Final Monograph

# General Recommendations for Quality Assurance Programs for Laboratory Molecular Genetic Tests

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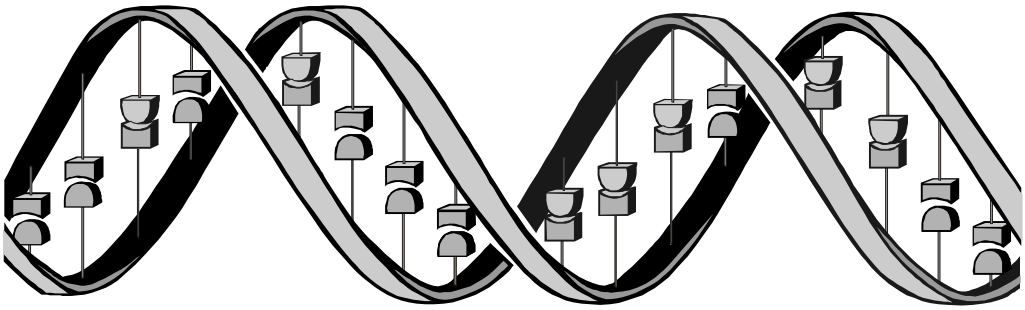

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## GENERAL RECOMMENDATIONS FOR QUALITY ASSURANCE PROGRAMS FOR LABORATORY MOLECULAR GENETIC TESTS

# GENERAL RECOMMENDATIONS FOR QUALITY ASSURANCE PROGRAMS FOR LABORATORY MOLECULAR GENETIC TESTS


## *Summary*

*A comprehensive year-long effort in gathering detailed technical information to characterize the focus of quality assurance (QA) and proficiency testing/performance evaluation (PT/PE) programs for molecular genetic testing (MGT) for human inheritable diseases, has resulted in practical recommendations that could significantly improve the quality of genetic testing laboratory practices. Significant input was received from clinicians, researchers, and laboratory scientists intimately involved with medical genetics and genetic testing in diagnostic laboratories. This effort was carried out in response to the recommendations of the Genetic Testing Work Group to CLIAC (Clinical Laboratory Improvement Advisory Committee, 1988) for CDC research into QA for MGT, with specific emphasis on PE/PT programs.*

*The findings, presented here, represent a compilation of the information gathered, with particular emphasis on efforts that might be addressed in the near future by the Centers for Disease Control and Prevention's Public Health Practice Program Office, Division of Laboratory Systems (CDC/PHPPO/DLS).*

*Critical examination of the technologies used in molecular genetic testing — nucleic acid amplification (NAA), DNA sequencing, Southern blot analysis (SBA), and fluorescence in situ hybridization (FISH) — has resulted in five major recommendations that form the basis of a long-term MGT/QA action plan: 1) conduct pilot research to develop positive controls and test samples for pilot performance evaluation (PE) programs; 2) develop PE programs to supplement what already exists, particularly for diseases and/or methodologies not covered by existing programs; 3) establish laboratory-oriented, disease-specific consortia to provide quality assurance (QA) support as a forum for information networking, and providing methods validation through results comparison; 4) establish and link laboratory oriented and disease-specific databases with other appropriate internet resources; and 5) improve training and continuing education for clinicians, laboratory scientists, and technicians.*


## INTRODUCTION

As a result of the Human Genome Project and other technological advances, MGT has emerged as an area with wide applicability for use in the clinical laboratory. While elements of standardization, proficiency testing, and performance evaluation are in need of improvement or development for diagnostic testing for the most common genetic conditions, the need for assurance of reliable data for rare and newly discovered diseases, as well as those considered as low frequency or regional tests, is equally important.

Despite its tremendous potential, the accuracy and reliability of MGT can be influenced by many factors. The diversity of MGT technology, its rate of evolution, the variety of applications, regional differences in the tests offered and the populations tested, low-volume testing, the lack of standardization inherent in in-house methods, and factors affecting performance, even of commercial test kits, make the assessment of testing quality for MGT both necessary and challenging. The objective of this CDC sponsored project was to characterize the focus of QA and proficiency testing (PT) methods in MGT for human inheritable disease (as specified by contract #200-98-0011), and to develop recommendations as to how MGT testing can be enhanced, so that laboratory practice may more closely approximate performance goals.

Through information gathering tasks, including literature searches, visits to testing laboratories, consultation with proficiency testing and other organizations, and the convening of panels of national MGT experts, recommendations to improve the quality of laboratory practices for the benefit of public health were developed and are presented in this report. From a group of 28 national MGT experts (*Appendix A*), panel meetings were convened on three different occasions for the purposes of identifying needs for laboratory improvement in MGT and making recommendations for future QA/PT programs [(1)] (See Panel Meeting Synopses, *Appendix B*). Panels consisted of representatives from the medical research and diagnostic laboratory testing areas, current QA/PT/PE program providers, a non-profit cell repository, and other stakeholders in the area of QA for MGT. Panelist affiliations included, among others, the Molecular Pathology Resource Committee of the College of American Pathologists (CAP); Biochemical and Molecular Genetics Committee jointly sponsored by CAP and the American College of Medical Genetics (ACMG); Quality Assurance Committee of ACMG; National Institute of Standards and Technology (NIST), Task Force on Genetic Testing/NIH-DOE-ELSI Working Group of the Human Genome Project; GeneTests [(2)] (formerly HELIX) Database Advisory Board; Writing and Review Committees for the new Standards and Guidelines for Clinical Genetics Laboratories of the ACMG[(3)]; co-authors of the proposed National Committee for Clinical Laboratory Standards (NCCLS) Guidelines for MGT[(4)] and the State of New York Proficiency Testing Program[(5)].

Results of those efforts provided an important resource through which QA/PT groups can design and implement programs to evaluate and maintain quality in molecular genetic testing. The primary objectives of the information gathering were:

- **Identify and characterize the focus of QA and PT programs for MGT, including an examination of test samples and positive controls.** Although the need for measuring accuracy and reliability in MGT is clear, evaluating the most productive methods for doing so is a major concern. Information compiled on representative PT and QA programs should provide CDC with background information necessary to address laboratory improvement through various potential initiatives.
- **Identify a test or group of tests that could be used to monitor quality in MGT.** This involved comparing the utility of performance measures as indicators of general proficiency in MGT, determining the degree of commonality between methods, identifying a test or group of tests that could be used to monitor quality, and determining the types of specimens that might be generically used for MGT proficiency testing.
- **Prepare a monograph summarizing findings and presenting recommendations.** This Information includes descriptions of existing QA and PT programs, as well as evaluation of current testing technologies and their critical pathways, and related performance measures used to gauge laboratory proficiency/performance, resulting in practical recommendations for QA in MGT that should significantly improve clinical diagnostics in the area of inheritable genetic disease.

While the panelists addressed the topic of generic testing early in discussions, and recognized its potential utility, they quickly focused on the fact that considerable and extensive pilot research would be required prior to efficient laboratory application and utilization.

# BACKGROUND

## Description of Technologies

Efforts focused on four technologies used in clinical MGT for human inheritable disease: nucleic acid amplification (NAA), DNA sequencing, Southern blot analysis (SBA), and fluorescence *in situ* hybridization (FISH). Often, laboratories use more than one technology serially (e.g., NAA followed by sequencing or SBA to characterize the amplified product). In other instances, two different MGT procedures are used to corroborate results (i.e., polymerase chain reaction (PCR) and SBA). MGT also is used as an adjunct to routine clinical tests (i.e., metaphase FISH and classical cytogenetics testing).

**Nucleic acid amplification.** NAA methods are based on the amplification of a target, probe or signal. PCR, a target amplification technique, is the most widely used NAA method. It can be performed on a variety of samples, including DNA or RNA derived from whole blood, frozen cell pellets, or tissues, and offers enormous potential as a diagnostic tool. Other examples of NAA methods are strand displacement amplification and self-sustaining sequence replication (target amplification); the ligase chain reaction and Qβ replicase methods (probe amplification techniques); and branched-chain-DNA (bDNA) assays, a signal amplification technique.

**DNA Sequencing.** Sequencing focuses on the order of nucleotides within DNA. Conventional sequencing technologies based on Sanger chain termination methods have been used pervasively by laboratories for many years. Consequently, the technology is well developed, somewhat standardized, and is available from commercial manufacturers in automated system formats. The Sanger sequencing method relies on enzymatic DNA synthesis from a specific oligonucleotide primer. The primer is annealed to the complementary sequence adjacent to the DNA of interest on a genetic element. DNA polymerase is used to extend the primer through the target segment, synthesizing a single strand of DNA while using the opposite strand as a template. DNA polymerases can also incorporate analogues of nucleotide bases. The dideoxy method of DNA sequencing developed by Sanger *et al*. takes advantage of this ability by using dideoxynucleotides as substrates. When a dideoxynucleotide is incorporated at the 3' end of a growing chain, chain elongation is terminated selectively at A, C, G, or T. Cycle sequencing is used to amplify the DNA and requires much less template DNA than single-temperature methods. This is a key benefit in the clinical laboratory where specimen quantity is limited. Cycle sequencing is a simple method in which successive rounds of denaturation, annealing, and extension in a thermal cycler result in linear amplification of the extension products.

**Southern blot analysis (SBA).** Southern blot analysis is the foundation of the applications of recombinant DNA towards the diagnosis of genetic disease. The transfer of electrophoretically separated DNA to membranes provides a highly sensitive method for detection of unique DNA sequences in a large background of unrelated sequences. However, the amount of sample and the length of time required for this technique are disadvantages in the clinical laboratory. Typically, DNA is extracted from cells of the patient and fragmented by one or more of the restriction endonucleases, resulting in many DNA fragments of varying length. The fragments are then separated according to size by electrophoresis, with the smaller ones moving more quickly through the pores of the gel. The fragments are then blotted onto nitrocellulose filter paper and overlaid with a labeled probe. The probe will seek out its complementary segment of DNA, which can then be visualized by autoradiography or other detection methods, such as fluorescence. The basic Southern blotting protocol can be adjusted to better suit a specific application or the desired aim of the analysis. Variable parameters include the restriction enzymes used for DNA digestion, the conditions used to prepare and run the agarose gel, the

transfer method, the type of membrane used for binding the DNA, the hybridization solution used, the type of label incorporated into the probe, and the detection method.

**Fluorescence *in situ* hybridization (FISH).** FISH uses fluorescently labeled nucleic acid probes to detect specific DNA or RNA targets in tissue sections, intact cells, or chromosomes, combining the specificity and sensitivity of nucleic acid hybridization with the ability to obtain cytogenetic and morphologic information. The basic underlying principle is the intrinsic ability of single-stranded DNA or RNA to anneal specifically to a complementary sequence and form a double-stranded hybrid. The nucleic acid targets remain localized in tissue sections, intact cells, metaphase chromosomes or interphase nuclei attached to glass slides. Hybrids between the target sequence and labeled probe are detected by microscopy and can be viewed in relation to chromosomal structure or tissue morphology. Metaphase FISH is adjunctive to the field of cytogenetics, and includes chromosome paints; chromosome markers; tests for translocations and mosaicisms; and tests for the presence or absence of markers/genes indicating aneuploidies, duplications, and microdeletions. Interphase FISH involves a variety of specimen types and procedures. It is used to detect numerical abnormalities in genes and chromosomes; gene duplications, deletions and rearrangements; sex chromosome constitution; mosaicism; and gene amplification.

## Applications Most Commonly Used

Inheritable disease MGT is used in the diagnosis of symptomatic individuals, carrier screening, prenatal testing, newborn screening, in tests for genetic factors that may be associated with adult onset disease, and to predict susceptibility to chronic disease. Several hundred applications are currently offered by clinical laboratories [(2)]. A few of the inheritable disease applications most commercialized and widely-available, and thus most commonly seen on laboratory test menus, are: cystic fibrosis, fragile X, and aneuploid FISH to detect abnormalities of chromosomes 13, 18, 21, X and Y. Applications of testing for chronic disease detection, such as the thrombophilia panel, continue to emerge.

