THE SECOND GENETIC TESTING REFERENCE MATERIALS PROGRAM (GeT-RM, formerly GTQC) EXPERT PANEL MEETING

November, 14-15, 2006, Orlando, Florida

Introduction

The Genetic Testing Reference Material Coordination Program (GeT-RM, formerly called GTQC) was created based on recommendations from three previous US Centers for Disease Control and Prevention (CDC)-sponsored QC Materials for Genetic Testing meetings held in 2003 and 2004. The goals of the GeT-RM are to coordinate a self-sustaining community based process to improve the availability of appropriate and verified materials for quality control, proficiency testing, test development/validation, and research purposes; as well as to facilitate information exchange between users and providers of reference materials.

The first GTQC Expert Panel Meeting, organized by the CDC, was held on November 29, 2005, in Turnhout, Belgium, in conjunction with the First International Symposium on Reference Materials for Genetic Testing organized by Eurogentest and the Institute for Reference Materials and Measurements (IRMM). A summary of this meeting can be found on the GeT-RM website under GTQC/GeT-RM Program Updates (http://wwwn.cdc.gov/dls/genetics/qcmaterials/default.aspx). The second GeT-RM Expert Panel Meeting, also organized by the CDC, was held on November 14-15, 2005 in Orlando, Florida. The 31 meeting participants included experts in genetics and genomic testing from professional organizations, government agencies, industry, commercial and academic clinical laboratories, and cell repositories. The main goals of the meeting were to:

1. Review progress of the GeT-RM program since November 2005
2. Discuss issues and obstacles of the current activities, and strategies for moving forward
3. Review and update, if needed, previously identified reference material needs and priorities
4. Explore the potential for new areas of reference material development such as infectious disease and/or molecular oncology
5. Discuss opportunities for coordination and collaboration nationally and internationally
6. Discuss evaluation of the success/effectiveness of the GeT-RM program and suggest improvements needed
7. Consider next steps (future activities and next meeting)

Meeting Summary

The first day of the meeting began with welcoming remarks and charge to the group by **Drs. Joe Boone** and **Bin Chen,** CDC. This was followed by a series of presentations describing the GeT-RM program, current and recently completed reference material mutation confirmation studies and updates and reports from Coriell Cell Repositories,

and European reference material activities. Subsequent discussion sessions were held to solicit input from the participants on the current projects and activities, potential for program improvements and opportunities for coordination and collaboration both nationally and internationally.

On the second day of the meeting, through several presentations and interactive discussion, the meeting participants explored the possibility of expanding the program to include reference materials for infectious disease and molecular oncology testing.

Presentations:

GeT-RM Program Overview
Dr. Lisa Kalman, CDC, presented an overview of the GeT-RM. She discussed the recent decision to use the term reference materials rather than QC materials, hence changing the name of the program from the Genetic Testing Quality Control Material Program (GTQC) to the Genetic Testing Reference Material Coordination Program (GeT-RM). Lisa then reviewed the process used by GeT-RM to develop reference materials and summarized the results of the fragile X, Huntington disease and Ashkenazi Jewish mutation confirmation studies. She also discussed recent activities to develop new cell lines, and described efforts to collect data and develop tables listing information on the genotypes of existing cell lines and genomic DNA that could be used as reference materials for pharmacogenetic testing. Lisa concluded her talk by presenting a list of the reference materials that will be confirmed in the near future and discussed reference material priorities for the coming year.

Development of Reference Materials for the Ashkenazi Jewish (AJ) Genetic Testing
In 2004, the American College of Obstetricians and Gynecologists recommended carrier screening for individuals of Ashkenazi Jewish (AJ) descent. **Dr. Kasinathan Muraliharan,** Quest Diagnostics, presented the final results of the GeT-RM (GTQC) project to develop reference materials for testing of the AJ disorder panel. Twenty seven Coriell cell lines representing clinically important mutations in 9 disorders commonly included in Ashkenazi Jewish screening (Bloom syndrome, familial dysautonomia, Canavan disease, Niemann-Pick disease, Tay-Sachs disease, Gaucher disease, glycogen storage disease, fanconi anemia group C and mucolipidosis type IV) were selected for the reference material panel. DNA was prepared by the Coriell Cell Repositories and aliquots were sent to each of 6 volunteer clinical genetic laboratories for mutation confirmation studies. A variety of testing platforms were utilized by these laboratories. The laboratories identified 21different disease causing alleles out of a total of 32 tested alleles. There were no discrepant results between the testing laboratories. The results of this study are presented on the Get-RM and Coriell websites and the materials are available from Coriell.

