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Molecular Genetics Laboratory: Detailed Requirements for Accreditation by the College of American Pathologists

Molecular Genetics Laboratory: Detailed Requirements for Accreditation by the College of American Pathologists

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Abstract

The Objectives of this review is to assimilate all known requirements in a single article for individuals or organizations interested in accrediting a molecular genetics laboratory by College of American Pathologists (CAP). The CAP checklists, which are sent to laboratories applying for accreditation, are series of questions designed to interrogate laboratory standards and all related aspects pertaining to quality. However it is by no means a fully detailed protocol to be followed to achieve full accreditation, hence the need for this review, and individuals or organisations are obliged to seek further supporting documentation and literature.

The accreditation program is dependent upon successful performance in the molecular genetics survey (proficiency testing) for each analyte tested and passing the on- site inspection. The on-site inspections are carried out by practicing laboratorians with expertise in molecular genetics, who uses a laboratory general checklist (covering general aspects related to all clinical laboratories) and molecular pathology checklist (covering specific requirements for molecular genetics). Once deficiencies cited during inspection are corrected, the laboratory will be accredited for a two-year period. Accreditation is maintained through continued successful participation in the proficiency testing and completion of a mandatory self-evaluation, which is done during the second year of the accreditation cycle. Accreditation is denied when the laboratory fails to meet the CAP standards for laboratory accreditation.

Introduction

In recent years the field of molecular genetics has matured dramatically to the point that techniques involved are now widely used in routine practices.

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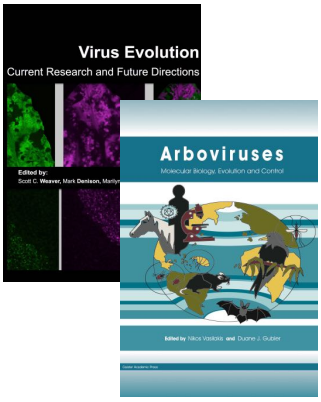

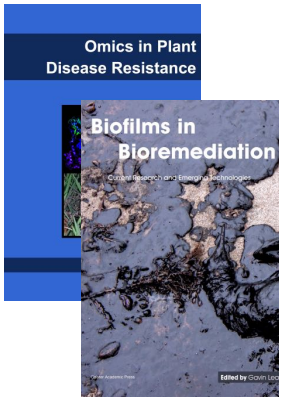

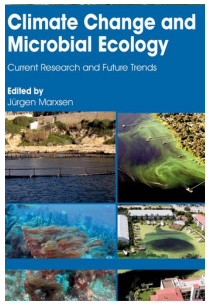

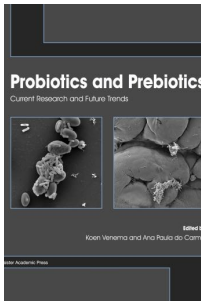

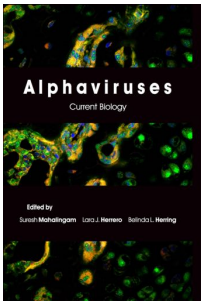

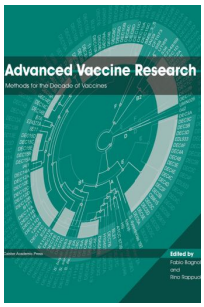

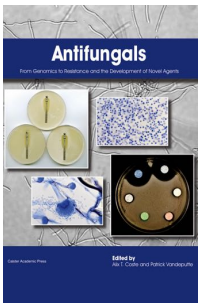

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Currently, molecular diagnostics is used in most major medical centers and numerous commercial laboratories -and can provide valuable information which impact positively on the well being of the individual.

Since the development of this technology, experts called for quality assurance measures, standards and recommendations governing genetic testing. The College of American Pathologists was amongst the first accreditation organizations to call for establishment of standards in such complex testing. These quality assurance measures and standards can now be achieved through the Laboratory Accreditation Program (LAP) run by CAP.

The history of LAP goes back to 1961, when it was first initiated by CAP and in 1967, the U.S. Clinical Laboratory Improvement Act (CLIA) came into effect, which recognized laboratories accredited by CAP. In 1970 the Joint Commission on Accreditation of Healthcare Organizations recognized the LAP offered by CAP, resulting in a large number of laboratories entering the program (Hamlin and Duckworth, 1997). LAP is voluntary in that each laboratory desiring accreditation must request it. LAP is widely recognized as the "gold standard" of laboratory accreditation programs and has served as a model for various state and private accreditation programs throughout the world. In fact, several governmental regulatory agencies (e.g. the U.S. Health Care Financing Agency) as well as private agencies (e.g. The Joint Commission on Accreditation of Health Care Organizations) accept the LAP in place of their own programs for laboratory accreditation. The program has now accredited more than 6,000 laboratories. Although the majority of laboratories accredited are in the USA or Canada the program has accredited several laboratories around the world (Merrick, 2000).

The program examines all aspects of quality control and quality improvement in the field of molecular genetics, including test methodologies, reagents, control media, equipment, specimen handling, procedure manuals, test reporting and internal and external proficiency testing. In addition, the LAP monitors all aspects related to personnel, safety, laboratory computer services, space, communications and overall management practices. The LAP uses an educational, peer-reviewed inspection process, which allows any laboratory to be inspected by knowledgeable working professionals who are in tune with the changing needs of the laboratory community. This serves the purpose of adding an educational experience to the inspection process and allows both inspectors and laboratory staff to share their knowledge and expertise (Merrick, 2000).

This review offers detailed updated requirements for accrediting molecular genetics laboratories by the Laboratory Accreditation Program of the College of American Pathologists. The review covers the accreditation requirements of molecular diagnostic methods for genetic diseases but not that for infectious diseases. Additionally, the overall process of accreditation by CAP will not be discussed here as it has been reviewed elsewhere (Abu-Amero *et al.*, 2001).

We hope that this review will be useful to professionals working in the field of

molecular genetics and to others considering accreditation by CAP. Although most accreditation requirements listed here are those for CAP, readers who are seeking accreditation by other agencies may find this review helpful. Readers should note that the CAP requirements for accreditation are constantly being updated and hence it is necessary to contact the CAP office to ensure compliance with the most up to date requirements.

Accreditation Requirements for Molecular Genetics Laboratories

CAP requirements for most operations carried out by molecular genetic diagnostic laboratories will be discussed below.

1. Requisitions and Specimen Receipt

All specimens should be accompanied by a requisition form which contains as much of the following information as possible: unique patient identification, sex, date and time of specimen collection, specimen type, race/ethnicity, unique identifier found on the specimen container, tests requested, patient location, reason for requesting the test, relevant clinical or laboratory information, pedigree (required for linkage analysis, recommended for all cases), referring physician or health professional and billing information.

All specimens received should be uniquely identified to minimize sample mix-ups, mislabeling etc. The system should allow to positively identifying all patient specimens, specimen type and aliquots at all times. A bar coding system is recommended, which will also help to ensure confidentiality.

2. Specimen Handling

The laboratory must have adequate instructions for specimen collection and handling before being received by the laboratory. These instructions will be for proper labeling of specimens, proper collection of specimens from all relevant sources, delivery of specimens, specimen preservation if processing will be delayed (e.g., refrigeration) and procedure for safe handling of specimens. All specimens received should be recorded in an accession book, worksheet, computer or other comparable record together with the date and time of receipt.

There should be a written criteria for rejection of unacceptable specimens. For every test offered, documentation describing appropriate and inappropriate clinical indications and the procedure for rejection of irrelevant samples should be in place. There should be a written policy that no aliquot is ever returned to the original container. Similarly a written procedure should be in place for safe aliquoting of samples in a way to prevent cross-contamination and schedule for retaining specimens.

