THE THIRD GENETIC TESTING REFERENCE MATERIALS PROGRAM (GeT-RM) EXPERT PANEL MEETING

November 6, 2007, Los Angeles, CA

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 characterized materials for quality control (QC), proficiency testing (PT), test development/validation, and research purposes; as well as to facilitate information exchange between users and providers of reference materials (RM).

This was the third in a series of annual Expert Panel Meetings following the founding of the GeT-RM Program in 2004. A summary of the first two meetings can be found on the GeT-RM website under GTQC/GeT-RM Program Updates (http://wwwn.cdc.gov/dls/genetics/qcmaterials/default.aspx).

Thirty-five experts in genetics and genomic testing from professional organizations, government agencies, industry, commercial and academic clinical laboratories, and cell repositories participated in this meeting, which was held on November 6, 2007 in Los Angeles, CA.

The main goals of the meeting were to:

1. Review progress of the GeT-RM program since November 2006
2. Discuss issues and obstacles related to 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, molecular oncology and biochemical genetics
5. Discuss opportunities for coordination and collaboration nationally and internationally
6. Discuss the success/effectiveness of the GeT-RM program and suggest improvements
7. Consider next steps (future activities and next meeting)

Meeting Summary

Presentations

The meeting was opened by a welcome and introductory remarks from **Dr. Joe Boone**, Acting Director, Division of Laboratory Systems, National Center for Preparedness,

Detection and Control of Infectious Diseases, Centers for Disease Control and Prevention.

GeT-RM Program Overview

Dr. Lisa Kalman, **Coordinator of the GeT-RM, CDC**, presented an overview of the GeT-RM's activities and accomplishments since the last expert panel meeting. Lisa recounted progress in some projects that had been previously completed. A manuscript describing the fragile X study was accepted for publication in the Journal of Molecular Diagnostics (for January 2008 issue) and another manuscript describing the results of the Huntington characterization study was published in the October 2007 issue of Genetics in Medicine. Lisa also mentioned that 4 newly acquired samples on the Ashkenazi Jewish panel were characterized. A manuscript describing this study is being prepared. Lisa reviewed several projects that were currently underway, including cystic fibrosis and newborn screening, which were also described by later speakers. The talk also described several challenges encountered by the GeT-RM over the past year, including lack of access to patient blood and new cell lines, errors by participating laboratories during characterization studies and ways around these problems. Potential new areas of reference material development, such as infectious disease, biochemical genetics and molecular oncology were introduced, a possible collaboration with the College of American Pathologists (CAP) was discussed. The talk ended with a presentation about possible reference material development priorities.

Cystic Fibrosis Characterization Project

Dr. Vicky Pratt, Quest Diagnostics began her talk with a description of the 2001, 2004 and 2006 American College of Medical Genetics/American College of Obstetricians and Gynecologists (ACMG/ACOG) professional guidelines recommending cystic fibrosis (CF) carrier testing to pregnant couples, recommending a revised mutation panel and recommending newborn screening for CF respectively. Vicky then chronicled the rise in participation in the CAP CF proficiency survey, and the change in CF test methods during this time. With the growing use of CF screening, many new analyte specific reagents (ASRs) for CF testing have become available, all of which test for the 23 alleles recommended by ACMG, and some for many additional alleles as well. Vicky described a recent GeT-RM CF characterization project that was carried out in 6 clinical genetic laboratories using 5 commercially available ASRs and a laboratory-developed assay. This study characterized genomic DNA extracted from 13 Coriell cell lines. Thirteen alleles commonly included in commercially available ASRs and laboratory-developed tests, but not included in the list of 23 recommended by the ACMG/ACOG were characterized.

Newborn Screening (NBS) Samples Collaboration

Dr. Madhuri Hegde, Emory University described the process for test development and validation she uses in her Clinical Genetics laboratory at the Emory University. She also described a number of assay techniques used in her laboratory, including DNA sequence analysis and comparative genomic hybridization (CGH) arrays. Dr. Hegde described a collaboration with the GeT-RM to sequence DNA from 22 Coriell cell lines with mutations in genes associated with disorders included on State Newborn Screening

Panels. She also discussed an IRB approved method to collect blood from consented patients for submission to Coriell for cell line development.

CAP Collaboration

The next talk was presented by **Dr. Vivianna Van Deerlin**, the CAP representative to the GeT-RM. Dr. Van Deerlin described the organization of CAP and the details of its many proficiency testing programs, especially those related to molecular genetics, molecular oncology and infectious disease. She discussed the production cycle for a PT survey, and reviewed the sources of PT samples as well as the limitations on the use of leftover PT samples and the limited potential for PT programs for rare disorders. CAP is addressing PT for tests performed in only a small number of labs with a new program that facilitates sample exchange for alternative assessment. Finally, Vivianna addressed ways in which CAP and GeT-RM could work together to help each other improve the supply of reference and PT materials.