The number of applications in testing for inheritable disease is expected to rise rapidly in the near future, because many technologies are already in place and continue to be developed and improved, new genes continue to be identified through the human genome project, and clinical significance data continue to accrue. As a result, a list of today's most common tests may soon be obsolete. For example, as recently as three years ago, laboratories tested for deletion 22 (a.k.a. DiGeorge Sequence, Velo-Cardio-Facial Syndrome (VCFS), and Shprintzen Syndrome) very rarely; today VCFS is recognized as one of the most common microdeletion tests performed. Conversely, linkage tests are expected to become less important as direct analysis methods (such as SBA, NAA, FISH) for specific mutations are developed and become available.

Considerable geographic variation occurs in both the test menus offered and number of tests performed for a given analyte. The most widely offered tests are probably those that are most standardized and relatively easy to perform technically. Yet, the number of requests for these tests may vary widely between laboratories due to the regional variations in the population base and other factors. Therefore some laboratories may have the capability of offering testing services which are infrequently ordered. "Pockets of testing" also exist in which laboratories specialize in tests for rare conditions because of their scientists' research interests. Furthermore, university laboratories sometimes work as regional genetic testing centers. Such specialization may be based on competition for business and/or individual laboratory expertise, particularly in university-based laboratories.

## State of the Art

**Quantitative PCR.** Quantitative PCR is an area with emerging applications of increasing interest to our expert panelists. As this technology is developed and refined, it is becoming increasingly sensitive and useful in diagnostics, particularly with respect to testing for infectious diseases and oncology where issues of sensitivity can be critical. (A sampling of vendors, compiled in 1999, currently supplying testing components is found in *Appendix C*.)

**Automated sequencing.** Current DNA sequencing technology is dominated by fluorescent dye terminator chemistry and continuous or discontinuous laser scanning in a polyacrylamide slab gel or capillary electrophoresis format, although autoradiographic visualization of isotope-labeled sequencing gels is still done in many laboratories. Automated sequencers are marketed by several commercial suppliers (*Appendix C*), and can determine up to 96 independent DNA sequences about 400 to 500 nucleotides long in approximately 8 hours. These sequencing technologies have proven themselves in daily practice, and are becoming sufficiently accurate for most diagnostic applications.

**Specialized and fast sequencing.** Increasingly, sequence analysis of polymorphisms can be an aid in prescribing treatment. For example, in sequencing tests for fragile X and Huntington Disease, the diagnosis and prognosis depend to some degree on the quantitation of the number of trinucleotide repeats. In addition, fast sequence analysis will allow laboratories to perform more tests, thus increasing throughput. Newly emerging clinical applications for DNA sequencing include testing for cardiac-related disorders and drug metabolism (which may be genetically-linked), infectious diseases, and oncology.

**Microarray (chip) technology.** Currently there are several commercially available FDA-approved diagnostic tests using Southern blot analysis (SBA). Experts in both the FISH and the SBA groups said that the microarray, or chip, technology will be of interest to many laboratories and ultimately replace other MGT technologies in some instances, since it will be automated, and will also allow for a large sample throughput. However, cost will likely limit its use to larger-volume laboratories, such as academic centers and reference laboratories. Many participants deemed the chip technology as the most important and widely applicable new technology in MGT, with direct applications in clinical genetics testing. A chip that tests for p53 has already been released (Affymetrix Gene Chip™, Hewlett-Packard Company) and has been designated as an analyte specific reagent. The expert panelists also referred to the minimicroarrays for telomeric sequences as an upcoming technology applicable to the clinical genetics laboratory. Chip technology will continue to develop and it is anticipated that the potential for widespread use will increase markedly in the next 5 – 10 years.

**Advanced FISH.** Laboratories are moving away from traditional FISH methods that use thin tissue sections (<5μm) and conventional fluorescence microscopy. Currently, many laboratories are using optical filters and detectors, combined with image analysis software to capture the results and measure fluorescent signals from FISH. Digital imaging equipment represents an improvement over traditional methods, however, questions arise as to how to validate the manufacturer's performance claims. Commercial test-kits and probes are available for laboratories performing FISH, including: 1) FDA-approved kits (centromeric probes, prenatal aneuploid testing, and HER2/neu for use in breast cancer patients); 2) analyte-specific reagent (ASR) probes, which are expected to rapidly increase in number soon; and 3) research-labeled probes. For the major microdeletions, expert panelists estimated that at least 50% of the probes used clinically are commercially-purchased, although this number may vary geographically. The recently FDA-approved HER2/neu kit has generated interest among laboratories, and is notable from a QA standpoint because it may be used widely by pathology laboratories that have not previously performed MGT.

Perhaps one of the greatest effects of the new and upcoming NAA technologies will be the quantity and scope of the resulting test information. As a result, laboratories will have to consider the problem of data management, as well as methods for handling and storing potentially sensitive information.

## Critical Pathways

The critical pathways outlined below for the four technologies were derived by considering the steps most prone to error; steps in which an error would be most likely to lead to adverse consequences; and steps for which performance could realistically be addressed and monitored. The degree to which steps in any of the technologies are prone to error vary and are based on factors such as the types, variety, and volume of samples received by the laboratory, laboratory experience, test volume, technician training, the level of sensitivity required, the use of commercial versus in-house reagents, the population being tested, the detection system used, and the question being asked.

**NAA.** The critical steps for PCR technology include: 1) DNA isolation, for which specimens, technologies, extraction product purity, reagents, and instrument manufacturers vary tremendously; 2) primers, whose performance can be impacted by population polymorphisms, reagents, primer source, and storage conditions (average shelf life 6 mos-1 yr if frozen); 3) amplification; 4) detection; and 5) interpretation. Although there is tremendous variability within NAA applications, the amplification step is crucial and requires thorough monitoring and QA. This is a most sensitive aspect of the procedure and must be monitored with controls throughout. As in all MGT, major sources of error can include volume/dilution errors, incorrect patient identification, condition and stability of DNA, technical error in mixing reagents, the presence of divalent cations, and variations in room temperature. In addition, for any NAA application, proper primer design and performance are critical, requiring appropriate controls, QC, and verification before use in a test.

**Sequencing.** Critical pathway steps for sequencing include: 1) Sample/specimen acquisition, including collection, transport, and sample tracking; 2) specimen preparation, which impacts the amount and purity of the DNA; 3) PCR (see NAA above); 4) sequencing, which can be impacted by the sequencing system, labeling method, the label itself, clean-up methods, and the hybridization step; 5) data analysis (reading and interpretation of the sequence) for confirming mutations, heterozygosity, or other abnormalities, and 6) results reporting. Virtually all sequencing is currently done using Sanger dideoxy methods and the majority of the testing performed is automated. However, a major source of variation between laboratories for the sequence runs is the primers used. As a result, the steps with the greatest degree of variation are DNA isolation and preparation for sequencing. A second source of error is inaccurate sequence readouts. In addition to sequencing the same template from both ends, laboratories are mitigating this problem in two ways, by 1) running the same control DNA every day, and 2) running replicates when possible. These two steps should be included in any DNA sequencing QA procedures. In general, the expert panelists considered redundancy as an extremely useful mechanism for monitoring the accuracy of the results. It should also be noted that often, expertise is required in reading and interpreting sequencing results, including those obtained through automated methods.

**SBA.** The critical steps for SBA include: 1) Specimen acquisition, including the verification of sample acceptability; 2) DNA extraction, including verification of presence and purity; 3) testing DNA, including the optimization of restriction enzymes and primers; 4) gel electrophoresis and transfer, in which agarose concentration, voltage, gel box variability, method of DNA transfer, and membrane type can affect results; 5) DNA hybridization and labeling of the probe; 6) use of controls, including size standards and hybridization controls; and 7) interpretation and results

scoring, including steps for handling unclear results. While each of these steps should be addressed in a comprehensive QA program, gel preparation and hybridization were described by the expert panelists as major sources of variation. The hybridization step and the stringency conditions must be carefully controlled in order to obtain the maximum positive signal with the minimum amount of noise. The labeling of the probe must be controlled for as well, and positive and negative controls are critical (e.g., cell lines). Obtaining high quality DNA is another error-prone step, involving DNA extraction, purification, and digestion. Performing these tasks requires attention to detail and consistency, and it can take three weeks to six months to adequately train someone to perform theses steps properly. QC checks required throughout a Southern blot include: 1) the use of high molecular weight DNA, 2) a post-digestion run on a mini-gel to monitor/verify digestion, 3) running a hybridization positive control, and 4) consideration of all controls and the appropriate number and position of bands for interpretation.

**FISH.** In FISH testing there are many variations in materials and technique. However, the common critical pathway for any FISH test would consist of the following steps: 1) pretest validation and implementation; 2) receipt of sample and clinician request; 3) sample preparation; 4) protease digestion; 5) DNA denaturation; 6) probe hybridization; 7) washes; 8) analysis; and 9) interpretation and reporting, which is particularly important for tests that are adjunctive to cytogenetics. Pretest validation and results interpretation are the most critical steps for many applications; however, these steps may also be the most difficult to assess for quality assurance purposes. During the pretest validation step, multiple factors should be considered by the laboratory, including: the use of reported reference ranges versus internal controls; allelic probe selection in relation to inherent polymorphisms in certain chromosomal foci; binding efficiencies of the internal control versus the target probe; and validation requirements for rare disease versus more common ones. Stringency optimization will vary with culture conditions, cell fixation conditions, slide-preparation procedures, slide storage conditions, specimen age, the equipment used, and the nature of the probe itself. In terms of determining the presence or absence of a signal and signal characterization, the interpretive component would be similar for all FISH analyses and thus could be included in a FISH QA/PT/PE program

## Current QA/PT Programs

A number of QA/PT programs have become available to molecular testing laboratories in recent years in the areas of infectious disease, oncology, and inheritable disease. The largest of these programs is cosponsored by the College of American Pathologists (CAP) and the American College of Medical Genetics (ACMG) but other programs are in operation as well, and smaller interlaboratory exchanges are also occurring.

College of American Pathologists (CAP) and American College of Medical Genetics (ACMG). CAP/ACMG sends out PT samples through its Surveys program as part of the accreditation process. For laboratories conducting DNA-based tests for inheritable disease, two Surveys are available: the MGL Survey (for molecular testing laboratories) and the CYG Survey (for laboratories performing FISH). For 1999, the MGL Surveys included ten analytes: cystic fibrosis, Duchenne Muscular Dystrophy/Becker, Factor V Leiden, spinal muscular atrophy, spinocerebellar ataxia type 3, fragile X, hemochromatosis, Huntington disease, Prader-Willi/Angelman syndrome, and prothrombin [6]. Two sample shipments are available per year and include ethanol-fixed cell lines or extracted DNA. Interpretive questions are also included. The CYG Surveys include congenital abnormalities shipped annually as metaphase slide preparations. In 1998, 147 laboratories were sent materials for the MGL-A Survey [7], and 121 requested materials for the CYG-A Survey [8]. CAP and ACMG also offer Surveys in cytogenetics, biochemical genetics, and FISH for neoplastic disorders. The Final Report of the Task Force on Genetic Testing cited CAP's estimate that the CAP/ACMG program includes

roughly 85% of molecular genetic testing laboratories [9]. In addition, CAP offers Surveys in molecular oncology, molecular microbiology, *in situ* hybridization, forensic identity and parentage testing.

Sample Exchange. MGT laboratories also participate in less formal sample exchanges for QA purposes. These range from small exchanges involving only a few laboratories to more highly-structured exchanges exceeding 20 laboratories. Laboratories within the geographic boundaries of the Great Lakes Regional Genetics Group (GLaRGG) have organized three of the larger exchanges in the area of FISH, resulting in two publications to date [10, 11].

New York State. New York State requires that laboratories performing diagnostic testing on its citizens (including out-of-state laboratories) meet specific mandatory standards and obtain permits. In 1990, the New York Clinical Laboratory Evaluation Program (CLEP) expanded its surveillance to include DNA and biochemical genetic testing (in addition to cytogenetic testing, which had been licensed since 1972). In 1996, permits were issued to 22 DNA laboratories involved in testing for more than 40 diseases [12]. Although the program for DNA-based diagnostic testing does not include sample sendout, each laboratory must establish its own program, for monitoring proficiency at least twice per year. The program may be internal or external (fee-for-service and/or voluntary testing programs). In addition, laboratories must participate in on-site inspections, submit validation data to obtain prior approval of each new assay, and meet a number of other requirements [13, 14].

