a prime example of successful harmonization. Creating a reference measurement system (RMS) for this key lipid over about 30 years (1970 – 2000) coincided with a major reduction in mortality rates for coronary heart

disease (CHD) in the US and also achieved a huge savings in healthcare dollars (1). The consequences of the lack of harmonization was demonstrated by an NIST report on calcium (Ca) that estimated the cost of a 0.1 mg/dL Ca bias can cost $8 - $31 for additional, but unnecessary, patient follow up testing (6). A bias of 0.5 mg/dL could results in an additional $34 - $89/patient. On an annual basis, a 0.1 mg/dL bias could translate into $60 - $199 million/year for about 3.55 million patients screened for Ca.

HARMONIZATION VS. STANDARDIZATION

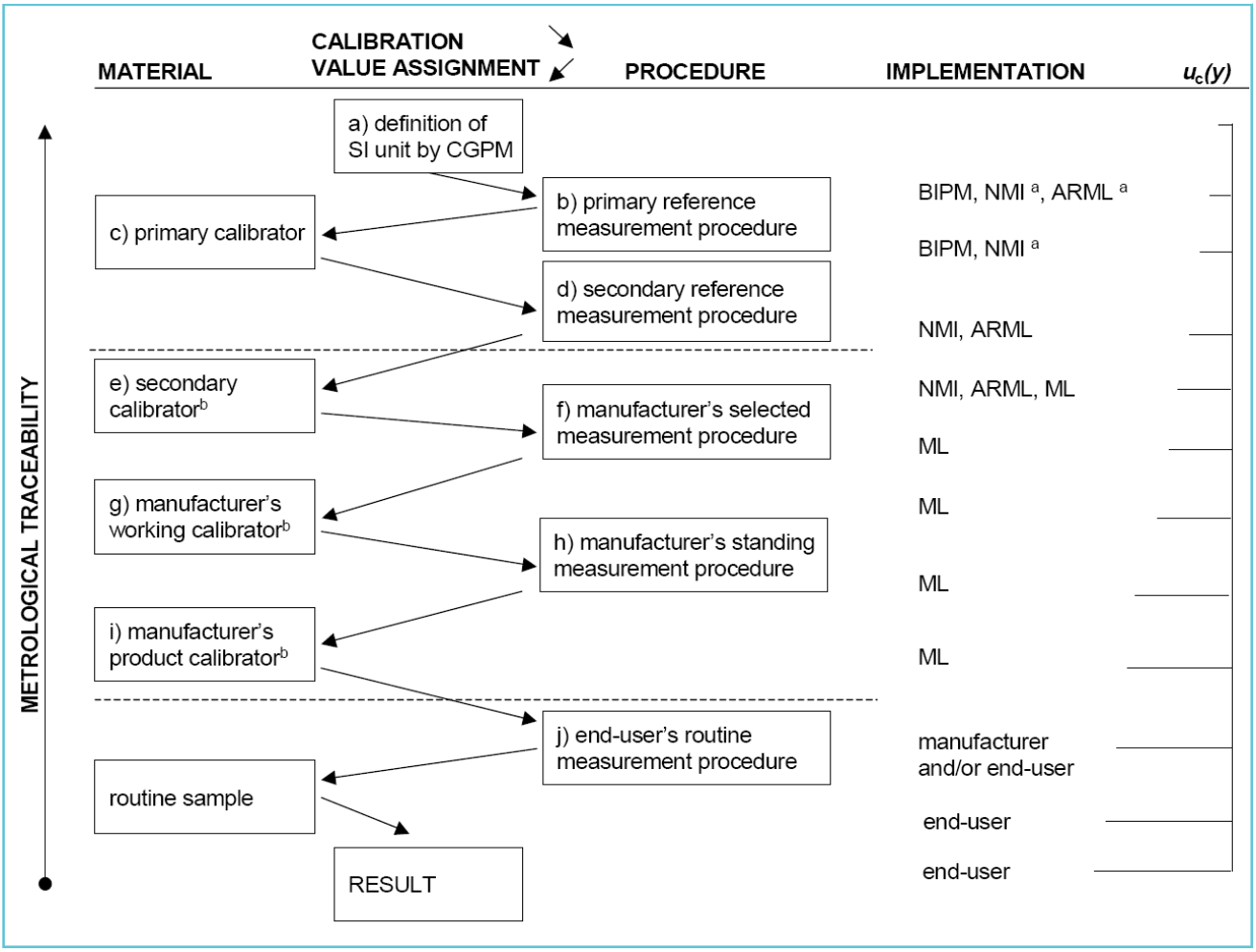
In this paper "harmonization" is used interchangeably with "standardization," though there is a distinction between the two (4). Standardization means results are traceable to higher metrological order reference materials and/or methods and ideally can be reported using SI units. Harmonization means results are traceable to some declared reference but accepted higher order reference materials and/or methods are not available and SI units are not applicable. Harmonization ensures comparability of results, enables application of clinical best practice guidelines and reference intervals, increases patient safety, and decreases medical care costs. Harmonization requires the cooperation of laboratories, academia, professional societies, metrological institutes, government agencies, EQA/PT providers, and industry. Two recent harmonization (actually, standardization) success stories mediated by Industry are creatinine and glycated hemoglobin (Hb A1c). Field assays for both of these analytes feature complete traceability chains and are firmly anchored by reference measurement systems. That said, ironically results for both assays are still typically reported in different units, creatinine in mg/dL ("conventional units") and mmol/L (SI units), and Hb A1c in % Hb A1c (NGSP units) and mmol/mol (SI units).

METROLOGICAL TRACEABILITY

The In Vitro Diagnostics Directive (IVDD) of 2003 applies to Europe for the purposes of the CE mark, but has global implications. It requires manufacturers to establish the metrological traceability and uncertainty of kit calibrators. "Metrological traceability is defined in the VIM, clause 2.41 as the 'property of a measurement result whereby the result can be related to a reference (a standard) through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty. (1)" The IVDD doesn't provide specifics but ISO 17511 (Metrological traceability of values assigned to calibrators and control materials) applies (7; see Fig 1.). It establishes a metrology infrastructure for assays. The IVDD requirements are incorporated in ISO 15189 (Medical laboratories- particular requirements for quality and competence) (8).

As White explains "Metrology, the science of measurement, provides laboratory medicine with a structured approach to the development and terminology of reference measurement systems which, when implemented, improve the accuracy and comparability of patients' results (9)." Metrological principles are a relatively new in the clinical laboratory. For example, the Tietz Textbook of Clinical Chemistry (third edition, 1999) made no mention of "uncertainty" or "commutability" (10). The fourth edition (2006) mentioned uncertainty and commutability but only a definition of commutability was given (11). The fifth edition (2011) includes a discussion of uncertainty along with commutability (12). As noted by De Bievre, "Discussions with analytical chemists have revealed that basic concepts in metrology, including 'traceability,' are generally not an integral part of university or college curricula and are not treated in most text books of analytical chemistry" (13).

Figure 1 General metrological traceability diagram from ISO 17511, *in vitro* diagnostic medical devices — Measurement of quantities in biological samples — Metrological traceability of samples assigned to calibrators and control materials, 2003



Abbreviations: ARML - Accredited reference measurement laboratory (such a laboratory may be an independent or manufacturer's laboratory); BIMP - International Bureau of Weights and Measures; CGMP - General Conference on Weights and Measures; ML - Manufacturer's laboratory; NMI - National Metrology Institute.

The symbol $u_c(y)$ stands for combined standard uncertainty of measurement.

Metrology must be adapted to the clinical laboratory, but a practical approach is advisable due to differences between the disciplines. For example, Metrology is a "pure science" contrasting with the mixed science of clinical chemistry (combines several diverse sciences/technologies). National metrology institutes are "ivory towers" in comparison to clinical laboratories ("the trenches"). Metrology tests pure, well-defined analytes in simple matrices but clinical labs test complex, ill-defined analytes in challenging matrices (serum, plasma, urine, etc.). Metrology estimates expanded uncertainty (bias eliminated) while clinical labs focus on Total Error Allowable (TEa = bias + imprecision). Metrology seeks "absolute scientific truth" by reference method analysis but clinical labs deal in "relative truth" by field method analysis.

Good metrology does not necessarily equal good clinical laboratory science but the clinical laboratory field needs to adapt Metrology concepts and "translate" them for practical application.

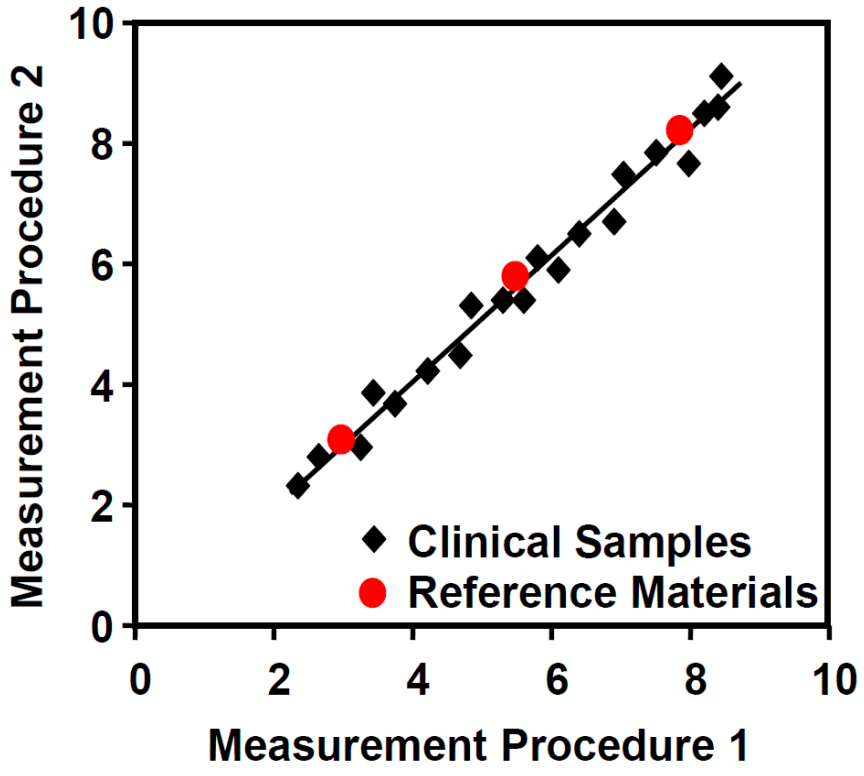
THE PILLARS OF HARMONIZATION

In anticipation of the IVDD, the Joint Committee for Traceability in Laboratory Medicine (JCTLM) was formed in 2002 (1). It established three pillars of traceability: 1. reference measurement procedures (RMP), 2. reference materials (RM), and 3. a network of reference measurement laboratories. The JCTLM maintains a searchable database for all three on the International Bureau of Weights and Measures (BIPM) web site (14). The laboratory community has identified three other "pillars" in response to harmonization: 1. universal reference intervals and medical decision levels (MDLs), 2. accuracy based grading EQA/PT programs to ensure traceability of field assays is maintained and analytical bias is minimized or meets established criteria (e.g., CAP PT requirement of +/- 6% of the NGSP target value for Hb A1c), and 3. harmonization of clinical laboratory practice and the total testing process (TTP), e.g., standardized nomenclature/terminology, reporting units, EBLM, etc.

The JCTLM goal is comparability of patient test results from different methods to ensure appropriate medical decision-making and optimal healthcare (15, 16). The components of a reference measurement system (RMS) are: 1. definition of the analyte, 2. RMP that specifically measures the analyte, 3. Primary and secondary reference materials, and 4. reference measurement laboratories. Analytes fall into two categories: 1. Type A (well defined; concentration in SI units; results not method dependent; full traceability chain), and 2. Type B (not well defined, heterogeneous, present in both bound and free state, not traceable to SI , rigorous traceability chain not available). The JCTLM provides a list of higher order RMs and RMPs and reference laboratories (17).

A requirement for harmonization is commutability. Commutability is defined as a property of a reference material, demonstrated by the closeness of agreement between the relation among the measurement results for a stated quantity in this material, obtained according to two given measurement procedures, and the relation obtained among the measurement results for other specified materials (4). In other words, fresh patient samples and materials such as calibrators need to provide an identical analytical response (see Fig. 2). Many secondary RMs are not commutable with native clinical samples and have failed to accomplish the intended goal of achieving harmonized results (4). Commutability is not a universal property of reference materials and must be proven with every field method. Well recognized by Metrology, commutability is not so widely appreciated in routine clinical laboratories. Historically, the commutability reference materials and calibrators prepared from them or traceable to them has not routinely been established. Noncommutability results in significant biases with field assays due to matrix effects, use of non-human forms of analyte, lack of antibody specificity, or other causes. The JCTLM now requires a commutability assessment of reference materials to be listed in its database. CLSI EP30 (Characterization and qualification of commutable reference materials for laboratory medicine) is a recent guideline (18). Metrology defines measurement uncertainty, or simply uncertainty, as a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used (4). It is roughly equivalent to imprecision but ideally assay bias is eliminated prior to estimating uncertainty. CLSI EP29 (Expression of

Figure 2 **Commutability is demonstrated if fresh patient samples and reference materials, e.g., calibrators, demonstrate an equivalent analytical response when tested by two methods**



Commutable: same relationship for clinical samples and reference materials.

measurement uncertainty in laboratory medicine) is another recent guideline (19).

The fourth "pillar" of traceability- universal reference intervals- cannot be erected without the adoption of reference measurement systems and assay harmonization. Reference intervals for some analytes can be affected by various partitioning factors, e.g., age, gender, ethnicity, BMI (body mass index), and thus universal ranges may not be feasible. But such decisions can't be made until harmonization has been achieved.

To meet the IVDD traceability requirement for result trueness and comparability requires the fifth "pillar:" validation of manufacturers' metrological traceability by EQA/PT. EQA/PT programs using commutable samples with reference method target values allow accuracy based grading (20). Horowitz notes "Far too many laboratories consider proficiency testing just a necessary evil, little more than periodic pass–fail exercises we perform solely to meet regulatory requirements. Even for central-laboratory techniques, traditional PT suffers from 'matrix effects,' in that samples used for testing often react differently from native patient samples. Therefore, comparisons must be made only to peer groups, rather than to the 'true value.' What if the peer group as a whole is wrong? (20)" EQA/PT has typically been used to measure proficiency at performing a test and not the trueness of the test method or its performance relative to other method. For

this reason, Miller concludes "Traditional PT materials are not suitable for field-based post-marketing assessments of a method's trueness (21)." In one study, commutable serum-based material was assigned target values by reference methods for six enzymes (ALT, AST, CK, GGT, LD, and amylase) and was tested by 70 labs in Germany, Italy, and The Netherlands using six field methods (22). Results were graded on accuracy based on biological variability targets. For ALT, results were deemed acceptable for > 94% of the six commercial assays. Performance for the other five enzymes was variable and all methods demonstrated significant bias for CK. "Overall, it appears clear that method bias should be reduced by better calibration to the internationally accepted reference systems (22)."

