



JCTLM SYMPOSIUM 2007

Activities and Challenges for International Standardization and Traceability in Laboratory Medicine

ABSTRACTS

11th Asian Pacific Congress for Clinical Biochemistry, Beijing International
Convention Centre, Beijing, China
16 October 2007 (10.00 am to 5.30 pm)

**Sponsored by the National Institute of Metrology, China (NIM) and
the National Institute of Standards and Technology, USA (NIST)**



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Date: 16 October 2007 (10.00 am to 5.30 pm)

‘Activities and Challenges for International Standardization and Traceability in Laboratory Medicine’

Session 1: International, Regional and National activities

Chair: J-C. Forest (IFCC)

10.00-10.25	Introduction to international activities	J-C. Forest (IFCC)
	JCTLM: Metrological traceability and Reference Materials, Methods and Laboratories for Laboratory Medicine and <i>In vitro</i> Diagnostics	R.I. Wielgosz (BIPM)
10.25 -10.50	IFCC Scientific Division Activities on Standardization and Traceability	M. Panteghini (IFCC)
10.50 – 11.35	Laboratory Medicine Standardization and Traceability activities in China	Representative of NIM CHEN Wenxiang (NCCL)
11.35 -12.00	JCCLS standardization and traceability activities	N. Hamasaki (JCCLS)
12.00-13.00	Lunch	

Session 2: Challenges for international standardization and traceability

Chair: W.E. May (NIST)

13.00 – 14.00	Next Generation Clinical Diagnostics: Detection of Organ-Specific Biomarkers	L. Hood (Institute for Systems Biology)
14.00 – 14.30	Cardiac Markers	R. Christenson (University of Maryland)
14.30 – 15.00	Hormones	L. Siekmann (IFCC)
15.00 – 15.30	Tea Break	
15.30 – 16.00	Microalbuminuria	M.J. McQueen (Mc Master University)
16.30 – 17.00	Biologicals	E. Gray (NIBSC)
17.00 – 17.30	Genetic testing	H. Schimmel (IRMM)
17.30	Close of Symposium	



JCTLM: Metrological Traceability, Reference Materials, Methods and Laboratories for Laboratory Medicine and *in vitro* Diagnostics

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The Joint Committee for Traceability in Laboratory Medicine (JCTLM) was established in 2002 by the International Bureau of Weights and Measures (BIPM), the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC), and the International Laboratory Accreditation Cooperation (ILAC). The JCTLM is committed to the goal of obtaining comparability of laboratory diagnostic test results by facilitating the adoption of common reference systems that can be established for worldwide use. A critical step in reaching this goal is to ensure the traceability of diagnostic test values to a universally recognized and accepted reference point such as the International System of Units (SI) or an internationally agreed upon protocol.

The traceability of diagnostic test results to materials and measurement procedures of higher metrological order came to the fore when the European Community issued its directive 98/79/EC, *In Vitro* Diagnostic Medical Devices. The requirement for traceability is not unique to the EC however. The US Quality System Regulation, which predated the IVD Directive, requires manufacturers who assign values to product calibrators to use calibration standards....traceable to national or international standards (21 CFR 820.72). The Joint Committee for Traceability in Laboratory Medicine (JCTLM) has addressed traceability requirements by creating lists of materials and measurement procedures that meet these requirements, i.e. formally identified as being of higher metrological order. Today there are more than 200 certified reference materials (CRMs) and 125 reference measurement methods and procedures (RMM/Ps) listed by JCTLM.

The JCTLM lists are updated annually and searchable using an on-line database on the website of the Bureau International des Poids et Mesures, (BIPM, www.bipm.org/jctlm/).

The JCTLM also provides a list of reference measurement service providers (RMS). The listed RMSs are provided by laboratories that employ materials and methods listed by JCTLM to assign values to proprietary calibrators and control materials from IVD companies. The RMS providers are compensated by the organizations that use their services. To assure adequate performance by RMS providers the IFCC operates a specialized external quality assessment scheme (EQAS) (<http://www.dgkl-rfb.de:81>) organization.