Development of Reference Materials for Huntington Disease CAG Repeat Sizing
Dr. Lisa Kalman presented the final results of GeT-RM's (GTQC) Huntington disease reference material project. Fourteen Huntington cell lines from Coriell, containing a large range of CAG repeat sizes and allele combinations representing important diagnostic

cutoffs were selected for study. Coriell prepared DNA from these cell lines, and CAG repeat size measurements were performed in 10 volunteer clinical genetic laboratories. These studies were coordinated by **Dr. Sue Richards,** Oregon Health Sciences University. The 10 volunteer laboratories for this project used PCR-based in-house developed assays with a variety of primers and detection methods. DNA sequence analysis was performed by the National Institutes of Standards and Technology (NIST). These materials are publicly available from Coriell and information regarding their CAG repeat sizes is listed on the GeT-RM and Coriell websites. A manuscript describing this study is in preparation.

Fragile X Reference Materials

Dr. Jean Amos Wilson, Focus Diagnostics, began her presentation with an overview of the need for fragile X reference materials. She then discussed the progress of a project sponsored by the Association of Molecular Pathologists (AMP), in conjunction with GeT-RM (GTQC) to develop reference materials for fragile X genetic testing. The Fragile X-perts, a group of individuals from the genetics community representing clinical testing laboratories, government, industry, and the Coriell Cell Repositories, selected sixteen fragile X cell lines, with a variety of CGG repeat lengths representing clinically important diagnostic cutoffs ranging from normal to full mutation for study. The CGG repeat lengths in each DNA sample was determined by consensus verification in 9 volunteer laboratories using both the laboratory's in-house method as well as a commercial prototype assay from Celera Diagnostics. This verification study is coordinated by **Dr. Amos Wilson**. DNA sequence analysis of the male cell lines was performed by NIST. Preliminary results indicate that the laboratories were able to reach a consensus CGG repeat length measurement for 12 of the 16 cell lines. Consensus was more difficult to achieve as the CGG repeat length increased, and one of the cell lines was found to be mosaic. When the data analysis has been completed, the estimated CGG repeat lengths and variance of each measurment will be listed on the GeT-RM and Coriell websites and the DNA and cell lines will be publicly available from Coriell. The results of this study will also be published.

Update from the Coriell Cell Repositories

Dr. Lorraine Toji, Coriell Repositories, presented an overview of Coriell's role in reference material development process. She provided information about the development of new cell lines from residual blood, information about cell lines that will be studied in the near future and some thoughts about the sales of previously studied materials.

Reference Material Development Activities in Europe

Dr. David Barton from the National Centre for Medical Genetics, Dublin, Ireland, presented a summary of reference material related activities in Europe. He discussed work by Eurogentest and reference material developers such as NIBSC and IRMM to prioritize and develop new materials. Dr. Barton summarized the results of a number of surveys in Europe and the US to prioritize reference material needs. Fragile X, cystic fibrosis, FVL/prothrombin, hemachromatosis and BRCA were the most commonly indicated disorders. In the coming year, Eurogentest plans to work on the development

of SCA and cytogenetic reference materials, develop guidelines for use of reference materials in molecular genetic testing, work to interpret the IVD directive and advise EU labs on the implications of the IVD directive.

Reference Material Needs for Molecular Oncology

Dr. Erasmus Schneider, NY State Dept. of Health**,** presented an overview of the reference material needs for molecular oncology molecular genetic testing. In his talk, Dr. Schneider discussed the characteristics of an ideal reference material, described the various types of molecular oncology assays currently in use, and outlined some of the issues and difficulties associated with these tests and the implications for reference materials. He then described the potential sources of reference materials and described the difficulties that may be associated with reference material development. There are many publicly available cell lines that can be used as reference materials. Dr. Schneider recommends developing a consensus for which cell lines should be used as reference materials for each test/assay. He suggested that the chosen materials should then be rigorously characterized and made available to the testing community.

Reference Material Needs for Infectious Disease

Dr. James Versalovic, Baylor College of Medicine, presented a discussion of the current reference material availability and needs for infectious disease testing. Dr. Versalovic described four categories of testing for molecular microbiology: qualitative testing, quantitative testing, genotyping or sequencing and molecular epidemiology. He presented the questions from the CAP checklist that apply to QC of these various categories of testing and outlined the difficulties labs face when they develop their QC and QA procedures. Although a number of vendors provide QC and reference materials for molecular infectious disease testing, there are still many unmet needs. In the future, new reference materials will be needed to meet the needs of novel testing technologies.

