THE FIRST GENETIC TESTING QUALITY CONTROL MATERIALS PROGRAM (GTQC) EXPERT PANEL MEETING

November, 29, 2005, Turnhout, Belgium

Introduction

The Genetic Testing Quality Control Material Program (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 GTQC 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 QC 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). The 14 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) Assess the progress of the GTQC in its first year of operation Nov. 2004 – Nov. 2005 including current QC material development projects (cystic fibrosis, fragile X, Huntington disease, Ashkenazi Jewish panel), website and communication efforts, and logistical issues including human subjects research issues and US regulatory oversight; 2) Identify current obstacles and discuss strategies to overcome these obstacles; 3) Discuss plans for the next year, including strategies to move current efforts forward, new QC material development priorities, and needs assessment projects; and 4) Discuss potential interactions and collaborations with the European QC material development efforts such as Eurogentest.

Meeting Summary

The meeting began with welcoming remarks and charge to the group by **Dr. Joe Boone** and **Dr. Bin Chen,** CDC. This was followed by a series of presentations describing the GTQC program, current QC material development projects and updates and reports from Coriell Cell Repositories, the US National Institute of Standards and Technology (NIST), and the US Food and Drug Administration (FDA). 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.

The participants expressed positive comments on the progress of the GTQC in its first year of efforts. The participants felt that the program should continue with its current efforts and discussed new areas of focus for the coming year.

Presentations:

GTQC Program Overview

Dr. Lisa Kalman, CDC, presented an overview of the GTQC. She reviewed the recommendations from the first three QC Materials for Genetic Testing meetings and described how each recommendation was incorporated into the structure of the GTQC program. The talk also described the process used by GTQC to develop QC materials, the GTQC website, human subject research issues, and the progress of QC material development for DNA-based testing of fragile X, Huntington disease, Ashkenazi Jewish panel and cystic fibrosis. QC material priorities established at the November 2004 meeting were reviewed and potential new priorities were presented.

Development of QC Materials for the Ashkenazi Jewish (AJ) and Cystic Fibrosis Panels

In 2004, the American College of Obstetricians and Gynecologists recommended carrier screening for individuals of Ashkenazi Jewish (AJ) descent. **Dr. Lisa Kalman,** presented the GTQC project to develop QC materials for testing of the AJ disorder panel. Based on analysis of AJ testing panels from a number of clinical laboratories, 30 Coriell cell lines representing clinically important mutations in 9 disorders (Bloom syndrome, familial dysautononia, Canavan disease, Niemann-Pick, Tay-Sachs, Gaucher, glycogen storage, fanconi anemia and mucolipidosis) were selected for the QC material panel. There are a number of clinically important mutations for these disorders that are not represented at the Coriell Cell Repositories and will need to be obtained. Coriell has prepared DNA from these cell lines and verification studies are being planned.

Lisa presented the QC material status for cystic fibrosis testing. Coriell has a set of 23 cell lines that represent the 23 CF mutations recommended for carrier screening by the American College of Medical Genetics. Although these materials are widely used as QC materials, only 6 of the 23 alleles have been independently verified. Analysis of clinical CF testing panels from 35 U.S. laboratories revealed that genomic QC materials exist for only 42 of the 102 CF mutations tested in the U.S. laboratories, while independently verified genomic QC materials are available for only 28 mutations. The potential QC material needs for CF testing and ways to prioritize these needs were discussed.

Development of QC Material for Huntington Disease CAG Repeat Sizing

Dr. Lisa Kalman presented an overview of GTQC's Huntington disease QC material development project. Based on consultations with the genetic testing community, 14 Huntington cell lines from Coriell were selected for development into QC materials. These cell lines contain a large range of CAG repeat sizes and combinations representing important diagnostic cutoffs. Coriell has prepared DNA from these cell lines, and verification studies, coordinated by **Dr. Sue Richards**, are currently underway. The 10 volunteer laboratories for this project use PCR-based in-house developed assays with a variety of primers and detection methods. DNA sequence analysis will be performed by the National Institutes of Standards and Technology (NIST). Upon completion of the verification studies, these materials will be publicly available from Coriell and

information regarding their CAG repeat sizes will be listed on the GTQC and Coriell websites. The results of this study will be published.

