

**Proceedings of the EuroGentest International Symposium on
Reference Materials for Genetic Testing
JRC-IRMM, Geel, Belgium, 29-30 November, 2005**

29th November

David Barton welcomed the participants and thanked IRMM for hosting the meeting. Hendrik Emons, who is responsible for the programmes on Certified Reference Materials (CRM), gave a brief introduction to the work of the Institute for Reference Materials and Measurements (IRMM), which has the mission to promote a common and reliable European measurement systems in support of EU policies. Many CRMs have already been developed, for instance in the food and clinical areas.

**EU Regulatory issues regarding Reference Materials (RMs)
for Genetic Testing (chair Hendrik Emons and Philippe
Corbisier)**

Implementation of the IVD Directive in Genetic Testing (John Brennan)

There are three directives concerning medical devices (the Active Implantable Medical device directive 90/385/EEC, the Medical Devices Directive 93/42/EEC amended in directives 2000/70 and 2001/104, and the in vitro diagnostic (IVD) medical devices directive 98/79/EC). The IVD Directive covers all aspects of the safety and performance of IVD medical devices; it is still relatively new and a Technical Specification Committee for IVD directive has therefore been created.

What is peculiar to IVDs is the need for common technical specifications such as sensitivity, along with vigilance and intervention measures. The Directive covers tests, rather than testing, and only those meeting the definition (having a medical purpose); therefore it does not include all IVDs. It excludes, for instance, forensic tests, 'in-house' tests, and certified reference materials. This is in contrast to the US, where bioterrorism tests would be included.

The main purpose of the Directive is to bring about the completion of a single market by introducing harmonised and statute-based controls to regulate the safety and performance of devices throughout the European Union. The CE marking means that a manufacturer is satisfied that his product conforms to the relevant Essential Requirements in the Directives and that it is fit for its intended purpose. The CE mark also means that the product can be freely marketed anywhere in the EU without further control.

At present DG Enterprise is reflecting on the Directive with responses expected from Member States in 2006. Modifications are thought unlikely as the Directive is already robust and no modification are foreseen for the coming 5 years.

In discussion, Helen Parkes asked how links were to be made between clinical and biological standards.

Ans. The Medical Devices Expert Group brings stakeholders together. If a need for a standard is identified by users or member states, the EC writes a mandate and the Group works with the European Committee for Standardization (CEN) (which develops standards at the European level). The output of such meetings comes via national standards organization. If there is a problem, a standard can be de-harmonized.

HP said CEN only looks at documented standards and is slow; reference material work tends to come more from group like the present one.

Ans. CEN is looking at ways of fast-tracking in genetic testing, for instance in SNP work.

Philippe Corbisier added that CRMs and materials for external quality control are not covered by the Directive, but kit calibrants are.

David Barton asked what was understood as "a material of higher order" in the IVD directive. A definition is not given in the Directive itself and a guidance document providing a method to recognise what is a material of higher order or a RM would be welcomed. This would allow consensus and avoid possible misunderstanding on the applicability of the IVD for certain materials.

ISO Standardization of Clinical Laboratory Testing and *in vitro* Diagnostic test systems (Klaus Stinshoff, Chair of ISO Technical Committee 212, also working in CEN Technical Committee 140)

According to ISO, 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 (e.g. 17511) and are widely applicable. The emphasis is on performance over prescription as this allows for technical development (this is based on a World Trade Agreement). So-called 'vertical' standards are more specific and there are none yet for genetic testing.

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

standards that could be taken up to EN-ISO). Reference material guidelines are dealt with by the standards section. Relevant 212 standards include 15193 (IVDs), 15194 (IVDs), 15195 (reference labs) A project, 15190, on specific requirements for quality and competence in genetic testing labs is also under consideration. It excludes generic QM standards, which are dealt with by Committee 176, and QM for medical devices. In short, the scope and structure of 212 qualify it to develop standards for reference materials.

Helen Parkes commented that CLSI is more advanced than the European equivalent and wondered about the situation elsewhere, e.g.. Japan.

Ans. ISO is working with other countries and Thailand and China are members of 212. But it is hard to get some developing countries involved.

HP responded that Singapore is active in genetic testing and it is important to learn to harmonize globally otherwise a 'two tier' world will result.