The sixth harmonization "pillar" is the Total Testing Process (TTP). Plebani observed "Although the focus is mainly on the standardization of measurement procedures, the scope of harmonization goes beyond method and analytical results: it includes all other aspects of laboratory testing, including terminology and units, report formats, reference intervals and decision limits, as well as test profiles and criteria for the interpretation of results (23)." Harmonization of reporting units would seem easy to achieve but that's not the case. "Even a change in the unit of hemoglobin (Hb) expression could potentially affect patient safety. Findings in a recent survey conducted in the UK revealed that 80% of laboratories were using g/dL, although g/L is the recommended unit … (23)." Harmonization of basic terminology and units is necessary but the international clinical laboratory community has yet to reach agreement. For examples of disharmony, see Table 1.

CHALLENGES FOR THE IVD INDUSTRY

Embracing metrological concepts and harmonization represents a paradigm shift for the *in vitro* diagnostics community. Manufacturers traditionally sought to differentiate themselves from competitors (e.g., by claiming a greater dynamic range, lower LoD, better precision, smaller sample size, etc.), and producing comparable patient results was not a priority. Lack of harmonization among field assays is evident from review of EQA/PT data, often of necessity reported by peer group (as opposed to accuracy based grading). In an era of

Table 1 The necesity of reaching agreement over harmonization of basic terminology and units in the international clinical laboratory community: some examples of disharmony

Analyte	"Conventional units"	SI units*
ALT	U/L	mkat/L
Bilirubin	mg/dL	mmol/L
Cl	mEq/L	mmol/L
Glucose	mg/dL	mmol/L
Creatinine	mg/dL	mmol/L
Hb A1c	% Hb A1c	mmol/mol

** SI = International System of Units (Système International d'unités)*

harmonization, results from different systems should be comparable. Manufacturers are responding by: providing calibrator traceability/uncertainty information, restandardizing assays, testing commutability, etc., and they work with many professional organizations and each other to attain harmonization, but this is a new approach and challenge for the industry. Manufacturers have an integral role in educating customers about harmonization of assays, harmonization and clinical laboratory practice in general. Of course the age old question remains: "Where do manufacturers' obligations end and the obligations of lab directors begin?" Manufacturers must provide "fit for purpose" tests, but labs must use the assays properly and effectively. When an assay "failure" occurs (and "failure" can apply to myriad issues and causes) does the fault lie with the manufacturer or with the lab and its use of the test?

A major challenge for manufacturers is to choose a total allowable error (TEa) goal from the many available options: CLIA requirements (U.S. specific); CAP; RCPA, RiliBÄK, or other EQA/PT provider specifications. A popular approach is to define TEa based on biological variability targets, but there are three targets from which to choose:

Minimum:

$$TE_a < 1.65(0.75\ CV_i) + 0.375(CV_i^2 + CV_g^2)^{½}$$

Desirable:

$$TE_a < 1.65(0.5\ CV_i) + 0.25(CV_i^2 + CV_g^2)^{½}$$

Optimum:

$$TE_a < 1.65(0.25\ CV_i) + 0.125(CV_i^2 + CV_g^2)^{½}$$

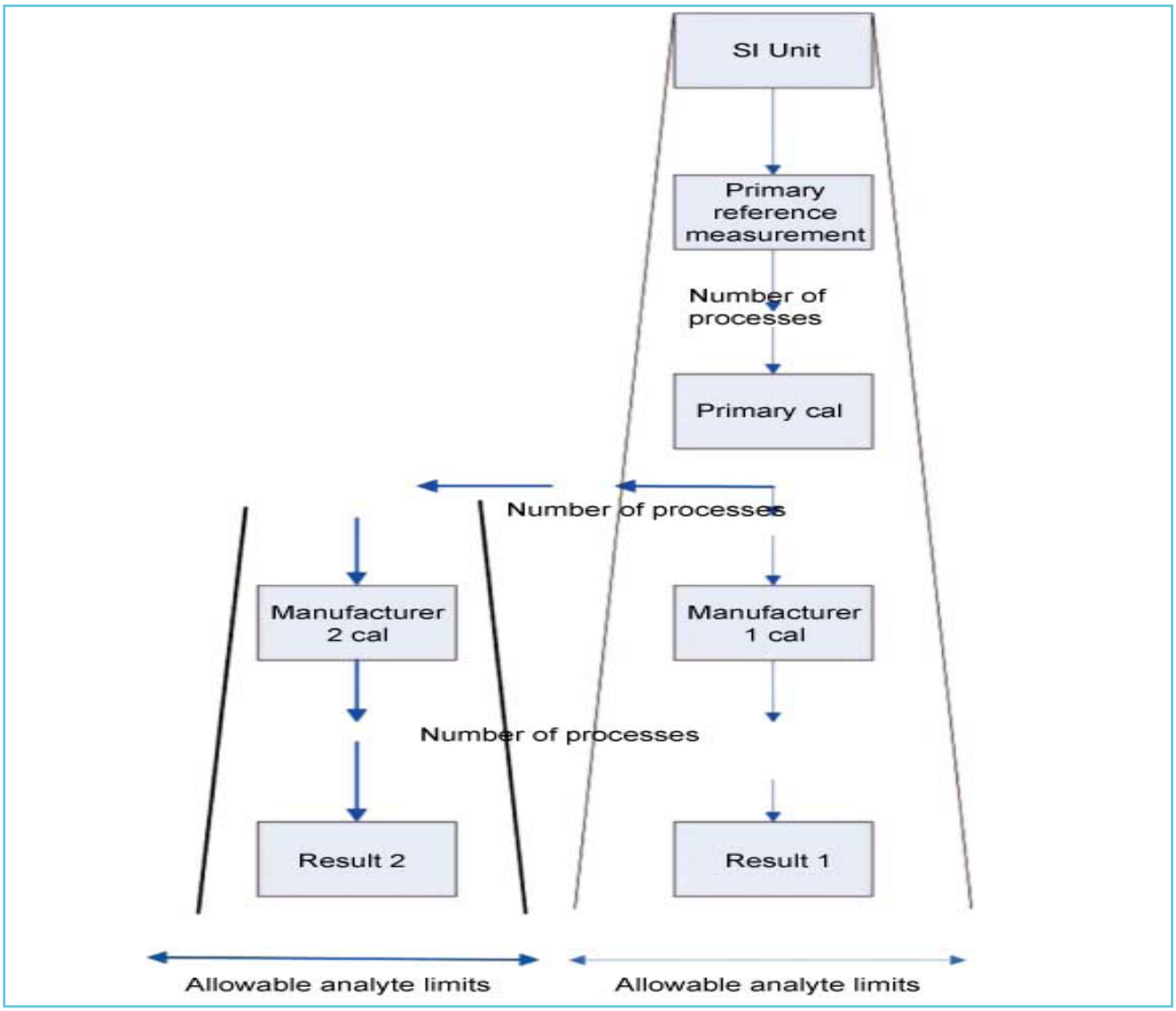
CV_i = *individual biological variability;*

CV_g = *group biological variability*

An IFCC initiative is the Working Group on Allowable Error for Traceable Results (WG-AETR). This group concluded "Although manufacturers are compelled by the European IVD Directive, 98/79/EC, to have traceability of the values assigned to their calibrators if suitable higher order reference materials and/or procedures are available, there is still no equivalence of results for many measurands determined in clinical laboratories" (24). For some common analytes, such as sodium, current assays are too imprecise to meet TEa targets based on biological variation. The aim of harmonization is equivalent results but unfortunately, due to cost and limited resources, IVD manufacturers don't always follow full traceability steps to value assign every new calibrator lot but rely on value transfer from an internally stored ("master") calibrator material. In most cases, this procedure is probably valid, but a common complaint is calibrator lot to lot variability. The WG-AETR noted that when there are two traceability paths for a measurand, calibrators from different manufacturers may both be derived from valid traceability chains but produce non-equivalent results, as illustrated by Fig. 3. Equivalent results from two systems may be possible by using a correction factor determined by a correlation study.

The international clinical laboratory community has embraced harmonization. A prime example is the AACC's ICHCLR (International Consortium for Harmonization of Clinical Laboratory Results) (2). The ICHCLR prioritizes analytes globally for harmonization and development of RMs and RMPs for listing in the JCTLM database, which will allow for comparable results irrespective of the laboratory, method, or the time when testing is performed. ICHCLR stakeholders include: clinical lab and medical professional societies, IVD manufacturers, metrology institutes, public health organizations, regulatory agencies, and standard-setting organizations. A similar initiative is Pathology Harmony in the UK (25).

Figure 3 Manufacturers may prepare calibrators starting with traceability to the same reference material and/or reference method, but the calibrator manufacturing process may diverge at some point, resulting in significantly different results for the same measurand in the same patient sample if tested by the two field methods, despite metrologically acceptable traceability for each assay's calibrators



Pathology Harmony states: "as we move towards full electronic reporting of pathology results, we appreciate more fully that variations in things such as test names, reference intervals and units of measurement associated with our results is something that hinders progress." In Australia, there is the RCPA (Royal College of Pathologists of Australasia) PITUS (Pathology Information Terminology and Units Standardisation Project) program that is dedicated to harmonization (26). PITUS in particular focuses on the interoperability of pathology test requesting and reporting. These initiatives and others are all supported by Industry.

MANUFACTURERS' ROLE IN THE 21ST CENTURY

Industry support can be optimized when the harmonization initiatives are coordinated and prioritized. From the industry perspective there are limitations, costs and tradeoffs which need to be considered. Device manufacturers all have substantial product development priority lists and development schedules and personnel and financial resources are committed over long term periods to achieve strategic goals. The development process for a new product can be measured over years in our highly regulated environment. Further, the cost for each project can run into the millions of dollars. Reprioritization is possible and welcomed by industry when the results will provide benefit to the clinician, patient and healthcare system. Stellar examples such as creatinine, hemoglobin A1c and cholesterol have been pointed out in this manuscript.

The global drive for harmonization creates competing project priorities for companies. As manufacturers sign on to support harmonization projects, timelines that reflect development cycles (years) allow companies to reprioritize resources while maintaining projects that drive innovation, product health and portfolio development.

Harmonization may also require worldwide re-registration of products. Meeting the criteria of country specific regulatory agencies comes with additional considerations and complexities beyond the harmonization initiative. Registration timing is not equivalent in all countries and multiple products for a given measurand may need to be supported for an extended period of time. This impacts manufacturing resources and production costs.

It is imperative there be close coordination of industry, professional bodies and the global leaders of harmonization initiatives to ensure harmonization is successful. If companies could contribute to the prioritization of projects, design of experiment and contribute to the inputs we would be assured changes requiring product re-registration would be successful. This would also avoid unintentional competitive imbalances.

A significant consideration is the traceability of the reference assay. Device manufacturer's typically register products using a predicate device to demonstrate product acceptance. In such cases proof of substantial equivalence is essential to demonstrate the assay is safe and effective. If a reference assay is a laboratory developed test the path to regulatory registration and the ability to commercialize the assay brings with it additional complications.

Lastly, a major consideration is whether the harmonization initiative provides benefit to the public. While accuracy is important, there are situations where existing assays may be relatively harmonized yet the reference method is very different from the commercialized assays. Under these special circumstances the cost of harmonization which includes physician education, patient safety and investment in product redevelopment must be carefully weighed to understand the benefit of harmonization.

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In this issue: Harmonization of Clinical Laboratory Test Results

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Opinion paper: deriving harmonised reference intervals – global activities

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Key words:
harmonisation, standardisation, reference intervals, transference, adult, paediatric

ABSTRACT

Harmonisation of reference intervals (RIs) refers to use of the same or common RI across different platforms and /or assays for a specified analyte. It occurs optimally for those analytes where there is sound calibration and traceability in place and evidence from a between-method comparison shows that bias would not prevent the use of a common RI. The selection of the RI will depend on various sources of information including local formal RI studies, published studies from the literature, laboratory surveys, manufacturer's product information, relevant guidelines, and mining of databases. Pre-analytical and partitioning issues, significant figures and flagging rates, are assessed for each analyte.

Several countries and regions including the Nordic countries, United Kingdom, Japan, Turkey, and Australasia are using common RIs that have been determined either by direct studies or by a consensus process. In Canada, the Canadian Society of Clinical Chemists Taskforce is assessing the feasibility of establishing common reference values using the CALIPER (Canadian Laboratory Initiative on Pediatric Reference Intervals) and CHMS (The Canadian Health Measures Survey) databases as the basis. Development of platform-specific common reference values for each

of the major analytical systems may be a more practical approach especially for the majority of analytes that are not standardised against a primary reference method and are not traceable to a primary or secondary reference material.

We encourage laboratories to consider adopting reference intervals consistent with those used by other laboratories in your region where it is possible and appropriate for your local population. Local validation of the adopted reference interval is also recommended as per CLSI guidelines.

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INTRODUCTION

Despite studies having shown that the variation in reference intervals (RIs) for chemistry analytes may be greater than the analytical inaccuracy of the measurement, differences in RIs persist between laboratories that use the same

Table 1 **Sequence of events to derive and validate common reference intervals (RIs) through an evidence-based approach and extensive data analysis**

Identify problem
Agree to address common RIs
Identify relevant groups
Seek formal co-operation (if external bodies involved)
Form working group
Describe problem in detail
Allocate a budget and determine sources of funding
Gather information (surveys, RI studies, data mining, bias study, calibration traceability, RI verification laboratory information, flagging rates)
Consider solutions
Produce discussion paper, etc.
Seek feedback from stakeholders
Revise recommendations
Obtain formal endorsement
Publish
Promote
Monitor introduction

platforms and the same reagents (1-3). This has implications for result interpretation and patient outcomes where the same values may be interpreted differently due to differences in RIs or decision limits hence leading to inappropriate over- or under-investigation or treatment of the patient.

One way to overcome this situation is to use the same interval. Harmonisation of RIs refers to use of the same or common RI across different platforms and /or assays for a specified analyte. Importantly, harmonisation of RIs occurs optimally for those analytes where there is sound calibration and traceability in place and evidence from a method comparison study shows that bias would not prevent the use of a common RI. The advantages of using a harmonised RI are less confusion and misinterpretation of results for both doctors and patients. Irrespective of the pathology provider or the method, provided the same RI, unit and terminology are used, an individual patient's results can then be amalgamated.