Fragile X QC Material Development

Dr. Jean Amos Wilson, Scientific Director-Genetics, Focus Diagnostics, began her presentation with an overview of the need for fragile X QC materials. She then discussed the progress of a project sponsored by the Association of Molecular Pathologists (AMP), in conjunction with GTQC to develop QC 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, formulated a "wish list" of fragile X QC materials. Fifteen fragile X cell lines, with a variety of CGG repeat lengths representing clinically important diagnostic cutoffs ranging from normal to full mutation, were generously donated by **Dr. Stephanie Sherman,** of Emory University, to Coriell for this project. DNA from these 15 lines and one additional cell line is currently being prepared by Coriell. The CGG repeat lengths in each DNA sample will be 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 will be performed by NIST. When the verification studies have been completed, the estimated CGG repeat sizes and 95% confidence interval of each cell line will be listed on the GTQC and Coriell websites and the DNA and cell lines will be publicly available from Coriell. The results of this study will be published.

Update from the Coriell Cell Repositories

Dr. Jeanne Beck, Director of the Coriell Cell Repositories, presented an overview of Coriell's role in QC material development and provided a summary of DNA sample shipments for use as positive controls. She also described the results of needs assessments surveys. Dr. Beck reported that large-scale DNA preparations have been completed for 8 of the 16 fragile X cell lines. Coriell has shipped 5 previously characterized fragile X DNA to the volunteer laboratories for use during the verification studies. DNA from all 14 cell lines on the Huntington QC material panel was prepared and shipped to 9 of the 10 verification laboratories as well as NIST. Analysis of shipment information indicated that approximately 18.8% (domestic) and 26% (international) of all DNA samples purchased from Coriell were intended as positive/negative controls. Twenty nine percent of DNA samples purchased in the US for use as positive controls were from CF, fragile X, FVL, MTHFR, HFE and HD cell lines. Thirteen percent of the DNA purchased in the US for use as positive controls was for cystic fibrosis. Dr. Beck presented the results of surveys conducted at 7 scientific meetings from 2001 to 2005. Survey respondents most frequently indicated that they would like to obtain characterized samples containing fragile X, CF, BRCA 1/2, FVL, and MTHFR mutations.

National Institute of Standards and Technology (NIST) Update

Kristy Richie, NIST, discussed plans to develop a standard reference material for Huntington disease (HD) testing. NIST has selected HD samples with 10 CAG repeat

sizes. These materials are also represented in the GTQC HD QC material panel. Ms. Richie and **Dr. John Jakupciak** are currently developing procedures to amplify the CAG repeats and isolate the PCR products. They plan to sequence the isolated PCR products to precisely determine the allele size. These data will be shared with the GTQC. NIST hopes to use these materials to develop a Standard Reference Material (SRM) for HD testing.

QC and Reference Material for Genetic Testing- Regulatory Aspects
Dr. Zivana Tezak, Food and Drug Administration (FDA), presented an overview of FDA regulations focusing on QC materials. She reviewed risk-based device classification, regulatory requirements for different types or classes of materials, regulatory strategies, and requirements for FDA submissions for QC materials. She shared with the group the type of regulatory oversight and requirements FDA has for QC materials, while clarifying that the use of control materials in clinical laboratories is required by the Clinical Laboratory Improvement Amendments (CLIA) regulations under the Centers for Medicare and Medicaid Services (CMS), not FDA. She also explained that QC materials that are subject to FDA review include unassayed controls, defined as materials that have no assigned values or are not linked to in vitro diagnostic (IVD) devices; and assayed controls that have assigned values and can be linked to specific IVD device.

Group Discussion

1. Current Projects and Activities

Cystic Fibrosis QC Materials
The group discussed the current status and availability of QC materials for CF genetic testing. The 23 CF alleles recommended by the ACMG are represented in the Coriell CF panel. The GTQC has been unable to recruit volunteers to perform DNA sequence analysis to confirm the mutations in these cell lines. The group suggested that these cell lines may have been sequenced by test developers and clinical laboratories during CF assay validation and for the FDA clearance of the TM Biosciences CF assay. It may be possible to obtain needed data from these sources.