Patent Issues in Reference Materials for Genetic Testing **(Pierre Kihn)**

Reference materials may involve gene sequences, so Directive 98/44/EC, which allows gene patents under certain circumstances, may potentially be 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 explicitly specifies the acts that are prohibited to third parties (i.e. potentially, 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 scope of protection of the patent is determined by the claims and the meaning of patent claims is ultimately decided by the courts. For the production of reference material, it is very difficult to determine general rules of what can be produced and what cannot be produced without infringing a patent. A case by case study is needed each time.

It was stressed that 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 that gene.

It is clear that patents on gene sequences often form an obstacle to producing RMs even if in some case the possibilities exist to bypass patents. It may be however better, to verify the territoriality of the

patent, its expiration date, or to get a license from the patent owner.

The publication of a new genetic test, or of a potential RM, in a scientific journal is a way to limit the patenting of such a test and prevent a commercial company from making a profit from a genetic test that can be important for public health.

Finally, patents are a useful source of information. More than 20 per cent of the R & D funds in the EU are spent to invent things that have already been invented. The fact that a product is not on the market does not mean that it has not already been described in the patent literature. Indeed, an OECD study has shown that 80 per cent of technical information is available exclusively through the patent literature. Patent literature can be found on several public websites such as www.espacenet.com , www.iprhelpdesk.org, www.delphion.com or www.depatistnet.de.

Defensive patents, such as the one taken by E. Bakker to block Myriad's patent on the breast cancer gene can be useful but requires a proper strategy including, for instance, uniform geographic coverage

Forming an EU Working Group on RMs for Genetic Testing

David Barton and Philippe Corbisier are currently running a working group on RMs for EUROAGENTEST. They will formulate a brief and circulate this by email – the idea is to meet 2/3 times a year.

Els Dequeker commented that labs are waiting for RMs. Could David Barton use the CRMGEN project to provide these?.

Rob Elles said that it was intended to take forward the RMs from CRMGEN via the National Genetic Reference Laboratories (NGRLs) and the National Institute for Biological Standards and Control (NIBSC).

Hendrik Emons pointed out that EUROAGENTEST is a network of excellence; its role is not to provide RMs. Labs that want these should make their needs clear to appropriate funding bodies. Egbert Bakker added that labs should make their own RMs and share them Elaine Gray reminded delegates that NIBSC is a WHO lab and if there is a public need for a RM they will make it. The hardest issues are obtaining patient consent and cell lines

According to Heinz Schimmel, RMs must comply with ISO standards before going onto the Joint Committee for Traceability in Laboratory Medicine list and also avoid redundancies with existing CDC materials.

Hendrik Emons said that EC interservice group for genetic testing is meeting regularly, and if prioritisation, needs and guidelines are identified, it might be justified in the group to ask for funding, but

the proposal should be detailed as to needs for materials, workload in man months, instruments needed, consumables, etc. However, discrepancies between sales and claims in projects for RM production must be identified first. The priority list for RMs should be reported to the next interservice group for genetic testing. Rob Elles commented that without certified RMs, we would be in breach of the Directive, so the EC ought to supply them.

30th November

On this day, European participants were joined by colleagues from the U.S.

Definitions of Type of Reference Materials and Controls (Philippe Corbisier)

The two basic characteristics of RMs are appropriate homogeneity and stability. RMs with no further characteristics are known as Quality Control Materials (or lab reference materials, lab controls, materials for EQAs, in-house materials).

In the ISO Guide 35, RMs are defined as materials sufficiently homogeneous and stable with respect to one or more specific properties, which may be qualitative or quantitative (the former is new).

A subgroup of RMs are CRMs. They carry a certificate which provides certified values, with uncertainties, and stated metrological traceability; (ISO Guide 35). Values can be quantitative or qualitative (the latter is new and can include sequence or identity; uncertainties can be expressed as probability)

Calibrants have additional properties with respect to QCMs; some CRMs are calibrants, some not.

The uses of RMs include

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

IVDs need a CE mark. CRMs and material for EQA do not have to have the CE mark, but calibrants (which must be traceable to a higher order RM, as defined in ISO 17511) and controls do, if sold as part of an IVD kit. The interpretation of a RM of higher order might be done according to ISO 17511. ISO 17511 covers IVD calibrators and gives a hierarchy of traceability:

1. SI Unit
2. Higher order reference measurement procedures
3. WHO conventional RM
4. In-house method and calibrants, QCMs

Needs of Stakeholders for Reference Materials for Genetic Testing

European Genetic Testing Labs (Christine Brady)

Three surveys have been carried out on the needs of users.