An organisational plan is required before setting out on the sequence of practical processes that are required to achieve a major national change in pathology RIs. This is not a trivial matter and the importance of a structured approach cannot be overemphasised. Table 1 outlines the sequence of steps required to derive and validate common RIs that was used for the Australasian RIs study (3). The four key areas are: 1) seeking the evidence; 2) consultation; 3) verification; and 4) implementation (Fig.1 A and 1B). The Australasian Association of Clinical Biochemists (AACB) and the Royal College of Pathologists of Australasia (RCPA) invited pathologists and medical scientists to harmonise RIs at the same time as other RCPA initiatives for standardisation of pathology units, terminology, and report formatting and flagging were being undertaken (4). The input by main stakeholders, i.e. pathologists, scientists, clinical societies and government

Figure 1A **Implementation plan for the introduction of adult common reference intervals**

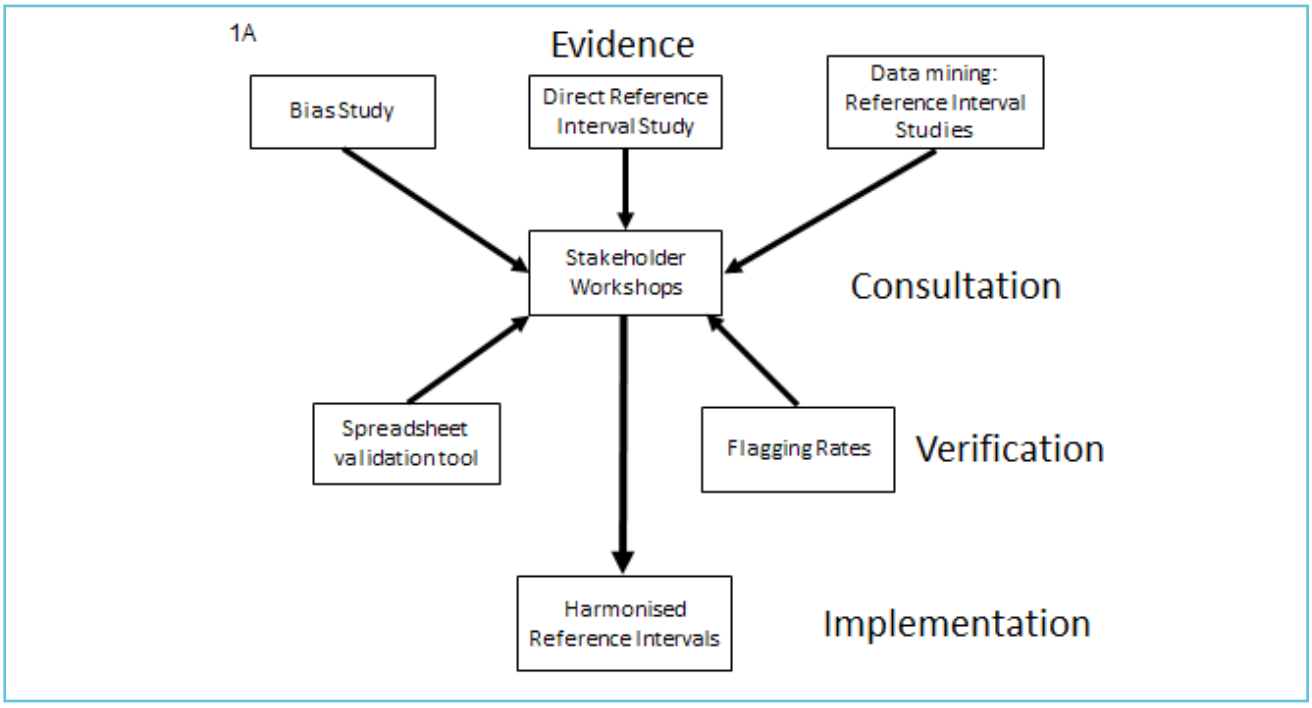
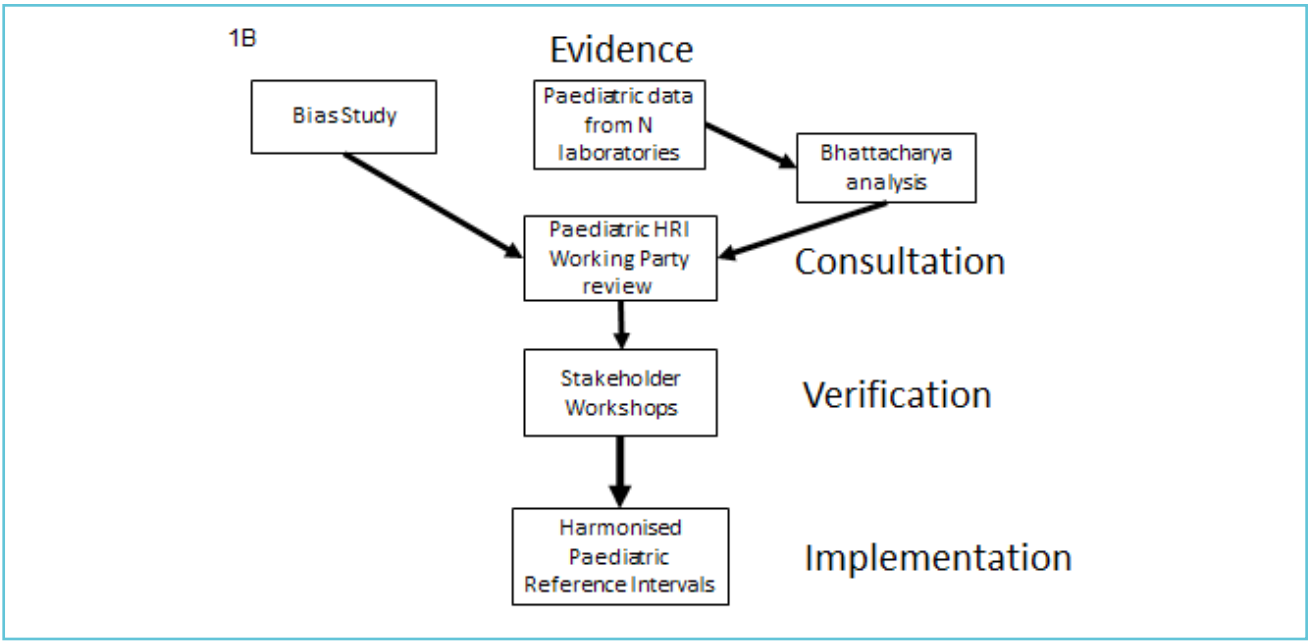


Figure 1B Implementation plan for the introduction of paediatric common reference intervals



bodies, is central to the success of any harmonisation project and can provide helpful advice and guidance as was the case for the UK Pathology Harmony RIs project (5,6).

REQUIREMENTS FOR USE OF HARMONISED REFERENCE INTERVALS

Seeking the evidence is paramount to the implementation of common RIs. One such approach used in Australasia to assess the feasibility of using common RIs was an evidence-based checklist approach. (7). It was based on the following criteria (8):

1. Define analyte (measurand)
2. Define assays used, accuracy base, analytical specificity, any method-based bias
3. Consider important pre-analytical differences, and actions in response to interference
4. Define the principle behind the RI (e.g. central 95%)
5. Describe evidence for selection of common RIs
   - data sources (literature, lab surveys, local RI studies, manufacturers' product information)
   - data mining
   - bias goal as quality criterion for acceptance
6. Consider partitioning based on age, sex, etc.
7. Define degree of rounding
8. Consider the clinical implications of the RI
9. Consider use of common RI
10. Document and implement

An example of the checklist approach is shown for creatinine (Table 2).

Assessment of method differences

Bias study

Any significant method bias will result in misclassification of too many patients. The expected information derived from the combination of assay and RI must meet the appropriate clinical sensitivity and specificity required for each test. Hence a key requirement for the use

Table 2 Checklist reference interval (RI) approach for creatinine

Analyte	Creatinine (plasma and serum)
Population RI	Based on healthy subjects not hospital patients. eGFR used for decision making.
Units	µmol/L
JCTLM-listed traceability or preferred method and reference material	ID-GC/MS and ID-LC/MS (some methods require instrument factors). SRM 914 (pure creatinine). SRM 909, 967 (human serum).
Pre-analytics 1. Serum/plasma 2. Sample collection 3. Interferences	 1. Interchangeable. 2. Increases with meat consumption.
Analytical differences	Analytically there are no differences.
Partitioning by 1. Gender 2. Age	 1. Gender differences. 2. Age-related increases above 60 years not agreed by Renal Physicians.
Reporting Interval	1 µmol/L

of common RIs is the effect of methodological differences on bias and if this would affect the sharing of a common RI. Method differences are best assessed for bias using commutable patient-based samples. In the case of the Australasian Harmonised RI study specified performance limits based on biological variation were applied to determine whether bias would prevent the use of a common RI by assessing if all results fell within the allowable limits of agreement and if regression lines were all within allowable limits for the tested measurement procedures (10). The allowable limits of performance or allowable error specify that the imprecision and bias of a method must be within stated limits. Of 27 tested analytes among eight platforms/assays, 19 gave acceptable bias for a common RI (11). Note that where a RI is shared the analytical variation for more analysers in more laboratories using more methods will be larger than a singly-derived interval, resulting in a wider RI (12).

Calibration traceability

An initial assessment of methodology and calibration traceability of laboratory assays to be used to establish the common RI is required. Laboratories need to assess the traceability claims made by manufacturers including the reference material and reference measurement procedures used to assign values to master calibrators from which product calibrators are traceable in routine assays. Preliminary information can be gathered from the manufacturer, external quality assurance (EQA) programs and other published data. If a laboratory uses a method known to be biased compared with the method used to set the RI, a common RI cannot be used. Rather, for analytes with established traceability, traceable assays should be used to both set and to use the interval (13). Ideally, analytes should have a complete reference measurement system or a reference material and/or a reference measurement procedure listed on the Joint Committee for Traceability in Laboratory Medicine (JCTLM) website (14).

Selection of reference intervals

Various sources of information on RIs should be searched including local formal RI studies, published studies from the literature, laboratory surveys, manufacturer's product information, relevant guidelines, and mining of databases. Pre-analytical and partitioning issues, significant figures and flagging rates, which provide an indication of the clinical considerations of the RI, should also be assessed for each analyte.

Common laboratory usage

A survey of local laboratories ideally through the national EQA provider provides the opportunity for laboratories to compare their RIs with those from other laboratories using the same and different methods. By linking RIs to results from measurements on commutable samples, it is also possible to see the effect of the intervals on between-laboratory differences. For the majority of common chemistry analytes the between-laboratory variation in RIs is usually greater than the variation in results (15). These types of data can be used to support the use of common RIs for many analytes.

Published studies

The Nordic Reference Interval Project (NORIP) established common RIs in apparently healthy adult populations from five Nordic countries for 25 of the most common clinical chemistry analytes (16). Results were traceable to higher-order reference measurement systems. More recently Nordic paediatric RIs have been determined for 21 common biochemistry analytes and intervals were suggested for combined age groups (17). In the United Kingdom, reference limits have been established by a survey of RIs in use followed by an assessment of analytical variability, any age and sex related variation, or other variances in populations where these were seen as relevant to the analyte (5,6). The aim was to remove unnecessary variation that was demonstrated to lack scientific validity prior to taking on new work to formally validate the consensus RIs (6).

Global formal reference interval studies

The CALIPER Initiative

The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) (18) was established by a Canadian team of investigators to develop a new database of biomarker reference values (stratified by age, sex and ethnicity) determined from a large, healthy population of community children and adolescents. The CALIPER project was initiated as a result of several detailed gap analyses evaluating the availability of pediatric RIs in four clinical subspecialties: bone markers (19), risk markers for cardiovascular disease and metabolic syndrome (19,20), hormones of the thyroid and growth

hormone axes (21), and markers of inborn errors of metabolism (22). These analyses revealed major gaps in data available to clinical laboratories and paediatricians and highlighted the critical need for new initiatives. Since its inception in 2009, the CALIPER program has made considerable strides in establishing and publishing a new RI database for biochemical markers (23-33), however, the reference values were initially established on a single analytical system, the Abbott Architect assay system. To address this limitation, a series of transference studies (34-37) have recently been completed by the CALIPER program, allowing transference of paediatric reference values from the Abbott database to four other major analytical systems including Beckman, Ortho, Roche, and Siemens. Additional transference studies are in progress to complete transference of the entire CALIPER RI database to all major chemistry assay systems allowing widespread application of CALIPER reference standards in clinical laboratories worldwide using any one of the five major biochemical assay systems.

Canadian Health Measures Survey (CHMS)

The Canadian Health Measures Survey (CHMS) is the most comprehensive, direct health measures survey ever conducted in Canada. The study was launched in 2007 by Statistics Canada, in partnership with Health Canada and the Public Health Agency of Canada, to collect population-representative health information from Canadians aged 3-79 years. An initial household interview collected information about general health including nutrition, smoking habits, alcohol use, medical history, physical activity, and socioeconomic variables. Respondents then visited a mobile examination centre, where direct physical measures of health were taken, such as height, weight and blood pressure, and blood specimens were collected and analysed for biomarkers of health and disease (25). Individuals were selected in a systematic manner to be representative of 96.3% of the Canadian population. Data from CHMS samples were then weighted to ensure that the study population was truly representative of age, geographical distribution and ethnic origin of the Canadian population. In a recent collaboration between CALIPER and CHMS, laboratory data from approximately 12000 Canadian children and adults were used to establish a comprehensive database of paediatric and adult reference intervals for 24 chemistry (38), 13 endocrine/special chemistry (39), and 16 haematology markers (40). These reference intervals provide a valuable description of the changes in key biochemical parameters within the Canadian population. The use of common patient selection, pre-analytical, analytical and post-analytical methods allowed for assessment of fluctuations in 'normal' levels over time and prevalence of disease risk factors. Together, these studies provide a comprehensive description of the changes in important biomarkers within the Canadian population throughout the course of a lifetime, from childhood to adulthood to geriatrics.