The Council of Regional Networks for Genetic Services (CORN). CORN fosters interaction among testing laboratories, resulting in exchanges ranging from a few laboratories to the larger GLaRGG studies cited above. CORN's Southeastern Regional Genetics Group (SERGG) also sponsored a PT program at one time, which has since been discontinued [15]. The bulk of funding for CORN was discontinued as of September, 1999.

Related Programs. Although not designed specifically for MGT laboratories testing for inheritable disease, several other relevant programs are currently operating. For example, the Association for Molecular Pathology (AMP) has sponsored sample exchanges for PCR-based assays among roughly 30 to 40 laboratories [16, 17]. Samples contained chromosomal translocations and immunoglobulin heavy chain and TCR receptor gene rearrangements. In addition, CDC offers performance evaluation (PE) programs for laboratories performing direct NAA detection of *Mycobacterium tuberculosis* [18, 19], as well as *M.tb* drug susceptibility testing [20], HIV viral load assays [21], and HTLV analyses [22] . The Association of Biomolecular Resource Facilities (ABRF) offers a program for research laboratories performing sequencing [23] that could potentially serve as a model for a method-based clinical laboratory program. In addition, the International Tay-Sachs Disease Data Collection and Quality Control Program sends out DNA-based samples to laboratories using enzymatic (non-molecular) methods [24]. Those samples could also be used by laboratories performing molecular-based Tay-Sachs testing.

The programs cited are valued by clinical testing laboratories for the purposes of assessing performance (test validation), continuing education, evaluating test methodologies, and defining test algorithms (*Appendix B*) [25, 26]. However, several (often interrelated) limitations remain (*Appendix B*) [25-27]. Specifically, they include: 1) the range of analytes offered cannot keep pace with the number of clinical tests being offered, partially due to the rapidly expanding menu of tests, and partially due to limited access to sample material, 2) only a limited amount of data is collected and analyzed with regard to pre- and postanalytic areas of testing (which can often be the most critical portions of the total testing process), 3) participation may not be cost-effective for laboratories testing analytes in low volume (This could become critical because of reimbursement issues.), 4) a PT/PE program does not currently exist for clinical testing laboratories in the area of sequencing; 5) PT/PE programs have limited personnel and financial

resources; 6) results turnaround times may be several months; 7) sample sendouts may occur relatively infrequently; 8) access to samples for which incorrect results were obtained is desired by some laboratories; and 9) "borderline samples" are not always available that challenge laboratories regarding test sensitivity, specificity, and other areas.

# RECOMMENDATIONS

The following recommendations were formulated based upon an assessment of current MGT methodologies, their identified QA needs, and existing proficiency testing/self evaluation programs.

## Immediate Needs

The expert panelists identified three immediate QA needs for the molecular genetic testing field that appear consistent with CDC/PHPPO's overall mission: 1) an emphasis on sample development (pilot research) and positive controls, 2) the development of performance evaluation (PE) programs to supplement what already exists, particularly for diseases and/or methodologies not covered by the CAP program, and 3) the establishment and support of disease-specific laboratory-oriented consortia, which could provide a forum for information networking and thus provide validation for different methods through results comparison.

## Detailed Recommendations

Specific methodological, program, and research recommendations were developed following intensive examination, discussion, and evaluation of three areas of critical relevance to molecular genetic testing: 1) an ideal QA program, 2) identification and prioritization of pilot research studies, and 3) involvement of CDC/PHHPO and others. Recommendations were developed in conjunction with an in-depth examination of the critical pathways for QA in MGT for each of the four technologies to identify critical points amenable to performance testing in the laboratory.

**Ideal QA Programs for MGT.** An ideal "global" QA program would encompass laboratory QA practices as well as system support such as that provided by government and professional organizations. Laboratory practices would include the appropriate use of calibrators, QC procedures, QA programs, technical support, and more. Many recommendations were made for system support for laboratories, which could be provided by government agencies, professional organizations, consortia, and combinations thereof. Collaboration with manufacturers in this total QA effort was advocated. The *preanalytic phase* includes information gathering, sample procurement, confidentiality and informed consent, the *analytic phase* includes the sample processing and assaying, and the *postanalytic phase* includes reporting of results, interpretation and confidentiality. Elements of an ideal QA program that addresses these critical phases are shown in *Appendix D*.

**Pilot Research.** Initial or preliminary areas of investigation relative to the improvement of MGT laboratory practices were identified and categorized according to 1) sample development, 2) program development, and 3) ancillary support. Elements of these were subsequently prioritized by expert panel consensus. The following information integrates the panelists' recommendations with these areas that might readily be addressed by CDC/PHPPO/DLS.

1. **Conduct pilot research to develop positive controls and test samples for pilot PE programs**. The issue of utmost urgency in the field of MGT today is the lack of positive controls/samples for both testing laboratories and QA/PT programs. Two approaches have been proposed to address this problem, specifically, 1) the use of existing immortalized cell lines, and 2) the investigation of alternate test material.

As an initial focus, the use of existing immortalized cell lines for positive controls is the simplest approach scientifically, but implies working with existing repositories to overcome currently existing logistical barriers. Some of these barriers include: informed consent issues, referencing and characterization of cell lines and other QA samples, establishing permanent sources of transformed cell lines and sources of other QA materials, distribution of QA materials and funding for these processes. If those barriers prove very difficult or impossible to surmount, research could be done into the possibility of establishing new repositories or commercial interests to circumvent them. Such an entity would be specifically designed to provide test specimens for use as positive controls in testing laboratories, test material for PE and PT programs, and test material for new test validation by diagnostic laboratories.

One approach to enhance access to immortalized cell lines would be to develop open-ended patient consent forms and/or open-ended permission for using the specimen for new and future applications. The research required to develop these forms should also determine how much information is needed on each normal or abnormal specimen to maximize its utility without violating patient rights.

As samples are identified and become available to meet QA/PT/PE needs, the panelists recommended the establishment and support of a limited number of test sites for validating/referencing new positive controls and maintaining their supply. A central testing laboratory may have some role in supporting this function.

The second major approach to developing positive controls is the use of material other than immortalized cell lines. Alternate material that could be investigated includes: 1) spiked, transfected, or engineered samples, 2) multiplex test samples (QA samples that could be used to check multiple steps along the testing process, as well as different analytes/disorders), and 3) disease-specific test samples.

2. **Develop PE programs to supplement what already exists, particularly for disease and/or methodologies not covered by existing programs.**

   As an example, one specific area of program development could be a pilot PE program for sequencing, an area in which no QA/PT programs currently exist for clinical laboratories. Components that should be linked to this program (to complement sample send-out) include: 1) the development and administration of a detailed laboratory methodology survey, and 2) the development of a comprehensive technology/methodology database.

   A method-specific pilot PE program also could be implemented to supplement existing programs as unmet needs are identified. Because MGT applications are evolving rapidly, a laboratory survey should be conducted prior to any program implementation, to identify appropriate representative tests. The survey would capture data on those tests and procedures currently performed, their relative frequency, and opinions on new and emerging tests. Such a survey might ideally be developed in collaboration with CAP and/or other stakeholders.

3. **Establish laboratory-oriented, disease-specific consortia to provide QA support as a forum for networking, providing methods validation through results comparison.**

   Disease-specific consortia emphasizing testing and laboratory issues could address a number of issues currently facing MGT laboratories. This is important because both test utilization and analytical issues (including interpretation) vary with each disease. Consortia could provide a mechanism for expanding the range of available clinical information, enhance communication between laboratories through disease-specific working groups, and establish databases of information useful to laboratorians. With regard to PE, consortia could participate in pilot research, provide a source of preliminary information for PE program

initiation, serve as a resource for identifying, contributing, or developing test samples, and serve as a discussion group for performance data. For optimal effectiveness, panelists felt that any databases developed by consortia or others should utilize refereed data entry with controlled access to the information therein. They stressed the importance of screening the input of data to eliminate irrelevant information.

Method-based consortia also would be useful in the area of MGT. These would address PCR, sequencing, FISH, and Southern blot technologies.

**4. Establish and link disease-specific databases with other appropriated Internet resources to meet the needs of clinical MGT laboratories.**

Disease-specific electronic resources could assist laboratories in several major areas. For example, a database of information on current methodologies could be developed based on a laboratory practices survey. Another suggested endeavor would be to develop a web page, database, or centralized index of information documenting test-specific variables such as DNA isolation procedures, primers, suppliers, technical details, thermocycler information, tests performed, technologies used, results, patient outcome data, genetic characterization of the disease, utility of technology in diagnosis, and other medical/technical parameters as needed. Such an information resource would be designed to: 1) assist laboratories with current technologies, novel technologies, and retrospective studies of genetic diseases; 2) foster communication among laboratories involved in genetic testing; and 3) facilitate the development and implementation of new tests. For any electronic resource that might be developed, information input should be controlled, and should follow an expert review by a qualified group before being entered into the database. CDC/PHPPO/DLS could possibly identify and convene such a group.

Another possible project for the CDC would be to develop and support a database of mutations and polymorphisms associated with genetic diseases and disease outcomes, treatments administered and their responses, and morbidity and mortality. For example, the CDC might then conduct long term follow-up required on a population-wide basis to assess the outcome of mutations and treatment for late-onset disorders such as hereditary breast cancer (BRCA). This polymorphism database would be useful for both ongoing MGT and retrospective studies. The database could also be used to record patient consent, confidentiality forms, and any permission given for use of the samples obtained.

**5. Improve training and continuing education for clinicians, laboratory scientists, and technicians.**

There exists a great need for training and continuing education (CE) for everyone involved in the total testing process. For example, clinicians and laboratory scientists could benefit from CE programs on the Internet, based upon or linked to disease-specific databases as described above. Also, an effective way to meet training needs at the technician level, for example, would be to conduct a laboratory survey to examine current needs, and forward the survey results to representatives of existing training/certification programs. The end goals would be to: 1) determine what level of training is needed, then ensure it is provided, and 2) coordinate efforts with existing technician-level training organizations. Continuing education also should be supported by some form of formal training, along with support of the certification of genetic laboratory personnel.

**Potential Involvement of CDC/PHPPO/DLS.** The mission of the CDC Public Health Practice Program Office (PHPPO), Division of Laboratory Systems (DLS) is to improve the quality of laboratory practices in support of continuous improvement of public health. A primary focus of the DLS is assessing the quality of laboratory testing, and conducting research into its improvement. In light of this mission and the information gathered on QA for MGT, especially from the panel of national experts, CDC/PHPPO/DLS could potentially play an immediate and significant role in improving the quality of molecular genetic testing for human inheritable disease through efforts in: 1) conducting laboratory surveys and pilot studies for PE programs, 2) supporting test sample development through exploration of existing resources and research of novel test material, and 3) expanding the CDC website to include targeted MGT databases and resource links,

1. **Conduct laboratory surveys and pilot studies for PE programs**. CDC/PHPPO/DLS laboratory surveys and pilot studies could lay the groundwork for a formal PE program. An important first step would be to identify as many prospective participant laboratories as possible using a variety of approaches, to include surveying the MGT panelists or other interested stakeholders, consulting with CAP, investigating the *GeneTests* database (an estimated 70% of laboratories offering human molecular genetic testing are listed), and checking the list of laboratories registered with the Health Care Financing Administration (HCFA).

   CDC/PHPPO/DLS should design and distribute a laboratory needs assessment survey to collect information on those tests currently being performed. Such a survey would separate acquired from inheritable disease, and would: 1) provide a general overview of the clinical genetics testing community and its QA/PE needs, 2) facilitate development of future programs based on actual need, 3) identify trends in testing, and 4) monitor trends in MGT technology development.

   CDC/PHPPO/DLS should develop and conduct a PE program with a broad scope. It is recommended that such an expanded program include: 1) samples that change intermittently, 2) appropriate staffing given the scope of the program, 3) technical support, and 4) consulting resources for participating laboratories. Through a pilot PE program, CDC could document performance as well as laboratory practices for a diverse group of tests that may be growing in utilization within the genetics testing community.