- The 2005 National Genetic Reference Labs (NGRLs) 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. 48 per cent said RMs are essential. Pure DNA was the preferred matrix (81 per cent). Download the survey from www.ngrl.org.uk/Manchester/pages
- The CRMGEN survey of demand for CRMs, initiated in 2004 and is ongoing, covers 71 respondents in 24 countries and found a need for RMs for muscular dystrophy, Fragile X (FraX) and Huntington disease testing. The majority thought RMs to be essential or useful and most would use them periodically. Download the survey from www.crmgen.org. However, there is a discrepancy between needs in UK and Europe, and basically each country has its own list of priorities for availability of RMs.
- The 2005 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 as possible or RMs for use with rare diseases.

In conclusion, there is a wide range of current practices and a wide variety in perceived need. Therefore prioritising which RMs need to be developed will not be easy.

Heinz Schimmel commented that RM needs were also dependent on different reference measurement system infrastructures and lab accreditation requirements. Helen Parkes mentioned that besides the RMs, the methodology that is used (reference methods) is also important to promote correct measurements.

Coriell Market Surveys (Jeanne Beck)

Coriell has been distributing cell lines and QCMs for many years. A survey of DNA shipments between 2002 and 2005 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. 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 and 8.5 per cent for diseases in the Ashkenazi Jewish panel.

Coriell has also carried out surveys at meetings and found that 60 per cent of respondents would use samples which characterized mutations. Most wanted controls for FraX, CR, BRA1/2, Factor V Leiden and MTHFR, which correlates well with what is seen from shipments.

Innogenetics (Gonda Verpooten)

Innogenetics makes diagnostics tests, for CF and HLA in transplantation, and so needs reference materials. In order of preference the company wants RMs in the form of

- Real samples – the best way to verify products, but hard to obtain
- Cell lines
- DNA

They may need to design a synthetic matrix that mimics real samples. The company is also setting up a databank with IEC/IRB approval, informed consent, with accessible and well-characterised samples

Roche (Hubertus Stockinger)

Roche is developing FV Leiden and Factor II (prothrombin) diagnostic kits and using their own RMs. There is a need for a lot of material to detect polymorphisms arising in real samples, which are currently not represented in the control materials used. Elaine Gray indeed reported that one kit for Factor II detection was missing all the homozygous samples and therefore to distinguish between wild type and heterozygote, the use of an appropriate RM was crucial. Synthesized material is preferred by Roche but the FDA wants real material. A RM cell line supply with polymorphisms would not solve this. A calibrator for DNA/RNA purity is desperately needed.

David Barton suggested going to a cell bank to find real material. Heinz Schimmel said that RM has to be fit for purpose and should be validated with the assay on the market.

Applera/Celera Diagnostics (Michael Zoccoli)

Celera uses RMs for many purposes, including assay development and optimisation, performance comparison, product demonstration and QC testing of reagents. The company is developing a FraX test

for female carriers and newborn screening. For normals, RMs with 5-44 repeats are needed, for full testing RMs with over 200 repeats are necessary. NIST has PCR products which can be used. However, Celera is looking for RMs that allow them to prove size accuracy. There is also a need for a CF mutation panel for some customers want expanded panels to cover ethnic populations and there are no reference DNAs for these. Synthetic controls can fill *some* gaps. However, when working with synthetic DNA as RM, the balance between mutated and wild type mutations is difficult to reach to mimic a real sample. The whole genome amplification method does not work consistently enough in order to circumvent this problem.

Qiagen (Edgar Setzke)

Whole genome amplification (REPLI-g) is an option with a very small sample (10ng of genomic DNA) or for a genome-wide study. Amplification bias is an issue but Qiagen has limited this, checking by locus representation. Low amplification bias has been demonstrated in many loci, applications and sample. The method has potential use for generation of standards and RMs when certified and approved RMs are not available (for rare samples, when there is no alternative). This method does not work well with fragmented DNA such as DNA extracted from paraffin-embedded tissues. Qiagen wants to collaborate on this and David Barton appreciated the offer.

