

Meeting Summary

International Meeting on Clinical and Laboratory Genomic Standards
May 3-5 2005, Paris

An increasing number of genetic tests are being carried out around the world. Some are single gene tests; others involve genomic information including expression profiling using microarrays. Testing is being done in the context of both research and clinical diagnostics, for single-gene disorders and for pharmacogenetics/pharmacogenomics. As genomic technology and clinical applications grow, there is a clear need to establish standard controls and best practice guidelines so that patients, clinicians, and researchers extract the maximum benefit. This meeting brought together stakeholders from around the world for the first time to discuss how to accelerate the establishment of clinical and laboratory genomic standard controls and to identify areas in which global harmonization in the development and application of the standard controls and guidelines are possible.

The meeting was chaired by Dr. Janet A. Warrington, VP Emerging Markets and Molecular Diagnostics R & D at Affymetrix, who described meeting goals and desired outcomes.

Goals

- Increase stakeholder interaction
- Accelerate development of guidelines and shared materials

Desired outcomes

- Identify areas where harmonization is possible and practical and areas where it is not
- Identify initiatives that would benefit from international community working together
- Identify areas that need to be addressed, where new working groups are needed
- Prioritize issues
- Identify next steps for moving forward

In the discussion below, the views and comments given are those of the individuals, rather than the organizations they represent

Some of the issues involved in standard setting in genetic testing were highlighted through four case studies which illustrate the range and scope of the work already being carried out in this area.

Towards gene expression profiling in leukemia diagnostics

Dr Torsten Haferlach, senior physician and head of the Laboratory for Leukemia Diagnostics, Grosshadern, Ludwig-Maximilians University, Munich, described the development of a new diagnostic tool for leukemia which relies solely on gene expression profiling of bone marrow or peripheral blood. Samples from 937 newly diagnosed cases covering 13 sub-types of AML, ALL, CML and CLL and from 45 normal controls were hybridized to an Affymetrix microarray and the top 100 differentially expressed genes analyzed. Cross validation studies verified the sub-type classification in 891 out of the 937 leukemia samples leading to an overall accuracy of 95.1%. A prospective study then achieved an accuracy of 94.5%. The findings suggest that gene expression profiling can identify all clinically and prognostically relevant sub-types of adult leukemia. Studies continue under the *Microarray Innovation in Leukemia* program, where Dr Haferlach is Principal Investigator. In discussion, Dr Edison Liu, Director of the Genome Institute of Singapore, pointed out that the choice of algorithm used to analyze the results in

microarray work is an important issue, with no single one being generally accepted.

Implementation of cyp450 testing in the clinical laboratory

Dr Daniel H. Farkas, Director of Molecular Diagnostics at The Methodist Hospital, Houston, Texas, described some of the scientific and regulatory challenges of introducing cyp450 genotyping into the molecular diagnostics laboratory.

A rating system is being developed that assesses several variables including economics, potential for adverse drug reactions and the availability of surrogate tests. The requirements for validation for this testing are set by the Clinical Laboratory Improvement Amendments (CLIA) and include, among other things, assay calibration and proper use of appropriate controls.

Validating cyp450 genotyping in the clinical laboratory may be problematic as the community may be faced with a lack of appropriate controls. Dr Farkas is involved in the CDC's Quality Control Materials for Genetic Testing Group which has met three times and made a number of recommendations; a paper entitled "Developing a Sustainable Process to Provide Quality Control Materials for Genetics Testing" has been submitted to *Genetics in Medicine* (author Bin Chen, Ph.D., at bkc1@cdc.gov).

Another relevant project is the Genetic Testing Quality Control Material Program website, which lists available QC materials, invites submissions, and has information on regulatory issues.

(<http://www.phppo.cdc.gov/dls/genetics/qcmaterials/default.aspx>)

Molecular karyotyping as an example of a high complexity DNA based test

Dr Joris Vermeesch, Head of the Constitutional Cytogenetics Unit of the Center of Human Genetics, Leuven, Belgium, outlined some of the challenges associated with molecular karyotyping .

These include the setting of threshold or cut-off values, how to agree the level of false positive/negatives which is acceptable, and how to map normal variations.

Various projects are ongoing to compile databases (CGHGate and Decipher) of human variation, unclassified variants and disease causing anomalies to aid karyotyping analyses.

Genetic test result reporting in cystic fibrosis

Dr. Ira Lubin, CDC, presented data from an ongoing study designed to assess how laboratories report genetic test results using DNA-based cystic fibrosis testing as a model. Considerable variability was soon so, following on from this, a guidance template including five key elements to be included in a report has been proposed.