For chorionic villi or amniotic fluid cells, there should be a back up cell culture available. The molecular genetic laboratory does not necessarily need to be responsible for the cell culture work provided that additional material for testing is readily available if required.

2.1 Parentage and Forensic Identity Testing

The following regulation should be strictly adhered to when handling these types of specimens. Verified identification of all individuals presenting themselves for testing should be documented (the use of photographs and/or fingerprints is strongly recommended). Procedures should be adequate to verify specimen identity, integrity and to maintain chainofcustody throughout all steps of the process beginning with specimen collection including packaging and transportation. Any tampering with the specimens upon arrival at the laboratory should be documented. In addition, specimens should be maintained in a secured area with limited access at all times (Tsongalis *et al.*, 1999).

3. Specimen Processing

Molecular diagnosis may be accomplished by any of several methodologies. CAP does not favor any particular technique over another of equivalent sensitivity and specificity, provided the laboratory can demonstrate reliable results and quality control with whichever technique is chosen.

3.1 Sample Identification

Sample identification should be assured through all applicable phases of analysis including nucleic acid extraction and quantification, restriction enzyme digest, electrophoresis, transfer, hybridization, detection, *in situ* hybridization, enzymatic amplification, photography and storage.

3.2 Nucleic Acids Extraction

Nucleic acids should be extracted and purified by methods reported in the literature; if not there should be documented evaluation of the method used. Extracted nucleic acids should be stored in a manner adequate to prevent degradation. Isolated DNA should be stored in a tightly capped container and kept at 4°C (stability of DNA can be guaranteed for many months at this temperature). Long-term storage should be carried out at -20°C or -70°C to prevent degradation. RNA should be stored at -20°C or -70°C once extracted, since RNA degrades quickly (Brown, 1991b).

3.3 Nucleic Acids Quantification

The quantity of nucleic acid should be measured and recorded. This is usually done using a spectrophotometer that has been properly calibrated with the use of proper controls and measuring the absorbance. This should be performed in clean, dry, quartz cuvettes within the linear range of the particular spectrophotometer being used. To determine the concentration of purified DNA, an absorbency reading at 260 nm (nucleic acid absorbs maximally at this wavelength) should be performed. An absorbance reading of 1 corresponds to approximately 50 mg/ml for dsDNA. Since proteins absorbs maximally at 280 nm, determination of the A_{260}/A_{280} ratio provides a qualitative measurement of the level of DNA in respect to the amount of contaminating protein in the sample. Ratios of 1.8 to 2.0 indicate high levels of DNA purity. If the ratio is

below 1.6, purity may be improved by re-extraction and precipitation (Brown, 1996).

3.4 Quality of Extracted Nucleic Acids

The quality (intactness) of high molecular weight DNA and RNA should be assessed. The laboratory should carefully follow established protocol and incorporate controls to verify proper performance for each extraction.

3.5 PCR Methodologies

3.5.1 Amplification

To assure PCR product specificity, all reaction conditions (reagents and thermocycling parameters) must be established for each test system. Reaction conditions must provide the desired degree of PCR product specificity. When amplification of a variable length sequence is assayed, the system should be tested with DNAs from individuals representing large and small amplification products to evaluate the impact of differential amplification (Brown, 1991a).

3.5.2 PCR Product Detection and Analysis

Detection systems (visual, restriction site, allele specific oligonucleotide, hybridization, etc.) employed in diagnostic testing are being rapidly adapted from established research and diagnostic protocols. Such systems should be well documented and published. The laboratory must demonstrate that a level of specificity characteristic of the selected detection system has been attained internally and that the level of specificity is adequate for detecting the expected products. Adequate care must be taken to guard against failure to detect PCR products.

3.5.3 Controls and Standards

For each PCR run, three types of controls should be included. A positive control, which will provide specific evidence of amplification for each mutation or genotype tested (positive controls must include individuals of known genotype for the locus being tested); a negative (normal) control which means running a DNA sample from a patient screened previously and found to be negative for the mutation or the disease being investigated and a blank control which contains all components of an amplification reaction except template DNA. The primary purpose of this final control is to detect contamination with DNA, especially amplicons from previous amplification reactions (Erlich, 1999). In addition, a known molecular weight marker that spans the range of expected product size should be used for each electrophoretic run, which will help in estimating the size of the PCR product. Controls for various types of assays are as follow:

Assays based on presence or absence of PCR products must include an internal control yielding a positive result to check for proper amplification and sizing of the PCR products and to ensure that a negative result is accurate (Rosenstraus

et al., 1998).

When specimens are analyzed for sequence variation (Restriction Fragment Length Polymorphisms (RFLP) sites, mutation specific sites, etc.) controls containing all alleles to be detected must be included.

Assays in which the result is based on fragment size [Variable Number of Tandem Repeats (VNTRs), microsatellites, etc.] must include size markers (sequencing ladders, etc.) covering the range of expected results during gel electrophoresis.

Assays based on changes in electrophoretic mobility (homo/heteroduplex analysis, single strand conformation analysis, etc.) must include appropriate controls to ensure correct interpretation of results. Any unexpected results require repeat of assay. Procedures for analysis of possible new mutations should be available.

3.6 Restriction Enzyme Digestion

Efficiency of restriction endonuclease digestion may be confirmed by including an undigested control sample, which contains DNA, restriction enzyme buffer and distilled water in the absence of restriction enzyme and electrophoresing alongside digested samples. The sum of all fragments sizes of the digested product should be equivalent to the size of the undigested fragment (Brown, 1991c).

3.7 Denaturing Gradient Gel Electrophoresis (DGGE) Assays

3.7.1 PCR Fragment Design

All sequences to be analyzed by DGGE should be amplified by PCR using protocols optimized for the amplicon in question. The specificity of the PCR reaction should be such that a single amplicon is seen on a stained gel. Each amplicon should be designed using available software or empiric analysis to produce a single melting domain throughout the region to be assessed. The primers used in the amplification step should be designed to include a 5'-clamp sufficient to stabilize the melting domain of the test DNA sequence (Fischer and Lerman, 1983).

3.7.2 Sample Preparation

DNA samples should be prepared, stored and amplified according to the previously mentioned guidelines. Samples should be heated and allowed to re-anneal prior to loading to permit heteroduplex formation. Time and temperature should be standardized. If a potential homozygous mutant condition is being analyzed, it may be appropriate to mix a known normal control and test sample to force heteroduplex formation.

3.7.3 Gel Electrophoresis

Appropriate denaturing gradient conditions should be established based on

calculated melting profile and empiric results observed with positive controls. A set of positive controls should include (whenever possible) samples containing mutations distributed throughout the region to be analyzed. Equipment used to form the gradients in the gels and to run gels under temperature-controlled conditions should be standardized within each laboratory. Any change in equipment will require a re-validation of the assay. Samples to be run on the same gel should be denatured, annealed, and loaded on the gel at the same time. A positive control sample should be analyzed simultaneously to provide a measure of the adequacy of the heteroduplex formation and the gel running conditions. A negative (normal) control sample can be used to aid in sizing of the observed bands. It is not necessary to run a sample of every known mutation in each gel. A single mutation control is sufficient to document the reproducibility of the system.

3.7.4 Data Analysis

Gels should be stained (or visualized based on labeled DNA) in a manner adequate to detect the entire banding pattern created. Heteroduplexes are often present in smaller amounts than the homoduplex forms and may produce a lighter signal. Samples on the gels should be identified by an unambiguous method clearly identifying positive and negative controls. Documentation of gel results by photography or other image storage system is necessary. Computerized image analysis may be helpful in identification of recurring mutations. The presence of putative mutations identified by DGGE must be confirmed by sequencing.