Affymetrix (Janet Warrington)

Affymetrix is moving into products from research. Their customers are international and they want to market new products internationally and need a set of controls for this. The company wants to develop RMs for its own and other platforms and RT PCR which is used to validate arrays. From the patient and regulatory perspective, there is a need for common information on tests done on different platforms.

The goals are to

- Increase stakeholder interaction – which is being done through the International Meeting on Clinical and Laboratory Genomic Standards (IMCLGS) project (www.imclgs.org – can deposit information here)
- Accelerate establishment of clinical and laboratory genomic standard controls (see IMCLGS)
- Identify areas for global harmonisation in development and application of standard controls and guidelines

Current activity of IMCGLS (which has quarterly conference calls) is focused on

- Pursuing recommendations for selection criteria and characterisation of DNA controls for evaluating technical performance (and their assisted metrics and information reported)
- Determining actions to facilitate the harmonisation of data standards initiated, including algorithm selection criteria, reporting issues and database standardisation.

ISO (Klaus Stinshoff)

ISO has the infrastructure for initiating the development of standards but has the disadvantage of focusing on horizontal/performance standards. Methods are laid down in the ISO Directives

Standards must behave like patient samples.

People are invited to participate in ISO.

FDA (Zivana Tezak)

The FDA's Office of IVD Devices for evaluation and safety was set up in November 2002. Medical devices are classed with respect to patient risk – I, II and III and cover genetic, genomic and molecular tests.

FDA wants the tests to be reliable and for the public to understand both their values and their limitations. QC has a contribution to make to reliability.

QC material is subject to various levels of regulatory oversight by the FDA, depending on the class of the related device. For instance, some is Class I and so exempt from some aspects of regulation. While FDA has oversight of the material, how it is handled in the lab is the responsibility of CLIA. The material may be regulated with the assay, or separately.

For clinical labs requirements for PCR-based devices include: positive/negative controls, two different concentrations, external control, internal control, amplification (inhibition) control

FDA does recognise some other standards, e.g. from WHO, NIST, NIH, CDC, CBER and so on

Current initiatives FDA is working on include (with CDC) QC in HD and FraX and, with ERCC, microarray controls.

For more information see <http://www.fda.gov/cdrh/ode/99.html>

Current situation as regards the availability & development of control materials for genetic testing

Availability of RMs for Genetic Testing in Europe (David Gancberg)

Current barriers to the supply of RMs are:

- 1. Lack of QA
- 2. Need for networking
- 3. Lack of certified RMs
- 4. Need for normative and regulatory framework application
- 5. Impact of patents

Expanding on these,

1. An EQA participation study has shown that 30 per cent of eligible labs participate in EQA for one genetic test but fewer do EQA for more than one test. Some do no QA or inspection. There is a need to harmonise EQA schemes.

2. Networking is made possible by CRMGEN, EuroGentest, IFCC and WHO

3. Ethical issues impede sourcing RMs from human donors. Choice and coverage of target populations needs to be looked into.

CRMGEN has a list of DNA-based RMs

4. Many labs lack accreditation (ISO 17025). The IVD Directive is an interesting development. There is a need for lab practice harmonisation.

5. On patents, stakeholders don't want to pay royalties. This issue might affect 'home brew' tests, kit controls and the development of control materials.

For more information see www.irmm.jrc.be
www.jrc.cec.eu.int

David Barton commented that different countries have different standards regarding accreditation and on issues such as whether the lab director needs to be certified. In Belgium and The Netherlands only certain people can do genetic tests.

Rob Elles said that OECD has looked at this issue (accreditation) and has data.

Development of RMs for Genetic Testing in Europe (David Barton)

CRMGEN was a four year feasibility for developing RMs (co-ordinated by DB), funded by the EU (funding expired in October 2005) and the final report is yet to appear. The project operated on a consortium design, where RMs were made and sent out in four forms: PCR products, cell lines, genomic DNA, synthetic DNA. The following diseases were covered:

- CF
- Haemochromatosis
- FraX
- Sickle cell anemia
- Thalassaemia

- Factor V Leiden
- HNPCC
- DMD

Issues explored included versatility of materials, stability, economics, storage, cost and ethics.

Four FraX and 6 HNPCC RMs have been generated in all formats. (Other diseases?) Know-how and guidelines have also been generated.

Meanwhile, EUROAGENTEST is a network for test development and is based on six different units. The sub-unit known as WP 1.6 deals with reference measurements and its work includes the identification of present and future needs for RMs, setting priorities, implementation of traceability and building up a network.