In discussion, it was noted that:

There has been little debate on how critical results should be communicated – labs tend to develop methods for themselves. Many of the same issues arise in the diagnosis of leukemia – where the prognosis may be very poor and great sensitivity is needed in communicating results to the patient and their family.

In Europe, test result reports are evaluated as part of the CF network and European Molecular Quality Network. This has been shown to be an effective mechanism for improving the quality of reports over time. Consideration should be given to how reports relevant to new technologies will be evaluated.

The presentations were followed by two roundtable panel discussions.

1. Technical Roundtable

[Participants: Dr. Catherine O'Connell (Tetracore), Dr. Carole Foy (LGC), Dr Rob Elles (National Genetics Reference Laboratory), Dr. Kunihiro Ueda (Kyoto University).]

Panelists spoke briefly about work being done towards standardization of genetic tests in their own organizations.

Catherine O'Connell While at NIST participated in a CDC led effort on quality in genetic testing and validation work on nucleic acid testing, e.g. for somatic mutations in cancer involving p53 gene and mitochondrial DNA.

Carole Foy Work on the DTI's Measurements for Biotechnology program, which is dedicated to improving the accuracy and reliability of biomeasurements and is co-ordinating its work with that of other countries (www.mfbprog.org.uk).

Rob Elles The NGRL provides advice to Government on policy and is a partner in EUROAGENTEST, a Framework VI project dedicated to improving the standards of genetic testing in European countries.

Kunihiro Ueda Efforts are being made to standardize genetic testing in Japan and they are working towards a new ISO standard for genetic testing either as a new ISO document or as an amendment to ISO 15189. Dr. Ueda introduced the idea of horizontal v. vertical standards. A concept analogous to the distinction between technical performance controls v. custom assay or analyte controls.

Discussion

Carole Foy pointed out that there is very little standardization at present in gene measurements. There is a need for

- Standard control materials
- Process definition
- Repeatability, reproducibility and quality metrics
- Discussion on format, analysis, interpretation and storage of information

Rob Elles added that quality in genetic testing depends upon many factors including

- Accreditation (the single most important factor)
- Best practice guidelines
- Training
- External quality assessment
- Validation of tests
- Reference materials

The issue of whether and what reference or control materials are needed in genetic testing generated much comment. For instance, some regulatory authorities do not like synthetic controls, preferring reference material to be as close as possible to actual biological material. WHO and NIBSC (UK) have both developed various reference materials; but it can, perhaps, be confusing to have many different types of standard materials. Helen Parkes (LGC) said that although WHO materials were seen as being 'less stringent' than certified reference materials it was maybe more a matter of a different approach. WHO materials are distributed not as working controls but as higher references. Meanwhile, Marc Salit of NIST pointed out that calibration and validation were very different issues when it came to understanding why (and if) standardization is important.

One important project in the context of standards is the External RNA Controls Consortium (ERCC) which is developing a set of external RNA controls for use in assessing technical performance in gene expression assays by microarray and by

QRT-PCR. The ERCC is preparing standard controls for assessing technical performance. Specifically, the effort is focused on producing standard controls, analysis tools and protocols. The controls will be established as a certified reference material and will be available via ATCC. In short, the ERCC, led by Janet Warrington and hosted by NIST, is working to foster broad, international participation within the community.

Ira Rubin commented that CDC also has a role as a facilitator in bringing interested parties together and in distributing QC materials.

Another important issue is setting variability and uncertainty levels in genetic testing – asking ‘what is normal?’ LGC is starting to develop calibrants to benchmark technologies as innovation continues.

Edison Liu noted the many different use of standard controls, in research, in development, in commercial products and in ‘in between’ applications.

Terminology clarifying application, assumed definitions is an area for future discussion.

Policy Roundtable

[Participants: Dr. Elettra Ronchi(OECD), Dr. Jean-Luc Sanne (European Commission), Dr Maria Chan (FDA), Mr. Noel Doheny(Qiagen).]

Elettra Ronchi said that genetic testing is part of OECD’s mission. She is especially interested in the challenge of pharmacogenetics and pharmacogenomics to health systems and the quality assurance of molecular genetic testing.

Maria Chan said that the FDA is very excited by the challenges posed by genetic testing and is involved in the development of standard controls and reference materials, especially for microarrays.