3.7.5 Validation

Each laboratory must validate the technique for each sequence to be analyzed. Validation with known mutations as well as normal samples is required. Results of validation studies for each gene analyzed must be available for review.

3.8 Heteroduplex Assays

PCR product sizes of approximately 150-300 bp are ideal for screening unknown mutations by heteroduplex analysis. Larger fragments can be used to detect specific mutations or polymorphisms once it has been established that a heteroduplex band can be consistently detected under standardized conditions. The location of the mutation/polymorphism of interest should be at least 40-50 bases from the ends of the DNA fragments. Thus, PCR primers in flanking intron sequences should be at 40-50 bases from the intron-exon junctions. PCR amplification of the regions of interest should be carried out according to all standard precautions. It is critical that each amplicon produce a clean, single band for use in heteroduplex analysis. Samples should be heat denatured and allowed to re-anneal to facilitate heteroduplex formation. The time and temperature for denaturation and annealing should be standardized. In case of potential homozygous mutations, PCR products from wild type controls should be mixed, denatured and re-annealed with the test samples to force the formation of heteroduplexes. The composition of the gel matrix to be used for heteroduplex analysis, the thickness of the gel, the length and time of the run,

and the electrophoresis equipment should be standardized within each laboratory. Samples to be analyzed on the same gel should be denatured, re-annealed and loaded on the gel run to validate the results for each gel. Heteroduplex gels should be visualized by staining or by autoradiography, depending on the detection system employed, to detect the entire banding pattern required for mutation detection. The detection system used to detect the heteroduplex bands (e.g., the specific staining protocol) should be standardized in each laboratory. Results should be scored unambiguously by comparison with the positive and negative controls. All putative positive results detected by heteroduplex analysis should be confirmed by sequencing to identify the mutation or polymorphism involved. The heteroduplex analysis technique should be validated by using known mutations, which should exhibit detectable and in many cases characteristic heteroduplex banding patterns for specific mutations, as well as normal control samples. For each gene analyzed by heteroduplex analysis, validation test results should be available for review (Glavic and Dean, 1995).

3.9 Southern Analysis

3.9.1 Restriction Digestion and Electrophoresis

Restriction endonuclease digestion of prepared DNA for Southern analysis must be done according to a standardized protocol, which will be documented in the laboratory manual.

Quality control of restriction digests must be done by one of the following: Run a test gel prior to electrophoresis. If incomplete, re-digest the specimen. Evaluate the analytical gel by visually comparing to size markers or to the patterns of all DNAs on the gel, including controls, for consistency of satellite bands as well as high and low molecular weight bands. Each test must include human DNA control(s) with documented genotype at the locus tested (Brown, 1993).

3.9.2 Membrane Preparation

Prior to transfer, the Southern gel must be photographed to provide a hard copy documentation of the gel. The method of transfer must be documented in the laboratory manual with appropriate references. Efficiency of transfer must be validated and documented either at time of transfer or at the end of the study by using photographic or autoradiographic film and appropriate control DNA, including human control(s), digested along side the samples. All Southern gels should include internal and external size markers to assist in the reading of the alleles. External markers may be excluded if appropriate heterozygotes or "all allele" controls are used.

3.9.3 Hybridization

Hybridizations must be carried out by accepted procedures and documented with appropriate references. Hybridization can be checked by scoring the known controls included on the Southern filter. For those markers new to the

laboratory, a previously used filter, if available, on which the DNA has been cut with the appropriate enzyme (or a test DNA of known genotype), shall be used as further quality control of the hybridization. The laboratory must retain a representation of the primary data (gel, film, autoradiograph, etc.) demonstrating the reported hybridization pattern.

3.10 Sodium Dodecyl Sulphate -Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Translation products are separated by discontinuous SDS-PAGE. Commercially available protein markers are usually used as molecular size standards. If the protein product of interest is very large, special standards may be required. A normal control must be run with each batch of test samples. Previously prepared (known product size) controls may be used as an external size indicator, but a simultaneously transcribed/translated control is also required (Maniatis *et al.*, 1989a).

3.10.1 Interpretation

A mutation is indicated by the presence of a novel band of lower-than-normal molecular weight representing a truncated peptide. If the band representing the full-length polypeptide is present in the same sample, it can serve as an internal control. Background" bands are often observed. Some of these are artifacts due to translation from internal AUG codons downstream from the authentic start codon or erroneous translation termination due to a non-optimized "*in vitro*" system. Other background bands present may represent proteins in the reticulocyte lysate or alternatively-spliced products from the gene of interest. Again, comparison of bands with those from a known normal control assayed simultaneously is essential. The presence of a truncated polypeptide is suggestive of an underlying genomic mutation. In most cases, the length of the truncated polypeptide (determined by using the protein markers as standards) can be used to localize the putative mutation. If the polypeptide is truncated due to a large deletion, the deletion site can be determined by restriction endonuclease mapping. The analytical specificity and sensitivity of the protein truncation assay is not known. It is essential to verify the presence of each mutation by either sequencing genomic DNA or sequencing cDNA followed by analysis of genomic DNA using RFLP or Allele Specific Oligonucleotide (ASO) methodologies.

3.10.2 Validation

Each laboratory must validate the technique for each gene to be analyzed. Validation with known mutations as well as normal samples is required. Results of validation studies for each gene analyzed must be available for review.

3.11 DNA Sequencing Analysis

Although the sequence assay shares elements in common with all other DNA diagnostic assays, there are unique concerns and areas that require separate attention. Unique issues that arise in DNA sequence assays result from the large number of analytical points measured in each particular assay (i.e., the

number of bases analyzed) and the relatively small signal strengths that are obtained from any base at any position. The technology for the generation of the sequence information is also generally complicated. Therefore, the sequence information must be verified and controlled at multiple points in the generation and interpretation of the sequencing data. One very positive aspect of the emerging use of sequencing for molecular diagnostics is that the likely errors will be biased very strongly towards the generation of false positives, rather than false negatives. This is a consequence of the fact that it is much easier to produce a sequence that looks as if it contains the wrong base(s) than a clear profile showing only the correct base. As each positive can and should be tested by an independent determination, this direction of bias is desirable. Potential for missing a heterozygous base substitution is a concern. To increase the sensitivity of heterozygote detection, both the sequencing chemistry and polymerase used should be optimized to produce uniform peak intensities in the case of fluorescent sequencing, since variations can result in false negatives. Both of these scenarios underscore the need to sequence both strands of the DNA region analyzed to optimize sensitivity and specificity of the assay (Maniatis *et al.*, 1989b).

3.11.1 Methodologies

Presently the most widely used method is the Sanger dideoxy chain termination, which can be applied in several forms. Manual sequencing requires a radioactive label (^{32}P, ^{33}P or ^{35}S) in one of the four dNTPs or at the 5´ end of a sequencing primer. The advantages over automated sequencing include good signal-to-noise ratio. However, the disadvantages are low throughput and requirement for radioactivity. Both manual and computer-assisted reading formats can be used, but computerized systems provide more accurate transfer of data. Fluorescent sequencing reactions can be performed using dye primers or dye-labeled primers or dye terminator chemistries and one of several polymerases. Data collection uses an imaging system and appropriate software. Automated fluorescent sequencing can be performed using automated sequencer formats providing automated gel running and data collection. Capillary gel electrophoresis for sequencing has been described and is superseding all currently used techniques.

3.11.2 PCR Amplification

The length of the region to be sequenced in a single run must be limited. An upper limit of accurately readable sequence exists for each methodology and gel apparatus type. The quantity of the DNA must be sufficient to generate adequate PCR product. This can be determined by meeting an expectation of PCR efficiency (e.g., an agarose or acrylamide gel separation of an aliquot of the PCR can be compared to a standard).