The CALIPER and CHMS initiatives also provide a unique opportunity to strive towards establishment of common RIs across Canada. A taskforce has recently been developed by the Canadian Society of Clinical Chemists and discussions have begun among a number of opinion leaders across the country to assess the feasibility of establishing common reference values using the CALIPER and CHMS databases as the basis. The Canadian common reference interval initiative is also examining the potential development of platform-specific common reference values for each of the major analytical systems. This may be a more practical approach especially for the majority of analytes that are not standardised based on primary reference method and not traceable to a primary or secondary reference material.

Asian Studies

Although not attempting to define population reference intervals, Ichihara et al. (41) found unexpectedly large variations between the results obtained from samples sourced from 6 Asian cities (Hong Kong, Shanghai, Seoul, Kuala Lumpur, Taipei and Tokyo) for the 13 analytes tested suggesting that harmonised RIs would be difficult between these countries that these cities represent .

In contrast to the study by Ichihara, a Japanese multicentre study by Yamamoto et al. (42) involving 105 laboratories across Japan and using 4 different chemistry platforms demonstrated no regional differences and concluded that the RIs established in this study were also suitable for adoption nationwide (Table 3).

Turkish Study: Similar to the Japanese study, a multi-centre study by Ozarda et al. (43) determining RIs for 25 commonly tested analytes showed similar results between the seven Turkish geographical regions in 28 laboratories where the samples were sourced. They concluded that the intervals determined by this study using the same Architect 8000 analysers were suitable for use in all Turkish clinical chemistry laboratories that used the same platforms (Table 3).

Australian Study

The Aussie Normals study was a formal reference interval study of 1876 male and female healthy adult Australians in the age group 18 to 95 years (44). Up to 91 biochemistry analytes were measured by Abbott Architect analysers. Partitioning was done according to the effects of gender, age and body mass index (BMI) on these RIs. For the most part these differences were statistically small such as for lactate dehydrogenase and phosphate where they were less than day to day biological variation. As shown in Table 3, reference intervals for the Aussie Normals formal RI study were in general similar to those for the Australasian common RIs study although somewhat tighter as they were determined using one platform only. However, γ-glutamyltransferase (GGT) upper reference limits were notably higher in the Aussie Normals study which demonstrated BMI differences with increasing age in men and women (44). For the 18-<45y age group and BMI <25 kg/m^2, GGT was 12-37 U/L for men and 9-38 U/L for women. However, it is difficult to adopt RIs in association with BMI at this stage as this parameter is not routinely provided to the laboratory.

Data mining

Expert groups can provide RI information through their data mining of millions of data points from primary care patients. This method has advantages over the direct RI validation process by providing large amounts of data on the local population being tested and reflects the actual analytical and pre-analytical conditions for the tested population. This approach is valid only if there is a majority of results from the primary care population such that the healthy distribution of values can be clearly identified in the midst of a smaller number of non-healthy values. Bhattacharya analysis to determine underlying distributions in the presence of outlier results can be used to assess proposed RIs. For example, in Australasia data mining of over 200,000 paediatric data points provided by 15 laboratories for the main general chemistry analytes from birth to 18 years of age was used for establishing partitioned paediatric RIs (3).

Final selection of the common reference interval

One approach to the setting of a common RI that was used in Australasia is described as follows. The starting point to develop a common RI was to do a national survey of laboratory RIs and determine the predominant RI in use. Then

a method comparison study across the major chemistry platforms using commutable samples from healthy subjects was used to assess if bias would prevent use of a common RI with acceptability based on the specified allowable limits of performance such as those based on biological variation for example (11). For analytes where bias may prevent use of a common RI for one or two main platforms, it may be possible for other platforms to share a common RI, e.g. lactate dehydrogenase methods that use pyruvate to lactate [P to L] rather than the IFCC-recommended [L to P] method cannot be combined.

The next step involves gathering supportive date for the proposed common RI using data from formal local RI studies, if available, and from data mining. In Australia values from the Aussie Normals adult RI study were used to confirm the common RIs recommended for use in Australia and New Zealand (44). Note that reference intervals are wider for the common RIs that have been established for eight platforms compared with those obtained using the one platform; inclusion of between-method variation results in wider intervals than for a singly-derived RI (Table 3). Further mining of hundreds of thousands of data points from primary care patients who are relatively healthy was then employed to show the biochemical physiology from childhood to adulthood through to geriatric age, according to age and gender (45). In order to compare partitioning according to the continuous variables of age and pregnancy, and whether merged or separate partitions will affect clinical outcomes, there must be an understanding of the physiological processes affecting an analyte. Without the knowledge of clinical outcomes and their association with partitioned RIs, the lesser approaches of clinical opinion, statistics or laboratory consensus are used to determine the suitability of partitioning (45).

Once RIs are agreed upon, the proposed reference limits should be supported by flagging rates which provide an indication of the clinical considerations of the RI. Excess flagging of results can lead to inappropriate testing due to decreased specificity of the RI. Horowitz suggests that laboratories should be mindful of excess partitioning which is due to minor changes in physiology not to pathology (46). Hence local laboratories should assess flagging rates to determine if a change to historical RIs will create higher flag rates. For example, the pre-analytical effect of delayed sample transport would impact on potassium levels and hence for pragmatic reasons laboratories may choose to have a higher upper reference limit (URL) of 5.5 mmol/L rather than 5.2 mmol/L (Fig. 2A) (3).

Final agreement by a majority of stakeholders is required to support the selected common RI and a laboratory's intention to implement it, as described in the next section. The consensus process for deriving common RIs is not perfect and there are limitations. As noted above, intervals are usually wider than for singly-derived RIs obtained on the same platform, pre-analytical issues can cause elevated flagging rates, and elevated BMI in the population is not factored into clinical interpretation by the routine laboratory of GGT for example. Traceable analytes with JCTLM-listed reference materials and reference measurement procedures are more likely to share common RIs. However, countries may not be using IFCC recommended methods for enzymes as is the case in Australia where non-pyridoxal-5'-phosphate (P5P) AST and ALT methods are predominantly in use (Table 3). A harmonised RI with non-P5P methods is better than no harmonised RI and a future goal is for Australian laboratories to use P5P methods for AST and ALT.

Table 3 Adult reference intervals (RIs) for chemistry analytes determined by direct RI studies or by consensus

Analyte	Unit	Australia[44] Cat 2a Direct	Turkey[43] Cat 2a Direct	Nordic countries[16] Cat 2a Direct	United Kingdom[5] Cat 4 Consensus	Japan[42] Cat 2a Direct	Canada[38] Cat 2a Direct	Austral-asia[3] Cat 4 Consensus
		Architect	Architect	Multiple platforms	Multiple platforms	4 main platforms	Architect	8 main platforms
Sodium (M)	mmol/L	136-145	137-144	137-145	133-146	137-144	16-49y: 137-142	135-145
							50-79y: 136-143	
Sodium (F)	mmol/L	136-145	137-144	137-145	133-146	137-144	16-49y: 137-143	135-145
							50-79y: 136-143	
Potassium	mmol/L	3.7-4.9	3.7-4.9	3.6-4.6	3.5-5.3	3.6-4.8	3.8-4.9	3.5-5.2
Chloride	mmol/L	101-110	99-107	-	95-108	101-108	30-79y: 102-108	95-110
Bicarbonate	mmol/L	20-29*	-	-	22-29	-	19-26	22-32
Creatinine (M)	µmol/L	<75y: 65-103	59-92	60-100	60-100	57-94	16-79y: 63-102	60-110***
		75+y: 47-120						
Creatinine (F)	µmol/L	<75y: 54-83	50-71	50-90	60-100	41-69	17-79y: 49-85	45-90***
		75+y: 40-91						

Calcium (M)	mmol/L	2.19-2.56	2.15-2.47	2.15-2.51	2.2-2.6 (adjusted)**	2.2-2.5	20-39y: 2.28-2.60	2-10-2.60
							40-79y: 2.24-2.56	
Calcium (F)	mmol/L	2.19-2.56	2.15-2.47	2.15-2.51	2.2-2.6 (adjusted)**	2.2-2.5	20-39y: 2.24-2.53	2-10-2.60
							40-79y: 2.24-2.56	
Magnesium	mmol/L	0.77-1.04	0.77-1.06	0.71-0.94	0.7-1.0	0.7-1.0	-	0.7-1.1
Phosphate (M)	mmol/L	0.83-1.36	0.80-1.40	<50y: 0.75-1.65	0.8-1.5	-	16-47y: 0.95-1.52	0.75-1.50
				50+y: 0.75-1.35			48-79y: 0.89-1.52	
Phosphate (F)	mmol/L	0.88-1.44	0.80-1.40	0.85-1.50	0.8-1.5	-	16-47y: 0.95-1.52	0.75-1.50
							48-79y: 0.99-1.54	
LDH (M)	U/L	130-230	126-220	<70y: 105-205	-	124-226 [JSCC]	-	120-250 (L-P [IFCC])
				70+y: 115-255				
LDH (F)	U/L	122-232	126-220	<70y: 105-205	-	124-226 [JSCC]	-	120-250 (L-P [IFCC])
				70+y: 115-255				
CK (M)	U/L	<45y: 52-340	48-227	<50y: 50-400	40-320	61-257 [JSCC]	-	<60y: 45-250
		45-65y: 55-357		50+y: 40-280				60+y: 40-200
		65+y: 49-207						

CK (F)	U/L	<45y: 37-247	34-131	35-210	25-200	43-157 [JSCC]	-	30-150
		45-65y: 39-230						
		65+y: 36-190						
ALP (M)	U/L	<75y: 43-112	43-116	35-105	30-130	122-330 [JSCC]	16-21y: 56-167	30-110
		75+y: 42-126					22-79y: 50-116	
ALP (F)	U/L	<45y: 32-96	<50y: 34-97	35-105	30-130	104-299 [JSCC]	16-29y: 44-107	30-110
		45-75y: 40-132	50+y: 47-133				30-79y: 46-122	
		75+y: 44-146						
ALT (M)	U/L	<75y: 11-41	9-57	10-70		10-42 [JSCC]	18-49y: 18-78	5-40 (no P5P)
		75+y: 9-48					50-79y: 20-62	
ALT (F)	U/L	<75y: 9-35	7-28	10-45		7-27 [JSCC]	12-49y: 14-41	5-35 (no P5P)
		75+y: 8-33					50-79y: 16-44	
AST (M)	U/L	<75y: 14-36	13-30	15-45		14-32 [JSCC]	18-54y: 18-54	5-35 (no P5P)
		75+y: 14-34					55-79y: 18-39	
AST (F)	U/L	<75y: 13-31	11-25	15-35		12-27 [JSCC]	20-54y: 18-34	5-30 (no P5P)
		75+y: 14-35					55-79y: 18-39	

GGT (M)	U/L	<45y: 9-63	11-69	<40y: 10-80		12-65 [JSCC]	20-35y: 12-62	5-50
		45-75y: 13-72		40+y: 15-115			36-79y: 13-109	
		75+y: 15-78						
GGT (F)	U/L	<45y: 9-49	7-33	<40y: 10-45		9-38 [JSCC]	18-35y: 12-38	5-35
		45-75y: 9-55		40+y: 10-75			36-79y: 10-54	
		75+y: 9-57						
Total Protein	g/L	62-79	66-82	62-78	60-80	66-80	20-29y: 65-83	60-80
							30-79y: 65-78	
Total Bilirubin (M)	µmol/L	5-20	3.8-24.1	5-25	<21	6.4-24.8	16-48y: 3-18	1-20
							49-79y: 2-20	
Total Bilirubin (F)	µmol/L	5-21	2.7-15.9	5-25	<22	6.4-24.8	16-48y: 1-16	1-20
							49-79y: 1-17	

** Bicarbonate measured prior to Abbott recalibration; ** Calcium is adjusted for albumin;*
**** Creatinine has harmonised RIs for adults up to the age of 60 y.*

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cat: category according to Stockholm Hierarchy; CK: creatine kinase; GGT: γ-glutamyltransferase; IFCC: International Federation of Clinical Chemistry and Laboratory Medicine; JSCC: Japan Society of Clinical Chemistry; LDH: lactate dehydrogenase; P5P: pyridoxal 5'-phosphate.

FINAL ACCEPTANCE, ADOPTION AND IMPLEMENTATION OF HARMONISED REFERENCE INTERVALS

Communication and discussion by all stakeholders

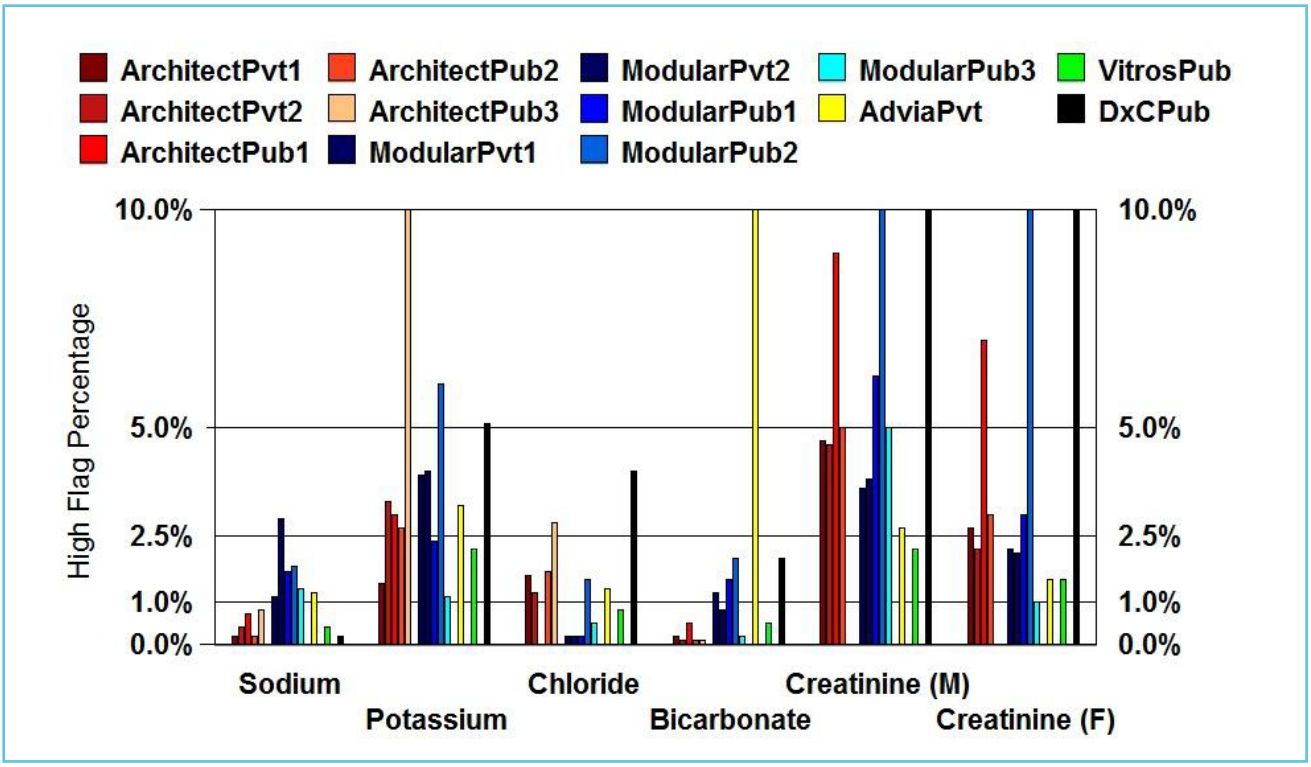
Laboratory acceptance should be sought at a national level prior to introduction of common RIs. Various approaches can be used to assess the likely adoption rates for the panel of RIs including a survey as to whether the laboratory is using the common RI already,

would accept the RI, or ask for comments and their reason if they do not accept the common RI. Representation is required from the whole nation and from public and private pathology, small and large laboratories and networks if harmonised RIs are to have any chance of being implemented. National acceptance of a change to pathology RIs requires that there is an on-going discussion by all involved stakeholders especially those at the highest management level who are responsible for patient pathology results and their interpretation. Harmonisation workshops provide a forum for presenting and discussing the evidence and reaching a consensus decision.

