International Symposium on Reference Materials for Genetic Testing
JRC-IRMM, Geel, Belgium, 29-30 November 2005

As genetic testing, and related technologies, begin to enter the mainstream of clinical practice, the need for appropriate reference materials (RMs) becomes increasingly urgent. Accordingly, *EuroGentest* for the EU and the *CDC* for the US, brought together an international group of stakeholders to discuss key issues, such as new regulations, current RM availability and prioritising future needs. The meeting was held, appropriately, at the *Institute for Reference Materials and Measurements (IRMM)*[i] charged with promoting a common and reliable European measurement systems in support of EU policies.

European and International Perspectives on RMs

The EU regulates genetic testing as a medical device, through the in vitro diagnostic (IVD) medical devices Directive (98/79/EC) which covers tests, rather than testing; moreover, its scope is limited to those IVDs having a medical purpose. The Directive deals with all aspects of safety and performance, taking on board the need for common technical specifications such as sensitivity - its main purpose is to introduce harmonised controls on these IVDs throughout the EU. At present, DG Enterprise is looking at the Directive with responses expected from Member States this year – although it is felt it is sufficiently robust not to require major modifications.

According to *ISO*, which looks at standards at the international level, a standard is a written document with rules and guidelines, covering consolidated technologies and processes. ISO prefers to concentrate upon 'horizontal' standards which cover general aspects and are widely applicable.

ISO TC 212 (whose secretariat is the *Clinical Laboratory Standards Institute – CLSI*) is focused on lab medicine and IVDs and will develop horizontal standards to apply to all IVDs and globalize regional standards where these have global impact.

As an international organisation, ISO is working with countries outside Europe and the Americas - Thailand and China are members of 212 – but it is proving hard to get some developing countries involved. Global harmonisation is important though, to avoid the emergence of a 'two tier' world as far as genetic testing is concerned.

BOX 1
Patent Issues in Reference Materials for Genetic Testing

Patent expert Pierre Kihn explained that an awareness of patents is important in work on RMs. For these may involve gene sequences, so Directive 98/44/EC, which allows gene patents under certain circumstances, is potentially an issue. Under Article 9, **'The protection conferred by a patent on a product containing or consisting of genetic information shall extend to *all material* ....in which the product is incorporated and in which the genetic material is contained and performs its function.'** Most patent law does specify the acts that are prohibited to third parties (which could mean those developing or using RMs). Generally, the patent owner has the right to prevent the third party from making, using, offering for sale, selling or importing the patented product. The actual meaning of patent claims is ultimately decided by the courts. For the production of reference material, there are no general rules - it is usual to proceed on a case by case basis.
The use of short sequences from a gene patented for its expression of a novel protein will not infringe the patent as in this particular case the **function** of the novel protein is patented and not shorter fragments of a that gene.
But patents on gene sequences can create a barrier to producing a RM. So it is always advisable to verify the territoriality of the patent, its expiration date, or perhaps get a licence from the patent owner. The publication of a new genetic test, or of potential RM, in a scientific journal is one way to limit the patenting of such a test and preventing a commercial company from making a profit from a genetic test that can be important for public health.

Patent literature is a rich source of technical information and can be found on several public website such as www.espacenet.com , www.iprhelpdesk.org, www.delphion.com or www.depatisnet.de.

On the second day of the meeting, European participants were joined by colleagues from the U.S.

What is a Reference Material anyway?

Philippe Corbisier began the day with a review of what we actually mean by the term 'reference material'. In the ISO Guide 35, RMs are defined as materials sufficiently homogeneous and stable with respect to one or more specific properties. If they have no further

characteristics they are known as Quality Control Materials (or, variously, as lab reference materials, lab controls, materials for EQAs, in-house materials).
In addition, Certified Reference Materials (CRMs) carry a certificate which provides certified property values, with uncertainties, and stated metrological traceability (ISO Guide 35).
The uses of RMs include

- Method development and validation; estimating the uncertainty of measurement
- Calibration
- Proof of a method's performance
- Proficiency testing

Needs of Stakeholders for Reference Materials for Genetic Testing

Contributors from both sides of the Atlantic shared observations on what people want when it comes to RMs. Christine Brady reported on three recent surveys on users' needs, revealing a wide range of current practices and perceived need across Europe. Prioritising which RMs need to be developed will not be easy!

- The *NGRL* survey of opinion on RMs in 111 UK genetic testing labs found that RMs are most wanted for tests for clotting disorders, cancer and core monogenetic diseases. See www.ngrl.org.uk/Manchester/pages

- The ongoing *CRMGEN* survey of demand for CRMs found a need for RMs for muscular dystrophy, FragileX (FraX)and Huntington's disease (HD) testing. Download the survey from www.crmgen.org.