NIBSC Activities in RMs for Genetic Testing (Elaine Gray)

NIBSC is involved in the WHO Biological Standardization Program, which was revised in November 2004 and carries ampouled material as RMs. NIBSC is one of the two (soon to be the only one) labs which holds and distributes these international standards.

WHO has guidelines on QA, use and datasheets for the RMs.

There are three type of RM

- International standards
- Reference reagents – with different characteristics and uses from the international standards
- International Standard and Reference Panels (a recent development) used in the evaluation of assays and diagnostic tests and intended to promoted global harmonisation

NIBSC has more than 95 per cent of these standards and accompanying technical information

The first WHO genetic testing RM was for FV Leiden from a well characterised patient. An EBV-transformed cell line was used to get a continuing supply and an international collaboration study on the RM has been carried out.

On this issue, NIBSC has been taking advice from the NGRLs, professional societies (e.g. the International Society on Thrombosis & Haemostatis) and clinical labs.

NIBSC also distributes a prothrombin standard [(G20210A) as freeze-dried genomic DNA] and it is working on RMs for FraX (coming early 2006, participants invited), Haemophilia A, hereditary haemochromatosis, HLA and, in future, will work on a bcr/abl test for foetal DNA from maternal circulation. Studies have shown the RMs are fit for use.

For more information visit www.nibsc.ac.uk

Development of GTQC Program (Joe Boone)

The Genetic Testing Quality Program is international and began by looking at pressing needs in genetic tests, one of which was improving the availability of positive controls. The Program has been coordinating the collection and verification of cell lines with mutations needed. These materials are intended to be daily quality controls, which are less highly characterized than certified reference materials, which are meant to be used as standards or calibrators. The CDC has organized four working meetings at which current and future needs for QC materials for genetic tests have been discussed, including ways of producing them. Coriell, and other cell banks, are acting as repositories.

In summary, materials can be obtained and made available at reasonable cost and sustainably. But there is still a need for guidance on the use of materials and clarification of regulatory requirements. Input is wanted, on ideas and needs, plus material donation, verification and support.

GTQC – QC Materials Development (Lisa Kalman)

Lisa Kalman, as the co-ordinator of the CDC's GTQC Materials Program, is charged with working with the genetic testing community to develop QC materials. This involves finding source materials, such as cell lines already in repositories, cell lines in research laboratories and patient samples. The mutations in these materials are then verified through testing in volunteer clinical laboratories. The verified QC materials are distributed to the genetics community through the Coriell Cell repositories. The program is developing QC materials for HD and Fragile X testing (through allele sizing) from a 'wish list' from lab directors. The Program is also developing QC materials for disorders on the Ashkenazi Jewish panels, which have been recommended by the American College of Obstetricians and Gynaecologists. Finally, the Program is working on CF and looking at QC material needs. Our research indicates that there are no available QC materials for many CF alleles, which raises the question of priorities.

It is difficult to get human material for development; a survey shows that it is not possible for many institutions to send anonymized residual blood to a cell repository without patient consent.

The GTQC is also working to

- improve information resources
- identify new targets for QC material development
- explore human subject and regulatory issues
- coordinate with Europe where possible.

For more information see
www.phppo.cdc.gov/dls/genetics/qcmaterials

Maine Molecular Quality Controls – a Case Study (Clark Rundell)

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.

The project is now heading for ISO. Key features include

- Precision manufacture
- Contains all analytes
- Affordability
- DNA extracted so controls are present in all steps
- Stability, traceability

For CF, a sequence of 180,000bp is validated. The RM consist of a group of 5 circular DNAs that contains 24 exons from the CF gene. The material has been sent out by E. Dequeker for testing to +/- 200 labs. CF, Factor V and G20210A Factor II, MTHFR materials are already available materials whereas hematologic translocation control and tuberculosis controls are under development.

For more information, go to www.mmqci.com

Helen White – Wessex NGRL (unscheduled contribution)

The lab has developed plasmid-based controls for HNPCC gene anomalies and breast cancer. Plasmid DNA is diluted in TE 0.1x at 10^4 cp/ μ L in a background of 50 μ g/mL tRNA This is based on blood from eight consenting 'normals'. They also have positive controls for all mutations and have done field trials on these.