3.11.3 Sanger Sequencing

Primers directed towards the end of the fragments are used. There are several chemistries available but each should be aimed at providing the best possible

sequence coverage of the fragment.

3.11.4 Gel Electrophoresis

Following the Sanger reaction, materials must be pooled (dye primer reactions) or purified from unincorporated materials. Normal care is needed to prevent sample mix-up. The tracking of individual samples on gels is a difficult and potentially error-prone step. Standard loading formats should be used to ensure this part of the process is accurate. Gel preparation using commercially available premixed solutions may provide additional quality control. If the supplier of the solutions changes, separation characteristics must be re-evaluated. The characteristics of each gel apparatus/power supply combination are unique. Therefore timing, voltage requirements and separation characteristics must be standardized for each individual set-up.

3.11.5 Primary Base Calling

The overall quality of the sequence reactions must be monitored. The concern is that poor sequence reactions containing artifacts such as "stops," compressions, or "Ns" will be difficult to interpret and will result in the classification of normal bases as mutant or vice versa. Every effort should be made to resolve any such regions. Routine analysis of the opposite strand sequence will be useful for that purpose. The use of a different sequencing chemistry or polymerase may resolve specific regions, since artifacts may not occur in identical spots under alternate conditions. Currently available criteria include the number of positions at which computer base calling is not possible.

A comparison of each test with a known standard (e.g., Gene bank) is required, including judgment of peak height. (Caution should be exercised, since not all sequences in Gene bank are correct.). Manual re-reading of areas where the software has had difficulty should be performed with caution. The chromatograms of both the forward and reverse strands should be evaluated and the consensus compared to the standard sequence.

3.11.6 Comparison of Sequence Data with a " Within Run" Standard

The comparison with a standard of a high quality sequence from the same run is also needed to identify base differences. Verification of readings using second strand and/or second aliquot sequencing is required. Some mutations may be missed if sequencing is performed in only one direction. Any positives should be confirmed by sequencing a second aliquot. For direct sequencing, a second PCR amplification product should be used for repeat sequence analysis.

3.11.7 Interpretation and Data Reporting

Base differences are correlated with the known gene structure and other relevant data and the likely effect of the base change on the gene is predicted. The report should note the exact base change and location by nucleotide position as referenced in Gene bank and the corresponding position change in the protein using standard nomenclature. For small deletions and insertion or

nonsense mutations resulting in a predicted protein truncation, the term "mutation" is appropriate. For missense alterations, one must consider whether these represent mutations, polymorphisms, or rare variants. For each genetic disease, the laboratory should first refer to a polymorphism and mutation database. If the base alteration has not been previously described, the nature and significance of the change may be unclear and should be stated as such in the report. For resolution, family studies and population based studies are appropriate. Reports in which no mutations are detected by sequence analysis should include multiple disclaimers, primarily that the sensitivity of the test is <100%. If sequencing was confined to the coding region of the gene, the possibility of mutations in the promoter or intragenic regions not covered by the test should be clearly stated. Sequencing will not detect large gene deletions or duplications. In addition, a mutation in a different gene that contributes to the disease, as well as misdiagnosis of the proband, constitute other possibilities.

3.11.8 Validation

Each laboratory must validate the technique for each gene to be analyzed. Validation with known mutations as well as normal samples is required. Results of validation studies for each gene analyzed must be available for review.

3.12 Single-Strand Conformation Polymorphism (SSCP) Assays

3.12.1 Assay Design

When screening for unknown mutations, DNA fragments between 150 and 300 bp are typically used. Larger fragments can be used if it is known that the specific mutation/polymorphism of interest produces an abnormal SSCP pattern in that DNA segment.

3.12.2 Polyacrylamide Gel Electrophoresis

Gels should be run for a sufficient length of time (depending on fragment length) to detect possible mobility shifts. In order to reduce the risk of missing mutations, samples should be run under two electrophoretic conditions that may differ in length of time, temperature, buffer concentration, cross-linking ratio, cross-linking reagents, and presence or absence of glycerol. It is preferable to standardize electrophoretic conditions for as many different mutations as possible. This can be done by using more than one control mutation (Orita, *et al.*, 1989).

3.12.3 Controls

Double-stranded DNA control should be run alongside single-stranded fragments to allow identification of both fragments. Some mobility shifts are observed only with double-stranded fragments. Optimal denaturation of double-stranded fragments should involve a dilution of the PCR product. This will

necessitate use of a sensitive detection method (fluorescence, radioactivity, or silver staining). The PCR product from at least one normal control should be included on every SSCP gel. The PCR product from at least one control sample containing a mutation should be included on each SSCP gel in order to ensure that the electrophoresis conditions are optimal for detection of at least one mutation. Inclusion of more than one control mutation is advisable to improve the accuracy and standardization of the assay. If screening for several known mutations in a DNA fragment, use of control samples for each is desirable to ensure that the sequence alteration produces an abnormal SSCP band under the conditions used (Orita, *et al.*, 1989).

3.12.4 Visualization of Results

For manual approaches to SSCP using ^{32}P-labeled or ^{33}P-labeled deoxynucleotides, multiple X-ray film exposures are recommended to visualize all signals. Some abnormal SSCP bands may be faint, requiring longer exposures than normal bands. For SSCP by automated fluorescent analysis, internal size markers help prevent artifactual lane shifting from influencing mobility shift data. It may be necessary to adjust the volume of sample loaded to achieve detection.

3.12.5 Interpretation of Results

All samples showing a mobility shift should be sequenced to determine the nature of the sequence change. It is possible for different sequence variations to produce similar SSCP results.

3.12.6 Validation

Each laboratory must validate the technique for each gene to be analyzed. Validation with known mutations as well as normal samples is required. Results of validation studies for each gene analyzed must be available for review.

3.13 Probe/Primer/Locus Documentation

All loci used for analysis in the laboratory need to be well documented by Human Gene Mapping Workshop, Geneatlas, Genome Data Base (GDB) or by publication in the peer-reviewed scientific literature. This documentation must be maintained in an up-to-date laboratory book and include the following: genome location, linkage data, literature references, cloning vector, cloning site, size of insert, enzyme used for the detection of the RFLP, the sizes of the alleles and any constant bands, the allele frequencies in each racial or ethnic group for which this information exists, new mutation rate (if known), how the probe was prepared as well as hybridization and wash conditions. For oligonucleotide probes or primers, documentation sheets also must include specific sequences. For primers, PCR conditions and the size of the expected positive result should be included. There must be internal documentation that the probe/primer used is consistent with the above data (i.e., a photograph indicating that the size of the insert isolated from the vector is the correct size or that the conditions used by the laboratory produce the appropriate

result) (ACMG, 1999).

3.14 Linkage Analysis

The laboratory must keep an up-to-date reference list documenting linkage relationships (i.e., location relative to locus in question, recombination fractions and/or q values at 95% confidence intervals) for each disorder analyzed by indirect linkage methods. The laboratory must have documented linkage relationships for all in-house generated probes prior to use in a clinical setting. In order for linkage analysis involving probes with significant recombination distances from the locus in question to be reported, the analysis must contain data from two informative flanking markers. If this is not possible, the reason must be stated so as to indicate that every effort was made to provide such. For linkage analyses involving probes with negligible recombination distances from the locus in question, it is only necessary to use only one highly informative marker. For each disease specific system examined, the number of informative markers to be used is dependent upon the informativeness of each marker, the disease specific recombination frequency and the availability of markers (Ott, 1991).