   CDC/PHPPO/DLS could also design and distribute a laboratory survey regarding: 1) policies and procedures for the initial acquisition of specimens, and the degree of standardization of related information, and 2) informed consent forms and their degree of standardization, as well as physician knowledge and training of when such forms are required.

2. **Support sample development**. CDC/PHPO/DLS involvement could include 1) encouraging and supporting the donation, referencing and characterization of existing cell lines (including addressing logistical barriers), and 2) conducting applied research into the potential use of novel test sample material. CDC/PHPPO/DLS might initiate a dialog with existing cell repositories relative to improving access to, and acquisition of, new cell lines, particularly relative to quantities necessary for a formal PE program and/or QA use in general. It might also conduct research into novel test sample material, such as pseudo-blood, engineered samples, or the use of disease-specific sample donors. Following identification or development, potential test samples would be pilot-tested in a large number of laboratories.

3. **Expand CDC website/create links, specific databases.** The CDC website could serve to bring together in one location, all existing MGT resources, through identifying and linking

existing websites, and perhaps developing new ones. A website might be developed specifically to address the needs of clinical MGT laboratories.

# DISCUSSION

In addressing the objectives of the project, with a focus on total QA in MGT, many of the foregoing recommendations could potentially be addressed by CDC/PHPPO/DLS at the present time. The recommendations are based heavily on input from the national experts who participated in the three Expert Panel Meetings (*Appendix B*), and additional resource information (See *References*). The discussions in each meeting were designed to build upon results of prior meetings, with an overall focus on reaching a consensus, and final recommendations for QA needs in MGT.

It was apparent that although various aspects of QA for MGT were focused upon at different times throughout the meetings, the same needs and priorities were repeatedly identified. The results can be summarized into four areas: 1) sample development, 2) program development, 3) ancillary support, and 4) coordinated effort.

## Sample Development

Sample development is of utmost importance to MGT laboratories due to the lack of positive controls for routine testing, and test samples for Quality Assurance/Performance Evaluation (QA/PE). Two approaches to this problem is proposed. The first approach requires pursuing the utilization of existing immortalized cell lines. This implies working with existing repositories and/or setting up repositories to be used specifically for QA needs. This would require work toward overcoming the logistical barriers to using currently available materials for multiple applications, as well as anticipated future applications. The second approach is to research the production of test material useful as positive controls or test samples for QA/PE. Suggestions for potential options included spiked samples, transfected cells, synthesized DNA, and multiplex specimens. Such samples are less representative of patient material than immortalized cell lines from patient specimens, but may prove as useful for QA/PE purposes in the absence of optimal material. Multiplex specimens (such as a cell line with several mutations inserted), or disease-specific specimens, would be useful for QA of procedures at multiple steps along their critical pathways, as well as testing for multiple diseases. A sample that can be tested by multiple laboratories, using their own probes, reagents, and protocols, would be particularly useful for a methodology-specific PE program. In further discussion regarding appropriate sample type for QA/PE, panelists noted that matrix effects should be considered; for example, the use of blood spots may be sub-optimal for QA/PE of some MGT procedures. Laboratories might also be able to use such samples, produced for QA purposes, to validate new tests they are implementing.

## Program Development

The focus should be on the establishment of PE programs and the development of disease-specific consortia. An important aspect of program development involves the establishment and piloting of PE programs. For example, a specific PE program for DNA sequencing is critical, since none currently exists. Components that should be linked to PE programs (to complement sample send-out) include: 1) administration of a detailed laboratory methodology survey and 2) development of a comprehensive technology/methodology database. In addition, novel pilot PE programs to supplement existing ones through a method-based approach should be initiated as needs are clearly defined. For example, laboratories not currently participating in existing PT/laboratory accreditation programs might be able and willing to participate in a method-specific PE program which allows them to monitor their performance through self-evaluations.

Method-specific programs applicable to sequencing, Southern blot, PCR, and FISH, would be useful to laboratories bringing new tests on-line or doing some tests on a very infrequent basis.

Disease-specific consortia could provide a needed support mechanism for genetic testing laboratories. Besides functioning as a resource of technical expertise for laboratories, these consortia might also address the problem of lack of positive controls by contributing samples and through networking. Working groups to establish disease-specific databases would be useful, particularly for laboratories implementing novel tests and testing for orphan diseases. Such a resource would help to integrate novel work groups with existing programs and organizations by expanding clinical information and directly enhancing communication between laboratories. Additionally, those groups would be an important component of the needed informatics support for MGT, with the consortia being instrumental in creating and supporting web-based databases that would serve as an important part of a centralized information source.

Finally, information gathering, in the form of a laboratory survey asking for general information, is needed in order to design a useful and effective PE program, and evaluate and assist with laboratory QA on routine and new tests. This could be done using a laboratory survey designed to obtain information regarding clinical genetics testing. Before widespread use, the survey must be pilot-tested on a small number of laboratories in order to establish data-fields for later PE surveys and databases. A disease-specific survey is initially recommended. Ideally, the survey and resulting database should be integrated with collaborative efforts on the part of CDC and qualified professional organizations (for example CAP) to maximize the data obtained, and recorded. The outcome of this survey would be the production of a database, compiling pertinent technical and clinical information for statistical analysis, and retrospective studies.

## Ancillary Support

Ancillary support is crucial to QA for MGT. Websites specifically intended to support laboratories, and genetics testing databases are in great demand by the testing community. A survey of current methodology designed to obtain information regarding clinical genetics testing laboratories could be used to establish a baseline of information regarding current laboratory practices. The information collected would be entered into the novel web-based database described above, containing technical and clinical information pertinent to testing issues. This web-site/database should be designed to function as a centralized information source.

Communication among the laboratories involved in genetic testing must be enhanced in order to facilitate the implementation of new tests, disseminate information to laboratories for comparative and self-evaluation purposes, and also for test validation. This could most easily and effectively be done through the support and continued updating of a centralized index of information, which includes existing genetic databases and resources. This could potentially be done most quickly and effectively through an expansion of existing databases and web pages, such as those of the CDC. Information submitted to such databases should be controlled, and follow an expert review before being entered into the database.

## Coordinated Effort

In order to ensure that any novel projects are effective in meeting the needs of the genetic testing community, and improving laboratory QA, it is critical that efforts towards designing an effective program of QA for MGT be done in collaboration with experts and stakeholders from the laboratory community. The formation of an advisory board for a QA/PE program comprised of existing members of current programs, and members from laboratories active in clinical genetics testing is strongly recommended. Also, in any program that is initiated, comprehensive reporting must be provided both to participating laboratories, and to program administrators. Laboratorians

want, and benefit from, timely feedback. Program administrators need to continually be informed about new tests, emerging testing needs, and technology trends in the laboratory community.

## CONCLUSION

A comprehensive year-long effort in gathering detailed technical information to characterize the focus of quality assurance and proficiency testing/performance evaluation programs for molecular genetic testing for human inheritable disease has been completed. Practical recommendations to significantly improve the quality of genetic testing laboratory practices were developed throughout this project and are presented in this monograph. Findings resulting from this project, generated through the interaction of genetic testing experts and the professional organizations represented, are already contributing to the advancement of the field.

A key outcome has been the organization of a group of recognized experts from the medical research and diagnostic testing areas that are available to sustain the momentum of this project. This network of experts is motivated and willing to work toward implementing the recommendations for QA for MGT detailed in this monograph, consistent with CDC/PHHPO's mission.

# REFERENCES

## References Cited


1. Williams, LO, EC Cole, IM Lubin, NI Iglesias, RL Jordan, LE Elliott, and DJ Boone (1999). Quality Assurance in human Molecular Genetics Testing: Results of CDC Expert Panel Meetings. The Second National Conference on Genetics and Disease Prevention, The Association of State and Territorial Health Officials (ASTHO), Baltimore, MD.

2. GeneTests: Genetic Testing Resource (Formerly HELIX): http://www.genetests.org

3. American College of Medical Genetics (1999*). "Standards and Guidelines: Clinical Genetic Laboratories, Final Draft 2nd Edition."* The American College of Medical Genetics Laboratory Practice Committee, Bethesda, MD.

4. NCCLS (1997). Molecular Diagnostic Methods for Genetic Diseases; Proposed Guideline. National Committee for Clinical Laboratory Standards, Publication MM1-P.

5. State of New York Department of Health (1998). Clinical Laboratory Evaluation Program Proficiency Testing Program Guide.

6. College of American Pathologists (1998a). SURVEYS – Comprehensive Solutions for Achieving Excellence (1999 SURVEYS Catalog).

7. College of American Pathologists (1998c). Participant Summary: ACMG/CAP Molecular Genetics Survey Set MGL-A.

8. College of American Pathologists (1998b). Participant Summary: ACMG/CAP Fluorescence In-Situ Hybridization Survey Set CYG-A.

9. Holtzman, NA and MS Watson, editors (1997). Promoting Safe and Effective Genetic Testing in the United States. Final report of the Task Force on Genetic Testing, National Institutes of Health, Bethesda, MD, p. 49.

10. Dewald, G, R Stallard, PI Bader, K Chen, J Zenger-Hain, CJ Harris, R Higgins, WT Hirsch Hsu, E Johnson, V Kubic, TW Kurczynski, JM Malone, DJ McCorquodale, K Meilinger, LF Meisner, JW Moore, S Schwartz, S Siembieda, PD Storto, G Vance, P Van Tuinen, A Wiktor, JF Yung (1996). Toward Quality Assurance for Metaphase FISH: A Multi-Center Experience. *Amer. J. Med. Gen.* 64:539-545.

11. Dewald, G, R Stallard, A Al Saadi, S Arnold, PI Bader, R Blough, K Chen, BR Elejalde, CJ Harris, RR Higgins, GA Hoeltge, W Hsu, V Kubic, DJ McCorquodale, MA Micale, JW Moore, RM Phillips, S Scheib-Wixted, S Schwartz, S Siembieda, K Strole, P VanTuinen, GH Vance, A Wiktor, L Wise, J Yung, J Zenger-Hain, and A Zinsmeister (1998). A Multicenter Investigation With Interphase Fluorescence In Situ Hybridization Using X- and Y-Chromosome Probes. *Am. J. Med. Gen.* 76:318-326.

12. Healy, NP and AM Willey (1996). Laboratory Quality Assurance in Cancer Predisposition Testing in Genesis (a quarterly publication of Wadsworth Center, New York State Department of Health).

13. State of New York Department of Health (1998). Clinical Laboratory Evaluation Program Proficiency Testing Program Guide.

14. Willey, AM, Director of Laboratory Policy Development, Wadsworth Center, New York State Department of Health. Personal communication (1999).

15. Matteson, K. *et al* (1990). American Journal of Human Genetics 46:182.

16. Association for Molecular Pathology (1998). AMP Newsletter 4 (3), pp. 8, 20.

17. O'Leary, T (1999). Department of Cellular Pathology, Armed Forces Institute of Pathology, Washington, DC. Personal communication.

18. Williams, LO, S Legois, PA Shult, MM Bussen, PJ Ward, GH Mazurek, B Metchock, and JC Ridderhof (1999). Performance of Nucleic Acid Amplification Tests for *Mycobacterium tuberculosis* (*M. tb*) in CDC's Performance Evaluation Program. The 39th Interscience Conference on Antimicrobial Agents and Chemotherapy; San Francisco, CA.

19. Williams, L, L Kubista, M Bussen, S Legois, P Shult, and J Ridderhof (1998). Sample Development for Use in Performance Evaluation Program for Rapid Nucleic Acid Detection of *M. tuberculosis*. American Society for Microbiology, Atlanta, GA.

20. Ridderhof, J, L Williams, and A Laszlo (1998). Comparison of External Quality Control Programs for *M. tuberculosis* Drug Susceptibility Testing. Interscience Conference on Emerging Infectious Diseases, Atlanta, GA.