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The JCTLM commitment to transparency demands that review processes occur according to written procedures, documented in the JCTLM Quality System. References to the relevant ISO standards, the processes used in review and the criteria for acceptance are available on the JCTLM web pages. As part of JCTLM's philosophy of continuous improvement, the Quality System undergoes periodic review and revision. A call for nominations for certified reference materials and reference measurement methods and services is issued by the JCTLM every year.



IFCC SCIENTIFIC DIVISION ACTIVITIES ON STANDARDIZATION AND TRACEABILITY

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The overall goal of the IFCC Scientific Division (SD) is to advance the science of Clinical Chemistry and its application to the practice of Laboratory Medicine. Within this context the SD seeks to identify research areas, technical innovations and diagnostic strategies and to assist translation of these to the profession. In addition, the SD aims to establish standards for scientific and technical aspects of good laboratory practice. The standardization of measurements is therefore of high priority. The SD, in collaboration with the IVD industry and with other organizations such as IRMM, NIST, and CLSI, is involved in developing reference measurement systems for measurands of clinical significance to reduce whenever possible uncertainty and to promote comparability of results for a better reliability of the information obtained from routine procedures. To illustrate the complexity and the impact of these activities on patient care, the work of SD Committees (C) and Working Groups (WG) related to the standardization of some analytes is presented. The C on Plasma Proteins is currently developing a new reference material for plasma proteins and investigating the possibility to establish common reference intervals for the most important proteins. The C on Standardisation of Markers of Cardiac Damage is currently working on the development of a troponin I secondary reference material and on the standardisation of BNP assays. The C on Reference Systems of Enzymes has developed and published reference methods for 6 enzymes, which will enable standardisation in this important field. This C has created a reference laboratory network which has demonstrated its competence to certify reference materials. The C on Reference Intervals and Decision Limits is promoting a standardised approach to establish common reference intervals obtained using methods traceable to validated reference systems. The C liaises closely with the C on Traceability in Laboratory Medicine, which supports all SD activities with respect to the implementation of the concept of traceability and, specifically, is responsible of the IFCC External Quality Assessment Scheme (EQAS) created to demonstrate the competence of reference laboratories as reference measurement service providers. The WG on Standardization of HbA1c has successfully developed a reference system for this measurand. It is now developing an implementation program to educate laboratory professionals and clinicians about the importance of international standardisation of HbA1c measurements for the benefit of diabetic patients. The WG on Standardisation of Glomerular Filtration Rate (GFR) Assessment is developing recommendations for creatinine measurement and accurate GFR estimation. The WG on Standardization of Thyroid Function Tests has embarked on an important program to standardize total and free T4. Hemoglobin A2, carbohydrate-deficient transferrin, cystatin C and urinary albumin are further markers for which SD is pursuing standardization. As can be seen, the SD standardization work seeks to address many issues of great importance to the profession, to clinicians and patients.

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Measurement Traceability of Clinical Medicine in China Review and Prospect

Fangdi Wu, Bei Xu, Peijun Zai

Measurement in clinical medicine provides information for diagnosis, therapy, preventive and healthcare. Accurate and comparable results are the requirement of mutual recognition.

History and Status

There are three organizations in China which are responsible for establishing reference system of laboratory medicine. NIM works in higher metrological level and develops both reference materials and reference procedures; NCCL works in medium metrological level and develops both reference materials and reference procedures, organized external quality assessment activities and established reference laboratory network; CNAS accredited clinic laboratories and reference laboratories, arranged proficiency testing schemes.