3.15 Assays Validation

Each laboratory must validate the analytical performance characteristics (sensitivity, specificity, reproducibility) of the technique chosen for analysis of each gene. Validation with well-characterized samples is critical. Where available, performance characteristics should be compared with an existing "gold standard" assay. In the absence of "gold standards" for comparison of results for new assays, the splitting of samples with another laboratory with an established clinical assay may be considered. Documentation of validation results must be available for review.

4. Reporting of Results

Laboratory reports should be designed to provide patient laboratory data effectively and completely. In general the report should include the following information: date of report, name of individual, date of birth, specimen collection date, specimen accession number or case number, indication for testing, test performed (including mutation tested), brief description of test methodology, test results, a statement interpreting the test results with clinical and genetic counseling indications and the signature of the laboratory director or other authorized individual. The final report should be easy to interpret and should include an appropriate summary of the methods, probes and endonucleases used, the loci or mutations tested, the objective findings and a clinical interpretation in an easytointerpret format (JAHCO, 1996).

The final report should be reviewed and signed by the director or a qualified designee if there is a subjective or an interpretive component to the test. When diagnostic reports are generated by computer or telecommunications equipment the actual signature or initials of the director need not appear on the report. Nevertheless, the laboratory must have a procedure that ensures

and documents that the report has been reviewed and approved prior to its release.

4.1 Molecular Inherited Disease Testing

In view of the recognized risks of genetic discrimination and stigmatization, confidentiality of molecular genetic test results is a critical consideration. Results should be communicated only to the referring physician, genetic counselor or in certain cases, the patient. Non-confidential media (e.g., fax) should be used with caution. Some patients, aware of the insurability risks will choose to pay for testing out of their own pocket and request that the results not be recorded in their medical record; such requests should be honored by the laboratory to the extent allowable under applicable laws. Under no circumstances should results be provided to outside parties, such as employers, insurers or other family members without the patient's express consent. Laboratory workers should even use caution when publishing or publicly presenting the results of such studies, as some family members have recognized their own pedigrees in published material and thereby derived otherwise confidential information (Holtzman and Watson, 1988).

Reports of genetic testing of complex disease genes with multiple possible mutations, the report should include (where appropriate) an estimate of residual risk of being a carrier for one of the mutations not tested for. The report should include a discussion of the limitations of the methods/tests and the clinical implications of the detected mutation (or negative result) for complex disorders with regard to recessive or dominant inheritance, recurrence risk, penetrance, severity and other aspects of genotypephenotype correlation. The report should also include an estimate of the risk of false negatives and false positives arising from recombination between the linked probe(s) and the disease gene, when linkage analysis is used.

The report should include a recommendation that appropriate genetic counseling be utilized to explain the implications of the test results, residual risks and uncertainties, and the reproductive or medical options it raises to the patient where appropriate. The genetic councilor will convey sensitive information to the patients in an understandable manner.

4.2 Paternity Testing

The report should include the individual paternity index for each genetic system, the combined paternity index, the probability of paternity as a percentage, and the prior probabilities used in calculations if there is a failure to exclude (Holtzman and Watson, 1988).

4.3 *In Situ* Hybridization

The interpretive report should include correlation with the morphological findings. *In situ* hybridization requires simultaneous re-evaluation of the histopathology or cytopathology on the actual hybridized slide, since sectioning or sampling may alter a focal lesion. Consequently, the correlation

between the morphological and molecular data must be described in the report (Nuovo, 1992).

Any report must ensure the confidentiality of the other family members whose studies were used to provide information to the proband. The format can be such that one copy is detailed and for the referring genetic expert, while a cover summary sheet is provided for the proband as long as no other family members' results are revealed.

4.4 Investigative Studies

A written report must be issued to the referring source and must contain the same information stated previously. However, there must be a qualifying statement clearly indicating that the results are based on an investigational study and may not be as accurate as a test recognized by the genetic community as an accepted or proven clinical service test.

5. Records

The laboratory must maintain all patient records of patient data and laboratory operations in a manner that permits timely review.

5.1 Patient Records

All patient testing laboratory records should be accessible and easily retrieved. Files should be retrievable by patient name and by a second unique identifier (e.g. laboratory accession number or case number). Files relating to individual or family studies should be cross-referenced. Records should be maintained in a manner that will preserve their confidentiality and integrity and released only with appropriate authorization. The records should be retained for a suitable period of time as required by applicable regulations. Critical records of genetic testing are often kept for one generation (i.e. 20 years) (Baer, 1993).

5.2 Laboratory Records

Records of all components of the internal quality improvement program, proficiency testing and internal quality control program should be complete and available. Copies of all outdated procedures and the dates for which they were in effect should be retained for reference. The laboratory records should include sufficient information regarding the individual specimen and assay conditions. A log of all stored specimens should be maintained to allow for prompt retrieval for further testing. Copies of each final report, all records of results, membranes, autoradiographs, gel photographs and *in situ* hybridization should also be retained in compliance with existing laws. All autoradiographs, gel photographs and *in situ* hybridization slides must be adequately labeled for identification and adequately cross-referenced in the case records.

5.3 Parentage and Forensic Identity Testing

This includes all technical, legal and administrative records available for review and use in legal proceedings. The records should reflect an adequate external

and internal chain-of-custody. Records pertaining to release of information should be maintained all the time (NCCLS MM1-A, 2000).

6. Reagents

The laboratory has the responsibility for ensuring that all reagents used, whether purchased or prepared by the laboratory are functional. Verification of reagent performance is required and must be documented. Any of several methods may be appropriate such as direct analysis with reference materials, parallel testing of old vs. new reagents and checks against routine controls. The aim is to check new reagents by an appropriate method before being placed in service. Documentation should exist that new reagent lots have been checked against prior lots or known standards before being placed in service. Reagents intended for use in a manner which do not follow manufacturer's recommendations requires that validation studies must be performed (CAP Laboratory General Checklist, 2001).

All reagents should be properly labeled with content and quantity, concentration, storage requirements, date placed in service and expiration date (all reagents should be used within their indicated expiration date). Upon visual inspection all reagents should be in satisfactory condition and should be stored appropriately as stated by the manufacturers.

Sufficient information should be documented regarding the nature of any probe or primer used in an assay to permit proper interpretation and troubleshooting of test results such as the type (genomic, cDNA, oligonucleotide or riboprobe) and origin (human, viral, etc.) of the probe or sequence; the oligonucleotide sequence and complementary sequence or gene region recognized; the appropriate restriction enzyme map of the DNA; known polymorphisms; sites resistant to endonuclease digestion and cross-hybridizing bands; the labeling methods used and standards for adequacy of hybridization or amplification. For linkage analysis recombination frequencies and map position must be documented. Loci should be designed as defined by the Human Gene Mapping Nomenclature Committee.

7. Instrument Maintenance

The laboratory should have an organized system for monitoring and maintaining all instruments. Function checks should be designed to check the critical operating characteristics to detect drift, instability or malfunction before the problem is allowed to affect the test results. All servicing and repairs must be documented. The procedures and schedules for instrument maintenance must be thorough and as frequent as specified by the manufacturer. Since certain equipment have no standard frequency or extent of maintenance, each laboratory should establish schedules that reasonably reflect the workload and specifications of its equipment. The following requirements should be followed for all equipment in the laboratory: i) written standard procedure for set-up and normal operation of all instruments in the laboratory; ii) regular schedule or system for checking the critical operation for all instruments in use; iii) function checks should be documented to detect trends or malfunctions; and

iv) instructions should be provided for minor troubleshooting and repair of instruments (this can be done by providing the manufacturer's service manual or notes) and records for each instrument repair history and preventive maintenance (these records should be available to and usable by the technical staff operating the equipment) (CAP Laboratory General Checklist, 2001).