The lab has 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). These materials would infringe existing patents in the US

Helen Parkes – LGC (unscheduled contribution)

LGC is the National Measurement Institute (UK) and does a lot of genetic testing. They have a number of programs, such as one on microarray performance indicators, including the development of reference materials. These are needed to look at variation within and between arrays; an inter-lab trial is underway. For more information, go to www.mfbprog.org.uk

Prioritization of Needs for Reference Materials for Genetic Testing

Determining Needs and Ongoing Monitoring – The US Experience (Bin Chen)

Factors recognized at the four CDC-organized “QC Materials for Genetic Testing” working meetings that drive the needs and demands include:

- Volume of testing
- Professional recognition of test use
- Laboratory standards
- Regulation by FDA, CLIA and state
- Need for testing standardization
- Test development

The current CLIA regulations provide general requirements for control materials and QC procedures in patient testing, including QC requirements for molecular amplification procedures. Other general QC requirements include the inclusion of a positive and a negative control material for each qualitative procedure and two levels of control materials for each quantitative procedure. For each in-house developed test, the laboratory must establish the number, type, concentration and frequency of controls. Control testing must be performed the same as patient tests. At present, CLIA does not contain specific requirements for molecular genetic testing, but a proposed rule to establish specialty requirements for genetic testing is being worked on and expected to be published by the end of 2006. In the U.S., there are two CLIA-exempt State programs in Washington and New York, which have State requirements that are equivalent or more stringent than the CLIA regulations. The QC Material Priorities workgroup, which was one of the workgroups formed at the 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, test development and

performance evaluation as areas that should be considered in assessing priorities.

Specific 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.

Mechanisms to monitor QC material needs include:

- Surveys
- Communication with Testing labs
- IVD manufacturers
- Test developers
- PT/EQA programs

See Genetics in Medicine October 2005

The Expert Panel of GTQC met on 29th November 2005 and reviewed the progress since November 2004. Further information was disseminated and updates were made to priority listings.

International co-operation was called for to ensure that:

- Projects are open
- Materials are accessible
- There are joint projects – for instance on developing consensus documents
- Filling in gaps
- Rare diseases

Next steps include continuous needs monitoring, exploration of additional projects (such as BRCA, Newborn screening disorders, , rare diseases, and pharmacogenetic testing), and obtaining input on other areas of testing, including biochemical genetic testing, molecular oncology, and molecular infectious disease testing.

For more information go to

www.phppo.cdc.gov/dls/genetics/default.aspx

Criteria for Prioritization – EU Perspective (David Barton)

In principle there is a need for RMs for every mutation in every gene, but this is not realistic. There is a need, instead, to get

maximum impact for the limited resources available. Important issues include

- The number of potential users of a RM
- Geographical distribution
- Current availability of a RM
- National and international guidelines
- The nature of the mutation
- Range of assays used
- EQA
- 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

There is a list of prototyped CRMGEN materials which are 'ready to go'.

International Meeting on Clinical and Laboratory Genomic Standards (Janet Warrington)

IMCLGS is looking at recommendations for selecting criteria and characterization of DNA controls for evaluating the performance of technologies on different platforms.

The view on RMs is they should be

- Fit for purpose
- Stable
- Homogeneous
- Renewable
- Traceable
- Have a range of concentrations
- Pure
- Complexity should be similar to sample

In discussion, Jean Amos-Wilson said that rare diseases had been talked about but priorities had not been assigned.

David Barton said it was important to produce guidelines for people working with rare diseases on how to validate materials.

Heinz Schimmel referred to ISO 17511 and the need to discuss the development of robust methods

Sue Richards said the American College of Medical Genetics had a consensus document from the QA Committee which had guidelines on testing for rare disorders and would be good as a starting point.

Larry Silverman said that newer trends, such as methylation assays, need to be looked at; furthermore, multiple loci being discovered within CF gene and will need also to detect accompanying expression patterns over and above the mutation – this raises the question of whether there is such a thing as a single-gene disorder. The next generation of controls must think about this.

David Barton commented that Prader-Willi, Angelman and Down Syndromes were not being addressed, while clotting disorders were well taken care of. In EUROAGENTEST, cytogenetic and biochemical tests also come up. Comparative genome hybridization is close to clinical application and may be difficult.