In general discussion, the two main topics raised as areas to address were standard controls/reference materials and working on global harmonization in their application.

Ira Lubin said that it is vital that standards emerge from an international effort because reference materials would be used at multiple locations. Marc Salit added that NIST was trying to get the private sector to set up controls; ERCC involves many array and reagent manufacturers.

It was suggested that a distinction be made between demonstrating that a device is performing properly and establishing the integrity of the analyte. Technical performance controls could be established that are generally useful across platforms as opposed to establishing a generic control for analyte performance. Controls for analytes would be developed in an assay and intended for use in a specific manner and a specific tissue type.

Ira Rubin commented that ‘one size does not fit all.’ A strategy needs to be developed regarding use of controls that will allow customization depending on regulatory environment, technical aspects of a specific assay, and intended use.

Noel Doheny said that good work had already been done in moving standard controls development along. However, a sponsor is needed as there is no ‘peer review society’ existing at present. Jean-Luc Sanne added that there is a need to co-ordinate genetic services within the EU; appropriate networks might be found through research. Michael Cannieux added that there is a need for validation and traceability of input samples.

Ira Rubin pointed out that when it comes to multiplex testing, complex algorithms may be used. But there is no consensus on measures to ensure that the results are meaningful to the clinician or on measures indicating how the platform performed.

Elettra Ronchi commented that the OECD is interested in the compatibility of regulations within and between member countries and the level of

harmonization that can realistically be achieved. Are existing regulations sufficient for genomics and genetics, and what strategies are regulatory agencies implementing? OECD carried out a survey of 18 countries doing molecular genetic testing and found an 'enormous amount' of variability. Two broad modes of regulation apply. In the US, the Government exerts direct control on genetic tests, whereas in the EU, responsibility is delegated to third parties. The Japanese, Indian and Chinese models of regulation also need to be reviewed.

Edison Liu pointed out that there were lots of assumptions from different professions: statisticians, biologists, clinicians. As we move towards massively parallel analysis in pharmacogenomics with thousands of SNPs, we have to deal with uncertainty in a bigger way. None of the regulatory authorities are prepared for this.

Jean-Luc Sanne added that where international variability stemmed from problems within labs, it was not the EU's problem but a national one; however networks could be organized to try to help raise standards.

Maria Chan pointed out that certification depends upon the lab and the devices they use; the position on the latter is evolving.

Edison Liu said that engineers define their technical endpoints very precisely in a way that is not done in diagnostics. Even CLIA is more 'accounting' based and it is not clear that it is easy to change national certification. However, professional organizations could do much to set 'engineering' standards.

Laura Reid said Expression Analysis sent RNA assays out multiple times to compare results across labs. The labs performing well have asked if they can be recognized or certified to distinguish themselves as running a quality lab. There is no clear owner of that process. It would be advantageous to the community if there was a way to identify the labs performing acceptably well.

Helen Parkes pointed out that there is no requirement to comply with CLIA; the certification procedures expected from the relevant European Directive are now being reviewed (these are not for labs, only kits).

In summary, Elettra Ronchi said that there is a consensus that the development of controls is essential and there is a need for policy to underpin this. There is a convergence between the US and Europe in at least some areas. There are specific challenges for expression analysis, where clinical validation is as important as platform validation. Dialog with regulators and public-private partnerships are essential. There are opportunities for the private sector to create initiatives but everything must be transparent. Models are available, but these must be validated. Management of uncertainty is important. Science and technology questions must be resolved to develop public policy. Peer review and third party organizations are also needed, but this must be an international concept.

At this stage of the meeting, the group was split into Working Groups: two technical groups, under the leadership of Dr. Kathryn Zoon and Dr. David Barton; and two policy groups, led by Dr. Edison Liu and Dr. Jean-Jacques Cassiman. After discussion, all reported back to the meeting on next steps to be taken.

Report of Technical Working Group 1/Kathy Zoon

Next steps concern the following:

Co-ordination of international standardization efforts should be directed towards reference materials, guidance/directives.

Current activities in this area should be catalogued: Who? Where?

What? How? (look towards FDA, ISO, EU, Japan for examples)

Prioritize these activities based upon analysis of information.
A model for standardization could be based upon the ICH (although they themselves are not keen to take on pharmacogenomics) or the efforts of the IMCLGS could be continued and formalized.

Standard control reagents for gene expression on microarrays are in development via the ERCC. Metrics and methods for measuring RNA integrity is an issue. Guidance is needed for sample quality, how to assess if they are 'fit for purpose'?