Electrophoresis Equipment

The electrophoresis apparatus in the laboratory should be clean and properly maintained (electrode and buffer tank intact, power supply electrodes fit snugly and no build up of dried buffer).

Power Supplies

The displayed voltage reading should be checked periodically with a voltmeter, to ensure that it is delivering the correct voltage.

Biological Safety Cabinets

The biological safety cabinet (or hood) should be certified at least annually to ensure that filters are functioning properly and that airflow rates meet specifications.

Fume Hoods

Fume hoods (or chemical filtration unit) should be available for any procedure utilizing volatile chemicals and the fume hood should be certified annually to ensure that airflow rates meet specification.

Pipettes

The pipettes should be checked for accuracy and reproducibility before being placed in service and should also be checked at regular intervals thereafter.

Thermometers

All thermometers in the laboratory should be checked against an appropriate thermometric standard device before being placed in service.

Temperature Dependent Equipment

The temperature should be recorded daily (and when used) and the ranges defined for the following equipments: water baths, heating blocks, incubators, ovens, refrigerators, freezers as well as the individual wells of thermal cycler should be checked for temperature accuracy and uniformity before being placed in service and periodically thereafter.

pH Meters

There should be written procedures for operation, calibration and function checks. High quality buffers (certified assay of content) should be used for calibration of the pH meter.

Centrifuges

Centrifuges in the laboratory should be cleaned and properly maintained. There should be a written protocol for maintenance of all centrifuges and the operating speeds should be checked periodically for the intended use.

Balances and Weights

The balances should be cleaned and checked periodically by qualified service personnel and all analytical balances should be mounted so that vibrations do not interfere with readings. Results of the periodic accuracy checks should be recorded. Standard weights of the appropriate American Society for Testing and Materials (ASTM) class should be available for calibration. ASTM Class-I weights are appropriate for calibrating high precision analytical balances (0.01 to 0.1 mg). ASTM Class-II weights are appropriate for calibrating high precision analytical balances (0.001 to 0.01g). ASTM Class-III weights are appropriate for calibrating high precision analytical balances (0.01 to 0.1g).

Volumetric Glassware

Volumetric glassware should be of a certified accuracy [Class A, National Institute of Standards and Technology (NIST) standard or equivalent] or if non-certified volumetric glassware is used all items are checked for accuracy of calibration before being placed in service.

Spectrophotometers

The absorbance and the photometric linearity should be checked periodically with filters or standards solutions if required by the instrument manufacturer. The filters (filter photometers) should be checked periodically to ensure they are in good condition (e.g., clean, free of scratches etc). The spectrophotometer wavelength calibration should be checked regularly with appropriate solutions, filters or emission line source lamps if so required by the manufacturer.

Film Processing/Photographic Equipment

The film processing (developing) equipment should be routinely serviced, repaired and appropriately replenished with reagents, if maintained by the laboratory. If the laboratory uses another department's film processing equipment, the quality of the autoradiographs produced must be monitored. The photographic equipment in the laboratory should be clean and properly maintained. Fixed camera mountings should be level and secure.

Signal Detection Instruments

Scintillation counters, luminometers, densitometers etc should have background levels checked and recorded daily, or with each use of the instrument if used less than daily. Written criteria for acceptable background levels should be included.

8. Personnel

For optimal patient care, only qualified personnel may be involved with molecular pathology testing.

8.1 Director

A board-certified pathologist, other physician, or a doctoral scientist qualified by training, expertise and experience in molecular genetics can serve as a director. When a non-pathologist physician or doctoral scientist serves as director, such individuals must have documented qualifications. The director shall be qualified to assume the professional, scientific, consultative, organizational, administrative and educational responsibilities for the scope of the services provided. In addition, the director shall have sufficient authority to implement and maintain the standards for laboratory accreditation listed elsewhere (CAP Standards for Laboratory Accreditation, 2001).

8.2 Technical Supervisor

The technical supervisor of the laboratory (or section) may be a pathologist, certified physician in a specialty other than pathology, or a doctoral scientist in a biological science, with specialized training and/or appropriate experience in molecular genetics. In the case of forensic identity testing the technical supervisor should have an appropriate degree, training or experience in forensic science (DHHS, 1988).

8.3 Technical Staff

The personnel performing the technical aspects of molecular genetics should qualify as one of the following: i) staff experienced in the field of molecular genetics under the direct supervision of a qualified director or supervisor; ii) medical technologist certified by the American Society of Clinical Pathologists (ASCP) or iii) BS degree holder in biological sciences with appropriate experience in molecular genetics methods. The person in charge of molecular genetics assays should be either a BS degree holder or medical technologist certified by the ASCP with at least four years of experience (at least one of which is in molecular genetics methods) under a qualified director. There should be adequate training programs for new technologists in addition to continuing medical laboratory education programs (DHHS, 1988).

9. Laboratory Computer Services

Laboratory computer systems are vital to accurate reporting of patient results and hence to patient care. The following regulations do NOT apply to small programmable technical computers, micro computers used solely for word processing, spreadsheets or dedicated microprocessors that are an integral part of an analytic instrument.

9.1 Environment

The computer facility and equipment should be kept clean, well maintained and

adequately ventilated with appropriate temperature and humidity control. All wires and computer cables should be properly located and/or protected from traffic areas. Computer components and storage areas must be readily accessible to fire-fighting equipment. The computer system must be adequately protected against unexpected power interruptions and surges using an Uninterruptible Power System (UPS) or similar device (NCCLS GP19-A, 1997).

9.2 Personnel and Security

Procedure manuals that are written and reviewed annually are necessary. The manual should have written procedures for the following: preservation of data and equipment in case of an unexpected destructive event (*e.g.*, fire, flood), software and hardware failure, security codes which define levels of security for those who are authorized to enter, access or change of patient results (information sent over a public domain, such as the Internet is considered in the public domain which is potentially accessible to all parties on that network and a system, such as "fire walls" and data encryption schemes should be in place) and documentation of training for all personnel.

9.3 Data Entry, Reports and Errors

There should be a written system to document errors in test reporting and transmission of patient results. A timely system for error correction that is convenient to use must be defined. Any error correction must be indicated on the test report with both the original and revised results reported and the operator indicated. Patient data on reports and video displays must be periodically compared with original input data to detect errors in data transmission, storage or processing. The laboratory director must approve at least annually a review of the content and format of the laboratory patient reports to ensure that they effectively communicate laboratory results and that they meet the needs of the medical staff. Reports that display corrected results must clearly indicate that the new results are replacing a previously reported incorrect result. This applies to all paper reports, as well as data that are displayed on video terminals or other systems receiving patient data. When a revised patient report is issued, both the original and revised results must be retained for at least 2 years. It is considered inappropriate to list only the last correction made, as the clinician may have made a clinical decision based upon erroneous data rather than the true result. All corrections should be shown in the patient report. In addition, there must be an audit mechanism, which allows the laboratory to identify all individuals who have entered or modified patient data, control files or computer programs.

9.4 Data Storage and Retrieval

Stored patient result data and archival information must be easily and readily reviewable within a time frame (less than 4 hours) consistent with patient care needs. The computer must be able to reproduce archived test results completely, which include the reference range originally given for that test, and any flags, footnotes, or interpretive comments that were attached to that

result. The tracing of results back to the original instrument on which the test was performed is important for both quality assurance as well as quality control. Data storage media such as tapes, disks, etc., must be properly labeled, stored and protected from damage or unauthorized use. A mechanism must be in place to minimize or prevent loss of patient information in case of hardware or software failure.