- Action item- Dr. Kalman will check with test developers and clinical laboratories to see if sequence data on the Coriell CF cell lines can be obtained. (Post meeting note: Dr. Michele Caggana has provided sequence data confirming the mutations in most of the 23 CF lines on the Coriell CF QC panel. She has kindly agreed to sequence DNA from the 5 remaining cell lines to complete the panel.)

The group also discussed prioritization of future CF QC material development. The group felt that priorities should be based on alleles included in commercial platforms, rather than those on specific laboratory panels. In addition, QC materials reflecting CF mutations found in at least 0.1% of the African American and Hispanic populations, as well as certain other alleles such as I507V, should be given priority. Further decisions and prioritization should be based on ACMG recommendations, mutations included on

CF IVD products, and allele frequencies from the CF foundation, Kaiser of California databases.

- Action item- Dr. Kalman will assemble a list of CF mutation priorities and circulate within the group for further insights.

Ashkenazi Jewish QC Materials

The group discussed the plans for verification of the AJ QC panel. Most laboratories in the genetics community seem to be using the TM Biosciences platform for AJ testing. The group felt that it was important to include a variety of assays in the verification studies to detect potential problems in test performance and control procedures. Mutations included in AJ testing panels that are not currently available from Coriell should be obtained. This will probably require collection of blood from consented carriers or patients. It was suggested that maple syrup urine disease (MSUD) be included in the AJ QC material panel and all mutations in the QC materials be confirmed by DNA sequence analysis. The group also felt that test developers may have already sequenced these mutations, or the data could be easily generated using platforms that employ DNA sequencing.

- Action item- Dr. Kalman will work with the genetics community to locate cell lines representing mutations in the AJ panel that are not available from Coriell. She will also do the necessary logistical work to secure, if necessary, collection of blood from consented patients or carriers. She will coordinate the donation of these materials to Coriell and arrange verification studies.

Huntington Disease QC Materials

The group was pleased with the progress of the HD QC material project. They suggested that alleles with expansions larger than those on the current panel (largest = 86 repeats) be developed. Dr. Beck indicated that Coriell may have some HD cell lines with alleles in the 200 repeat range that could be used. She will check to see if the submitter indicated whether the expansions are associated with juvenile onset Huntington disease. These materials will not be included in the current project, but will be developed separately.

- Action item- Dr. Kalman will coordinate the verification of a Coriell Huntington cell line with approximately 200 CAG repeats. This sample will first be tested by Dr. Sue Richards. If appropriate, other testing laboratories will be contacted to verify this sample.

Fragile X QC Materials

The group expects the fragile X test volume to increase in the near future due to a new report published by Dr. Stephanie Sherman in Genetics in Medicine on female carriers of FMR1 premuations and premature ovarian failure and the possibility of newborn fragile X screening.

There was discussion of the clinical cutoff near the top of the gray zone. Fragile X alleles with 59 CAG repeats have been shown to expand during female meiosis. The possibility of full expansion from 56 repeats has been suggested but has not been proven. The group decided that it is important for laboratories to be able to accurately size alleles in the 56-59 repeat range. The current fragile X QC material panel has a male with 54 repeats and

a female with 55 repeats. It would be desirable to obtain additional cell lines with 56 and 59 repeats. We should also try to obtain cell lines with repeat sizes between 120 and 200. The group agreed that controls for methylation are also desirable, however, it is unclear whether methylation status would be stable in cell culture.
The verification studies have not yet begun. Celera Diagnostics is training each of the 9 laboratories on the use of their assay. So far, the repeat length measurements from the home-brew and the Celera assays seem to agree.

- Action item- Dr. Kalman will work with the genetics community to collect and submit cell lines or patient samples representing fragile X alleles with between 56-59 repeats as well as other needed repeat sizes. She will coordinate appropriate verification studies.

Website and Information Dissemination
The group discussed ways for the GTQC to more effectively disseminate information about its activities and QC material availability to the genetics community. The suggestions include:

- Create links in related websites (including the American College of Medical Genetics (ACMG), the Association of Molecular Pathologists (AMP), NY Dept of Health, The International Meeting of Clinical Laboratory Genomic Standards (IMCLGS), the College of American Pathologists (CAP), and the Human Genome Variation Society (HGVS)) to the GTQC website.
- Seek to have the program and its activities featured in publications such as CAP Today and AMP News
- Improve linkage between the Coriell website and the GTQC website
- Publish a paper describing the third QC Materials meeting (November 2004) and the GTQC program
- Publish papers describing the development of QC materials for Huntington disease, fragile X, Ashkenazi Jewish disorders and CF.