Works related with reference system in clinical medicine in China stated in 1990's. Now NIM has

1. Certified Reference Materials

- Pure materials—Cholesterol, Uric acid, Urea (JCTLM database, list I)
- Matrix materials—frozen human serum (K, Mg, Ca, Se, Fe, Cu, Zn etc)
- Matrix materials—frozen human serum (Cholesterol, Glycerides), four levels shared with NCCL

2. Reference Methods

- K, Mg, Ca, Se, Fe, Cu, Zn (ID-ICP-MS)
- Cholesterol(GC/MS), Glycosides(GC/MS), Uric acid (LC/MS)
- Creatinine(LC/MS), Progesterone(GC/MS and LC/MS/MS)

3. Technical Specification for Medical Devices

So far we have about 20 technical specification documents. Such as the Verification Regulation of Electrocardiograph and Electroencephalograph (JJG543-1996), the Verification Regulation of Blood Gas Acid-Base Analyzer and the Ultrasonic Source for Medical Ultrasonic Diagnostic Equipment, etc.

Chinese National Joint Committee for Traceability in Laboratory Medicine

CJCTLM was established in March, 2007. The aim of the committee is promoting the concept of traceability of measurement results to the SI or, to other internationally agreed references and coordinating and giving guidance in the establishment of Chinese national reference system for measurement in clinical medicine.

1. Structure of the Committee

The committee has one president, four vice presidents, one secretariat of three persons and four working groups. The working groups are reference materials and reference

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procedures, reference laboratory network, technical specification and laboratory accreditation. The total number of the members is 35.

2. Mission of the Committee

- a. Drafting metrology technical specification in clinical medicine
- b. Evaluating reference materials, reference methods and reference laboratories
- c. Organizing intercomparisons for reference laboratories
- d. Providing support for Clinical Laboratory and Reference Laboratories preparing for accreditation
- e. Participating in the activities of JCTLM
- f. Organizing proficiency testing for clinical laboratories
- g. Transporting knowledge in metrology and training

3. Work Plan in 2008

- a. Technical specification drafting—respiratory device, syringe pump, baby incubator and blood glucose analyzer
- b. Intercomparison of Enzyme Reference Laboratories

Under the coordination of the committee we will get more and more government support to promote the establishment of Chinese national reference system for measurement in clinical medicine.



Standardization Activities at the National Center for Clinical Laboratories

Wenxiang Chen, MD, MSc

National Center for clinical Laboratories and Beijing Hospital Institute of geriatrics

Founded in 1982 by the Ministry of Health, the National Center for Clinical Laboratories is an institution and a government agency that has a mission of improving the quality of clinical laboratories in China. One of the major activities at the Center is the operation of a national EQA program for clinical laboratories. Over the years various variations in the test results have been observed. The variations may be caused by the number and the diversity of analytical systems that have been used in the laboratories. The number of manufacturers is large and clinical laboratories may make combinations of and modifications to the methods that generate numerous analytical systems. This situation often make the EQA program difficult. It has become more and more clear that reference measurements are needed, among many other purposes, for assigning target values and investigating the properties of the EQA materials. Development of reference methods has become an area that the Center takes as a priority. The Center carries out this task in collaboration with university, institution and hospital laboratories and the national metrology institute and with the Chinese Committee on Clinical Laboratory Standards and the Chinese Society of Laboratory Medicine. The US CDC reference method for total cholesterol and designated comparison method for HDL-C have been established and now the laboratory is a member of the CDC CRMLN. Reference methods for red and white blood cell counting have also been established. Some progresses have been made in the establishment of the IFCC reference procedures for enzymes and in ID/MS measurement of metabolites/substrates, electrolytes and hormones. The reference system activities have been supported by the National Key Technologies R&D Program and the National High-tech R&D Program (the 863 Program) and more reference methods and materials for important common clinical laboratory tests are expected.



JCCLS standardization and traceability activities

N. Hamasaki, M.D., Ph.D.

Vice President

Japanese Committee for Clinical Laboratory Standards (JCCLS)

Since 1970's, we have been collaborating with JSCC (Japan Society of Clinical Chemistry) and JACRI (Japan Association of Clinical Reagents Industries) to standardize laboratory measurement more than for 30 years in Japan. We have tried to establish the external quality control system and validation, and to provide reference materials for density items. Our society is also carrying out various projects for the establishment of a variety of reference materials including enzyme reference materials, and diverse reference materials are supplied by organizations and groups in Japan.