9.5 Maintenance

A schedule of maintenance procedures and training for employee should be devised. A system to monitor computer resources and test system integrity should be in place. Testing should be done after any modifications and results documented. Approval of new programs and changes have to be documented by the medical director. Policies and procedures must be in effect to minimize downtime and that any downtime or backup procedures must be documented. Written contingency plans must be developed to handle services in the event of a computer system failure. Emergency services for both computer hardware and software must be improved. Records must be maintained to document regular maintenance and allow operators to trace any work done on the computer system.

10. Proficiency Testing

Proficiency testing involves the performance of test procedures on common samples by multiple participant laboratories. Laboratories must participate regularly in a CAP approved proficiency testing program for each patient reportable analyte whenever an appropriate program is available. Each separately accredited laboratory must be enrolled in such a program under its own CAP number. It is preferable that the laboratory has a performance history of one or two shipments of proficiency testing before an initial inspection. If proficiency testing for an analyte is not commercially available, is not formally graded, or is not compatible with all methods, the laboratory is still required to perform some type of external, alternative or comparable testing at least every six months. This may be accomplished through blind testing of specimens with known results, exchange of specimens with other laboratories, or other equivalent systems specifically recommended and approved by the laboratory director (NCCLS GP27-A).

For molecular genetics testing the CAP currently runs the Molecular Genetics Survey program. This program was established with the American College of Medical Genetics. The Survey consists of two shipments per year, which are sent to participating laboratories. The shipments comes as ethanol fixed cell lines or extracted DNA and include the following disease samples: Cystic Fibrosis, DMD/Becker, Factor V Leiden, Fragile X Syndrome, Friedreich's Ataxia, Hemochromatosis, Hemoglobin S/C, Huntington disease, Methylenetetrahydrofolate reductase, Myotonic dystrophy, Prader-Willi/Angelman syndrome, Prothrombin, Rh, Spinal muscular atrophy and Spinocerebellar Ataxia. These specimens should be tested in the same manner as patient's specimens and by the same personnel and the results are sent to

the CAP office within the specified period of time.

The survey intended results are then sent to the laboratory. These should be actively reviewed by the laboratory director and laboratory personnel and compared to the actual laboratory results. Testing of the survey samples should be rotated among all technical personnel when possible (Merrick, 2000).

11. Procedure Manual

The laboratory should have a procedure manual, which includes protocols for all assays it performs in sufficient detail to permit qualified laboratory personnel to perform them consistently and accurately. The procedure manual should be written in compliance with the NCCLS GP2-A3 (NCCLS, 1997) without having to precisely copy it. The procedure manual must include: principles of the test, clinical significance, specimen type, required reagents and equipments, quality control, procedural steps, calculations, reference ranges, interpretation and protocol for reporting of results. Also, there must be documentation on DNA probes and PCR primers, which include the size, complete or partial nucleotide sequence, allele frequencies of certain mutations in various ethnic groups, recombination frequencies, cloning vector, relevant restriction enzymes sites, method of preparation and relevant literature citations.

The procedure manual should also be available to all personnel at the workbench. It should be reviewed at least annually by the laboratory director and any changes initialed and dated by the director. When a procedure is discontinued a copy is maintained for at least two years recording initial date of use and retirement date (CAP Molecular Pathology Checklist, 2001).

There should be a written procedure to prevent specimen loss, alterations or contaminations. The procedure manual should document that all analysts acknowledge the contents (including changes) of procedure manuals relevant to the scope of their testing activities.

12. Laboratory Safety

12.1 Chemical Hazards

Many of the procedures used in molecular genetics laboratories involve the use of chemicals which are toxic or mutagenic. Appropriate precautions are defined in the Material Safety Data Sheets (MSDS) for each chemical and should be included in a separate file for all chemicals in use by the laboratory. In addition, the laboratory should have a Chemical Hygiene Plan (CHP) which defines the safety procedures for all hazardous chemicals which are in use by the laboratory and should contain the following elements: i) responsibilities of lab director and supervisors; ii) designation of a qualified chemical hygiene officer; iii) policies for all operations that involve hazardous chemicals; iv) criteria for the use of personal protective equipment and control devices; v) criteria for exposure monitoring when possible levels are exceeded; vi) provisions for medical consultations and examinations; vii) provision for training employees in the elements of the CHP and documentation that each chemical used in the lab

been evaluated for carcinogenic potential, reproductive toxicity. Finally, there should be an annual review and evaluation of the effectiveness of the CHP (NCCLS GP17-A, 1998).

12.2 Biological Hazards

All human blood specimens are to be treated as infectious and handled according to the standard precautions. The laboratory should have a documented policy for infection control that complies with the Occupational Safety and Health Administration (OSHA) standards on occupational exposure to blood-borne pathogens and to the hospital's exposure control plan. Personnel expected to have direct contact with body fluids should receive education on precautionary measures, epidemiology, modes of transmission and prevention of HIV, Hepatitis-B and Hepatitis-C and the application of universal precautions to their work practices. For detailed specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood borne exposure refer to the NCCLS document M29-Protection of Laboratory Workers from Instruments Biohazards and Infectious Disease Transmitted by Blood Body Fluids and Tissue (NCCLS M-29, 1997).

12.3 Radiation Safety

The institute policy for handling radioactive material should be readily available to all members of staff. There should be an up-to-date manual for radiation safety, which should include a section on decontamination and handling of radioactive waste. Radiation survey instruments should be calibrated regularly.

In all areas or rooms where radioactive materials are being used or stored, there should be a sign to indicate the presence of radioactive material. Wipe test should be carried out for different radiation areas and records for such test are maintained. In addition, all workbenches and sinks should be surveyed and if necessary decontaminated each day of use. A policy should be in place, which includes procedures for inspections, monitoring of shipment and notification in the event of damaged or leaking radionuclide shipment. In addition, all shipments should be logged and the amount used and disposed should be documented. The laboratory should have a radiation safety officer who is responsible and actively monitors radiation safety.

12.4 Ultraviolet Hazard

Proper eye and face shields are required when viewing eithidium bromide-stained DNA in gels on a UV-trans illuminator box. Severe burns and eye damage can result from even short exposure to the UV light

12.5 Electrical Hazards

Electrophoresis power supplies present an increased shock hazard due to the proximity of the electrical supply to liquids and to the operator. All power supplies should be examined for worn power cords or other signs of damage

before use. Sequencing gel electrophoresis presents a special shock hazard due to the extremely high voltage used (1,500 to 2,500 volts). This type of electrophoresis should not be performed if personnel are not present to monitor the equipment. Unsupervised outside personnel, such as cleaning staff, should not be allowed in areas where high voltage gels are being run.

13. Quality Control

The laboratory must have, at a minimum, a written program defining the general quality control policies and procedures in use. The quality control program should be under surveillance by the section supervisor and reviewed monthly by the director or designee.

DNA should be extracted and purified using standard methods and should be stored in a manner to prevent degradation. There should be a procedure to document recovery rates of DNA extraction procedure. There should be written guidelines for handling insufficient or low quantity samples as well as specimens, which do not meet quality standards (Otter and Cooper, 1999). Control specimens should be tested in the same manner as patient specimens and by the same personnel. Tolerance and acceptability limits should be defined for all control procedures, control materials and standards. Results for controls should be verified for acceptability before reporting of results. There must be documentation of all corrective actions taken when controls, instruments, temperature, etc exceed defined tolerance limits. Records of controls, instrument function and maintenance, temperature etc should be documented for routine procedures (Husiman, 1994).

13.1 Controlling False Positive Nucleic Acid Target Amplification Reactions

Extreme care and measures should be taken to avoid false positive results when dealing with diagnostic nucleic acid amplification methods. These measures can include the following:

Reagents and Solutions

All reagents used in nucleic acid amplification should be prepared, divided into aliquots and stored in an area that is separate from the specimen preparation or post-amplification area. Dedicated equipment and supplies should be used. Oligonucleotides should be synthesized and purified in a clean, amplification product-free environment. Once reaction conditions have been optimized reagents can be premixed into master mixes. These master mixes can be divided into aliquots of the volumes required for each reaction run. This will minimize the number of samplings and reduce the potential for contamination. The reagent lot number should be recorded so that if carryover does occur the source can be easily identified (Kitchin, *et al.*, 1990).

