Genetics inMedicine | ACMG STANDARDS AND GUIDELINES

American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders

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Microarray methodologies, to include array comparative genomic hybridization and single-nucleotide polymorphism–based arrays, are innovative methods that provide genomic data. These data should be correlated with the results from the standard methods, chromosome and/or fluorescence *in situ* hybridization, to ascertain and characterize the genomic aberrations of neoplastic disorders, both liquid and solid tumors. Over the past several decades, standard methods have led to an accumulation of genetic information specific to many neoplasms. This specificity is now used for the diagnosis and classification of neoplasms. Cooperative studies have revealed numerous correlations between particular genetic aberrations and therapeutic outcomes. Molecular investigation of chromosomal abnormalities identified by standard methods has led to discovery of genes, and gene function and dysfunction. This knowledge has led to improved therapeutics and, in some disorders, targeted therapies. Data gained from the higher-resolution microarray methodologies will enhance our knowledge of the genomics of specific disorders, leading to more effective therapeutic strategies. To assist clinical laboratories in validation of the methods, their consistent use, and interpretation and reporting of results from these microarray methodologies, the American College of Medical Genetics and Genomics has developed the following professional standard and guidelines.

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Neoplastic processes are a complex group of disorders that develop as a result of the accumulation of genetic alterations including gene mutations, chromosomal rearrangements, gain and loss of genetic material, epigenetic changes, loss of heterozygosity (LOH), and various other genetic changes. Defining and understanding the genetic alterations of specific neoplastic disorders influences the diagnoses, prognoses, and therapeutic choices for patients with both malignant and benign neoplasms.[1–7]

Published clinically applicable data now show the utility of DNA microarray analysis in the assessment of multiple neoplastic disorders.[8–13] Data indicate that microarray technologies provide information about gain and loss of genetic material in neoplastic disorders, including hematologic malignancies and solid tumors.[14–17] These gains and losses, represented as an increase or decrease in the proportion of genetic material as compared with a reference genome, are collectively referred to as copy-number variants (CNVs). Microarray methodologies are appropriate complementary methods to standard methods of chromosome and fluorescence *in situ* hybridization (FISH) analyses for detection of genetic anomalies in neoplastic disorders.

DNA microarray technologies should confirm genetic imbalances identified by conventional and molecular cytogenetic or FISH analyses and provide further detail of the aberrations.[15–17] However, additional important information about the genetics of specific disorders may be revealed, e.g., leukemia with normal cytogenetic and FISH analyses.[18–23]

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DNA MICROARRAY PLATFORMS

Different types of DNA microarray platforms currently available for clinical testing include bacterial artificial chromosome–based array comparative genomic hybridization, oligonucleotide-based array comparative genomic hybridization, oligonucleotide plus single-nucleotide polymorphism (SNP)-based arrays that contain both copy-number (intensity-only) and SNP (allele-differentiating) probes, as well as SNP-only–based arrays.[9,24–26]

For comparative genomic hybridization–based microarrays, patient DNA and reference DNA are labeled with different fluorochromes and hybridized to probes on the microarray. SNP-based arrays use a single color dye compared with an *in silico* reference. A scanner measures differences in the intensities of the fluorochromes, and the data are expressed as having more or less signal as compared with the reference. For genomic regions with two copies of the DNA sequence, copy-number data are graphed as a $\log_2$ ratio with the expected normal copy number equaling "0." Duplications will have signals of greater intensity ($\log_2 > 0$) and deletions less intensity ($\log_2 < 0$). Microarrays that incorporate SNP probes allow simultaneous detection of DNA copy-number changes and absence of heterozygosity (AOH) by providing information about the intensity of the signals at the loci. AOH may be due to LOH, hemizygosity, or homozygosity.

Advantages of DNA microarray analysis

Advantages of DNA microarray analyses include:

- The ability to use any sample that yields DNA of sufficient quality,
- Assessment of the genome at very high resolution,
- Interpretation of raw data using objective biostatistical algorithms,
- The ability to detect copy-number-neutral runs or regions of homozygosity (ROHs or AOH) with SNP-array technology, and
- A ready interface of the digital data with genome browsers and Web-based genome-annotated databases.