- The EuroGentest survey on positive controls among assessors of EQA schemes run by the *European Molecular Genetics Quality Network* found that most respondents were using in-house RMs. They wanted as many RMs as possible, especially for rare diseases.

Meanwhile, in the US, the Coriell Cell Repository has been distributing cell lines and QCMs for many years. Jeanne Beck discussed a survey of their DNA shipments between 2002 and 2005 which shows that 20 per cent, of a total of 80,000, are for positive controls, mostly for cystic fibrosis (CF) or FraX. In fact, 38 per cent

of these positive controls shipped outside the US are for genetic disease. Other findings included:

- More than 550 DNA samples shipped outside the US (20 per cent of the total shipped as positive controls) have been for CF, FraX, Factor V Leiden/MRHFR, HFE and HD.
- 33 per cent of DNA samples purchased by non-US researchers as positive controls were for CF, 11.5 per cent for FraX, one per cent HD, 8.5 per cent for diseases in the Ashkenazi Jewish panel.

Companies using/needing RMs

Delegates from a number of companies shared their experience of RMs

- *Innogenetics* wants RMs for its CF and HLA (transplantation) diagnostic tests and is setting up a databank with accessible and well-characterised samples
- *Roche* uses its own RMs for its Factor V Leiden and Factor II (prothrombin) diagnostic tests and wants material that can detect polymorphisms which are not currently represented in control materials
- *Applera/Celera Diagnostics* is developing test reagents, and would like to have RMs available for Fragile X and expanded CF mutations
- *Qiagen* has been working with whole genome amplification (REPLI-g) for very small samples and genome-wide studies and is offering collaboration opportunities
- *Affymetrix* wants to develop RMs for its own and other platforms as it moves from research to products. The company has been active in the *International Meeting on Clinical and Laboratory Genomic Standards* (www.imclgs.org) which is working to accelerate the establishment of clinical and laboratory standard controls and global harmonisation in this area.

Box 2
FDA update

Zivana Tezak reported that the *FDA* had set up an *Office of IVD Devices* in November 2002. FDA is committed to improving the reliability of such tests and knows that QC material has an important contribution to make. While FDA has oversight of these materials, how they are handled in the lab is the responsibility of *CLIA*. QC material may be regulated with the assay, or separately.

Current initiatives *FDA* is working on in this area include (with *CDC*) QC in HD and FraX and, with the *External RNA Control Consortium* (*ERCC*), microarray controls.
For more information see http://www.fda.gov/cdrh/oivd/. For the FDA guidance on Quality Control Material, see http://www.fda/gov/cdrh/ode/99.html.

Current availability & development of control materials for genetic testing

David Gancberg listed the current barriers to the supply of RMs, which are:

- Lack of QA
- Need for networking
- Lack of certified RMs
- Need for normative and regulatory framework application
- Impact of patents

(see www.irmm.jrc.be and www.jrc.cec.eu.int)

However, progress is definitely being made. David Barton gave a summary of *CRMGEN*, a four year EU-funded feasibility study on developing RMs which he co-ordinated. *CRMGEN* made and sent out RMs in four forms: (PCR products, cell lines, genomic DNA, synthetic DNA) for the following diseases

- CF
- Haemochromatosis
- FraX
- Sickle cell anemia
- Thalassaemia
- Factor V Leiden
- HNPCC
- DMD

with the result that four FraX and six HNPCC RMs have now been generated in all formats.
Meanwhile, EuroGentest continues to work on identification of present and future needs for RMs, setting priorities, implementation of traceability and building up a network. The *National Institute for Biological Standards and Controls* (*NIBSC*) is involved in the *World Health Organisation* (*WHO*) Biological Standardization Program and is one of the two (soon to be the only one) labs which holds and distributes these international standards.
The first *WHO* genetic testing RM was for FV Leiden from a well characterised patient. *NIBSC* also distributes a prothrombin standard and is working on RMs for FraX, Haemophilia A, hereditary

haemochromatosis, HLA and in future hopes to work on a reference material for BCR/ABL detection. (see www.nibsc.ac.uk)

Joe Boone spoke about the international *Genetic Testing Quality Control Material Program,* which started by looking at pressing QC material needs for DNA-based genetic tests and has been coordinating the collection and verification of cell lines with the mutations needed. The Program welcomes input – needs, ideas, material donation, verification and support. Co-ordinator Lisa Kalman added that the *GTQC* is developing QC materials for HD testing (through allele sizing), Ashkenazi Jewish panels (nine disorders), FraX and CF. Next steps include