Group Discussion

1. Program Issues

A. Change of program name and use of new terminology

The name of the program has been changed from GTQC to GeT-RM (Genetic Testing Reference Material Coordination Program, an acronym suggested by Vicky Pratt). This was done at the request of the FDA to avoid misrepresenting the nature of the materials characterized by this program, to harmonize with the terminology used internationally and to reflect the fact that these materials are useful for many purposes including QC, PT, test development and validation. The group agreed with these changes and endorsed the new acronym.

B. Communication

The group suggested many ways to increase the visibility of the GeT-RM program and communicate the need for genetic reference materials. The ideas expressed included working with the AMP professional relations committee and CLIAC, educating legislators though Kathy Hudson's work with the Genetics and

Public Policy Center and the Annual Legislative workshop in New York. We should also try to increase our visibility by seeking publication of articles in CAP today and the Eurogentest Newsletter, postings in CHAMP, and links to AACC, AMP, ASM and organizations focused on molecular oncology.

2. Completed/current projects and activities

The group discussed the projects that have been recently completed (Huntington, Ashkenazi Jewish) or are in progress (fragile X). Overall, the group was pleased with the outcomes of the studies. We discussed obstacles that arose during these studies, including transcription errors, over-interpretation of data and difficulties obtaining raw data from the participating labs. It was also noted that it is important for the data collection sheets to be user friendly.

3. Efforts for 2007

Pharmacogenetics

Pharmacogenetics tests are becoming more common on genetic laboratory menus even as the list of important polymorphisms is constantly changing. There is one commercial source of genomic DNA reference materials for these tests, but not all genes/polymorphisms are available.

The group discussed ways to monitor pharmacogenetic testing trends. Suggestions included tapping into the AACC, AMP Clinical Practice Committee, and AMP Test Directory. Vicky Pratt, Debs Payne, Elaine Lyon, Jean Amos Wilson and Beth Rohlfs volunteered to start a pgx reference material workgroup to explore the reference material needs of the pgx community and to discuss potential roles for the GeT-RM in the process. (note: Amy Brower Third Wave Technologies, Steve Wong ??? and Saeed Jortani, U. Kentucky, Louisville have also joined the workgroup).

Reference Materials for inherited genetic disorders not previously addressed by GTQC

The group considered where future GeT-RM efforts should be directed. We discussed which tests had the highest need for reference materials, ways to ascertain needs such as looking at test volumes and other considerations such as patent and commercial issues. The group was unable to reach a consensus on reference material needs for inherited genetic disorders. There are a lot of potential targets, however, reference materials for many of the high volume tests such as CF, HD, FX, NBS and AJ panel, have already been addressed by the GTQC. It was suggested that perhaps there are more obvious needs and opportunities for reference material development in the areas of infectious disease and molecular oncology than in inherited genetic disorders. The group agreed to wait until next year to redefine reference material priorities for tests for inherited genetic disorders.

New Areas-

1. **Molecular Oncology**

   Following the presentation by Erasmus Schneider, the group discussed ways to assess which reference materials are needed for molecular oncology, potential sources of these materials, potential contacts and stakeholders and

who should be involved in the process. Several tests, including Jak2 and B and T cell cancers were mentioned as obvious priorities. Quantitative reference materials for BCR/ABL were also mentioned and are being addressed by an international consortium. Many potential material sources were mentioned, including: the National Cancer Institute (NCI), Children's Hospital, Children's Oncology Center and ATCC. Potential stakeholders and collaborators include: commercial developers, NCI, CAP, AMP, and numerous cancer groups. The group agreed that the first step should be to determine what potential reference materials currently exist and to assess, through a survey, what reference materials are needed.

2. **Infectious Disease**

The group agreed that there is a need for reference and PT materials for molecular infectious disease testing. Needs and potential needs discussed included: influenza, vaccine strains, various types of HPV, drug resistant MTB, HIV (drug resistance testing, genotyping and phenotyping), quantitative CMV (being addressed by NIST), MRSA and Vanco-resistant SA. Proficiency testing materials for HIV are getting harder to find as more patients have lower viral loads due to drug therapy, thus there is a need for viral stocks. The participants noted that selection of reference materials should take into consideration the molecular target (gene etc) that is detected by the tests currently used to detect a particular organism. Other considerations include choosing the best type of material for a particular test technology, the needs of the community and whether there is enough demand for commercial producers to develop materials. Participants also pointed out that reference and PT materials are also needed for FDA approved tests, which often detect many more genotypes than are represented in the QC materials included in the kits. The group agreed that information should be gathered about what materials are currently available and what the current and future needs may be. From this list, we can define the focus of further activities. This information should be made available on the GeT-RM, AMP and ASM websites. Potential collaborators and sources of information mentioned include: CLSI, ASM, AMP, CAP.