Limitations of DNA microarray analysis

Limitations of DNA microarray analyses include:

- Inability to detect molecularly balanced chromosomal rearrangements,[27]
- Inability to detect tumor-specific changes (acquired clonality) with a low ratio of tumor cells to normal cells,
- Inability to determine the chromosomal mechanisms of the genetic imbalance, e.g., insertion, tandem duplication; chromosome and/or FISH studies may be needed,
- Inability or difficulty in detection of tetraploidy or other ploidy levels; platforms that include SNP probes may facilitate detection, and
- Inability to characterize clonal and subclonal populations; the $\log_2$ ratio may provide an indication of clonal heterogeneity.

Because of these limitations, results using microarray technologies at diagnosis should be correlated with other established methodologies (chromosome analysis, FISH). Microarray analysis is neither established nor recommended as a method for posttherapy follow-up or for minimal residual disease detection.

It should be understood that the current copy-number genomic microarray technologies are not designed to detect point mutations, gene expression levels, methylation anomalies, and microRNA anomalies, all of which may contribute to tumorigenesis. Detection of a "small" insertion or deletion, e.g., intragenic, will be affected by platform resolution, probe spacing, gene coverage, laboratory software parameters, and sample DNA quality.

Microarray platform design and verification

The laboratory should choose a microarray design with probe coverage suitable for detection of known copy-number aberrations associated with the neoplasm of interest. Microarray platform design may be (i) targeted to specific regions of the genome for detection of known cancer-associated unbalanced genomic alterations, (ii) genome-wide with a specified distribution and spacing of probes, or (iii) both targeted and genome-wide, with varying distribution and spacing of probes in specific regions and across the entire genome.

Manufacturers of microarrays should verify the identity of each clone or probe on the platform used for clinical testing. Probes selected from the public domain should be listed with their physical and cytogenetic positions on the human genome. All probe descriptions and annotations should be openly accessible. Details regarding the microarray design, the synthesis verification, and all quality control (QC) steps taken to validate and assess the performance and reproducibility of the microarray should be documented and provided by the manufacturer. Additional information may be found in the American College of Medical Genetics and Genomics recommendations for the design and performance expectations for clinical genomic copy-number microarray devices.[28]

Microarrays should be designed with consideration of the statistical algorithms to be used for determining abnormal thresholds. The number and density of probes within a given region of interest, i.e., within a region known to be associated with a cancer gene or feature, should provide the sensitivity needed for detection of a copy-number variation.

VERIFICATION AND VALIDATION OF HARDWARE, SOFTWARE, REAGENTS, AND PROCESSES

Definitions

Verification. Verification is a confirmation, through provision of objective evidence, that specified requirements have been fulfilled. This is a one-time process completed to determine or

confirm test performance characteristics before the test system is used for patient testing. Verification is a quality assurance process to determine that instruments, software, and associated data are accurate per the manufacturer's description and specifications, i.e., does the system (hardware, software, probes) function as described by the vendor/manufacturer?

Validation. Validation is a confirmation through the provision of objective evidence that requirements for a specific intended use or application have been fulfilled. Validation is a QC process to determine that the data from test samples are accurate for the intended use when compared with a validated method, i.e., does the system (processes) provide the correct (accurate, reproducible) result(s) when test samples or test data are analyzed?

Platform

Initiation of microarray technologies requires the laboratory verify that the instrumentation, software, and probes perform as specified by the vendor. All platforms intended for clinical testing must be verified and validated. The method and scope of the verification and validation must be documented. A new platform is defined as any new methodology or microarray type introduced into the laboratory. A single microarray vendor may produce multiple similar platforms, but each must be assessed independently. A new version is defined as a minor modification to probe coverage, either through manufacturing of the microarray or by *in silico* probe filtering.

Laboratory with little or no experience with microarray technologies

The laboratory with little or no experience with microarray technology should become familiar with all aspects of the new technology through the verification process, consultation with vendor support, and if possible, other laboratories with demonstrated proficiency using the same platform before beginning the validation process. Familiarization includes understanding the processes, features, and capabilities of the technology selected. The laboratory should gain experience with the instrumentation, platform design, software, reagents, methodologies, technological limitations, workflows, and DNA quality parameters by experimental sample runs. Similarly, the laboratory should become familiar with the features of each sample type the laboratory will process.