Recently, cooperating with JSCC, JACRI, JAMT (Japan Association of Medical Technologist), and JSLM (Japanese Society of Laboratory Medicine), we, JCCLS, have established a nationwide project for the standardization of laboratory measurements including 27 clinical chemistry/immunology and 5 hematology analytes. In this project, we introduce a new internet system which can daily monitor the intra-laboratory quality control data for assessing inter-laboratory data difference. As a results, inter-institution variation has been confirmed to be in 5% CV for chemistry/immunology analytes and in 10% CV for hematology. It indicates that the standardization of laboratory measurements for the major analytes has been reasonably established in Japan. Thus, we will continue our efforts to increase the number of measured analytes and to establish a real-time monitoring system which could monitor all measurements from nationwide institutions.

This study was subsidized by the Japan Keirin Association through its Promotion funds form KEIRIN RACE and was supported by the Mechanical Social Systems Foundation and the Ministry of Economy, Trade and Industry.



Systems Biology and Medicine: From Reactive to Predictive, Personalized, Preventive and Participatory (P4) Medicine

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Abstract: The grand challenge for biology and medicine in the 21st century is complexity. A currently emerging paradigm change is the idea that biology is an informational science and that most biological information is mediated by dynamical biological networks. The systems approach to biology and medicine is a general category of approaches that appear to be very effective in dealing both with biological circuits and hence with biological complexity. Systems approaches require a truly cross-disciplinary environment and the effective integration of biology, technology and computation/mathematics. I will discuss my views of systems biology. Then I will discuss a systems approach to one disease, prion disease in mice, and demonstrate how it profoundly alters our views of disease—with regard to understanding disease pathophysiology as well as new approaches to diagnosis, therapy and eventually prevention. Then I will talk about the emerging measurement technologies that are the foundation of P4 medicine, as well as some of the pioneering computational and mathematical tools that will be necessary to usher in this revolution in medicine. The view of biology as an information science, the systems approach to disease, the new measurement and visualization technologies and the evolving mathematical/computation tools will catalyze this paradigm change in medicine. I will make five predictions: 1) our current largely reactive medicine will be transformed to a predictive, preventive, personalized and participatory (P4) medicine over the next 10 to 20 years, 2) this will lead to the digitalization of medicine (extracting information from single molecules and single cells) with even more profound implications for society than the digitalization of communications and information technologies, 3) systems medicine and its digitalization will dramatically turn around the slope of ever increasing healthcare costs to the point that the developed world will be able to export its P4 medicine to the developing world, 4) P4 medicine will necessitate fundamental changes in the business plans of virtually every sector of the healthcare industry and 5) this new world of medicine will be propelled forward by carefully chosen strategic partnerships—across all sectors of science—academia, industry, government laboratories, independent research institutes, etc—and that these partnerships will be international. ISB hopes to play an important role in catalyzing a series of these strategic partnerships.

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Standardization and Harmonization of Cardiac Troponin I Assays: Where have we been? Where are we now? Where are we going?

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There has been need for standardization and harmonization of cardiac troponin I (cTnI) measurements because interassay differences were documented as 20- to 40-fold. Toward this goal, the American Association for Clinical Chemistry formed a cTnI Standardization Committee in collaboration with the National Institute of Standards and Technology (NIST).

In the first step of this standardization and harmonization effort, the committee endeavored to identify an appropriate cTnI reference material. Initially ten candidate reference materials (cRMs) were proposed and evaluated using rigorous criteria. After this evaluation, the number of cRMs was narrowed from ten to two. These remaining candidates, a CIT complex from human heart tissue and a CI complex from recombinant technology, were supplied to NIST for assessment of composition, purity, and cTnI value assignment. Characterization studies at NIST showed that the CIT and CI cRMs were of specified composition. These cRMs and six cTnI-positive human serum pools were shipped to manufacturers of 15 cTnI assays. Commutability of the materials was examined using an accepted method which consisted of determining the numerical relationship for the two cRM preparations between each manufacturer-specified “field” method and each of the other 14 field methods. These relationships were then compared to the corresponding numerical relationships for the human serum pools. The proportion of cTnI methods that demonstrated commutability for the CIT cRM was 45%; for the CI cRM, 39% of methods demonstrated commutability.