Reference Material Development Activities in Europe

Dr. David Barton, National Centre for Medical Genetics Our Lady's Children's Hospital Crumlin, Dublin, Ireland gave his annual "What is happening across the pond" summation of reference material activities in Europe. David reviewed completed or in progress reference material development plans for 2007, which include work on a Spinocerebellar Ataxia (SCA) RM panel, further trials of CF multiplex RMs and guidelines for use of RMs. Plans for 2008-2009 include work on RMs for new technologies including molecular cytogenetics, collection of new cell lines and discussions with the International Standards Organization (ISO) about RM for quantitative tests. The UK National Genetics Reference Laboratory, Manchester, is working on the development of RMs for Prader-Willi/Angeleman Syndromes and SCA. They are currently looking for potential samples. The UK National Genetics Reference Laboratory, Wessex is currently working with a number of BCR-ABL testing labs worldwide to develop suitable reference reagents to allow accurate conversion of BCR/ABL RQ-PCR data to an international scale. In 2008 the National Institute for Biological Standards and Control (NIBSC) and the World Health Organziation (WHO) are expecting to complete the development of international standard reference materials for Fragile X, Prader-Willi/Angelman, HLA-DRB1. Development of international standards for hereditary nonpolyposis colorectal cancer (HNPCC), CF, Red Cell Blood Grouping and hereditary hemochromatosis (HH) is expected in 2009.

Molecular Oncology Needs Assessment Survey

The results of a Molecular Oncology needs assessment survey conducted by the Association for Molecular Pathology (AMP) was presented by **Dr. Erasmus Schneider, New York Department of Health**. The survey collected data on current and anticipated test methods, RM and QC material usage, needs and sources for 57 molecular oncology tests. Thirty four responses (~25%) were obtained. Data from the survey indicates that for the most part, the laboratories have been able to identify a source of QC/RMs for all tests. A few needs were identified, mostly for quantitative tests. **Dr. Shannon Barker**, a new **Association for Prevention Teaching and Research (ATPR) fellow** with the GeT-RM program at CDC, discussed the possible ways that the GeT-RM may help to improve the availability of characterized reference materials for molecular oncology. Shannon suggested that the

GeT-RM could Identify needs for new reference materials (RMs) including cell lines and genomic DNA, characterize new and existing RMs and prepare a comprehensive listing of available RMs on the GeT-RM website. Shannon presented a list of available reference material sources, which includes a number of cell repositories and commercial manufactures and asked the group to consider whether and how GeT-RM could move into this area.

Infectious Disease Needs Assessment Survey

Dr. Aaron Bossler, University of Iowa presented the results of an infectious disease RM needs assessment survey that was distributed on the AMP member listserv (CHAMP). The purpose of this survey was to identify areas of need with reference materials and proficiency testing in molecular infectious disease testing and to identify labs that might have resources for new RMs. Information gained from this survey may be used to help guide future efforts of the GeT-RM reference material development. The survey asked, for each bacteria or virus tested- what technology is used for the test, the target gene or sequence for the assay, the current source of RMs for the test, the source of that material and what RMs were needed. A total of 28 labs responded to the survey indicating testing of a number of pathogens. The results of the survey indicated that RMs of one sort or another were available for most tests, although a few needs, mostly quantitative, were identified. **Dr. Shannon Barker** presented a talk discussing the potential role of the GeT-RM in development of RMs for infectious disease testing. She presented a list of available RM sources and discussed the creation of a database of infectious disease RMs. She also solicited the group to discuss whether the GeT-RM should try to develop RM in this area and if so, what RMs would be needed.

<u>Group Discussion</u>

Reference Material Priority-The group made many suggestions of potential reference material development projects and discussed the issues and feasibility of each. These include-

- Myotonic dystrophy (repeat sizes near the cutoff), suggested collecting patient samples at clinics
- Lysosomal storage diseases- will be added to newborn screening panels
- Samples for technology-based QC and PT (eg. sequencing, aCGH)
- Samples representing differences in DNA methylation, epigenetic changes

The group discussed methods by which potential reference material development projects could be identified and prioritized. They suggested developing an algorithm. Possible criteria for the algorithm could include: risk, test volume, unmet needs, long repeats, clinical utility, prenatal, range of mutations, risk of error, platform (new platforms, multiplex, difficult assays). In addition, feedback from PT schemes may be useful to help identify priorities.

Lisa and Shannon will develop an algorithm with the help of members of the Expert Panel. A draft of this algorithm will discussed at next GeT-RM meeting.