It is strongly suggested that laboratories use a combination of data from well-characterized controls and/or data from public databases to gain and broaden their experience. Sample exchanges with a laboratory proficient with a similar microarray platform can provide a good source of samples for validation. Exchange of validated data sets between laboratories provides additional experience in data analysis. Samples chosen for validation studies should have aberrations that challenge the technical limits of detection for reportable deletions and duplications.

Laboratories must be able to recognize nonperforming probes, technically induced artifact and quality issues. Laboratories should become familiar with benign and/or common CNVs and resources to aid in recognition and interpretation of CNVs, whether in a constitutional or neoplastic setting.

The laboratory should demonstrate expertise in technical aspects of the processing of sample types to be used for clinical testing, technical performance of the microarray, reproducibility of results, and data analysis and interpretation. Expertise should be documented for each microarray platform used for clinical testing, regardless of whether the laboratory has prior experience with a different platform.

New platform

A minimum of 30 samples should be processed and interpreted by the laboratory to verify and validate any new platform. This includes changing to a platform of the same type from a different manufacturer or a different platform type, e.g., array comparative genomic hybridization to SNP. Samples with known abnormalities should be used to gain expertise with the new methodology and assess performance.

New/different version of an established platform

Analysis of a minimum of five known abnormal samples should be run on a new platform version. Data from a new version should be compared with data from the established version to determine if the platform and software perform as expected to detect known CNVs. New probe additions for enhanced coverage or improved performance should be investigated with samples known to have variation in the region of new content (when possible).

New versions of established platforms will vary with the manufacturer and platform type. A manufacturer may define minor upgrades as new versions. There are no definitive criteria for a new version; however, a different version should be limited to minimal probe changes, e.g., removal and/or replacement of probes to improve performance and/or coverage over a limited number of genomic regions. These types of changes to an established platform are likely to be rare, with most changes of platforms requiring a full validation.

Validation of a new clinical test or assay

Any assay intended for clinical diagnosis must be verified and validated before offering as a clinical test. Proficiency in test performance, analysis, and interpretation must be demonstrated.

It is understood that the microarray platform employed by the laboratory may be used to analyze multiple sample types and multiple neoplastic disorders. Inherent differences in obtainable results from different biological materials require that the laboratory determine the performance characteristics of the microarray for each sample type, e.g., bone marrow/blood, fresh or frozen tissue/tumor, formalin-fixed paraffin-embedded (FFPE) tumor, to be used for clinical testing. A surgical pathologist should be involved in the collection of optimal solid tumor samples to ensure a minimum of 25% tumor in the sample.

Laboratories that plan to offer clinical testing for different neoplastic disorders using different sample types should

prepare by processing and analyzing a sufficient number of each type to establish proficiency. Disease-specific samples for which clinical testing will be offered should be included in the validation sample collection. The laboratory should run technical replicates of multiple samples during the validation process to ensure that the assay results are accurate and reproducible. Discrepancies between replicates should be investigated and documented.

Each laboratory should use judgment and experience to determine the number of samples of a particular type of neoplastic disorder to include in their preclinical testing validation. Laboratories will also need to use judgment and experience to determine differences and issues of processing various sample types and adjust sample numbers of each type accordingly, with the goal of optimizing quality and analytic interpretation of results.

Sample assays for a specific diagnosis may be validated by comparison of results with those obtained by other methods, e.g., conventional cytogenetics, FISH, or another validated microarray assay. During the validation process, all genomic imbalances identified by standard method(s) should be detected by the microarray within the limits of clonality detection levels established by the laboratory for the diagnosis or sample type. Reportable abnormalities, e.g., CNVs or LOH detected by microarray but not by cytogenetic analysis, should be confirmed by another method, e.g., multiplex ligation-dependent probe amplification (MLPA), quantitative PCR (qPCR), FISH, or a different microarray platform, during the validation process to gain sufficient expertise and confidence in data interpretation.