Using the human serum pools, interassay cTnI variability for the field methods was determined to be 88%. Harmonization of methods was accomplished by determining regression parameters relative to the analytical system yielding values closest to the median for each of the six pools. These regression parameters were used to recalculate pool-specific values in an effort to harmonize the assays. After harmonization in this way, variability of the serum pools for the cTnI methods was reduced to 15.5%. Therefore, a simple strategy using serum pools can improve harmonization of field cTnI methods by over 5-fold. The CIT cRM was selected by the committee and a new lot was classified as the cTnI certified reference material Standard Reference Material (SRM) 2921 by NIST.

A third study was conducted by the committee to demonstrate the extent to which cTnI standardization can be achieved, and to propose a strategy for evaluating consistency for each cTnI method using SRM 2921. In this study, eight serum pools prepared in accordance with Clinical Laboratory Standardization Institute guidance to have cTnI target concentrations between 0.05 and 8.0 $\mu\text{g/mL}$. These pools were measured

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in duplicate on 11 cTnI field methods and the data were used to harmonize cTnI methods by determining regression parameters relative to consensus cTnI values from assays having quality specifications of low imprecision and values within 20% of the overall median. For establishing long term stability, each method's analytical response to RM 2921 was characterized by measurement of gravimetrically prepared solutions of SRM 2921 having concentrations of 0.5, 1.0, 2.0 and 4.0 $\mu\text{g/mL}$ in a cTnI positive pool. The characterization factors were validated utilizing the 8 serum pools calibrated with SRM 2921 in a human serum matrix.

Before harmonization, measurement of the serum pools demonstrated results that were between 40% and 100% for the 11 participating methods. After harmonization, CVs for the methods ranged from 1% to 25% for the pools. Characteristic factors for the response of each cTnI method to RM 2921 were determined for each of the 11 methods. Independent validation of the characteristic factors revealed values that were within 5% of each (range: 1.05 to 0.85). In this way, a simple strategy using serum pools can harmonize cTnI methods to a level within about 18%. Each cTnI method showed a characteristic analytical response to SRM 2921, which can be used for assuring cTnI method stability over time.

Based on this work, the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) is currently refining a strategy for widespread standardization and harmonization of cTnI assays. This will be an important contribution because cTnI measurement has been accepted by the cardiology, emergency medicine and laboratory medicine specialties as the cornerstone for diagnosis of myocardial infarction and these procedures are critical for risk stratification, patient monitoring and guidance of treatment and intervention. Harmonized results will improve utilization of cTnI measurement in patient care.



Challenges for International Standardization and Traceability in Laboratory Medicine - Hormones

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Standardization and quality assessment of hormone determinations in human body fluids is closely related to the concept of **measurement traceability**. Traceability provides probably the most important strategy to achieve **standardization** in laboratory medicine aimed at **comparable** measurement results regardless of the method, the measurement procedure (test kit) or the laboratory where analyses are carried out.

In view of the low concentrations of hormones in human blood, highly sensitive and specific techniques are necessary to measure these substances. The most commonly applied analytical principle of hormone measurements in daily practice is based on immunological techniques. Immunoassays are highly sensitive. However, their specificity is limited due to many known as well as unknown cross reactions. Consequently, considerable dispersions can be observed when comparing results from different routine test procedures.

It can be demonstrated that this situation can be significantly improved when reference methods of higher metrological order are applied for calibration, validation and quality assessment of test procedures for hormone determinations. This is a challenge for manufacturers of commercial test kits and also the organizers of proficiency testing schemes. Reference materials, reference methods and reference measurement service providers have been identified and listed on the BIPM – JCTLM website.