21. Blumer, SO, WO Schalla, TL Hearn, and RJ Fehd (1998a). Evaluation of Laboratory Performance in Determining HIV-1 Viral RNA Levels Present in CDC Performance Evaluation Samples. 12th World AIDS Conference, Geneva, Switzerland.

22. Williams, LO, SO Blumer, WO Schalla, P Robinson, JH Handsfield, RJ Fehd, JC Hancock, HB Lipman, and TL Hearn (1998). Laboratory Performance in Human T-Lymphotropic Virus Types I/II (HTLV) Analyses. American Society for Microbiology, Miami, FL.

23. Grills, G, MK Dolejsi, S Hardin, D McMinimy, P Morrison, J Rush, and PS Adams (1998). ABRF DNA Sequence Research Committee Study: Assessing the Current State of the Art in DNA Sequencing and Creating a Quality Control Resource. ABRF'98: From Genomes to Function: Technical Challenges of the Post-Genome Era: An International Symposium of the Association of Biomolecular Resource Facilities, p. 32.

24. Kaback, M, J Lim-Steele, D Dabholkar, D Brown, N Levy, and K Zeiger (1993). Tay-Sachs Disease – Carrier Screening, Prenatal Diagnosis, and the Molecular Era. An international perspective, 1970 to 1993. The International TSD Data Collection Network. JAMA 270(19):2307-2315.

25. First Expert Panel Meeting on QA/PT Programs in Molecular Genetic Testing, December 3, 1998.

26. Second Expert Panel Meeting on QA/PT Programs in Molecular Genetic Testing, February 10-11, 1999.

27. Third Expert Panel Meeting on QA/PT Programs in Molecular Genetic Testing, May 5-6, 1999.

# ADDITIONAL REFERENCES AND RESOURCES

The following is a list of resources that may be of value to clinical laboratories performing MGT tests. It is not meant to be exhaustive, but provides information that can be used for the purposes of overall laboratory quality improvement in MGT and to identify further sources of information.

## Standards, Guidelines, and Recommendations

American College of Medical Genetics and American Society of Human Genetics Working Group on ApoE and Alzheimer Disease (1995). Statement on Use of Apolipoprotein E testing for Alzheimer Disease. JAMA 274:1627-1629.

American College of Medical Genetics (1996). *"Standards and Guidelines: Clinical Genetic Laboratories."* The American College of Medical Genetics Laboratory Practice Committee, Bethesda, MD.

American College of Medical Genetics (1999). *"Standards and Guidelines: Clinical Genetic Laboratories, Supplement 1."* The American College of Medical Genetics Laboratory Practice Committee, Bethesda, MD.

American Society of Human Genetics/American College of Medical Genetics Test and Technology Transfer Committee (1996). Diagnostic Testing for Prader-Willi and Angelman Syndromes: Report of the ASHG/ACMG Test and Technology Transfer Committee. Am. J. Hum. Genet. 58:1085-1088.

Association for Molecular Pathology (1999). Association for Molecular Pathology Statement. Recommendations for In-House Development and Operation of Molecular Diagnostic Tests. Am. J. Clin. Pathol. 111:49-463.

Holtzman, NA and MS Watson, editors (1997). Promoting Safe and Effective Genetic Testing in the United States. Final report of the Task Force on Genetic Testing, National Institutes of Health, Bethesda, MD.

Jacky, PB, et al (1991). Guidelines for the Preparation and Analysis of the Fragile X Chromosome in Lymphocyte Preparations. American Journal of Medical Genetics 38:400-403.

Kiechle, FL, LM Chambers, RS Cox, et al. (1996). Patient Preparation and Specimen Handling: Reference Guide for Diagnostic Molecular Pathology/Flow Cytometry. Fascicle VII. Northfield, IL: College of American Pathologists.

Mitelman, F, ed. (1995). *ISCN 1995: An International System for Human Cytogenetic Nomenclature.* S. Karger Publishers.

## Technical Publications

Adams, PS, MK Dolejsi, S Hardin, S Mische, B Nanthakamur, H Riethman, J Rush, and P Morrison (1996). DNA Sequencing of a Moderately Difficult Template: Evaluation of the Results from a *Thermus thermophilus* Unknown Test Sample. BioTechniques 21:678.

Adams, PS, MK Dolejsi, G Grills, S Hardin, DL McMinimy, J Rush, and P Morrison (1997). Effects of DMSO, Thermocycling and Editing on a Template with a 72% GC Rich Area: Results from the 2nd Annual ABRF Sequencing Survey Demonstrate that Editing is the Major Factor for Improving Sequencing Accuracy. Ninth International Genome Sequencing and Analysis Conference. *Microbial and Comparative Genomics* 2(3):198 (abstract).

Andrews, LB, JE Fullerton, NA Holtzman, and AG Motulsky, Eds. (1994). Assessing Genetic Risks: Implications for Health and Social Policy. National Academy Press, Washington, DC.

Association for Molecular Pathology (1998). AMP Newsletter 4 (3), pp. 8, 20; October 1998.

Blumer, SO, WO Schalla, TL Hearn, RJ Fehd (1998a). Evaluation of Laboratory Performance in Determining HIV-1 Viral RNA Levels Present in CDC Performance Evaluation Samples. 12th World AIDS Conference, Geneva, Switzerland.

Blumer, SO, WO Schalla, TL Hearn, RJ Fehd (1998b). Evaluation of Laboratory Performance in Determining HIV-1 Viral RNA Levels Present in CDC Performance Evaluation Samples. 1998 Conference on the Laboratory Science of HIV; Atlanta, GA.

Check, W (1998). Who Does Cytogenetics, Where and on What? CAP in the News, CAP TODAY Archive, April 1998.

Clinical Laboratory Improvement Advisory Committee (CLIAC). Summary report of the Subcommittee Meeting on Genetics. September 10, 1997.

Clinical Laboratory Improvement Advisory Committee (CLIAC). Genetic Testing Working Group Report to CLIAC. September, 1998.

College of American Pathologists (1998a). SURVEYS – Comprehensive Solutions for Achieving Excellence (1999 SURVEYS Catalog).

College of American Pathologists (1998b). Participant Summary: ACMG/CAP Fluorescence In-Situ Hybridization Survey Set CYG-A.

College of American Pathologists (1998c). Participant Summary: ACMG/CAP Molecular Genetics Survey Set MGL-A.

College of American Pathologists (1998d). Participant Summary: ACMG/CAP Molecular Genetics Survey Set MGL-B.

Dewald, G, R Stallard, PI Bader, K Chen, J Zenger-Hain, CJ Harris, R Higgins, WT Hirsch Hsu, E Johnson, V Kubic, TW Kurczynski, JM Malone, DJ McCorquodale, K Meilinger, LF Meisner, JW Moore, S Schwartz, S Siembieda, PD Storto, G Vance, P Van Tuinen, A Wiktor, JF Yung (1996). Toward Quality Assurance for Metaphase FISH: A Multi-Center Experience. *Amer. J. Med. Gen.* 64:539-545.

Dewald, GW, AR Brothman, MG Butler, LD Cooley, SR Patil, IA Saikevych, and NR Schneider (1997). Pilot studies for proficiency testing using fluorescence in situ hybridization with chromosome-specific DNA probes. Arch Pathol Lab Med 121:359-367.

Dewald, G, R Stallard, A Al Saadi, S Arnold, PI Bader, R Blough, K Chen, BR Elejalde, CJ Harris, RR Higgins, GA Hoeltge, W Hsu, V Kubic, DJ McCorquodale, MA Micale, JW Moore, RM Phillips, S Scheib-Wixted, S Schwartz, S Siembieda, K Strole, P VanTuinen, GH Vance, A Wiktor, L Wise, J Yung, J Zenger-Hain, and A Zinsmeister (1998). A Multicenter Investigation With Interphase Fluorescence In Situ Hybridization Using X- and Y-Chromosome Probes. *Am. J. Med. Gen.* 76:318-326.

Farkas, DH, editor (1993). Establishing a Clinical Molecular Biology Laboratory, in Molecular Biology and Pathology: A Guidebook for Quality Control. Academic Press, San Diego, CA.

Garrett, CT and A Ferreira-Gonzalez (1996). FDA Regulation of Analayte-Specific Reagents (ASRs): Implications for Nucleic Acid-Based Molecular Testing. Diagn. Mol. Pathol. 5:151-153. Cited in Grody and Pyeritz, 1999.

Grills, G, MK Dolejsi, S Hardin, D McMinimy, P Morrison, J Rush, and PS Adams (1998). 1998 ABRF DNA Sequence Research Committee Study: Assessing the Current State of the Art in DNA Sequencing and Creating a Quality Control Resource. ABRF'98: From Genomes to Function: Technical Challenges of the Post-Genome Era: An International Symposium Sponsored by the Association of Biomolecular Resource Facilities, p. 32 (abstract).

Grody, WW (1994). Proficiency Testing in Diagnostic Molecular Pathology. Diagnostic Molecular Pathology 3:221.

Grody, WW, RJ Desnick, NJ Carpenter, and WW Noll (1998). Diversity of Cystic Fibrosis Mutation-Screening Practices. American Journal of Human Genetics 62:1252-1254.

Grody, WW and RE Pyeritz (1999). Report Card on Molecular Genetic Testing. JAMA 281(9):845-847.

Healy, NP and AM Willey (1996). Laboratory Quality Assurance in Cancer Predisposition Testing in Genesis (a quarterly publication of Wadsworth Center, New York State Department of Health), Winter/Spring 1996.

Hoeltge GA (in press). Setting the standards for cytogenetic laboratories In: Mark HFL, ed. Cytogenetics for Pathologists, 1st ed. New York: Marcel Dekker, Inc. Cited in Mark (1998).

Hoeltge, GA, D Dewald, CG Palmer, MA David-Nelson, I Saikevych, S Patil, S Schwartz, NR Schneider and M Herrmann (1993). Proficiency testing in clinical cytogenetics. A 6-year experience with photographs, fixed cells, and fresh blood. Arch Pathol Lab Med 117:776-779.

Holmes, LB and RE Pyeritz (1998). Screening for Cystic Fibrosis. JAMA 279:1068-1069. Cited in Grody and Pyeritz, 1999.

Kaback, M, J Lim-Steele, D Dabholkar, D Brown, N Levy, and K Zeiger (1993). Tay-Sachs Disease – Carrier screening, prenatal diagnosis, and the molecular era. An international perspective, 1970 to 1993. The International TSD Data Collection Network. JAMA 270(19):2307-2315.

Mark, HF and L. Wells (1998). A laboratorian's view on the College of American Pathologists' Laboratory Accreditation Program for Clinical Laboratories. Ann Clin Lab Sci 28(2):115-23.

Mark, HFL, T Kelly, MS Watson, G Hoeltge, WA Miller, and L Beauregard (1995). Current issues of personnel and laboratory practices in genetic testing. J. Med Genet 32:780-786.

McGovern, MM, MO Benach, S Wallenstein, RJ Desnick, and R Keenlyside (1999). Quality Assurance in Molecular Genetic Testing Laboratories. J Am Med Assoc 281(9):835-840.

Moore, DH, L Epstein, J Reeder, L Wheeless, FM Waldman, and the NCI Bladder Tumor Marker Network (1996). Interlaboratory variability in fluorescence in situ hybridization analysis. Cytometry 25:125-32.

Nakamura, RM (1993). College of American Pathologists: Conference XXIV on molecular pathology: Introduction. Arch Pathol Lab Med 117:455-456.

Newmaier, M, A Braun, and C Wagener (1998). Fundamentals of Quality Assessment of Molecular Amplification Methods in Clinical Diagnostics. Clinical Chemistry 44(1):12-26.

Schalla, WO, SO Blumer, TL Hearn, and RJ Fehd (1998). Analysis of Results Reported by Laboratories Participating in the CDC Model Performance Evaluation Program's First Performance Evaluation for HIV-1 RNA Determinations. 13th Annual Conference on Human Retrovirus Testing; Association of Public Health Laboratories; San Diego, CA.

Wagner, LR (1997). The College of American Pathologists, 1946-1996. The surveys program. Arch Pathol Lab Med 121(6):646-51.