Exchanging samples with another laboratory conducting similar assays in a blind, split-sample comparison using both normal and abnormal samples and comparing results at the appropriate detection levels declared by the laboratories can provide valuable feedback during the validation process. After the validation period, sample sharing can be used for external proficiency testing (PT). All validation data for each disease and sample type, including discordant results and limitations, should be documented.

Clonality detection and limits

Samples from neoplastic disorders can be expected to have varying amounts of nonneoplastic cells admixed with neoplastic cells. The proportion of clonal and nonclonal cells may or may not be clinically relevant but will affect assay sensitivity. Detectable clonality can be influenced by several factors including microarray platform used, sample source, DNA quality, size and copy-number state of the abnormality, and probe coverage. Noise from poor-quality DNA may mask clonality. Each laboratory will need to challenge their microarray with mosaic, aneuploid, and clonally diverse samples to gain experience in their detection. The various factors should be considered with data analysis.

Visual inspection and manual review of the data should be employed to detect clonality and gain experience with data interpretation. The software may not flag low-level clonality. A call made by visual/manual inspection, when the call was not made by the software, should be verified by another method, e.g., interphase FISH, qPCR.

Determination of levels of detectable clonality

Methods to evaluate levels of detectable clonality will differ with sample type, e.g., fresh, fixed, or FFPE. Dilution studies are one method that may be used to create different levels of clonality for test purposes.[29] Flow cytometric analysis and interphase FISH analysis of fresh (uncultured) samples provide reliable methods for confirmation of clonality level(s). Conventional cytogenetic analysis of metaphase cells provides information about clonal populations but does not reliably reflect levels of clonality.

Dilution studies for SNP arrays require nonneoplastic and tumor DNA from the same patient. Buccal cells or blood may provide a source of nonneoplastic patient DNA.

Assessment of levels of neoplastic to nonneoplastic cells or sizes of different clonal populations in fresh or fixed (FFPE) tissue samples is more difficult. Dissection of fresh tumor with an inverted microscope can reduce the amount of nonneoplastic tissues. Microdissection of FFPE tumors can enrich the DNA sample for tumor. Estimation of clonality in tumor tissue samples can be useful when analyzing data from these tumor types.[11,29]

Determination of ploidy

Polyploidy may be detected by microarray analysis but may be difficult to appreciate. The allelic states of SNP probes can assist in determining ploidy levels. The validation process should include samples with varying levels of ploidy to gain experience in analysis and recognition of different ploidies. The manufacturer should provide the method used for normalization. The laboratory must understand the effect that normalization may have on polyploidy detection and subsequent interpretation of gains and losses in the context of polyploidy.

Clonal diversity

Clonal diversity, common to neoplastic disorders, should be visible by microarray when the cell populations of different clones reach the threshold for detection. However, determination of the composition of clones or the sequence of progression of clonal evolution will not be possible. Correlation with conventional cytogenetic analysis may facilitate interpretation of the microarray results.

Software experience and evaluation

Software may not be specifically designed for analysis of cancer specimens. Laboratories may choose to design their own software programs or modify parameters of the platform's standard software program. The laboratory should recognize software limitations and the need for manual and visual inspection of the data for aberration and clonality detection.

A comprehensive evaluation of any software to be used to analyze microarray data should be performed. The laboratory

must determine and document the ability of the software to define accurately the limits of copy-number variations, i.e., deletions, duplications, and/or amplifications, according to software rules and parameters. When applicable, the laboratory should also determine the ability of the assay to define the end points of copy-neutral ROHs according to the software settings. Limits should be reestablished whenever the microarray platform, probes, software, or analysis rules change. The laboratory should challenge the software with a variety of aberrations, especially copy-number variations that help define the limits of detection. The limits, rules, and parameters for detection of clonality should be determined. The laboratory should document the software parameters and rules used in the analysis of the microarray, as well as all limitations of the analysis program.

REFERENCE DNA

Comparative genomic hybridization–based microarray analysis requires comparison of sample DNA to reference DNA. Selection of an appropriate reference DNA is essential. Constitutional DNA from blood or normal tissue from the same individual may be used. Constitutional patient DNA will mask constitutional CNVs and reduce the complexity of postanalytic interpretation. However, novel underlying germline abnormalities that could contribute to disease will not be detected.