In practice the technique of isotope dilution mass spectrometry (IDMS) had been developed initially as a reference measurement procedure for the highly accurate measurement of low molecular (steroid- and thyroid-) hormones before this principle of measurement became also popular for many other measurands - not only in laboratory medicine.

The proficiency testing system for low molecular hormone determinations of the German Society for Clinical Chemistry and Laboratory Medicine (DGKL) is based on certified reference method target values which form the basis for evaluation of the results of routine laboratories participating in such surveys. It may be of interest to elucidate how the introduction of the concept of traceability improved the performance of diagnostic tests since 1988.

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For **proteo- and peptide hormones**, a different strategy for introducing traceability must be applied. Usually, we find a large scatter of test-kit dependent results in ring trials for such peptide hormones. This is not necessarily due to a lack of specificity of different test procedures but to the fact that essentially different molecular entities of these types of hormones exist. In fact, different commercial test procedures measure different measurands of the same family. The definition of the proteo-hormone measurand is very critical and several aspects must be taken into consideration as it concerns the sub-unit to be measured (β -chain or complete molecule), the epitope to be detected and, finally, the glycosidic structure of the molecule.

Before it is possible to establish reference systems (reference procedures, materials and laboratories) and to use reference procedure values as targets in proficiency testing surveys, the measurand under consideration must be clearly defined. Whenever possible, a global consensus on the definition of the measurand should be achieved.



Challenges for International Standardization of Microalbumin

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Many of these challenges were recognized at a joint NKDEP/IFCC Conference held in Washington, March 27-28, 2007 to address standardization of urine albumin/creatinine, measurement and reporting. Microalbuminuria is an early marker of kidney damage and is now recognized as a risk factor for cardiovascular disease. It is increasingly being considered as a risk factor for prognosis and as a surrogate outcome for kidney disease progression and CVD risk reduction.

Most assays in use are immunoassays and there are many immunochemical methods, the most frequently used being turbidimetric and nephelometric. There are also labeled immunoassays, e.g. RIA, enzyme immunoassay, fluorimmunoassay, chemiluminescence immunoassays, electrochemiluminescence immunoassays. Size-exclusion HPLC with UV detection and chip electrophoresis are more recently introduced procedures. At present there is no internationally recognized urine reference material and no reference method. A plasma preparation (CRM470) is being diluted and used as a urine reference material. Currently there are variations in traceability schemes, details of dilutions, diluents, plasma versus urine matrix, and in value transfer protocols. Although there are many different methods, essentially the same recommended reference intervals are being applied across all the methods.

Recently there has been some dispute relating to the variable reactivity of different microalbumin assays with fragmented or modified albumin. It appears that assays based on competitive assay formats or which use monoclonal antibodies have more variable reactivity to modified albumin than those with polyclonal antisera. A recently described HPLC-MS method for the quantitation of urine albumin has potential as a reference method. The next step is to improve its low end sensitivity and to do clinical studies. The Japanese Institute for Standardization has a working reference material and primary reference material should be prepared for the establishment of traceability chain in the future.

There is a need to define a full reference system for urine albumin which involves defining the measurand, reference material and reference method measurement procedure. The completion and publication of data from the Japanese Standardization Project is needed to see if it will demonstrate that albumin (urine) equals albumin (serum) to validate their application of CRM470 as reference material.