Watson, MS (1995). Current Activities Involving Economic, Regulatory, and Practice Issues in Molecular Genetic Testing. Diagn Mol Pathol 4:233-234

Yost, J (1998). Genetic testing and CLIA '88: Present and future. American Association of Clinical Chemistry 8th Annual Clinical Chemistry Forum, "Issues in Genetic Testing", November 3-4, 1998.

## Personal Communication

First Expert Panel Meeting on QA/PT Programs in Molecular Genetic Testing, December 3, 1998.

Centers for Disease Control and Prevention, Division of Laboratory Systems, Public Health Practice Program Office, Model Performance Evaluation Program (MPEP) for laboratories performing HIV-1 Ab, HTLV-I/II Ab, TLI, HIV-1 RNA, and HIV-1 p24 Ag testing.

Centers for Disease Control and Prevention, Division of Laboratory Systems, Public Health Practice Program Office, Mycobacterium tuberculosis (M.tb) Nucleic Acid Amplification (NAA) Performance Evaluation Program for laboratories performing direct detection of M.tb in patient specimens using FDA-approved test kits

Centers for Disease Control and Prevention, Division of Laboratory Systems, Public Health Practice Program Office, M.tb/NTM Susceptibility Testing Performance Evaluation Program for laboratories performing drug susceptibility testing in patient specimens.

Kaback, M. Director, NTSAD Laboratory Quality Control Program. Personal communication (1999).

Laxova, R. (1999). Chair, CORN Quality Assurance Committee, personal communication.

O'Leary, T. Department of Cellular Pathology, Armed Forces Institute of Pathology, Washington, DC. Personal communication, 1999.

Second Expert Panel Meeting on QA/PT Programs in Molecular Genetic Testing, February 10-11, 1999.

Schalla, William O.; Chief, CDC Model Performance Evaluation Program, personal communication, 1999.

Third Expert Panel Meeting on QA/PT Programs in Molecular Genetic Testing, May 5-6, 1999.

## Websites

American College of Medical Genetics Laboratory Practice Committee Standards and Guidelines for Clinical Genetics Laboratories (1993): http://www.arvo.org/genetics/acmg (accessed July 15, 1999).

Association of Biomolecular Resource Facilities: http://www.abrf.org (accessed June 24, 1999).

Association for Molecular Pathology: www.ampweb.org (accessed June 24, 1999).

Association of Genetic Technologists (AGT): www.agt-info.org (accessed July 13, 1999).

Centers for Disease Control and Prevention, genetics website: http://www.cdc.gov/genetics/ (accessed July 13, 1999).

Centers for Disease Control and Prevention, Public Health Practice Program Office (CDC PHPPO): www.phppo.cdc.gov (accessed July 13, 1999).

Center for Medical and Molecular Genetics within the Military Health Services System: http://www.afip.org/OCLAB/V1n1.html (accessed January 4, 1999).

College of American Pathologists: http://www.cap.org/ (accessed July 13, 1999).

Coriell Cell Repositories: http://locus.umdnj.edu/ccr/ (accessed July 15, 1999).

Council of Regional Genetics (CORN): http://www.cc.emory.edu/PEDIATRICS/corn (accessed July 15, 1999).

Cystic Fibrosis Genetic Analysis Consortium: http://www.genet.sickkids.on.ca/cftr/agreement.html (accessed July 15, 1999).

Cystic Fibrosis Mutation Data Base: http://www.genet.sickkids.on.ca/ (accessed July 15, 1999).

Division of Laboratory Quality Certification Clinical Laboratory Evaluation Program (CLEP) Wadsworth Center, New York State Department of Health: http://www.wadsworth.org/labcert/clep.html (accessed July 15, 1999).

GeneClinics: http://www.geneclinics.org (accessed July 13, 1999).

GeneTests: Genetic Testing Resource (Formerly HELIX): http://www.genetests.org (accessed July 1, 1999).

Genesis Newsletter (prepared by the Genetics Network of New York State, Puerto Rico and the Virgin Islands) Wadsworth Center, New York State Department of Health: http://www.wadsworth.org/publish/genesis/index.htm (accessed July 15, 1999).

Heriditary Disease Foundation (offers guidelines for the molecular genetic predictive test for Huntington's Disease): http://www.hdfoundation.org/testread/testwfn.htm (accessed July 15, 1999).

National Certification Agency – offers examinations for Certified Laboratory Specialist in Molecular Biology (CLSp[MB]): http://www.applmeapro.com/nca/ (accessed July 13, 1999).

National Committee for Clinical Laboratory Standards (NCCLS): http://www.nccls.org (accessed July 1, 1999).

National Institute for Standards and Technology (NIST), DNA standard information: http://129.6.16.1130/somcatalog/tables/105-8.htm (accessed July 13, 1999).

National Tay-Sachs & Allied Diseases Association, Inc.: http://www.ntsad.org/ntsad/labsus.htm (accessed July 15, 1999).

NIH Consensus Development Conference Statement. Genetic testing for cystic fibrosis: http://odp.od.nih.gov/consensus/cons/106/106_statement.htm. (accessed July 15, 1999).

NIH-DOE Joint Working Group on the Ethical, Legal, and Social Implications of Human Genome Research Task Force on Genetic Testing: http://www.nhgri.nih.gov/ELSI/TFGT_final/ (accessed July 15, 1999).

Wadsworth Center, New York State Department of Health: http://www.wadsworth.org

# APPENDIX A
## MOLECULAR GENETIC TESTING EXPERT PANELISTS

# Molecular Genetic Testing Expert Panelists

| Panelist | Affiliation and Related MGT Activities |
|---|---|
| **Steven Anderson, PhD**<br>Senior Technical Director, Center for Molecular Biology and Pathology<br>Associate Vice President | Laboratory Corporation of America (LabCorp)<br>1912 Alexander Drive<br>Research Triangle Park, NC 27709<br><br>American Society for Human Genetics<br>NC Society of Medical Genetics |
| **Hans Andersson, MD**<br>Clinical Biochemical Geneticist<br>Assistant Professor, Pediatrics | Tulane University Medical School<br>Human Genetics Program<br>Hayward Genetics Center<br>1430 Tulane Avenue<br>New Orleans, LA 70112<br><br>American College of Medical Genetics<br>Southeast Regional Genetics Group |
| **Jaya Bansal, PhD**<br>President | Cell Technology, Inc.<br>7798 Jessup Road<br>Jessup, MD 207994 |
| **Jeffrey Bartlett, PhD**<br>Research Assistant Professor<br>Associate Scientist, Gene Therapy | University of North Carolina – Chapel Hill<br>Gene Therapy Center<br>7109 Thurston-Bowles Building<br>CB#7248<br>Chapel Hill, NC 27599<br><br>American Society for Gene Therapy<br>Mammalian Genome Society |
| **Louis Elsas, MD, FFACMG**<br>Professor of Pediatrics<br>Director, Division of Medical Genetics | Emory University School of Medicine<br>Dept. Of Pediatrics,<br>Div. of Medical Genetics<br>2040 Ridgewood Dr., Rm. 1030<br>Atlanta, GA 30322<br><br>Trustee, American College of Medical Genetics<br>Founding President, Society for Inherited Metabolic Disorders |
| **Richard Erbe, MD**<br>Chief, Division of Genetics<br>Professor of Pediatric Medicine, S.U.N.Y. Buffalo | Children's Hospital of Buffalo<br>Division of Genetics<br>936 Delaware Avenue<br>Buffalo, NY 14209<br><br>Founding Fellow, American College of Medical Genetics<br>American Society of Human Genetics<br>Society for Inherited Metabolic Disorders |

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| **Glen Evans, MD, PhD**<br>Director, McDermott Center for Study of Human Growth and Development<br>Director, NHGRI Genome Science and Technology Center<br>Professor of Internal Medicine | University of Texas Southwestern Medical Center<br>McDermott Center HGA<br>6000 Harry Hines Blvd., NB10.204<br>Dallas, TX 75235<br>NIEHS Working Group-Environmental Genome Project<br>Associate Editor, Genomics |
| **Mary Jo Evans, PhD**<br>Director, Molecular Diagnostics | Children's Hospital of Buffalo<br>936 Delaware Avenue<br>Buffalo, NY 14209<br>American Society for Biochemistry & Molecular Biology<br>American Association for Cancer Research |
| **Andrea Gonzalez, PhD**<br>Assistant Professor, Molecular Diagnostics Division | Medical College Of Virginia of VCU<br>Pathology Department<br>1101 E. Marshall Street<br>Sanger Hall, 4024<br>Richmond, VA 23298-0248<br>NCCLS Subcommittee on Quantitative Molecular Methods in Infectious Disease<br>Association for Molecular Pathology |
| **Wayne Grody, MD, PhD**<br>Director, Diagnostic Molecular Pathology Laboratory | UCLA School of Medicine<br>Dept. of Pathology<br>10833 LeConte Avenue<br>Los Angeles, CA 90095-1732<br>College of American Pathologists, Molecular Pathology Resource Committee<br>American College of Medical Genetics, Biochemical & Molecular Genetics Committee<br>NCCLS Subcommittee on Molecular Genetics |
| **Suzanne Hart, PhD**<br>Director, Clinical Genetics Laboratory | Wake Forest University School of Medicine<br>Watlington Hall, 3rd Floor<br>Medical Center Boulevard<br>Winston-Salem, NC 27517<br>American Society of Human Genetics<br>American College of Medical Genetics, Fellow<br>NC Medical Genetics Association, Chair, DNA Application Committee |
| **Robert Johnson, PhD** | Coriell Cell Repositories<br>401 Haddon Avenue<br>Camden, NJ 08103 |

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|---|---|
| **Kenneth Kidd, PhD**<br>Professor, Genetics and Psychiatry | Yale University<br>Dept. Of Genetics, SHM, I-353<br>333 Cedar Street<br>New Haven, CT 06520-8005<br>The Human Genome Organization (HUGO)<br>American Society of Human Genetics |
| **Robert Lyons, PhD**<br>Assistant Professor, Biological Chemistry<br>Director, DNA Sequencing Core | University of Michigan<br>1150 West Medical Center Drive<br>2560 MSRBII, Box 0674<br>Ann Arbor, MI 48109-0674 |
| **Karla Matteson, PhD**<br>Associate Professor, Dept. of Medical Biology/Dept. of Pathology<br>Director, Biochemical and Molecular Genetics Laboratory | University of Tennessee<br>Developmental and Molecular Genetic Laboratory<br>1930 Alcoa Highway<br>Suite 435<br>Knoxville, TN 37920<br>American Board of Medical Genetics<br>NCCLS Subcommittee on Molecular Genetics |
| **Roger McLendon, MD**<br>Associate Professor, Pathology<br>Head, Section of Neuropathology | Duke University Medical Center<br>DUMC 3712<br>Davison Bldg, Rm. 216B<br>Erwin Road<br>Durham, NC 27710<br>American Board of Pathology, Test Committee for Neuropathology |
| **James Miller, PhD**<br>Director, Biochemical Genetics Laboratory<br>Research Assistant Professor, Biochemistry/Human Genetics | Tulane University Medical School<br>Human Genetics Program<br>1430 Tulane Avenue<br>New Orleans, LA 70112<br>State of Louisiana Genetic Diseases program Advisory Committee<br>State of Louisiana Newborn Screening Subcommittee |
| **Catherine O'Connell, PhD**<br>Research Biologist<br>Acting Group Leader, DNA Technologies | National Institute of Standards & Technology<br>Bldg. 227, Rm. A243<br>Gaithersburg, MD 20899-8311 |
| **Vicky Pratt, PhD, FACMG** | Laboratory Corporation of America (LabCorp)<br>1912 Alexander Drive<br>Research Triangle Park, NC 27709 |