Pipettes

Separate pipettes should be used for reagent preparation, specimen preparation and post-amplification analysis. Pipettes used for nucleic acid

amplification set-up should always be separated from amplified products and should remain in the area in which they are used. Positive displacement pipettes or barrier pipette tips should be used to prevent contamination of pipette barrels by aerosols. All pipettes should be cleaned at regular intervals.

Gloves

Disposable gloves should be worn and changed when entering or re-entering the amplification-preparation area. Disposable gloves may be changed between samples to prevent cross-contamination between samples.

Laboratory Coats

Laboratory coats should be dedicated to areas and changed when traveling among reagent preparation, sample preparation and amplification and detection areas.

Uncapping Reaction Tubes

To force any liquid down from the sides it is recommended that the tubes be subjected to a quick centrifugation before uncapping. Tubes should be uncapped carefully to prevent aerosolization.

Addition of Reaction Components

Non-sample components (mineral oil, dNTPs, primers, buffer and enzymes) should be added to the amplification reactions before addition of the sample. When possible, before proceeding to the next tube, each tube should be capped after the addition of the sample.

Reagent Blank

These controls contain all necessary components of the amplification reaction except the template DNA and should be included in each assay.

Workflow and Laboratory Design

Ideally, three physically separate areas of the laboratory should be available for reagent preparation, specimen preparation and amplification and product detection. The reagent preparation area, for those laboratories using only commercially available kits, is considered to be the site of the manufacture. In a laboratory where enzymatic or chemical means of inactivating amplified products are used, the demands for physical separation of pre- and post amplification procedures may be somewhat reduced, but good laboratory practice should still be diligently exercised. The flow in the laboratory should be in a unidirectional workflow from clean to dirty areas. The use of a unidirectional workflow will reduce the opportunity for contamination to occur. Color-coded equipment, reagents and supplies may help to ensure that a unidirectional workflow is maintained (NCCLS GP18-A, 1991).

In the event that laboratory space is not available to separate pre- and post-

amplification a Class-II biological safety cabinet should be used for specimen preparation. Class I safety cabinets do not provide protection for material contained within them. Dead air boxes with ultraviolet light attachment can provide a clean bench area for specimen preparation in a dedicated specimen-preparation laboratory.

14. Quality Improvement

The quality improvement (QI) program in molecular genetics must be under active surveillance by the supervisor with documented review at least weekly. Secondary review should occur at least monthly by the laboratory director or designee. The QI program must provide the system design and evaluation of proper patient identification and preparation; specimen collection, identification, preservation, transportation, processing and accurate result reporting. This system must ensure optimum patient specimen and result integrity throughout the pretesting, testing, and posttesting processes. Opportunities for system improvement are identified based on such evaluations and corrective plans are developed and implemented. Evidence of active review of results of controls, instrument maintenance and function, temperature, etc. for routine procedures should be available on all shifts. There must be a written system in operation to detect and correct significant clerical and analytic errors that could affect patient management. Failed nucleic acid isolations should be recorded, and documentation should be maintained for all corrective action(s) taken. The submitting physician (or requester) should be notified promptly, when a specimen is inadequate or if insufficient nucleic acid is isolated. Failed hybridization reactions should be recorded and the corrective action documented. There should be evidence that the laboratory monitors sample turnaround times and that they are appropriate for the intended purpose of the test (Hoeltge, 2000). Preliminary reports should be promptly generated when indicated. Discrepancies between preliminary and final reports should be investigated and documented. Discrepancies between the molecular genetics laboratory's final results, other laboratory findings, and the clinical presentation should be investigated and documented, along with any necessary corrective action. All abnormal prenatal testing results should be confirmed after birth or termination of pregnancy, where feasible. There should be statistical records on all molecular genetics laboratory results (*e.g.*, percentages of normal and abnormal findings, allele frequencies, etc). These records should be maintained and appropriate comparative studies performed. These records should be reviewed at regular intervals by the laboratory director or designee and appropriate corrective action taken should be taken when indicated. A log of unusual, difficult, or instructive cases should be maintained.

The Accreditation Fees

A deposit to initiate the application process is currently set at 500 US dollars, which goes towards the first year's accreditation fees. In addition there are fees for Checklist sections dependent on the number of Checklist sections used to inspect the laboratory, which is currently as follows:

1-4 Checklist sections / Year $ 950

5-8 Checklist sections / Year $ 1,580

9 Checklist sections /Year $ 1,860 plus $ 270 per checklist > 9

The inspection team expenses such as airline tickets, hotel accommodation and meals are paid for by the CAP central office. There are no hidden costs for the accreditation process. However, it has to be borne in mind that the cost of participating in the molecular genetics survey (proficiency testing), which is currently $ 1,200, should be added to the overall cost of accreditation.

Granting / Denial of Accreditation

Accreditation is formally granted to molecular genetics laboratories that: Successfully meet the standards for laboratory accreditation set forth by the CAP, correct and document correction of all deficiencies, cited during inspection, within the specified time frames, Successfully participate in a molecular genetics survey for all tested analytes, participate in mid-cycle self-evaluation processes and resolve all issues and questions to the satisfaction of CAP technical associates and regional commissioners. Laboratory staff is required to respond to all communication concerning requests for additional information by CAP, personal consultation with laboratory directors and re-inspection of specific laboratories or laboratory areas (CAP Standards for Accreditation, 2000). Denial or revocation of accreditation is possible when the laboratory does not respond to the deficiencies cited at the on-site inspection, fails to correct and document major deficiencies, fails to meet the CAP standards for laboratory accreditation or does not participate in a self-evaluation. Denial or revocation requires a vote of the entire commission on laboratory accreditation or a vote of the executive committee of the commission. The commission or executive committee is presented with facts surrounding the inspection, after which a vote is taken. Denial is followed by a certified letter to the laboratory director, effective immediately and reported to the appropriate oversight agencies. The laboratory may appeal the decision within 60 days of notice. Documentation of compliance with all standards must be submitted to the commission. The director may be invited to present the information at a commission meeting if facts not previously reviewed are provided that may affect the decision. Should the commission adhere to its original decision to revoke or deny accreditation, the laboratory may appeal to the college board of governors. Three members will review the documentation, and, if the appeal is considered valid, will refer the final decision to the entire board of governors (Merrick, 2000).

Evaluation and Feedback

To ensure that the accreditation program meets the members needs, an evaluation is included as part of the process. Each facility is requested to complete a post assessment questionnaire to provide feedback on the accreditation process. By doing this, the CAP can ensure that the laboratory accreditation program is meeting the set goals and that modifications and

improvements are implemented as necessary.

Conclusion

This review has brought together, in one document, all the up-to-date information concerning requirements for CAP accreditation in molecular genetics laboratories. The CAP accreditation is dependent upon successful performance in the molecular genetics survey and passing the laboratory inspection. The inspection is carried out by practicing laboratorians who have experience in the field of molecular genetics. They examine all activities carried out in the laboratory ranging from specimen receipt to reporting of results, and all aspects related to laboratory safety, equipment and computer databases. Once all requirements for laboratory accreditation are met, the laboratory will be accredited for a two-year period. Although the accreditation requirements mentioned in this review are those for CAP, readers who are looking for accreditation by other agencies or simply looking for a document summarizing good laboratory practices in the field of molecular genetics may find this review helpful.

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