Validation of reference intervals by local laboratories

Responsibility for adoption of common RIs lies with each laboratory. Advice on how to do this is found in guidelines from the Clinical and Laboratory Standards Institute (CLSI) (47). Key questions are: 'Is this RI suitable for my method and for my population?' Validations of RIs may be by subjective assessment assuming the same method and the same population are used or by a simple validation using 20 normal subjects representing the local population (47,48). Alternatively, you can mine your laboratory's existing data. The most useful parameter is the midpoint of the extracted data, which can be used to assess

Figure 2A **Typical high flagging rates for the first measurement in outpatient adults (18y – 60y) for sodium, potassium, chloride, bicarbonate, creatinine (M), creatinine (F)**



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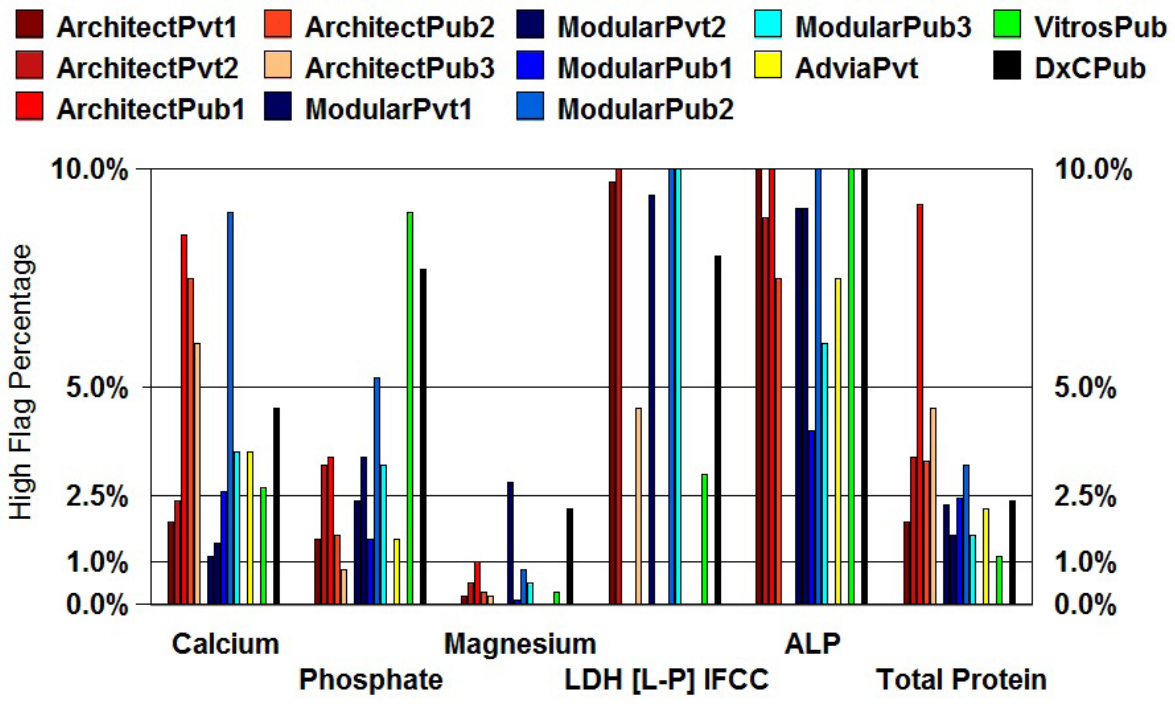
analytical or population bias by comparison with the corresponding midpoint of the data used to set the reference interval. Bhattacharya analysis can also be used to assess the proposed intervals (12).

Validation of flagging rates for local population

Based on the principle of minimum, desirable and optimal categories used to define allowable bias limits, flag rates may range from 1.0% to 1.8% for low flagging rates, and 5.7% to 3.3% for high flagging rates, respectively. Flag rates however, may be quite complex to interpret depending on the population used to derive them. For example in the Australasian common RIs project, a URL of 110 U/L for alkaline phosphatase may result in a flag rate of 7-8% (3). However, the clinical benefit of using the URL of 110 U/L is to detect pathology in postmenopausal women. Increasing the URL to 115 U/L did not have any significant impact due to the logarithmic distribution of reference values. In contrast, the flag rate at the URL for sodium was 1% indicating that hypernatraemia is uncommon (Fig. 2A and 2B). Data mining of local population values also allows for an assessment of the expected number of results outside the RI (12). The laboratory can then compare the expected flagging rates with their current rates.

Figure 2B Typical high flagging rates for the first measurement in outpatient adults (18y – 60y) for calcium, phosphate, magnesium, lactate dehydrogenase, alkaline phosphatase, total protein



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CLOSING THE HARMONISED REFERENCE INTERVAL LOOP

Following the endorsement of common RIs by pathologists and scientists, formal endorsement by the profession is sought from the National Pathology College and National Clinical Chemistry, Biochemistry or Laboratory Medicine Society. Support by the National Testing Authorities for Laboratory Medicine, who should be included in meetings on harmonisation, is via formal recommendations to laboratories that they use these intervals, or if not, to provide supporting evidence for other references.

Continuing work is required to produce and validate common RIs, to manage ongoing issues, e.g. problems with implementation of RIs by the local laboratory Information Technology unit into the Laboratory Information System. These issues may be changes to reporting units, significant figures, rounding, report formatting, etc. Consultation with clinical societies and education of local clinicians are imperative if the new RIs are to be used. Other flow-on effects can be those regarding the reimbursement of pharmaceutical benefits according to national government benefit schemes that use specific RIs or decision limit values when assessing the provision of a treatment drug.

The level of uptake of common RIs can be readily surveyed through EQA programs. One such scheme that also surveys the bias of methods within the reference interval measuring range by using commutable samples from healthy subjects is the RCPA Quality Assurance Program Liquid Serum Chemistry program (15). The scheme allows assessment of the between-laboratory variation in results, RIs and the information transmitted by the combination of these factors. For most common chemistry analytes, use of common RIs has improved the variation seen in the information produced by different laboratories.

CONCLUSION

Consideration should be given by laboratories to adopting RIs consistent with those used by other laboratories in the region where it is possible and appropriate for the local population. These may be common RIs for use across several major platforms in the region, e.g. United Kingdom, Nordic countries, Japan, Australasia, or for use with one specific platform, e.g. Canada, Asia, Turkey. Scientific evidence supports the use of common RIs for many general chemistry analytes especially those with sound calibration and traceability in place. For other non-harmonised immunoassay analytes where either there is currently no secondary reference material or reference measurement procedure for value assignment, it seems logical to use platform-specific RIs and decision limits across regions, provided that laboratories have acceptable assay precision, until such time when methods become harmonised internationally.

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In this issue: Harmonization of Clinical Laboratory Test Results

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Critical risk results – an update on international initiatives

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ARTICLE INFO

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Key words:
harmonization, critical risk results, critical laboratory results, alert table, alert threshold, critical laboratory notification

ABSTRACT

Direct communication of significant (often life-threatening) results is a universally acknowledged role of the pathology laboratory, and an important contributor to patient safety. Amongst the findings of a recent survey of 871 laboratories from 30 countries by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), only 3 tests were noted to be common to 90% of alert lists, and only 48% of laboratories consulted clinicians in developing these alert lists despite ISO15189 recommendations to do so. These findings are similar to previous national surveys demonstrating significant variation worldwide in how critical risk results are managed and also in how these protocols are developed. In order to promote "best practice" and harmonization of critical risk results management, guidelines and recommendations have been published, most recently by Clinical and Laboratory Standards Institute (CLSI) and Australasian Association of Clinical Biochemists (AACB). These statements in particular have placed strong emphasis on patient risk and risk assessment in the management of critical risk results. This focus has resulted in recommendations to adopt new terminology, the

consideration of risk assessment when compiling alert tables, consultative involvement of laboratory users in setting up protocols, and the need for outcome-based evidence to support our practices. With time it is expected that emerging evidence and technological improvements will facilitate the advancement of laboratories down this path to harmonization, best practice, and improve patient safety.

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INTRODUCTION

Direct communication of significant (often life-threatening) results which require timely clinical attention is a universally acknowledged role of the pathology laboratory. Accreditation standards formalise the requirement for laboratories to manage these "high risk results" but only offer very general guidance on how this should be achieved. Not surprisingly, there is evidence of wide differences in practice between laboratories both internationally and within the same country. These differences are seen in all aspects of high risk results management including the nomenclature and definitions used; which critical tests and thresholds are included in alert tables; specification of who can receive results and by what mode of communication; what information should be conveyed with the result; how receipt of the result is acknowledged; escalation protocols for failed attempts at communication; and how communication events are recorded. Lack of agreement is evident not only in *what* is contained in laboratory protocols but also in *how* these protocols are developed.

It is now increasingly recognised that successful management of high risk results is an important contributor to patient safety[1]. As such, harmonization in this area cannot simply be a matter of shared definitions and procedures, but must involve the determination and implementation of best practice. The challenge is to define best practice and to obtain the evidence required to support this. This review discusses the work currently being undertaken by a number of professional organisations worldwide to harmonize and bring best practice to the management of high risk results.

WHAT IS THE CURRENT SITUATION?

Existing practices

Information on how laboratories manage high risk results is largely provided by national surveys [2-13], most of which have been questionnaire-based with voluntary participation. Although their findings are limited by the response rate and potential selection bias inherent to this method of data collection, these surveys remain the best source of information we have on existing practices. In 2011, the Australasian Association of Clinical Biochemists (AACB) undertook a survey of laboratories representing a mixture of large private and public pathology networks from key providers in the region, servicing community and hospital patients[2]. Between September 2012 and March 2013, the European Federation of Clinical Chemistry and Laboratory Medicine (ELFM) invited its members, affiliates and provisional member countries to complete a modified version of the Australasian survey, adapted for the European professional environment. Eight hundred and seventy one laboratories from 30 countries responded and these results[3,4], in combination with the Australasian findings, have provided a comprehensive insight into international state-of-the-art practice in this area.

One finding common to all surveys has been the lack of uniformity in alert lists, both in their contents and how they are compiled. Only 41% of Australasian and 48% of European laboratories consulted clinicians in this process despite the recommendation within ISO 15189 that

clinical agreement be sought. However, this rate varied between nations with Norway and the Netherlands reporting high consultation rates (72% and 88% respectively) comparable to the 73% of U.S. laboratories previously described[10]. It is known from other national surveys that clinical consultation rates can be significantly lower[12,13]. Alert lists solely derived from the laboratory run the risk of being detached from clinical practice. A Canadian laboratory found that when their laboratory-derived alert lists were presented to their hospital physicians, only 36% of adult and 61.5% of paediatric alert thresholds were considered acceptable and did not require modification[14,15]. "Published literature" is another commonly cited source of critical thresholds (listed by 59% of Australasian and 66% of European laboratories) but what laboratories interpret this term to mean is often not explored. A previous survey of UK laboratories found that only 2 out of 94 laboratories actually quoted literature to support the thresholds in their alert table[6].

Surveys have consistently highlighted variation in the content of alert lists. In Europe, only 3 tests (potassium, glucose and sodium) were common to the alert lists of more than 90% of survey respondents. In comparison, a U.S. report found 8 common tests (potassium, sodium, calcium, platelets, hemoglobin, activated partial thromboplastin time, white blood count and prothrombin time), again shared by more than 90% of the surveyed laboratories[7]. How many tests should we expect to be common on alert lists is not clear. The answer is likely to be complicated when considering the patient population serviced by individual laboratories, the tests performed and whether there is evidence of clinical risk from outcome studies.

When the numerical alert thresholds used are compared between laboratories, the findings are varied. Some analyte thresholds do show harmonization probably as a consequence of the wide adoption of thresholds from a single source (e.g. guidelines), rather than consensus regarding clinical risk. This can be seen amongst laboratories measuring the drug carbamazepine. In the Australasian survey, 22 out of 26 laboratories reported a high critical threshold for this drug, the median of which was 15 mg/L (range 9-20). This same median high threshold (15 mg/L) was found in a US survey of 36 internet-published alert lists for therapeutic drugs (range 11-20)[16]. Fifteen mg/L was also the mean high threshold (range 10-20) found in a survey of UK laboratories[6]. In contrast, there is little agreement with C-reactive protein thresholds in adults. Its inclusion in alert lists can be seen in 28% of Australasian alert lists with a median value of 100 mg/L (range 80-300) and in 30% and 43% of European adult and pediatric alert lists, respectively. Forty-three percent of Norwegian laboratories use CRP on their alert lists[17] with a median applied alert threshold of 200 (10 and 90 percentiles; 50-200) mg/L. Of interest, only 35% of responding general practitioners actually wanted to be alerted of CRP values above 120 mg/L (10 and 90 percentiles of responses were 50 and 200mg/L, respectively). Further variation in alert list content has been described as a result of some laboratories using customized thresholds and modified policies based on the patient age, location, individual provider or practice group requesting the test, or the disease type where known[7]. Sixty-one percent of European laboratories use children-specific alert thresholds, and 19% apply unique thresholds for specialist wards.