In non-invasive pre-natal diagnosis (now semi-routine) – there is a need for controls to ensure that truly foetal (for female) DNA is being used. MfB has been working on this issue; CRMGEN can also share experience and EUROAGENTEST can also collaborate on this and other new technologies. It would also be useful to have sensitivity level controls for maternal cell contamination studies, where in-house controls are currently used.

Open Discussion/Future Plans

- Joint EU/US Efforts. So far there has been shared discussion, attendance at meetings, participation in email discussion and conference calls, but no formal collaborations. It is important to ensure work is complementary – rather than duplicated - in as far as the regulatory system will allow (it is not clear whether EC marked materials would be acceptable as controls in the US, or if Coriell material is allowed as controls in Europe - more clarity on this is needed). A 'think tank' may be needed to link EU/US together to ensure no unnecessary duplication and ensure we stay coordinated/connected because there is a lot to do and we're still playing 'catch up' in a situation when there are also many new things emerging
- Formation of a Working Group. EUROAGENTEST needs an advisory working group on reference materials (currently only PC and DB). An email invitation is to be sent out to recruit discretely and the scope and role of this group will be further defined.

Helen Parkes commented that many reference materials had been discussed at this meeting and invited nominations of some 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. Heinz Schimmel added that many IVD manufacturers use materials reviewed and approved by JCTLM and a new call is to go out for nominations. More information available on www.bipm.org/en/committees/jc/jctlm/ and www.ifcc.org. HP will send around invitations to nomination. As far as higher order is concerned, this refers to fit for purpose, high quality, internationally accepted – basically, if the group declares it as such (no ISO

definition of higher order) (this in response to a query on whether RMs always need to be higher order)

Future Plans

The current meeting is part of the CDC series (no 4) and EUROAGENTEST (first). A CDC meeting is proposed in 2006 – prior to the Association for Molecular Pathology meeting in Orlando in November. Ireland will host the next EUROAGENTEST meeting, around May 2007.

Thanks

Joe Boone thanked David Barton and Philippe Corbisier on behalf of the US delegation. In turn, DB thanked PC, Heinz Schimmel and the team at IRMM for hosting the meeting and expressed his gratitude to all the delegates.

Annex 1: Current availability of RMs for genetic testing (draft).

Illness	QC Material available at CDC	Coriell Material with Characterised mutations	Coriell cell lines with identified mutations	gDNA with identified mutations or deletions (Δ)	Synthetic DNA	PCR Product
Angelman Deficiency ¹			2 chr Δ	3 chr Δ	0	0
Bloom ² syndrome		13	25	Coriell (4)	0	0
Breast Cancer BRCA1 BRCA2		23 8	25 9	Coriell (29) Coriell (17)	0 0	0 0
Cystic Fibrosis	6 cell lines 28 gDNA	47	109	Coriell (92)	MQCS InnoGenetics (CFTR)	

¹ Approximately 70% of cases of AS have a deletion of 15q11-q13 in the maternally contributed chromosome 15.

² Bloom syndrome is a disorder of DNA repair characterized by chromosomal instability, predisposition to malignancy, growth deficiency, and sun-sensitive facial telangiectasias. Bloom syndrome, which is an autosomal recessive disorder, is caused by mutations in the RECQL3 gene and has an increased incidence in the Ashkenazi Jewish population.

Factor II		2	6	Coriell (3) WHO/NIBSC (1)	IRMM (3) Roche Diag (1)	
Factor V Leiden		1	117	Coriell (7) WHO/NIBSC (1)	Roche Diag (1)	
Down syndrome			110	Coriell (8)		
Fragile X	16 cell lines 8 gDNA	1	55	Coriell (55)	NIST (1)	
Galactosemia		6	44	Coriell (6)		
Hereditary Haemochromatosis		7	27			
HLA			27	Coriell (2)	Innogenetics	
Huntington Disease	14 cell lines 9 gDNA (NIST)	1	3	Coriell (13)		
Muscular dystrophies	0	1		Coriell (1)		
Prader Willi Syndrome ³		2 chr Δ	1	Coriell (1)		
Sickle cell anemia		0	12	Coriell (12)		
Thalassaemia		9				

³ Prader-Willi syndrome (PWS) is a complex disorder whose diagnosis may be difficult to establish on clinical grounds and whose genetic basis is heterogeneous. Slightly >70% of cases are due to a 15q11-q13 deletion in the paternally contributed chromosome.