- Completion of current verification projects
- Developing improved information resources
- Identifying new targets for QC material development
- Exploring human subjects and regulatory issues
- Co-ordination with Europe (see www.phppo.cdc.gov/dls/genetics/qcmaterials)

Box 3
Maine Molecular Quality Controls

The following are QC material requirements

- Monitoring all steps of the test
- Monitor associated genotypes
- Assure a constant value over time
- Should be easy to manipulate and use
- Acquire multiple genotypes per sample
- Rare QC genotypes should be easily generated

All of these are best tackled by synthetic molecular quality controls. The *Maine* program involves generating in vitro mutated DNA, validating sequence, stabilising the construct and then producing wild type and mutant alleles. The controls can be handled just like whole blood and have been tested in 7 major platforms.
For CF, a sequence of 180.000 bp is validated. The RM consists of a group of 5 circular DNAs containing 24 exons from the CF gene. The material has been sent out by Els Dequeker to around 200 labs for testing. CF, Factor V and G20210A Factor II, MTHFR materials are already available and haematologic translocation control and tuberculosis controls are under development.
(see www.mmqci.com)

From the UK, Helen White of the *Wessex NGRL* reported on their development of plasmid-based controls for HNPCC gene anomalies and breast cancer. Plasmid DNA is diluted in TE 0.1x at 10^4 cp/µL in

a background of 50 µg/ml tRNA This is based on blood from eight consenting 'normals'. They have positive controls for all mutations and have done field trials on these.
The lab has also constructed 52 plasmids for BRCA1, BRCA2, MLH1 and MLH2 to be tested after sub-cloning in pUC18 (originally the sequences were all cloned in pCR2.1). Meanwhile, *LGC,* the UK's National Measurement Institute has a number of programs in genetic testing, including one on microarray performance indicators. For more information, go to www.mfbprog.org.uk

The meeting concluded with discussion on prioritising needs for RMs

The US Experience

Bin Chen discussed the drivers of QC material needs in the U.S. including

- Volume of testing
- Professional/practice recommendation of test use
- Laboratory standards/ professional recommendations
- Regulation by *FDA*, *CLIA* and state
- Need for test standardization
- Test development

The *QC Material Priorities workgroup*, which was one of the workgroups formed at the CDC-organized QC Materials for Genetic Testing working meetings, was charged to identify genetic tests in urgent need of QC materials and make a priority list. Thus far, the group has identified intra-lab testing processes (method validation, testing process, lot validation), test development and performance evaluation (PT/EQA) as priorities.
Other priority focus includes:

- CFTR mutant analysis (genomic and synthetic supercontrols)
- FraX controls for, normal, permutation, full mutation and methylation status for PCR and Southern blots
- Disorders included on the Ashkenazi Jewish panel
- Galactosemia, acyl-Coenzyme A dehydrogenase deficiency, biotin deficiency
- Pharmacogenetic testing
- Mitochondrial disorders
- Other trinucleotide repeat disorders

There is an ongoing need to monitor and improve QC material availability for rare disease DNA and biochemical genetic testing and emerging technologies such as microarrays and genomic screening.
(See Genetics in Medicine October 2005 and www.phppo.cdc.gov/dls/genetics/default.aspx

The EU Experience

David Barton listed the following issues which need to be considered when prioritizing which RMs to develop.

- The number of potential users of a RM
- Geographical distribution of testing
- Current availability of a RM
- National and international guidelines
- The nature of the mutation
- Range of assays used
- Feedback from EQA/PT schemes
- Availability of source materials for RM (e.g., patient consent)
- IP issues – such as the cost of licensing, legal assessment, potential of these to block RM development

Looking to the future

The EU and the US want to work together on the RM issue, although so far there are no formal collaborations. Ensuring work is not duplicated is important – in so far as the respective regulatory systems will allow (but it is not clear whether EC marked materials would be acceptable as controls in the US, or if validated Coriell material is allowed as controls in Europe). Maybe a joint EU/US 'think tank' is now needed.
EuroGentest also needs an advisory working group on reference materials and is to carry out some discrete recruitment. Helen Parkes of LGC invited nominations of some the many RMs mentioned at the meeting to *JCTLM* (*Joint Committee on Traceability of Laboratory Materials* http://www.cstl.nist.gov/jctlm.htm), a non-governmental organization on which several of the current delegates serve. See also www.bipm.org/en/committees/jc/jctlm/ and www.ifcc.org.

Future Plans

The current meeting was part of the CDC series (no 4) and EuroGentest (first). A CDC meeting is proposed in 2006 – possibly Brisbane in August, which will give more of an international feel, or associated with the AMP meeting in Orlando, Florida in November. Ireland will host the next EuroGentest meeting, around May 2007.

[i] Important groups and organisations in the world of reference materials are all italicised