- Action item- Dr. Kalman to address the above suggestions and in addition will continue to monitor website traffic and, if possible, try to find out the number of hits coming from Coriell and other relevant websites (with permission).

2. Program Improvement Issues

Professional Guidance Issues
The need for clarification of professional guidance on the use of QC materials was discussed. CLIA requires that laboratories perform QC procedures adequate to monitor testing process and detect immediate errors, but does not prescribe the specific type of QC materials to be used. The group felt that the use of genomic vs. synthetic CF QC materials should be examined in light of the possible FDA clearance of CF supercontrols. In addition, cost issues associated with commercially available control materials were discussed.

Human subject research issues surrounding submission of residual patient blood specimens to cell repositories need to be examined further.

- Action item- The GTQC will continue to seek guidance from the CDC IRB.

Information Dissemination

The group felt that the information provided by GTQC may not be reaching all who could use it. In addition to current information dissemination strategies, we should try to provide information to hospital laboratories and others who may not be a part of AMP. The group suggested information dissemination through organizations including CAP, CLIA and the Joint Commission for Accreditation of Health Care Organizations (JACHO).

Coordination and Collaboration with European Groups and/or Colleagues
Collaboration

The group discussed ways in which GTQC could collaborate with our colleagues in Europe. Suggestions include:

- Share QC needs list with Europeans and encourage them to submit needed cell lines or patient blood samples to Coriell, especially for rare diseases.
- Encourage Europeans to share proficiency testing (PT) data to assess QC material needs
- Encourage Europeans to provide input on QC material needs
- Cooperate on joint QC material development projects
- Development of consensus/agreement documents
- Work together to link analytical data to clinical information
- Work together to develop QC materials for rare disease testing (submission, validation, access)

National and International Needs Assessment and Monitoring

Many possible ways for the GTQC to assess QC materials needs were discussed. The suggestions include:

- Working with the international community to determine what kinds of QC materials are most needed and develop a consensus on how to select needed materials. This collaboration should include Europe as well as Australia and Asia.
- Formation of groups that work in parallel to GTQC to address QC materials needs/development for biochemical genetic testing, molecular oncology and infectious disease.
- Conducting a survey through AMP (and ACMG?) to assess QC material practices and needs. Evaluate results from European QC needs surveys.
- Consulting with CAP and other organizations to assess needs

The GTQC will explore these ideas

3. Next Steps

QC Material Development Priorities

The group suggested tests they felt are in immediate need of QC material development. These include:

- Pharmacogenetic tests (UGT1A1, CYP2D6, CYP2C19, CYP2C9, etc)
- Disorders included on CAP surveys
- Disorders on newborn screening panels including:
    - MCAD - Medium Chain Acyl-Coenzyme A Dehydrogenase Deficiency
    - Galactosemia,
    - MSUD- Maple Syrup Urine Disease
    - PKU – Phenylalanine Hydroxylase Deficiency
    - CAH – Congenital Adrenal Hyperplasia
    - Biotinidase deficiency
- Other genetic disorders including:
    - BRCA1/2 - Hereditary Breast/Ovarian Cancer
    - SMA - Spinal Muscular Atrophy
    - Cx26 - Conexin 26
    - APC - Associated Polyposis Conditions
    - MEN2 - Multiple Endocrine Neoplasia type 2
    - Rhett Syndrome

Researchers and institutions that have special interest in many of these disorders, and who may be able to help our efforts, were identified by the meeting participants.

- Action item- Dr. Kalman will explore the needs of the genetic testing community with respect to testing for the above disorders. She will coordinate development of appropriate QC materials.

QC Practice and Material Needs Assessments

The participants supported the idea of conducting an AMP sponsored survey to identify current QC practices, perceived barriers to proper QC procedures, and current/future QC material needs. Similar surveys have been conducted in Europe, so international collaboration may be possible.

The group suggested that the second GTQC Expert Panel Meeting be held in spring or early fall, 2006. NIST was suggested as a possible location for this meeting.