Laboratories may establish their own reference DNAs. Reference DNA may be from a set of normal individuals with common CNVs identified for a specific type of microarray. The laboratory should characterize any reference DNA to identify CNVs that may have an effect on the interpretation of patient data.

Male and female controls should be established. Laboratory policies should detail how reference DNAs will be used, i.e., for mismatched opposite-sex or same-sex comparisons, as single male or single female references, or as pools from multiple male or multiple female DNA samples. The laboratory should document the rationale for the use of reference DNA types and have provisions for use in different situations. The advantages and limitations of different approaches should be understood and considered during interpretation of data.

Each new reference DNA or new lot of purchased reference DNA for array comparative genomic hybridization should be compared by microarray analysis to the previous lot of reference DNA.

SNP-based microarray analysis requires comparison of the sample result with established references or an *in silico* reference library. If sufficient data are available for a control population, a laboratory may establish its own *in silico* reference that mimics the typical study population. New reference data should be established for new SNP-based array designs.

QUALITY CONTROL

Identification

For each microarray, the slide ID, sample sex, control sex (when appropriate), and sample-tracking control (for multiplex microarrays) should be verified. Discrepancies in the documentation from the physical sample should be investigated and resolved before processing.

Sample requirements

The laboratory should establish parameters for the minimum DNA quality and quantity requirements for each sample type used for clinical testing. The laboratory should demonstrate proficiency in sample preparation, DNA extraction, and DNA purification for each sample type. Fresh or frozen tumor tissue is preferable to fixed tumor tissue for quality. FFPE tumor samples should be evaluated by a surgical pathologist to assess the quality and quantity of tumor in the sample used for microarray analysis. A minimum of 25% tumor is recommended to prevent masking of clonal changes by normal tissue DNA.

DNA extraction, purification, measurement, and amplification with different sample types

DNA extraction methods should ensure the highest-quality DNA possible from the sample type(s) tested by the laboratory. Samples from neoplastic disorders present unique challenges for generating high-quality, tumor-specific DNA. Written protocols should be available in the laboratory procedure manual and/or quality management program for optimizing DNA extraction and labeling, DNA quantification (e.g., fluorometer, spectrophotometer), DNA quality and concentration (e.g., examination by gel electrophoresis), DNA fragmentation (e.g., via sonication or digestion), fluorescent labeling (e.g., examination by gel electrophoresis, visual inspection, ultraviolet/visible spectroscopy), and amplification (e.g., significant increase in product). For any labeling method, acceptable ranges should be determined for proper dye incorporation. Protocols for optimization, e.g., reextraction, repurification, tumor cell enrichment for hematological samples (cell sorting or concentration), and/or microdissection for paraffin-embedded tumor, should be available as appropriate. Laboratories should be aware that fixatives other than formalin may influence DNA quality and that decalcification of bony tumors may adversely affect DNA quality.

Suboptimal samples

The laboratory should establish sample adequacy requirements. Samples that do not meet the laboratory requirements should be rejected with a repeat sample requested from the referring physician.

When a repeat sample is not available, whole-genome amplification may be a reasonable alternative if the laboratory has expertise with the method and if potential biases inherent in the technique are detailed in the report. Laboratory policies and protocols should describe when and how whole-genome amplification is performed.

Equipment calibration, maintenance, and QC

Equipment, instrumentation, and methodologies employed during the validation and use of microarray platforms should be calibrated, receive regular maintenance, and be monitored for QC. Quality metrics should be established for each step of the assay.

QC metrics

Every microarray platform has defined quality metric values, e.g., adequate dye incorporation and/or amplification, fluorescence intensities variance, signal-to-background-noise ratio, and SD or error. Standard cutoff values and acceptable limits should be established for these metrics to ensure that the generated results are reliable and sufficiently precise to be used for a clinical assessment. Quality metrics should be monitored for DNA labeling, hybridization efficiency, data generation and analysis, and other platform-specific parameters. QC metrics should be incorporated into the laboratory quality assurance and quality improvement programs to monitor analytical variables.