Also needed:

For equipment, calibrants and performance criteria

Reference reagents for isolation of RNA

Batch-to-batch RNA controls

Bench-marking for lab performance

In an ideal world, all of this would be done but clearly there is a need to prioritize.

A working group is proposed on:

Uncertainty versus validation [and, to this, might be added traceability]. The former has an important meaning in microarrays, as hundreds of genes are involved, so a different approach to validation may be needed.

A quality guidance document compiled with manufacturers/regulatory authorities is proposed

Standardization efforts for data reporting/output

Finally, the development of standard controls and guidelines should facilitate, rather than impede, technical developments.

Report of Technical Working Group 2/David Barton

Shared control materials for single gene disorders are desirable. The group suggested 'aiming low' with this project to identify something that would move the field forward now. The following points are important:

- 'Everyday' controls on each run
- No-one group can generate or supply the necessary controls
- Many genetic disorders have advocacy groups or consortia for research and diagnosis - a resource that should not be overlooked

This group therefore suggests drafting a proposal to CLSI for a guidance document on the production of DNA controls for assessment of technical performance. The controls would aid in supporting the following:

- ✓ Sourcing/traceability
- ✓ Validation/inter-lab comparison
- ✓ Re-validation

It was noted, however, that there could be difficulties with accreditation if this was not a CLSI or ISO standard document. The proposed action is to develop a proposal to submit to the Molecular Methods Area Committee of CLSI.

Controls developed through this initiative would need to be broadly available. It would be desirable to have CDC, LGC, NIST and the FDA involved in the subcommittee. Six – ten people indicated an interest in involvement in this initiative.

There was also discussion in the group on synthetic controls for multiplex assays and support for keeping this on the agenda by the CDC and EUROAGENTEST, key stakeholders, and manufacturers.

Report of Policy Working Group 1/Ed Liu

The key issues discussed were:

Education of scientists, policy makers and the public on gene testing and expression arrays

The need for international collaboration.

Building upon existing guidelines and recommendations (i.e. don't 're-invent the wheel'). Examples come from the International Federation

of Clinical Chemists, WHO, ICH, which has a Joint Committee for Traceability of Materials in Medicine
Realize that vertical controls (single genes) are different from horizontal controls (pattern recognition in arrays)
How to accept uncertainty in interpretation in a test. What level of uncertainty is acceptable?
Validation of algorithms. A workshop on this would be a good idea.

Report of Policy Working Group 2/Jean-Jacques Cassiman

Policy focus should be on the clinical and regulatory aspects of genetic and genomic tests (not research). There needs to be:

- Development of standards in terms of reagents, calibrants, protocols/SOPs, consensus specifications

- Policies that focus upon platforms rather than specific tests

When it comes to platform standards, there is a need to look at:

- Pattern recognition versus specific values

- Algorithms versus detection technologies

- Prognostic versus diagnostic values

- Acceptable data mining approaches, as more data is accumulated

For RNA, standards are needed for high and intermediate multiplex assays.

Precision requirements differ for qualitative, semi-qualitative and quantitative analyses.

For DNA, standard controls for technical performance and analytical performance need to be considered. For analytical performance, controls for functional variants (mutations) versus linked or associated variants (polymorphisms) must be considered in terms of clinical validity. Multiplex versus singleton assays need to be compared, along with large-scale genomic changes.

Phase IV clinical trials should be considered as part of the framework program for genomics.

The number of diagnostic tests is increasing dramatically and there is a need for mechanisms to deal with this.

Finally, looking to the future, the entire IMCLGS group noted:

- There is tremendous advantage to developing standard controls recognized and used internationally, including Europe, the US and Asia
- There was consensus on pursuing the development of a proposal to the MM committee of CLSI focused on a guidance for the development of DNA controls for evaluating technical performance

- A consortium or network model such as the ERCC may be the most effective method for moving forward. It is possible that the OECD could act as a catalyst and guide.

- The effort needs to be open and inclusive, the IMCLGS needs to identify other experts to invite, consult about proposal. Other organizations to invite include IFCC, WHO, EUROAGENTEST and AMP.

Action Items

- An email distribution list of the attendees at this meeting will be created to help further this work. (McKenna) (Complete: see www.imclgs.org)

- A meeting summary will be issued in 3-4 weeks (Warrington, Aldridge)

- Dr. Warrington will follow up with several individuals to review action items and a proposed agenda for the first conference call. A follow-up conference call for all interested parties will be scheduled to put together a plan.