Many surveys also described diversity in the communication policies around high risk results. Around 65% of European and 80% of Australasian laboratories would not actively communicate a critical risk result if it was not significantly different from a previously delivered result for that patient. In U.S., only 36% laboratories had a policy allowing for these repeat critical results not to be called[18]. Furthermore, a

College of Pathologist Q-Tracks study suggested that reporting all critical values, including repeat ones, was actually valuable as it may indicate a higher degree of vigilance in the critical value reporting system[19].

Where results are successfully conveyed by verbal communication, the procedure of asking recipients to "read-back" results to confirm successful transmission was practiced inconsistently between countries[2,5,7,11] ; only 46 % of laboratories surveyed both in France and Australasia compared to 79% of U.K. laboratories. Rates at which this "read back" was formally documented and records kept also varied between nations; 10% of Australasian and 23% of European laboratories.

There is also diversity in escalation policies when a responsible clinician cannot be contacted. Only 38% of responding laboratories in the European survey had an existing formal protocol. Some laboratories contact the patient either directly (64% of French and 23% of Australasian laboratories) or via the police or ambulance service (15% of Australasian laboratories). Thirty four percent of European and 39% of Australasian laboratories formally documented occurrences where delivery of a critical result had to be abandoned. Information regarding the average time to abandonment of communication attempts is sparse but has previously been reported amongst U.S laboratories to be 20.2 minutes for inpatients and 46.3 minutes for outpatients[10] .

Available evidence

For patient safety, laboratories should follow procedures that are considered best practice and based on high level evidence. However, in most aspects of high risk results management, the evidence required is often lacking. Contributing to this problem is the inconsistency in terminology and definitions used in the literature. There has been disagreement on terminology since the original phrase "panic values" was first coined by Lundberg[20]. Commonly used terms including "critical", "significantly abnormal", "life-threatening" and "urgent" have all been criticised because of their inability to include all results that require timely notification, and because of the ambiguity caused by their use in other areas of medicine and everyday language. Their generic use creates a problem when these phrases are used as search terms; searching the NIH PubMed website (accessed 4/11/2015) with "laboratory AND critical AND results" yielded over 22,500 articles, the top 50 of which were not relevant to our intention. Likewise, use of the term "value" itself has also been discouraged as it seemingly excludes semi-quantitative or non-quantitative results such as microbiological cultures[21].

Failure to distinguish "critical tests" from "critical test result" also creates confusion. A "critical test" is a laboratory test that influences clinically urgent patient management decisions irrespective of whether the result is normal, abnormal or critical. Thus any result for a critical test should be rapidly communicated. It is distinct from a "critical test result" which refers to a test result that requires timely communication only because it falls outside a pre-defined risk alert threshold. If critical tests are not clearly defined, the lack of associated thresholds to assist in their identification may lead to results being overlooked and therefore not communicated nor acted upon.

Recent discussion around the evidence required for alert list design has suggested that alert thresholds should be considered "clinical decision limits" given that their purpose extends beyond merely indicating illness, but to trigger clinical action. A modified Stockholm Hierarchy has been proposed for clinical decision limits which assigns Level 1 evidence as "clinical outcomes in specific clinical settings"[22]. Such evidence is best attained with randomised

control trials as they explicitly investigate the relationship between an exposure (e.g., a critical risk result) and an outcome (e.g., mortality or serious morbidity) and enable calculation of the outcome risk specifically associated with that exposure. However, even if it were possible to induce a pathological state to generate critical risk results within a random selection of subjects, it certainly would not be ethical. Consequently, the critical risk result outcome studies reported in the literature are generally retrospective observational studies. The main, and often impossible, challenge in the design of such studies is separating the contribution to the risk of adverse outcome posed by confounding variables (characteristics of the study subjects other than the critical risk result) in order to assess the independent effect of the critical risk result.

A further limitation of retrospective observational studies is that they typically have not been designed for the purpose of identifying the optimal alert threshold. A number of retrospective observational studies published for serum potassium show relatively congruous results with increased mortality risk observed when potassium concentrations are below 3.0-4.1 mmol/L or above 4.3-4.5 mmol/L, despite diverse study populations (general hospital, patients with chronic kidney disease, acute myocardial infarction, head trauma or on peritoneal dialysis) and varying timeframes observed for mortality (during inpatient admission, 1 year or longer term)[23-27]. However, these thresholds cross into commonly quoted reference intervals for potassium and therefore would be impractical for laboratory alert lists. While studies that explore the continuous relationship between test result values and outcome are important, a decision must be made as to when the risk of adverse outcome becomes unacceptable and hence where clinical action should be taken. Unlike potassium (and sodium), only a small number of studies addressing clinical decision limits exist for many other analytes. This likely reflects the difficulty of studying analytes with assay-related variations in measurement and where a clearly associated clinical outcome has not been identified.

INITIATIVES

Terminology

The need for harmonization and the implementation of best practice in high risk results management is now widely acknowledged and has provided a common goal for laboratories and pathology organisations worldwide. Addressing the variation in terminology has been an important first step. It is vital that the language used must not only be common but it must correctly convey the intention so that there is shared understanding of the concepts underlying the process.

Recently, the term “high-risk results” has been proposed as an umbrella term to include “critical-risk results”; results requiring immediate medical attention and action because they indicate a high risk of imminent death or major patient harm, and “significant-risk results”; results that are not imminently life-threatening, but signify significant risk to patient well-being and therefore require medical attention and follow-up action within a clinically justified time limit[28]. Emphasising the clinical *risk* to the patient rather than the timeframe required for notification or the need to initiate clinical action, is an important distinction. It underscores the need for clinicians to assess and consider the risk of harm in an individual patient with a particular result, and to then decide on an appropriate course of action. Although it might be argued that this change in terminology is purely cosmetic, it reminds us that critical values are not “one-size-fits-all”; that results notification is a *trigger* for clinical assessment. Common use

of this terminology in clinical trials and publications would facilitate the transferability of findings as well as helping to collate evidence in a more systematic manner.

CLSI GUIDELINES

The terminology and concepts of "critical-risk" and "significant-risk" have already been adopted by some professional bodies in their guidance documents[29,30]. The Clinical and Laboratory Standards Institute (CLSI) in its recently released guideline for management of laboratory results that indicate risk for patient safety[29] has introduced these terms to emphasize that the appropriate steps for reporting a laboratory result can be defined by the degree of risk for adverse patient outcome. Degree of risk in this context is differentiated by immediacy, probability and/or severity of potential patient harm, as well as likelihood of harm due to undetected breakdowns in communication. "Critical-risk" results signify probable, immediate risk of major adverse outcomes in the absence of urgent clinical evaluation. The guidelines stress that such results should be actively communicated to responsible caregivers without delay, and that there should be documentation that the caregivers received this information accurately. "Significant-risk" results indicate risk of important adverse outcomes that can be mitigated by timely clinical evaluation (although the risks are not necessarily immediate, highly probable or life-threatening). Unless routine reporting systems have safeguards against breakdowns in communication, significant-risk results should also be actively reported to responsible caregivers with documentation of successful and accurate communication. However, the time frame(s) for reporting such results do not need to be the same as for critical-risk results, as long as they permit appropriately timely clinical evaluation.

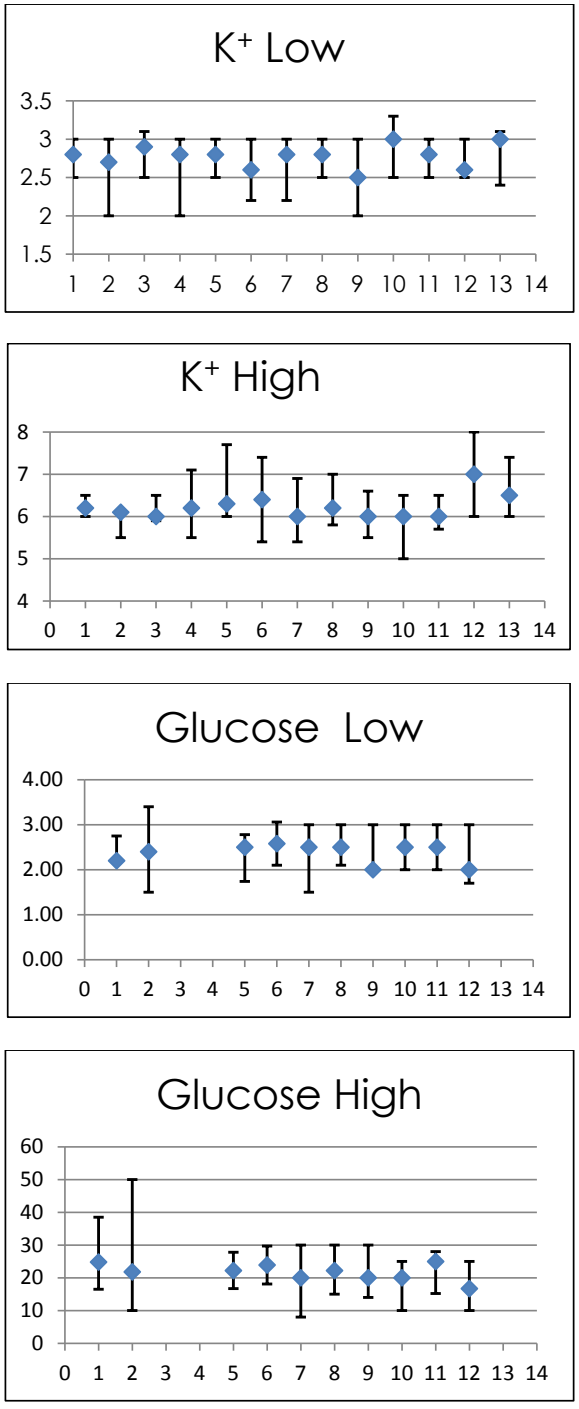
The CLSI guideline recommends that a laboratory or healthcare organization conduct local risk analysis to determine which laboratory results should be defined as "critical-risk" or "significant-risk". In addition, risk analysis should determine the most reliable processes to communicate results to responsible caregivers, and how to monitor these processes for effectiveness. The analysis should focus on the following initial questions:

1. Do the laboratory results indicate a significant risk for adverse patient outcome?
2. Can the caregiver act on these results to significantly reduce patient risk?
3. Will active communication from laboratory to caregiver reduce patient risk or promote better care?

To address these questions, organizations should consult with local laboratory and medical staff leadership, and review locally applicable regulations and accreditation standards. In addition, the organization can refer to the growing number of international surveys on the reporting of abnormal laboratory results. The surveys, while revealing substantial practice variations, have identified a core list of results that the majority of peer institutions define as "critical-risk"; these results would likely be applicable for the organization, with modification as needed based on local risk analysis or feedback from laboratory and medical staff. Examples of common critical-risk results include very abnormal potassium or glucose concentrations in serum/plasma (See Figure 1), or cell counts in whole blood.

In contrast to critical-risk results, significant-risk laboratory results are not specifically addressed in regulatory and accreditation standards, and there are few published surveys for reporting these results. Therefore, the organization's reporting procedure can be determined by local risk analyses. To use a specific example,

Figure 1 **Alert thresholds of the two most frequent blood parameters on adult alert lists in different surveys**



Surveys included: 1. US 2002 Median (p10-p90), 2. UK 2003 Mean (range),3. US 2007 Median (p5-p95),4. Italy 2010 Median (p10-p90), 5.Spain 2010 Median (p10-p90),6. Thailand 2010 Mean (±SD), 7.Australia 2012 Median (range), 8. China 2013 Median (p5-p95),9. Norway Median (range), 10. Norway GP's Median (range),11. EU adult Median (p10-p90),12. EU paediatrics Median (p10-p90), 13.EU dialysis Median (p10-p90),14. EU obstetrics Median (p10-p90).

the organization might consider how to report unexpected, early-stage adenocarcinoma in a routine appendectomy specimen. This result is significant for prognosis and therapy, but does not indicate immediate risk of severe adverse events, and does not require immediate clinical intervention for appropriate care. However, a delay in recognition and treatment could result in a significantly worse outcome for the patient. Therefore, this result might meet criteria for "significant-risk" depending on an organizational risk analysis. If routine pathology reports cannot be verified for receipt and acknowledgment, the organization should classify the unexpected finding of malignancy as a significant-risk result, and require the pathology laboratory to actively notify caregivers in a clinically appropriate time frame (for example, within 24 hours). On the other hand, if routine pathology reports are monitored to verify acknowledgment by responsible caregivers within an appropriate time frame, the organization might choose to rely on standard reporting in this situation.

Policies and procedures for reporting critical-risk and significant-risk laboratory results should include the following:

1. The definition of critical-risk and significant-risk results, and timeframes for reporting. These should be established through consensus between laboratory, medical and administrative personnel.
2. The laboratory should identify personnel responsible for reporting critical-risk and significant-risk results.
3. The organization should identify caregivers authorized to receive reports of critical-risk and significant-risk results. Final recipients should be responsible clinicians who can direct patient care based on the laboratory results. It may be reasonable for the laboratory to report results to intermediaries, who relay the report to the responsible clinician. However, the accuracy and timeliness of the communication must remain appropriate for patient care.
4. Reports of critical-risk and significant-risk results should be documented to identify the patient or patient's sample, the laboratory result, the reporter and recipient, the time of report, and verification of accurate communication. If intermediary personnel are involved in the report, each leg of communication should be documented.
5. The reporting of critical-risk and significant-risk results should be continually monitored for effectiveness. Root cause analyses should be conducted if performance targets are not met, in order to identify potential sources of risk.

AUSTRALASIAN RECOMMENDATIONS

A guidance document on the communication and management of high risk results has also been recently published by the AACB in conjunction with the Royal College of Pathologists of Australasia (RCPA)[30]. It contains recommendations which reflect "best practice" based, where possible, on available literature but ultimately reflects the consensus view of a specifically formed working party comprising of pathologists and laboratory scientists with interest and expertise in this area. The statement has been written in a general manner so as to be able to be applied to all disciplines within pathology. Before publication, an open invitation to comment on the draft was sent to the wider laboratory community, clinicians and patient interest groups. This wide consultative process acknowledged the importance of agreement amongst these three groups in order for the successful management of high risk results.

The document features 8 key recommendations for laboratories, namely to:

1. compile an alert list(s) in consultation with its users;

2. have procedures to ensure that high risk results are reliably identified;
3. specify, in agreement with its users, the modes of transmission for the communication of high risk results;
4. specify, in agreement with its users, who is authorised to receive high risk results;
5. define what data needs to be communicated to the recipients of high risk results;
6. develop a system for the acknowledgement of the receipt of high risk results to confirm that results were accurately and effectively communicated;
7. ensure that every high risk result notification is appropriately documented;
8. have procedures that involve its users in maintaining and monitoring the outcomes of its high risk result management practices.