Microarray content

It is not feasible for a laboratory to validate the identity and copy-number responsiveness of every probe on a microarray. The laboratory should obtain documentation from the microarray manufacturer that the probes on each microarray are the intended sequence, located appropriately by the software, empirically selected for appropriate copy-number responsiveness and/or SNP allele specificity, and stable for these assessments from lot to lot.

Data quality

Detection of genomic aberrations is dependent on the size of the DNA targets, the probe density, the probe performance, and the distance between the sequences naturally located on the chromosome. The quality of the data will affect the ability to detect genomic aberrations; thus, the laboratory needs to understand the within-array metrics provided by the analysis software and how each metric reflects the quality of the data. One metric that provides a measurement of noise or random variance unrelated to genomic location in the data is the derivative log ratio. The derivative log ratio is the difference between the log ratio values of consecutive probes (derivative log ratio spread), i.e., the spread of the derivative log ratio values after outlier rejection. For SNP arrays, quality may be assessed using data from such parameters as call rates and variability (spread) of allele frequency.

Other features to be kept in mind when assessing copy-number changes are the appropriate log ratio difference between patient and control samples, presence of nonrandom contiguous probe behavior, sharp copy-number state transitions, and supportive SNP allele states (when applicable). The software manufacturer should provide confidence metrics for a copy-number call or SNP allele state/genotype based on the algorithms used by the software for aberration calls.

The laboratory should establish acceptable ranges for each QC metric chosen to assess data quality. The manufacturer often provides these ranges; however, the laboratory may want to modify these ranges based on their experience with the microarrays during the validation process. The laboratory should establish criteria for next steps should the data fall outside of these established ranges.

Custom and public annotations/databases are integral to data analysis. Because these annotations are critical for interpretation, it is important that these tools are carefully constructed and applied by the software manufacturer. Manufacturers should provide updates to these annotations as they become available. The laboratory should check any inconsistencies with an additional data source, e.g., compare results from the University of California, Santa Cruz (UCSC) genome browser with those from the Ensembl browser. Custom annotations from the laboratory should be verified.

Laboratories should ensure that the software manufacturer provides documentation and safeguards such that data are processed and summarized in a consistent manner for every clinical analysis. Most analysis software provides a hierarchy of users with customizable permissions, which enables the laboratory to prevent modification of analysis settings so that all specimens are analyzed consistently. Any changes to data processing should be validated and documented.

Verification of new lots of microarrays and/or reagents

Verification should ensure that new lots of microarray slides and/or reagents perform in the same manner as the previous lot. The manufacturer should supply documentation of the QC comparison between lots of microarray slides, e.g., oligo synthesis verification, accuracy of SNP calls, or other defined control parameters. A new lot of microarray slides should be tested to ensure equivalency by testing, either before or concurrently with new patient specimens, preferably using a patient specimen with an abnormal result that has been tested on a previous lot. Manufacturers may include a normal control and request that it be run. New lots of reagents, e.g., new labeling kits and consumables, should have documented equivalency between runs. This may be accomplished by documenting that the QC metrics meet certain set parameters for the new lot of reagents.

QUALITY ASSURANCE

Laboratory accreditation and personnel qualifications

Laboratory personnel must have documentation of education, degrees, and certifications as appropriate for the level of testing, as well as training, competency assessments, and continuing education as required by appropriate regulatory bodies, e.g., College of American Pathologists (CAP), Clinical Laboratory Improvement Amendments (CLIA), Center for Medicare and Medicaid Services (CMS). The testing laboratory must have CLIA certification and state certifications as required to provide clinical testing. CAP accreditation is strongly encouraged.

Indications and ordering for microarray analysis of neoplastic disorders

Microarray analysis of tumors should be limited to specimens that contain ample tumor, e.g., diagnostic or relapse. The sample should be accompanied by an appropriate indication for the test. Clinical testing should be limited to neoplastic disorders for which unbalanced genomic anomalies are well documented to have diagnostic, prognostic, and/or therapeutic implication(s).