Challenges for International Standardisation and Traceability: Biologicals

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Since the 1920's, the World Health Organization (WHO) ensures the safety and efficacy of biological medicines and the diagnostic procedures by the provision of relevant guidelines and international standards and reference reagents. WHO defines biologicals as “substances which cannot be completely characterised by physic-chemical means alone and therefore requires the use of some form of a bioassay”. The exact nature and mechanisms of action of biological are not always known. A biological measurand does not necessarily refer to a single defined analyte; it can refer to a group of components, antigen epitopes, immunoreactivity or infectivity. This presents challenges in the traceability of biological reference materials. In the majority of cases, biological activities cannot be measured directly and are dependent on the comparison with activities of reference materials such as the WHO international standards. The WHO standards are usually value assigned with an arbitrary unit (international unit, IU) by multiple methods. This gives accurate measurement by minimizing assay bias and imprecision. The IU is related to the physical existence of that standard and since it is not always possible to replace a biological standard with exactly the same material, there may be a discontinuity of unit and hence traceability path and uncertainty of measurement cannot be clearly defined. This is in contrast to the internationally accepted metrological principles of measurement (the SI system) which is based on direct measurement of an analyte by primary measurement methods, thereby traceable to a primary measurement method and calibrant and allows the assessment of uncertainty of measurement. The WHO has recently recognise that it is possible to measure well characterised biologicals such as growth hormones by physical chemical methods and have established international standards and reference reagents with SI traceability. The 2004 revision of the WHO guidelines on preparation, characterization and establishment of international and other biological reference standards (WHO Technical Report Series, No 932, 2006) include considerations given to calibration of biological standards (if appropriate) via the SI system.



Challenges in Genetic Testing

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Genetic tests can be highly predictive for the future health of the individual and can be carried out at any stage of life even in the embryo before implantation. They are relevant to healthy people as well as to those showing symptoms of an unhealthy condition and may have also important implications for the relatives of the person tested. The genotype established by a single laboratory test is usually not repeated and forms a permanent part of the medical record of the patient. Consequently, molecular genetic testing requires a high level of data reliability based on proper quality assurance of laboratory operation and measurement procedures applied in accordance with ISO 15189 and ISO 17025. As the field develops, the need for appropriate reference materials which are required to establish this reliability combined with a thorough assessment of the performance and stringent quality assurance of methods applied in the testing laboratories become increasingly relevant.

Several national and international institutions like the European Commission, the FDA (US), the NIH (US), the OECD, the WHO and the CLSI (US) have realized the significance of the problem and the importance to edit clear guidelines addressed to the concerned parties.

The EU regulates *in vitro* diagnostics for genetic testing through the *in vitro* diagnostic (IVD) medical devices Directive (98/79/EC) and the 93/42/EEC Directive on medical devices. The IVD Directive deals with all aspects of safety and performance and requires common technical specifications for high risk assays. Its main purpose is to introduce harmonised controls on these IVDs throughout the EU.

The type of reference material which is required to perform a proper analysis of the sample of interest depends on the analytical problem. Several groups can be distinguished:

- 1) In the field of genetic testing, currently most applications are qualitative analyses which are directed to the identification of variants or abnormalities in a nucleic acid sequence such as mutation(s), translocation(s), duplication, amplification and deletion(s).
- 2) The counting of the number of nucleotide repeats or the determination of the length of nucleic acid sequence in terms of base pairs or number of nucleotide repeats could be considered as quantitative application, although it could be also described in qualitative terms by determining the sequence.
- 3) A measurement procedure producing a signal within a dynamic range and which is turned into a qualitative format by defining a cut off value or by setting up the measurement procedure in a way that it gives at a certain concentration level of the analyte a detectable signal. Tests for pathogens are for example mostly falling into this group. Although the results of such tests are expressed in qualitative terms, the ability to distinguish positive and negative sample populations can be best described by the limit of detection, which is a quantity value.



4) Measurement procedures of which results are expressed quantitatively. Currently quantitative measurement procedures are not widely applied in the genetic testing area. However, with increasing understanding of the function of the human genome, quantitative applications such as gene expression measurements can be expected to become highly relevant.

For these groups the types of reference materials required, their characteristics and possible use as well as aspects related to the accompanying quality assurance procedures to be implemented in the laboratory will be discussed.

Due to the limited availability of human material, reference materials in the genetic testing area are usually synthetic or highly processed DNA/RNA or DNA derived from cell lines. They are either pure or added to a matrix, the latter in order to simulate the conditions in a real patient sample. In addition they may have been designed to work with a dedicated platform. Due to these limitations the reference materials may not perform identically to a patient sample. Consequently, in addition to verifying their homogeneity and stability, all these materials have to be tested in ring trials, covering most of the methods currently applied, so that their fitness for purpose has to be demonstrated.