Further details of how each recommendation should be achieved, including some examples, are explored within the body of the paper.

The consensus statement aims to incorporate a number of important concepts for harmonization and best practice. Laboratories are encouraged to adopt the newly proposed international terminology and are also encouraged not to develop their procedures in isolation but instead to collaborate with their laboratory users (that is, medical practitioners, nurses and other health care professionals directly involved in patient care). Although the guidance document represents what is considered best practice, it recognises that individual laboratories, due to unique circumstances, may struggle with some recommendations. To address this, the terms "needs to", "should" and "may" are purposely used to give an indication of the strength of each recommendation, providing laboratories with an understanding of which recommendations must be adhered to, and which can be viewed as suggestions. It is also important that laboratories see the management of high risk results as a dynamic process requiring monitoring and updating in light of changing circumstances and technology.

These recommendations are an initial step towards harmonization. The working party hopes to compile a "starter" alert list with thresholds based on outcome studies and expert opinion, framed by the risk assessment model proposed by the CSLI. Laboratories could expect to use this list as a foundation for discussion with their clinical users.

FUTURE DIRECTIONS

Future directions in the area of high risk results management will be influenced by emerging evidence and advances in technology. There is a clear need for more outcome studies. These studies should use consensus terminology and be designed to not only demonstrate where the risk of harm to patients starts but also determine the threshold level(s) where clinical action can eliminate or diminish this risk. With stronger evidence will come harmonization of alert thresholds and protocols for laboratories and their users. Studies should also look at specific populations or scenarios to allow for alert lists to better cater for individuals thus generating less false positive clinical notifications. While having more exceptions or rules seems unmanageable today, it is reasonable to expect improvements in technology that will assist the way we identify and communicate high risk results.

Laboratories will also need to adapt their procedures and protocols as new opportunities are presented by improving technology. Already, the use of electronic text messaging as an alternative form of communication to the traditional phone call has been described with success[31,32]. Further advances in the way laboratories identify high risk results and notify clinicians are certain. However, it is important that

the underlying principles of best practice remain, so that in the example of text messaging, receipt of the result must be acknowledged and documented and where this does not occur, an escalation procedure implemented.

CONCLUSION

High risk results management is recognised as an important contributor to patient safety. Wide variation in laboratory practices worldwide has been identified, and the need for harmonization is universally acknowledged. Recent initiatives towards harmonization have focussed on patient risk and risk assessment. This approach has framed proposed new terminology, discussions around the design of alert tables, the need for outcome-based evidence and best practice recommendations for laboratory procedures. With time it is expected that emerging evidence and technological improvements will further advance laboratories down this path to harmonization and best practice, and improve patient safety.

DEFINITIONS

Critical test: A test that requires immediate communication of the result irrespective of whether it is normal, significantly abnormal or critical.

Critical risk result: Results requiring immediate medical attention and action because they indicate a high risk of imminent death or major patient harm.

Significant risk result: Results that are not imminently life-threatening, but signify significant risk to patient well-being and therefore require medical attention and follow-up action within a clinically justified time limit.

High risk results: A collective term used to denote results that require communication in a timely manner; i.e. critical risk results, significant risk results and results of critical tests.

Alert threshold: The upper and/or lower threshold of a test result or the magnitude of change (delta) in a test result within a clinically significant time period, beyond which the finding is considered to be a medical priority warranting timely action.

Alert list: A list of critical tests and tests with alert thresholds for high risk results ideally reflecting an agreed policy between the laboratory and its users for rapid communication within a pre-specified time frame and according to a procedure.

Escalation procedure: An ordered list of alternative steps to be followed when the appropriate recipient(s) of a high risk result cannot be reached in a clinically appropriate time frame.

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Analytical challenges in the genetic diagnosis of Lynch syndrome – difficult detection of germ-line mutations in sequences surrounding homopolymers

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Key words:
diagnosis, Lynch syndrome, analytical challenges

Competing interests:
The authors declare no competing interests.

Ackowledgements:
We thank the Hereditary Cancer Program of the Valencian Region.

ABSTRACT

A genetic diagnosis is essential in families with a suspicion of Lynch syndrome, as it allows the use of proper and specific surveillance programs for high-risk individuals who carry a pathogenic mutation. The prediction and prevention schemes reduce the impact of cancer in high-risk families in a cost-effective manner. Genetic tests for LS are well standardized and broadly used, although there remain some specific difficulties that need to be addressed to reach an optimal diagnosis. In this report, we addressed the problem raised by the detection of mutations at intronic-splicing consensus sites located near mononucleotide repeats. A standard procedure was applied for LS diagnosis in all cases. PCR and Sanger sequencing results of the whole coding sequences and intron–exon boundaries of the *MSH2* gene were analyzed. Moreover, we designed quality-control procedures to verify the attainment of the intended quality of results regarding sequences located in complex contexts. We found eight families with point mutations at intron 5 of the *MSH2* gene located near the BAT26 mononucleotide marker, which could be missed in a regular diagnostic process. Four families had the c.942+2T>A mutation, and the remaining four families had the c.942+3A>T mutation. In conclusion, the detection of pathogenic

mutations located near microsatellite sequences is especially difficult and requires the implementation of specific quality controls to optimize diagnostic methods.

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INTRODUCTION

Lynch syndrome (LS) (MIN No: 120435) is an autosomal dominant hereditary condition that predisposes to colorectal, endometrial, and other tumors. The syndrome is caused by germ-line mutations in one of the mismatch repair (MMR) genes: *MLH1*, *MLH2*, *MSH6*, or *PMS2*[1]. A genetic diagnosis is essential in families with a suspicion of having LS, as it allows the use of proper and specific surveillance programs for high-risk individuals who carry a pathogenic mutation. Thus, high risk individuals are advised to stay within the normal weight range and refrain from smoking since a high BMI and smoking increase the risk of developing adenomas and colorectal cancer in Lynch syndrome. Regular colonoscopy leads to a reduction of colorectal cancer-related mortality. Hysterectomy and bilateral oophorectomy largely prevents the development of endometrial and ovarian cancer and is an option to be discussed with mutation carriers who have completed their families especially after the age of 40 years[2].

The prediction and prevention schemes reduce the impact of cancer in high-risk families in a cost-effective manner.

In general, genetic tests for LS are well standardized and broadly used, although there remain some specific difficulties that need to be addressed to reach an optimal diagnosis. In addition to the postanalytical limitations in the interpretation of the clinical significance of some genetic variants, there are other analytical challenges, such as the difficult study of the *PMS2* gene because of the high number and homology with several pseudogenes, or the detection of variants located in the proximity of a homopolymer sequence in the MMR genes.

In this report, we addressed the problem raised by the detection of mutations located at intronic-splicing consensus sites, near mononucleotide repeats. In this particular sequence context, the detection of mutations is a real analytical challenge.

METHODS

Our laboratory performs genetic testing for the diagnosis of LS covering a population of over five million people in the southeast of Spain. These genetic tests are requested from the five genetic counseling units of the Public Health Hereditary Cancer Program of the Comunidad Valenciana[3].

The present study was conducted in compliance with the ethical principles for medical research involving human subjects of the Declaration of Helsinki. Informed consent was obtained from all subjects, and the study received the approval of the Ethics Committee of the Elche University Hospital.

A standard procedure was applied for the diagnosis of LS in all cases. Fulfillment of the revised Bethesda Guidelines or loss of expression of MMR genes during universal screening for colorectal and endometrial tumors is required for referral to the Genetic Counseling in Cancer Units[4,5]. Before gene mutation analysis, the tumors of the probands were studied for microsatellite instability and MMR protein expression (MLH1, MSH2, MSH6, and PMS2), to confirm MMR implication and select the target gene/s for mutation analysis.

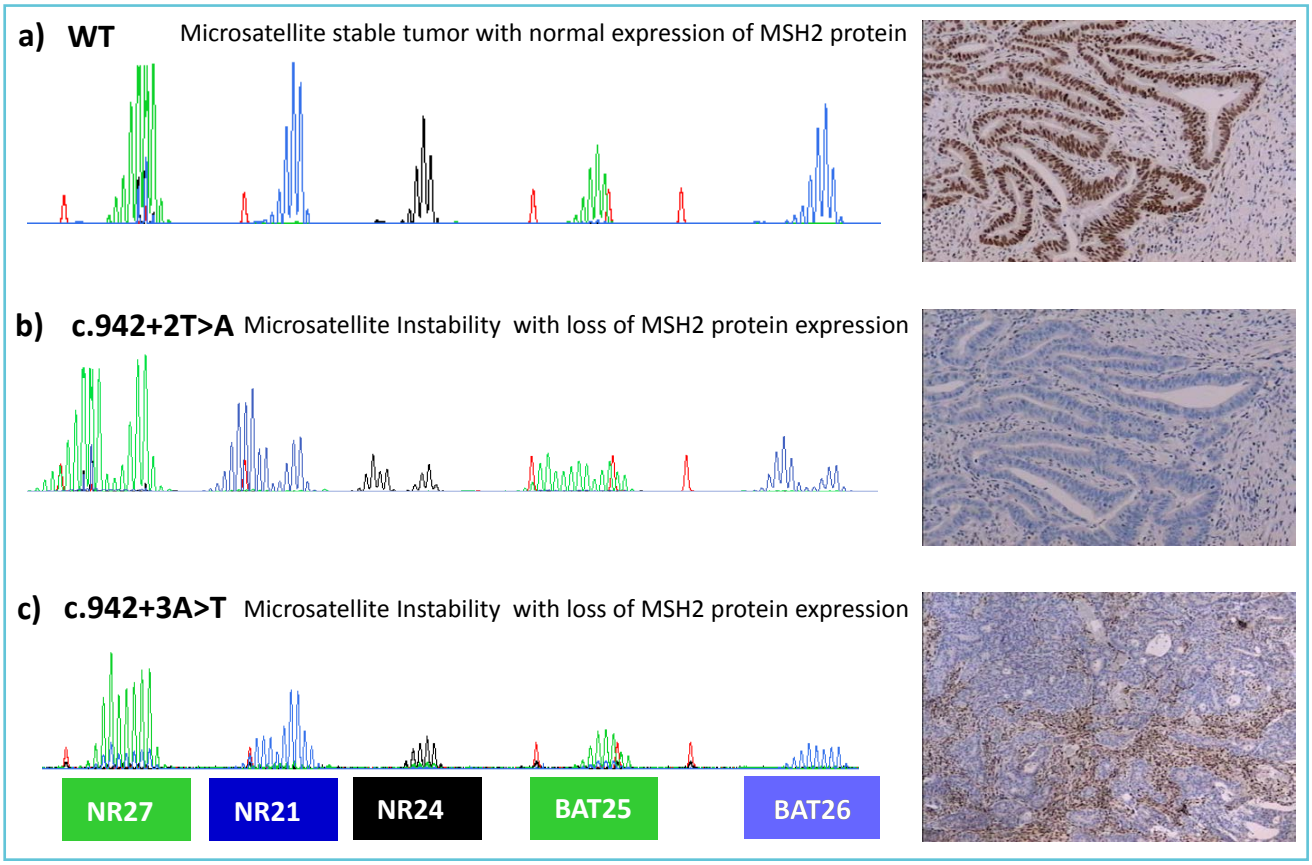
Mutation testing using the probands' blood DNA was then performed to assess the causative germ-line mutation in their families. PCR and Sanger sequencing results of the whole coding

sequences and intron–exon boundaries of the *MSH2* gene were analyzed. The PCR primers and conditions used were reported by Wahlberg et al.[5]. The suspicious genetic variants detected were confirmed by an independent sequence analysis of both DNA strands. The clinical significance of the variants was assessed according to the *InSiGHT Variant Interpretation Committee: Mismatch Repair Gene Variant Classification Criteria, Version 1.9 August 2013* (http://insight-group.org/criteria/)[7]. Pathogenic mutations and variants of unknown clinical significance were deposited in the *InSiGHT* database (*International Society for Gastrointestinal Hereditary Tumors*: http://www.insight-group.org). Once a causative mutation of LS was detected, the patient received the corresponding genetic counseling and genetic predictive tests were offered to at-risk relatives. Genetic predictive tests are usually performed using PCR and sequencing of both strands of the amplicon that contains the mutation detected in the family.

RESULTS AND DISCUSSION

After 10 years of testing experience of genetic diagnosis for LS, we have found eight families with mutations in homopolymer surrounding sequences. All tumors analyzed in these families showed high microsatellite instability with positive results for the five mononucleotide markers analyzed, as well as loss of inmunohistochemical

Figure 1 **Microsatellite Instability analysis and loss of expression of MSH2 protein by Immunohistochemistry in colorectal tumors**



a) wild-type control, b) and c) cases with c.942+2T>A and c.942+3A>T mutations, respectively. NR27, NR21, NR24, BAT25 and BAT26: microsatellite markers.

Table 1 Clinical data of probands and families with pathogenic mutations located near the BAT26 marker

Id.	Sex	FH	Variant	Protein	Proband´s neoplasms	Ages	Relatives* +/-
1	F	AM II	c.942+2T>A	p.Val265_Gln314del	EC, ***CRC***	41, 43	6/13
2	F	BG			***CRC***	33	5/0
3	F	BG			***OC***	45	3/2
4	M	AM II			***CRC***	51	0/12
5	F	AM II	c.942+3A>T		***EC***	42	0/2
6	F	BG			***CRC***, GC	50, 50	0/2
7	F	AM I			***CRC***, BC	48, 49	0/0
8	M	BG			***CRC***	47	0/2

**Relatives: number of predictive tests performed to date in the family with positive/negative results.*
Sex: F, female; M, male.
FH, family history; AM II, Amsterdam Criteria II; AM I, Amsterdam Criteria I; BG, Bethesda Guidelines.
Neoplasms: BC, breast cancer; CRC, colorectal cancer; EC, endometrial cancer; GC, gastric cancer; OC, ovarian cancer.
In bold italic letters: tumors in which microsatellite instability and MMR protein immunohistochemistry were detected.