Microarray analysis is not indicated for tumor surveillance or detection of minimal or residual disease because of insensitivity of the test for low levels of disease. Alternative methods should be recommended to monitor patient response to treatment and for residual disease detection, e.g., FISH, qPCR. A clonal abnormality identified and confirmed at diagnosis may be used for follow-up. The same method used for confirmation, e.g., qPCR or FISH, is recommended for use in follow-up studies. Alternatively, DNA or cells may be saved and used as a control when follow-up samples are assessed for residual disease.

Laboratories may facilitate appropriate ordering by providing a directive or disease-specific testing menu. The test requisition should provide sufficient clinical and/or pathological information for the laboratory to assess the appropriateness of the test order.

Proficiency testing (PT)

The laboratory should participate in PT for sample types and tumor types that are included in the laboratory test menu by participating in an external PT program when available through an appropriate-deemed organization, e.g., CAP. In addition, the laboratory may establish external PT of normal and abnormal specimens by the exchange of DNAs, in a blinded manner, with another laboratory performing microarray testing for neoplastic disorders.

The laboratory should also establish internal PT of normal and abnormal samples as part of the laboratory internal quality assurance program and ongoing quality improvement program. Correlation between microarray results run in parallel on different microarray platforms or correlation of microarray results with conventional cytogenetic and/or FISH results may be sufficient to provide ongoing proficiency. PT should be performed according to the CLIA '88 guidelines.

Documentation of participation and the performance results of internal and external PT must be retained by the laboratory and made available to all accreditation agency inspectors.

Failure to achieve agreement on external or internal proficiency tests should be documented and followed by investigation of the discrepancy with resolution. If indicated, appropriate remediation should be undertaken.

Turnaround time

Laboratory policies should define acceptable standards for microarray analysis test prioritization and turnaround times. Turnaround time should be clinically appropriate so the results are available for patient care management decisions.

It is suggested that 90% of cases should have a final written report by 21 calendar days. A longer turnaround time is acceptable when custom probes, oligos, or primer sequences must be designed, ordered, validated, and used. Normal or preliminary abnormal results should be available within 14 calendar days.

Documentation of problems

A logbook, database, or sample processing form should be created and used to track problems that may occur throughout the processing of samples for neoplasia, from sample intake to final report, e.g., sample adequacy and/or errors. Data from the QC metrics program can provide information for oversight of all processes. Ongoing collection of sample or process variances allows patterns or trends to be recognized and promptly addressed.

ANALYSIS OF DATA INCLUDING ANALYTICAL SOFTWARE ALGORITHMS

Analytical software algorithms differ between platforms. Microarray software is designed to determine gain, loss, or long ROHs in a chromosomal region. Most software manufacturers provide standard algorithms to set cutoff values for calls. However, each laboratory should thoroughly test the rules or filters during the validation process and determine the parameters for cutoff values, e.g., the number of consecutive probes deleted or amplified and the $\log_2$ ratio to call a CNV, depending on probe density. It may be necessary to set different parameters for different chromosome regions or specific genes of interest while keeping in mind the potential for a false-positive call.

Ratio values for mosaic cases will be less than expected for nonmosaic cases and may fall below the standard cutoff value. Clonality may be apparent by visualization or by examination of the moving averages across the chromosomes. The sensitivity of the microarray for detection of clonality should be determined during the platform validation process.

The laboratory must be familiar with the principles of the software program for any platform used. However, the laboratory should never depend solely on the software for analysis. A visual inspection of the moving average across each chromosome and a review of the allele frequency for SNP arrays should be done to identify appropriate and inappropriate results for the disorder being tested. Analysis should be continued until all inconsistencies are resolved.

RESULTS EVALUATION AND INTERPRETATION

The laboratory should be consistent in the analysis, interpretation, and reporting of microarray results. The laboratory should have a record of and be familiar with the microarray coverage, including known cancer-associated genes and regions, benign and/or common population CNVs, and common genetic disorders caused by genomic CNVs and/or LOH.

Systematic evaluation and interpretation of DNA microarrays

The laboratory should establish the methods for microarray result analysis and interpretation using the following recommendations.