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|---|---|
| **Theodore Puck, PhD**<br>Founder and Director | The Eleanor Roosevelt Institute for Cancer Research<br>1899 Gaylord Street<br>Denver, CO 80206-1210<br><br>American Society of Human Genetics<br>Genetics Society of America |
| **Stuart Schwartz, PhD**<br>Professor, Dept. of Genetics<br>Associate Professor, Oncology | Case Western Reserve University School of Medicine<br>Center for Human Genetics<br>Dept. of Genetics<br>105424 Euclid Avenue, 6th Floor<br>Cleveland, OH 44106<br><br>American Board of Medical Genetics |
| **Karen Snow, PhD**<br>Co-Director, Molecular Genetics Laboratory<br>Assistant Professor, Dept. of Laboratory Medicine | Mayo Clinic<br>Laboratory Genetics/HI 970<br>200 First Street, SW<br>Rochester, MN 55905<br><br>American College of Human Genetics<br>Human Genetics Society of Australia<br>British Society for Human Genetics/Clinical Molecular Genetics Society |
| **Timothy Stenzel, MD, PhD**<br>Director, Molecular Diagnostics Laboratory<br>Fellow, Clinical Molecular Genetics | Duke University Medical Center<br>Duke South Hospital, Rm. 4340<br>Research Drive<br>Durham, NC 27710<br><br>CAP/ACMG Biochemical & Molecular Genetics Resource Committee<br>CAP/Laboratory Accreditation Program Inspector<br>American Society of Human Genetics |
| **Jay Stoerker, PhD**<br>Director of Assay Development and QC | Genzyme Genetics<br>DNA Laboratory<br>One Mountain Road<br>Framingham, MA 01701<br><br>American Society of Human Genetics |
| **Petros Tsipouras, MD**<br>Professor, Pediatrics | University of Connecticut Health Center<br>Dept. of Pediatrics<br>263 Farmington Avenue<br>Farmington, CT 06030<br><br>American College of Medical Genetics<br>American Society of Human Genetics |

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|---|---|
| **Linda Wasserman, MD, PhD**<br>Director, Molecular Genetics Laboratory<br>Assistant Clinical Professor | University of California-San Diego<br>Division of Medical Genetics, Dept. of Medicine<br>Molecular Genetics Laboratory<br>9500 Gilman Drive<br>LaJolla, CA 92093-0639 |
| **Michael Watson, PhD**<br>Director, Diagnostic Cytogenetics Laboratory<br>Associate Professor, Pediatrics<br>Assistant Professor, Genetics | Washington University School of Medicine<br>One Children's Place<br>St. Louis, MO 63110<br>ACMG Laboratory Practices Committee<br>NCCLS Subcommittee on PCR-Based Assays in Molecular Hematology<br>NIH-DOE Task Force on Genetic Testing |
| **Ann Willey, PhD**<br>Chief, Laboratory of Clinical Genetics and Genetic Epidemiology | New York State Department of Health<br>Wadsworth Center<br>PO Box 509<br>Albany, NY 12201-509<br>American Board of Medical Genetics |

# APPENDIX B
## EXPERT PANEL MEETING SYNOPSES

**First Meeting – December 3, 1998, Atlanta, GA**
**Second Meeting – February 10-11, 1999, Dallas, TX**
**Third Meeting – May 5-6, 1999, Raleigh, NC**

# First Expert Panel Meeting

# Synopsis

**Participants.** On December 3, 1998, in Atlanta, GA, DynCorp convened and facilitated the first of three expert panel meetings, with nationally recognized experts in molecular genetic testing (MGT) for human inheritable diseases. The 23 panelists included clinicians, researchers, and laboratorians, routinely involved in the many aspects of MGT, to include test method research and development, clinical testing, quality assurance (QA), proficiency testing (PT), and laboratory accreditation. Centers for Disease Control and Prevention (CDC) Project Officers, Richard A. Keenlyside, MD, and Laurina O. Williams, PhD, as well as other CDC personnel, were in attendance

**Objectives.** Based on background information previously gathered by DynCorp, the meeting was organized and conducted to review and evaluate the spectrum and complexity of MGT QA and PT programs now offered in the US, and specifically work toward identifying a test or group of tests that could be used to monitor quality in MGT. The panelists looked at defining points in the critical pathway of each technology relative to QA, and discussed commonalities within and between technologies. The meeting format included discussions in four technology Focus Groups (PCR, Sequencing, FISH, Hybridization), as well as general sessions.

**Recommendations**. The panelists determined that: 1) method-specific QA would facilitate laboratories in validating their results with respect to their specific applications, and that there is a particular need for such a program in the area of sequencing, 2) method-specific QA tests would be complimentary to current disease-specific QA programs, and ideally, they would monitor both the process in the laboratory, as well as the results obtained, 3) before a disease-based QA/PT program is implemented, a survey should assess the need and feasibility of the proposed QA test, 4) any QA/PT program must have feedback mechanisms, so that laboratories not only assess current performance, but understand how to monitor new tests, 5) a successful QA/PT program should be designed with input from the potential participants that utilize the specific technology, 6) positive controls must be available, potentially through a cell repository, 7) a web-based information exchange would be beneficial to participating laboratories, 8) standardized guidelines are needed for incorporating new tests into the clinical laboratory, 9) standards or guidelines are needed for personnel training within all the technologies, and 10) a steering committee should be formed for any future MGT QA/PT programs, and composed of existing members of current PT programs, and from laboratories active in clinical genetics testing.

**MGT Applications Most Commonly Used in Clinical Genetics Testing.** Panelists concluded that after infectious disease and oncology, testing for inheritable diseases is the next most frequent utilization of MGT, and the conditions most often tested for are: cystic fibrosis, Fragile X, and abnormalities of chromosomes 13, 18, 21, X and Y. They then generated an extensive list of representative diseases most commonly tested for using MGT. There was consensus that much geographic variation exists for the most common tests, and low-volume tests for rare disorders may not lend themselves to QA/PT programs. However, once a test is adopted by multiple clinical genetics laboratories, it can be standardized, and then potentially incorporated into QA/PT programs. The panelists also considered all of the factors relative to decision making as regards the area of referred testing.

**State of the Art: New Tests and Technology.** The experts discussed new MGT tests being done, as well as new applications and developments of current MGT for each of the technologies: PCR, Sequencing, FISH, and Southern Blot. They indicated that the microarray or CHIP technology will be the most important and widely applicable new technology in MGT, with direct applications to clinical genetics testing. They identified quantitative PCR as an emerging and powerful tool with a great deal of utility in the clinical laboratory, and stressed the need to utilize PCR to test for more mitochondrial disorders. The

panelists also identified the need for greater automation of testing, and the desire to move away from radioactive testing.

**Commonalities and Differences Within MGT Technologies Based on Defining Critical Pathways.** The panelists discussed commonalities within each major technology, relative to potential common QA samples, and identified nucleic acid extraction/amplification, and automated sequencing as most common. Gel preparation and hybridization were seen as major sources of variation in southern blot testing, and while FISH testing requires many variations in materials and techniques, some steps may be amenable to a QA program. They identified some major sources of error in all MGT, such as volume/dilution errors, incorrect patient identification, error in mixing reagents, presence of divalent cations, and variations in room temperature. The availability and use of positive controls was deemed crucial.

**Resources and External Program Participation.** The panelists cited a number of resources that are currently available to them, to include task force reports, detailed guidelines and standards, and commercial cell repositories, as well as identified external QA programs (such as ACMG/CAP) and informal sample exchanges in which they participate.

# Second Expert Panel Meeting

# Synopsis

**Participants.** On February 10-11, 1999, in Dallas, TX, DynCorp convened and facilitated the second of three meetings of nationally recognized experts in human genetic testing. The panelists included clinicians, researchers, and laboratorians, who are routinely involved in the many aspects of MGT, to include test method research and development, clinical testing, quality assurance, proficiency testing, and laboratory accreditation. The meeting was based on background information previously gathered by DynCorp on QA/PT programs in molecular genetic testing (MGT) throughout the US, as well as summary information from the First MGT Expert Panel meeting in December, 1998. Personnel from the Centers for Disease Control and Prevention (CDC) in attendance included Project Officers, Richard A. Keenlyside, MD, and Laurina O. Williams, PhD, in addition to Ira Lubin, PhD.

**Objectives.** The meeting was held in both Focus Group and general session format, and addressed total QA needs for each of the four standard MGT technologies, with a specific focus on the potential for a test or group of tests that could be generically used to assess performance in human genetics testing. The panelists were specifically charged to address: 1) the best way to measure competency of laboratories performing MGT, and 2) those areas of QA research that might be needed to clarify current problems in MGT. In focusing on those areas, they were specifically asked to address the potential utility of generic test material, particularly as applicable to method-specific testing, standardization of test samples, and disease-based testing.

**Consensus.** The panelists identified three major needs for QA for MGT: sample development, program development, and ancillary support. Considering the meeting objectives, they specifically stressed the need for research in : 1) using spiked and multiplex samples, 2) using transfected cells and immortalized cell lines, 3) method-based testing of DNA extraction/quantitation, and 4) sample development utilizing synthesized or constructed DNA to contain specific restriction sites. They stressed development of: 1) pilot/PE programs for PCR, Southern Blot, Sequencing, and FISH, and 2) a comprehensive technology/methodology database. Additionally, they advocated needed support to : 1) administer a detailed laboratory methodology survey, 2) sponsor both disease-specific and method-specific consortia, 3) establish a website/LISTSERV for sharing MGT information, 4) create a centralized index of genetic databases, 5) enhance communication between laboratories for facilitating new tests, validation, and comparison, and 6) facilitate training for laboratory scientists. The panelists advocated the formation and development of organizations that would be complementary to existing programs such as the MGT PT Program of the ACMG/CAP. It was determined that generic testing may have a niche in laboratories that: 1) haven't performed MGT before, or 2) that may be taking on a new MGT test for which no PT program is offered.

# Third MGT Expert Panel Meeting

# Synopsis

**Participants.** This third and final meeting was held May 5-6, 1999 in Raleigh, NC. It included our core group of molecular genetics testing (MGT) experts from the medical research and diagnostic laboratory testing areas (to include CAP), along with the addition of others from industry, a non-profit cell repository (Coriell), and the National Institute of Standards and Technology (NIST). Among the panelists were Dr. Wayne Grody, representing CAP and its Surveys for MGT, Dr. Michael Watson, Chair of the writing and review committees for the new Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics (ACMG), and Dr. Karla Matteson, a co-author of the proposed NCCLS guidelines for MGT. The participants were enthused regarding this project, and very knowledgeable regarding current QA needs and other concerns in MGT. Personnel from the Centers for Disease Control and Prevention (CDC) in attendance included Project Officer Laurina O. Williams, PhD, and Ira Lubin, PhD.

**Goals and Objectives.** The meeting was held in both Focus Group and General Session format to maximize the productivity of the discussions. It addressed four major goals: 1) to prioritize pilot research ideas generated in the 2nd Expert Panel Meeting, based on total QA needs for MGT, and urgency to the laboratory community; 2) to describe an ideal, total QA program for MGT including pre- and postanalytical testing issues; 3) to suggest potential involvement of CDC/PHPPO and others to conduct pilot research and/or otherwise provide support of QA in MGT; and 4) to examine the critical pathways of the test methodologies with respect to quality assurance and performance evaluation.

**Consensus.** Building upon the findings of the two previous Expert Panel Meetings, and addressing the objectives of the meeting, the panelists were able to reach consensus on QA needs and issues that potentially could be addressed by CDC/PHPPO at the present time. It was interesting that, as different aspects of QA for MGT were focused upon, the same priorities were identified each time. The overwhelming consensus identified three immediate QA needs for the molecular genetic testing field that appear consistent with PHPPO's overall mission: 1) an emphasis on sample development (pilot research) and positive controls, 2) the development of Performance Evaluation (PE) programs to supplement what already exists, particularly for diseases and/or methodologies not covered by the CAP program, and 3) the establishment and support of disease-specific laboratory oriented consortia, which, could provide a forum for information networking and thus provide validation for different methods through results comparison.

**Pilot Research Prioritization.** The experts categorized pilot studies into the general areas of sample development, program development, and ancillary support. The consensus was that sample development and the need for positive controls is of the utmost importance, focusing first on existing repositories and resources, and secondarily researching novel sample development (i.e. spiked samples, transfected cells, synthesized DNA, etc.). Program development follows in importance, with the need to develop PE programs and sponsor disease-specific consortia. Ancillary support was deemed important in terms of web sites created to support laboratories, as well as the establishment and maintenance of genetic testing and methodology databases. All panelists expressed their willingness to participate in any further research activities in molecular genetic testing.