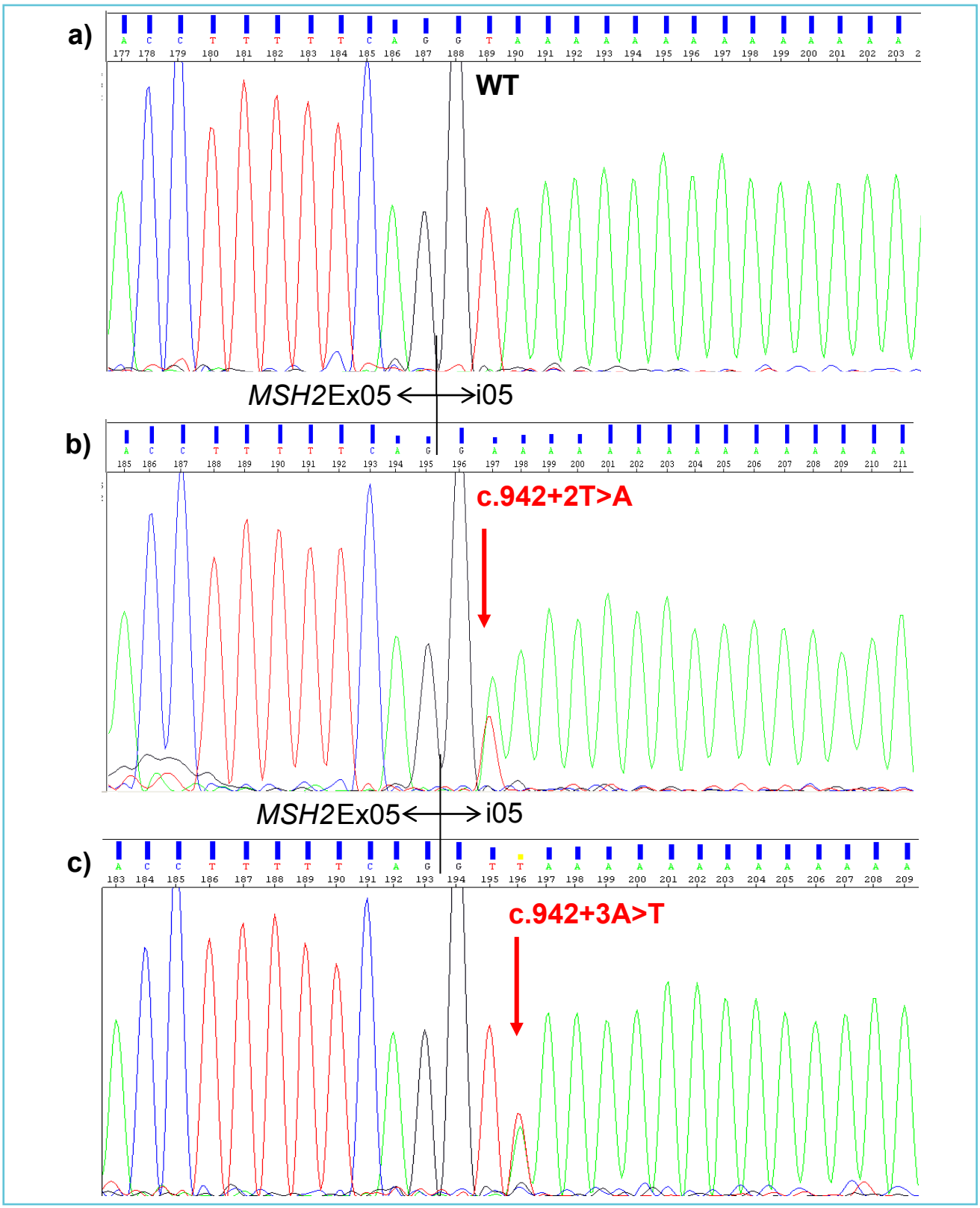
expression of the MSH2 and MSH6 proteins (Figure 1, Table 1).

Four of these families had the c.942+2T>A mutation, whereas the remaining four families had the c.942+3A>T mutation. Both at intron 5 of *MSH2* gene just by BAT26 mononucleotide marker [$(A)_{26}$] (Figure 2, Table 1). These mutations had the same effect at the protein level, i.e., exon 5 skipping (p.Val265_Gln314del). Consequently, important functional domains of the protein were affected, such as MutS II and III, which are connector and lever domains, respectively. These domains play different roles in holding the DNA that is to be repaired. Therefore, both mutations were pathogenic and causative of LS.

The special sequence context of these mutations hampers their detection. In our series, these mutations represent about 18% (8/45) of *MSH2* point mutations and 5% (8/148) of all point mutations detected in the four MMR genes. In addition, and to date, 47 at-risk relatives from these families have also been tested, which led to the identification of 14 mutation-carrier individuals. These high-risk individuals are currently benefiting from a specific surveillance and monitoring program aimed at minimizing the impact of cancer[2].

Up to nine intronic large homopolymer sequences (over 10mer long) are located in the proximity of exons and around splice sites in the MMR genes. To date, pathogenic mutations at eight out of these nine sites have been described in the *InSiGHT* database (Table 2). The splice sites are conserved and essential for exon definition and appropriate splicing. Mutations in those consensus positions generate aberrant transcripts and loss of protein function and are, consequently, pathogenic.

Figure 2 Sanger forward sequence of the MSH2 gene: Ex05–i05 boundary



a) wild-type sequence, b) c.942+2T>A mutation, and c) c.942+3A>T mutation

Table 2 Mononucleotide repeats (>10mer long) located in the proximity of consensus splicing sites of the MMR genes and mutations detected at those sites

Gene	Intron-exon boundaries	Homo polymer	Mutation	Class*	# families Our Lab	# families InSiGHT
MLH1	i04-E05	$(T)_{21}$	c.1039-2A>G	4	0	2
			c.1039-2A>T	4	0	1
			c.1039-1G>A	5	0	4
MSH2	i01-E02	$(T)_{13}$	c.212-2A>G	4	0	2
			c.212-2A>G	5	0	7
	E05-i05	$(A)_{26}$	c.942+2T>A	5	4	6
			c.942+3A>T	5	4	161
MSH6	i06-E07	$(T)_{13}$	c.3556+3_3556+13del	3	0	2
PMS2	i04-E05	$(T)_{13}$	None	-	-	-

**Class: variant classification according to their clinical significance (InSiGHT database): 5, pathogenic; 4, probably pathogenic; 3, unknown.*

It is important to note that mutations that occur in the proximity of a large mononucleotide repeat can be detected only by sequencing of the DNA chain that contains the variant in the 5' side to the repeat. Confirmation by sequencing of the complementary chain is unfeasible; for this reason, special care is needed to detect and confirm these variants.

Currently, the vast majority of tests used for the genetic diagnosis of hereditary cancer syndromes in Europe are laboratory-developed tests (LDT). As stated by the international standard ISO 15189:2012(E) (Medical laboratories, requirements for quality and competence), the laboratory should design quality-control procedures that verify the attainment of the intended quality of the results. Standard operating procedures that include the approaches that are necessary to overcome these specific difficulties are mandatory. For the analysis of probands by Sanger sequencing, validated PCR and sequencing conditions for all amplicons that are needed to cover the regions of interest are required. The use of visual inspection, in addition to the bioinformatics tools used for sequencing analysis, is highly recommended. When there is reason to suspect the presence of mutations, a double check by a second experienced observer and a confirmatory analysis using the same DNA sample are required. A positive-control sample should also be tested in parallel. When next-generation sequencing (NGS) platforms are used for non-validated diagnostic testing, confirmation by Sanger sequencing is compulsory. Furthermore, for predictive testing of at-risk individuals, at least two independent PCR-Sanger sequencing experiments that include positive

and negative controls of the genetic variants in question are recommended.

In conclusion, the detection of pathogenic mutations located near microsatellite sequences is especially difficult and requires the implementation of specific quality controls to optimize diagnostic methods.

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The first green diagnostic centre and laboratory building in Indonesia

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ABSTRACT

Like every human activity, clinical laboratories produce a carbon foot-print which they have a societal obligation to reduce. The renovation or construction of a new laboratory provides an opportunity to achieve this. A new, environmentally-friendly diagnostic centre in the city of Surabaya, Indonesia, was recently constructed under the supervision of a LEED (Leadership in Energy and Environmental Design) - certified architect incorporating the three basic tenets of good environmental practices, i.e. to reduce, reuse and recycle. Sustainable practices that were adopted in the construction of the building involved its architectural features, the location and the construction materials used. The building has been designed for energy and water conservation in the long-term. The cost for these green features was an additional 30% compared to that of a conventional building. It is expected that this extra cost will be recouped in the long run through cost-savings.

INTRODUCTION

All human activity consumes resources, leaves a carbon footprint and produces waste. The clinical laboratory is no exception to this rule and it impacts the environment in several ways. They therefore have a societal obligation to reduce their environmental impact. Laboratories may do so by adopting policies and activities that are sustainable and environmentally friendly. The construction of a new laboratory building or renovation of an existing one provides an excellent opportunity to make it environmentally-friendly with regard to the utilization of building materials and recurrent resources such as energy and water and waste production. (1)

LEED or Leadership in Energy and Environmental Design, is a green-building certification program that recognizes best-in-class building strategies and practices. It is the most widely recognized and used green-building program across the globe (2). The first green laboratory and diagnostic-centre building in Indonesia , the Grha Prodia, was opened on 14th March 2015 in the city of Surabaya. This centre performs an average of about 60,000 tests a month of which clinical chemistry, including urinalysis, accounts for about 43,000.

The building was designed and constructed under the supervision of a LEED-certified architect. LEED accreditation for the building is being sought. The three fundamental tenets of good environmental practices, namely, to reduce, reuse and recycle, underscored its design and construction. The building has also been designed to make the internal environment mentally conducive for the staff.

The sustainable practices that were adopted for this building have involved consideration of the following aspects:

- The location and features of the building;
- The construction materials used;
- Energy conservation;
- Water conservation.

The incorporation of green features to the building has resulted in a 30% increase to the overall cost compared to that without these features.

LOCATION, BUILDING FEATURES

The key features of the building are stated in Table 1. The building has a recycling facility. Initially this will be for paper and plastics. It is located in one of the main arteries of Surabaya close to amenities such as banks, schools and

Table 1 Building parameters

Feature	Parameter
Main orientation	north-south
External dimensions	18.75 by 56.45 metres
Floors (including ground floor)	11
Total gross square footage / gross floor area	4406.577 sqm
Total parking floor area	1.027,601 sqm
Total floor space (include basement parking)	5434.178 sqm

other facilities. This will enable the approximately 213 staff members to use these facilities without having to travel long distances and thereby reduce their carbon footprint. In addition clients and suppliers will also have easy access to the building. The parking lots are reserved for staff who car-pool. There are bicycle racks and three showers for staff who commute by bicycle.

Orientation and space

The main axis of the building has an east-west orientation to reduce the heat from the sun. This means that most of the external surface area faces the north-south axis and is not directly exposed to the heat from the tropical sun. To further reduce the heat inside, the building has been constructed with sun-shading horizontal fins made from aluminum panels.

The building occupies 50% of the land area belonging to it. Of the remaining area surrounding the building, 41% has been turned into garden space, which is 3 m wide. This contrasts with the city requirement of 10% for green space for public buildings. The local species of grass and trees that make up the garden requires minimal maintenance. In addition, there is a roof top garden.

Indoor environmental quality

The building has been designed such that 95% of all spaces that are regularly occupied have a view of the outside. This is important for eye and mental health, which should yield the consequent benefit of improved work performance. The top floor where meetings are held has glass walls on 3 sides with a view of the city.

BULIDING MATERIALS USED

Recycled materials were used in the construction of the building. Most of the materials were manufactured in nearby regions. The envelope of the building contains rock-wool that provides both thermal and sound insulation. The steel and aluminum used has recycled content; the latter was produced very close to the building. Fly-ash was used in the construction of the concrete structure. It is a recycled material composed of the fine particles which is one of the residues generated by coal combustion. Care was taken to ensure that the paint used had low volatile organic compounds (VOC) since organic compounds used in conventional paint have VOCs that are carcinogenic.

The waste materials generated during construction were glass, ferrous metals, non-ferrous metals, gypsum, concrete, wood, cardboard and plumbing fixtures. They were kept in separate bins and sold for recycling.

Floor covering

The floor spaces that are carpeted use square tiles (50 x 50 cm) to permit replacement of the tiles in the event of damage or wear and tear. The tiles were made of 100% recycled materials. In areas where linoleum is used as in the laboratories, it is made from linseed oil. This is a rapidly renewable material that can be harvested and put to use for manufacturing in less than 10 years from planting. Both the linoleum and carpet tiles were imported.

ENERGY CONSERVATION FEATURES

Natural lighting

The rooms have been designed to maximize the use of natural light. The windows are adequately sized to allow the maximum amount of natural light to enter a room. All rooms in the building have windows with a view of the outside. The building is insulated with double-glass windows which have high-performance low-emissivity glass on the inside and tinted glass on the outside. With a window height of 3 meters, it is calculated that light can penetrate 6 meters into the space.

Lighting

The building uses light-emitting diode (LED) lighting with motion sensors in every room which automatically switch off the lights when the rooms are not occupied. LED lighting is approximately 3 times more expensive than conventional lighting.

Air-conditioning

The temperature inside the building is set by thermostat at 24° C. The air-conditioning uses a Variable Refrigerant Volume® (VRV) system from Daikin (Japan), a concept that is similar to inverter air-conditioners that are commonly available. The amount of cool air that is produced will depend on the number of persons in the room. The VRV system is 3 to 4 times more expensive compared to conventional air-conditioning systems.

WATER CONSERVATION MEASURES

The city of Surabaya faces a water-shortage in the dry season and receives excessive rain that results in floods during the wet season. The building expects to obtain 42% of its water requirements from processed sewage and rain-water and will depend on the city for only 58% water supply. Rain-water will be harvested from the roof and stored in a tank with a 11,000-litre capacity which is located in the basement floor. The building contains a sewage treatment plant. The water salvaged from the plant is used for the toilets and for watering plants.

Toilets

All toilets have a dual-flow capability which use 4.5 and 3 L of water, respectively, per flush as against single-flow conventional toilets that use 6 L. The urinals in the building are of two types: waterless urinals for the staff and low-flow urinals for the public who may not be familiar with the former. All faucets have sensors that provide the same flow for less water. These water-saving devices are approximately 150% more expensive than the conventional ones but use 43 % less water.

CONCLUSION

The construction of this green facility is recognition of the need for sustainable practices by laboratories. It is a visible act of corporate social responsibility that should yield intangible rewards in the future. It is expected that the additional cost of the building will be eventually recovered through lower energy costs and other sustainable practices.

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Publisher: IFCC Communications and Publications Division (IFCC-CPD)

The eJIFCC is a member of the **Committee on Publication Ethics (COPE).**

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