Cystic Fibrosis- Vicky's presentation generated much discussion. The group debated whether we were inadvertently endorsing CF mutation panels that went well beyond the 23 alleles recommended by ACMG. There is almost no data on the majority of these alleles to indicate which are significant in the population, which are truly pathogenic and which are not (clinical validity). Are we sending the wrong message to the labs when we provide reference materials for alleles not on the ACMG screening panel? It was also noted that the purpose of the GeT-RM program is to help labs with the analytic validity of their assays. If the allele is included in an assay, then we should promote the availability of reference materials needed to assure the quality of the assay.

The group noted that functional studies of all 1500 CF mutations are being done, which will help labs to know which mutations are pathogenic. ACMG needs to update the frequency data on the various mutations.

Idea for manuscript
The suggestion was made to write a paper describing the accomplishments and lessons learned during the first few years of the GeT-RM program. The paper would essentially start where the Genetics in Medicine article by Chen et al., which describes the first two CDC sponsored Quality Control for Genetic Testing meetings. A workgroup was formed to develop an outline and write the paper.

Information
It may be possible to use information, such as PT challenge results from previously uncharacterized, publicly available cell lines to initiate the development of new RMs. It might also be possible to obtain data from labs that use publicly available samples for assay validation. Perhaps this information could be posted on the GeT-RM website without indicating its source.

Sources of Potential Reference Materials
PT providers have a number of material sources. ATCC is now allowing limited use of its samples for PT. The German Cell repository (DSMZ) does not allow their samples to be used for PT, but are being asked to consider it. Other sources, such as the National Cancer Institute Repository, have limited access, but might be useful as well. The group also considered the idea of collecting data about the genotypes of patients in various patient registries and possibly approaching them to collect blood samples for cell line development. This approach would facilitate collection of samples from both affected patients and carriers.

Molecular Oncology
The results of the AMP molecular oncology reference material survey indicated that labs seem to feel that they have most of the reference materials that they currently need. The majority seem to be using residual patient specimens, so they may not have an urgent need for RMs until their supply runs out! It was suggested that perhaps the survey focused on QC materials and did not mention validation materials. This may have influenced the responses. Respondents may have been reluctant to answer the survey completely for fear of admitting that they are violating intellectual property laws.

The group agreed that the molecular oncology community does need controls for quantitative tests and also sensitivity controls. There is an international effort currently underway to develop a BCR-ABL calibrator using a quantitated mixture of DNA from several cell lines. They hope to produce a certified reference material (CRM) and also get WHO certification. Suggestions were made for controls for BCR-ABL with defined breakpoints.

There are several sources of cell lines or tissue samples that could be used to develop reference materials for molecular oncology, none of which are ideal. The DSMZ Cell Repository in Germany has a number of highly characterized cancer lines, and great quality control, however, they are unwilling to sell to commercial labs, which would be a problem for our purposes. The ATCC repository also has a good collection, however, it has been difficult to reach an agreement with them for use of the two JAK2 cell lines. A company called InVivoScribe also sells a sensitivity control for BCR-ABL, however, the cell lines used to make this product vary with each batch and labs have complained that it is not consistent enough to use as a standard. Coriell Cell Repositories has only 26 cancer cell lines and cannot accept more unless they represent inherited diseases.

Cancer cell lines are often unstable and the group suggested that it may be useful to consider alternatives. The group discussed options to genomic DNA controls. Synthetic DNA could be useful as a reference material for point mutations, but won't work for B and T cell assays because labs use different primers in their assays. Armoured RNA and/or plasmids may be a practical alternative. Controls for sensitivity testing could be created by spiking BAC clones into normal DNA.

The group suggested that we talk with other organizations that might be of help. Possibilities suggested include C-Path and the National Cancer Institute.

A small group of volunteers was organized to work on these issues.

Infectious Disease (this discussion was opened up to the AMP membership)
A number of suggestions were made by the expert panel members as well as the AMP attendees present. These include:

- Need to provide sources of information about existing reference materials
- Need a number of RM types including:
    - Quantitated standards
    - Matrix appropriate controls
    - Sensitivity controls
    - Quantitative cytomegalovirus (CMV), BK virus, Epstein-Barr virus (EBV) standards
    - High, medium, low level standards for quantitative assays
    - Standards for lower limit of detection
    - Metapneumovirus (hMPV)
    - Sepsis markers
    - Human host factors

- Materials in proper matrix

The group discussed that none of the available controls are traceable to any standards. A representative from Assuragen mentioned that they sell controls that are traceable to materials from NIST.

All agreed that communications between labs and commercial manufacturers is essential.

Next meeting- AMP 2008 in Grapevine TX