**Ideal QA Program.** There was overall consensus that within an ideal QA program, there would be tremendous support toward ensuring the quality of the preanalytic, analytic, and postanalytic steps. An ideal preanalytic focus would utilize standardized request forms and informed consent agreements, and a database of disease-specific information relative to specimen acceptance/rejection criteria, along with other clinical and technical information. For the analytic phase, ideally there needs to be adequate information about test methods and performance, along with personnel training in all areas of the diagnostic process, while the postanalytic phase needs the standardization of results reporting and

interpretation. The experts strongly recommended the establishment and support of disease-specific databases and consortia that would provide valuable information to assist laboratories bringing up and validating new tests, provide information regarding appropriate tests and new methods, and assist with interpreting and reporting results effectively. This would be an important resource to testing laboratories throughout the entire diagnostic process.

**Involvement of CDC/PHPPO and others.** The experts discussed potential roles they would like to see CDC/PHPPO or other agencies and organizations fill in the general area of quality assurance for molecular genetic testing, in order to support the laboratory community. They focused on identifying roles and activities associated with 1) laboratory improvement, 2) performance evaluation, and 3) pilot research projects discussed during the meeting. Panelists envisioned a number of possible roles for CDC/PHPPO in assisting clinical MGT testing laboratories. Those included: 1) the assistance of CDC in sample development and research, and the need to expand the availability of suitable positive controls, 2) a survey of clinical laboratories to determine the extent of molecular genetic testing for human inheritable diseases, including the major QA needs, as an initial step toward implementing an MGT PE program, 3) the linking of appropriate internet resources through an expanded CDC website that addresses specifically the needs of clinical MGT laboratories, and the development of an MGT PE Program to complement programs currently available.

**Examination of Critical Pathways.** The expert panel examined the critical pathways for QA in MGT for each of the four technologies, PCR, FISH, sequencing, southern blot, to identify critical areas amenable to performance testing in the laboratory. They considered what steps were most prone to error; at which points an error might lead to adverse consequences for the patient; and what steps could realistically be monitored or otherwise addressed. The key steps they delineated in each pathway would be an integral part of any proposed method-specific or disease-specific PE/QA program.

**Conclusion.** As the final MGT Expert Panel Meeting concluded, the panelists thanked Dr. Williams and CDC for the opportunity to participate in such an extensive information gathering program focusing on quality assurance in molecular genetic testing. Unanimously, they were in agreement that the momentum toward improving diagnostic testing for human inheritable diseases that the intensive year-long program had produced, should proceed with the continued support of CDC, along with their own involvement. Each of the participants acknowledged their availability to assist CDC, particularly by participating in the recommended sample development and other pilot study research areas.

# APPENDIX C
## SUPPLIERS OF MOLECULAR GENETIC TESTING COMPONENTS

### A Sampling of Vendors

The following is a sampling of vendors that manufacture and/or market instrumentation and reagents for MGT, including products for FISH analysis, automated DNA sequencers, DNA sequencing kits, DNA primers and/or DNA amplification kits and thermal cyclers for PCR. Several vendors also provide genomic microarrays for research use only. In general, the average shelf life for most of the DNA primers and kits can be from six months to a year when stored under optimal conditions, i.e. in their commercial buffers at -20°C. Complied in August, 1999.

Amersham Parmacia Biotech, Inc.
800 Centennial Avenue
PO Box 1327
Piscataway, NJ 08855-1327
800-323-9750
http://www.apbiotech.com
Products include: DNA sequencing kits and reagents, automated sequencers, PCR kits and reagents

BioRad Laboratories
Life Science Research
2000 Alfred Nobel Drive
Hercules, CA 64547
800-4-BIORAD
http://www.bio-rad.com
Products include: DNA sequencing kits, primers

Boehringer Mannheim/Roche Molecular Biochemicals
PO Box 50414
Indianapolis, IN 46250-0414
800-262-1640
http://biochem.boehringer.com
Products include: DNA sequencing and PCR primers, kits and reagents

Clontech
1020 East Meadow Circle
Palo Alto, CA 94303
800-662-2566
http://www.clontech.com
Products include: DNA sequencing primers and kits, PCR amplification kits

Epicentre Technologies Corp.
1402 Emil Street
Madison, WI 53713
800-284-8474
http://www.epicentre.com
Products include: DNA sequencing primers, DNA sequencing kits

Genetic Microsystems, Inc.
34 Commerce Way
Woburn, MA 01801
781-932-933
http://www.geneticmicro.com
Products include: genomic microarray products and services

LI-COR Inc.
4308 Progressive Avenue
Lincoln, NE 68504
800-645-4267
http://www.licor.com
Products include: IR2 System DNA sequencers, DNA sequencing primers, DNA sequencing kits

Life Technologies, Inc.
9800 Medical Center Drive
Rockville, MD 20850
800-338-5721
http://www.lifetech.com
Products include: DNA sequencing and PCR primers, kits and reagents

Perkin-Elmer Applied Biosystems
800-762-4000
Products include: automated DNA sequencers, DNA sequencing kits, thermocyclers, PCR kits

Pharmacia
Global Medical Instrumentation, Inc.
3874 Bridgewater Drive
St. Paul, MN 55123
800-858-5945
http://gmi-inc.com
Products include: ALF Express DNA sequencer, DNA sequencing primers, DNA sequencing kits

Promega Corp.
2800 Woods Hollow Road
Madison, WI 53711-5399
800-356-9526
http://www.promega.com
Products include: PCR and DNA sequencing reagents, Molecular Diagnostics

Qiagen
28159 Avenue Stanford
Valencia, CA 91355
800-426-8157
http://qiagen.com
Products include: automated DNA sequencers, DNA amplification kits, DNA purification kits

Sigma-Aldrich
3050 Spruce Street
St. Louis, MO 63103
800-325-3010
http://sigma-aldrich.com
Products include: PCR and DNA sequencing reagents and accessories, molecular biology reagents

Visible Genetics, Inc.
700 Bay Street
Suite 1000
Toronto, Ontario
Canada M5G 1Z6
http://www.visgen.com
Products include: OpenGene System: Microgene Clipper (2 dye sequencer), Microgene Blaster (1dye sequencer), OpenGene Software, DNA sequencing kits

Vysis, Inc.
3100 Woodcreek Drive
Downers Grove, IL 60515-5400
800-553-7042
http://www.vysis.com
Products include: FISH probes, reagents, and instrumentation, genomic microarrays

# APPENDIX D
## ELEMENTS OF MOLECULAR GENETIC TESTING ADDRESSED BY AN IDEAL QA PROGRAM

**Preanalytic Phase**
**Analytic Phase**
**Postanalytic Phase**

The ideas summarized in these tables for an ideal "global" QA program target needs in laboratory QA practices and system support. Laboratory practices would include the appropriate use of calibrators, QC procedures, QA programs, technical support, and more. Support could be provided by government agencies, professional organizations, consortia or combinations thereof.

## PREANALYTIC PHASE

| AREA REQUIRING IMPROVEMENT | HOW AN IDEAL QA PROGRAM/SYSTEM WOULD ADDRESS THIS AREA |
|---|---|
| Obtaining patient-related information from physician | Develop standardized forms to capture appropriate patient information<br>Educate physicians regarding MGT by providing disease-specific clinical information regarding patient-related information |
| Obtaining test request from physician | Develop standardized test request forms<br>Educate physicians regarding MGT by providing disease-specific clinical information and peer-reviewed materials |
| Obtaining patient consent | Develop standardized consent forms<br>Address the consent issue for various uses of patient materials, such as for QA |
| Accepting sample for testing | Determine what laboratory's responsibilities are as "gatekeeper" – i.e., should the laboratory refuse to test samples that lack complete paperwork?<br>Formulate disease-specific criteria for sample acceptance<br>Support continuing education for technicians and other technical personnel |
| Preanalytic phases of test development and implementation | Certify clinical genetics laboratories through outside regulatory bodies and/or professional organizations |
| Developing/implementing new tests in the laboratory | Develop centralized resources to provide controls/samples for test validation.<br>Develop and frequently update disease-specific databases that includes outcome data (see Analytic Phase). A performance evaluation (PE) database would play a role here.<br>Create a centralized resource for patent information and vendor requirements.<br>Provide laboratories with technical expertise when validating new tests. |

## ANALYTIC PHASE

| AREA REQUIRING IMPROVEMENT | HOW AN IDEAL QA PROGRAM/SYSTEM WOULD ADDRESS THIS AREA |
|---|---|
| Lack of positive controls | Provide laboratories with the option and ability to maintain an adequate stock of internal controls.<br>Facilitate collection and distribution of test specimens and standards through disease consortia or other mechanisms<br>Instruct centralized facilities as to what is needed in terms of controls, test specimens and standards through consortia or other mechanisms. |
| Limitations on existing QA/PT programs | Provide support through state health departments, cooperative agreements, or other mechanisms to: 1) monitor areas of the total testing process; 2) allow laboratories to obtain aliquots of samples for which they got incorrect results on the ACMG/CAP surveys, to provide for troubleshooting and correction of problems; 3) provide more "borderline" samples; 4) provide more challenges per year and for more diseases, 5) establish results-based test validation criteria. |
| Lack of standardization in testing among laboratories | Facilitate and support establishment of a mechanism for promoting exchange of information among laboratories.<br>Capture and report information on sample processing, including sample handling, preparation, identity verification, and storage.<br>Develop and frequently update disease-specific databases that include: 1) methodology; 2) outcome data; 3) summarized results of PT/PE programs such as ACMG/CAP; 4) information from member laboratories regarding procedures, reagents, and instrumentation; and 5) information on personnel training.<br>Follow database variables and correlate with laboratory results to provide information regarding the reliability and reproducibility of methods used.<br>Standardize and verify laboratory methods, by comparison of results, through disease-specific consortia or other means.<br>Increase standardization and monitoring of commercial products.<br>Utilize standardized guidelines for writing standard operating procedures. |
| Inconsistent use of external quality assurance measures by laboratories | Ensure that in any laboratory's QA program, confidentiality is maintained and the correct information is disseminated to the appropriate parties.<br>Support laboratory use of: 1) licensing of personnel, including technicians, 2) resources currently available, including daily QC, CAP surveys, and any other available resources.<br>Facilitate offsetting of cost for PE/PT especially for laboratories doing small volume testing |
| Inconsistent use of internal quality assurance measures by laboratories | Support laboratory use of *Good Laboratory Practices* as a model for written standards for QC/QA<br>Pursue continuous "quality improvement" with QA being continuously redefined, standardized and optimized in the field<br>Continuously update QA/QC standards for improving testing efficiency and effectiveness<br>Participate in proficiency/performance testing programs. |
| Rare disease testing | Address the need for QA procedures for rare disease testing because of the relatively low frequency with which it is performed. |
| Inadequate personnel training | Further develop/support licensing for MGT to improve the quality of testing in the laboratories.<br>Provide training to personnel involved in the MGT process. |

## POSTANALYTIC PHASE

| AREA REQUIRING IMPROVEMENT | HOW AN IDEAL QA PROGRAM/SYSTEM WOULD ADDRESS THIS AREA |
|---|---|
| Interpretation | Ideally laboratories should report PE/PT results as they would for patient specimens.<br>Ensure that in any laboratory's QA program the laboratorian and the physician signing out the report correctly interpret the results. This step is typically addressed by clinical audits, but may not be done as thoroughly or as often as needed for total QA.<br>Develop guidelines to standardize results interpretation, realizing that these may be both mutation-specific and disease-specific.<br>Address utilization and requirements for reporting depending upon purpose of testing<br>Develop guidelines with recommended algorithm pathways to indicate when: 1) a test should be repeated, or 2) when a secondary test is needed.<br>Develop and support consortia/websites/databases to provide and share outcome data; continuously update and correlate test results with the clinical data. |
| Reporting | In PE/PT programs, laboratories should ideally report results as they would for patient specimens.<br>Develop disease-specific standards for reporting results<br>Develop model report forms |