Disease-associated genetic aberrations

Analysis and interpretation of microarray data from a neoplastic disorder should take into account the working diagnosis, the clinical information provided, and other disorders in the

differential diagnosis. The indication or working diagnosis may prove to be incorrect after the diagnostic workup is complete; thus, the laboratory should be aware of other disorders that may be in the differential.

The laboratory should be familiar with recurrent, clonal aberrations associated with particular diagnoses. In addition, the laboratory should be familiar with specific genes known to be pathogenic or to contribute to the pathogenesis of a particular disorder. The medical literature should be used to stay abreast of current disease-specific genetic aberrations, as well as the diagnostic, prognostic, and therapeutic significance of aberrations.

CNV interval size and cancer-associated genes

The size of a CNV is relevant, as larger CNVs encompassing multiple genes are more likely to have a clinical impact; however, very small CNVs that interrupt or delete an established cancer-associated gene may be clinically significant. A single laboratory-established CNV size cutoff or threshold for determination of inclusion of a CNV in a clinical report should not be used as the sole determinant of a call. The laboratory should establish methods for detection of clinically significant CNVs that fall below laboratory-established thresholds, particularly in regions of known cancer-associated genes.

Genomic content in CNV interval

The genomic content of the CNV should be carefully examined for genes relevant to disorders in the differential diagnosis, gene-rich sequences, or genes known to have a clinical association. CNVs encompassing known oncogenes or tumor suppressor genes may have significance, although the implications of the CNV for the particular disorder or patient being studied may not be clear based on current literature.

Copy-number-neutral ROHs detected by SNP analysis

Thresholds or minimal criteria to identify clinically important ROHs consistent with LOH (LOH or AOH) should be established. ROHs associated with parental consanguinity or uniparental disomy should be distinguished from acquired LOH. Distinction of acquired versus constitutional AOH may be facilitated by detection of the clonal aberration in affected tissue (acquired LOH) and/or detection (or not) of the aberration in unaffected tissue (constitutional LOH). Homozygosity in a region that contains a tumor suppressor gene may be associated with an inherited cancer predisposition syndrome. Constitutional analysis should be recommended as appropriate.

Comparison of CNV to internal and external databases

Public databases and the medical literature should be used in determining the significance of CNVs. Available databases include (all last accessed 26 January 2013) the following:

- Database of Genomic Variants (http://projects.tcag.ca/variation/),
- Online Mendelian Inheritance in Man (http:www.ncbi.nlm.nih.gov/omim/),
- DECIPHER (http:www.sanger.ac.uk/research/areas/),
- dbVar—database of Structural Variation (http:www.ncbi.nlm.nih.gov/dbvar),
- dbGaP—database of Genotypes and Phenotypes (http:www.ncbi.nlm.nih.gov/gap),
- Memorial Sloan-Kettering Cancer Center (http://cbio.mskcc.org/CancerGenes),
- The Cancer Genome Anatomy Project (http:www.ncbi.nlm.nih.gov/ncicgap/),
- UCSC Genome Bioinformatics (http://genome.ucsc.edu/cgi-bin/hgGateway),
- The Cancer Genome Atlas (http://cancergenome.nih.gov/),
- Ensembl (http://uswest.ensembl.org/Homo_sapiens/Gene/Summary),
- The International Standards for Cytogenomics Arrays Consortium (https://www.iscaconsortium.org/), and
- Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/genetics/CGP/cosmic/).

Laboratories should document pathogenic CNVs, CNVs of uncertain significance, benign CNVs, and CNVs thought or determined to be constitutional. The intralaboratory data should be used along with external data as a reference for interpretation of data from new studies.

Categories of clinical significance

Using the guidelines outlined above for systematic investigation of a CNV for clinical significance, it is recommended that the interpreting laboratory geneticist use the following categories for reporting. Consistent terminology will facilitate unambiguous communication of clinical significance. Taking into account that tumors may be genetically complex, it may not be feasible to provide a detailed interpretation of every CNV and/or AOH region detected. In such cases, a narrative to describe variants and their clinical significance and interpretation should be provided to communicate the desired information. When feasible, the laboratory should provide details of specific CNV and AOH anomalies.

Pathogenic

Acquired. The CNV is a documented clinically significant and/or disease-associated clonal genetic aberration.

Constitutional. Microarray analysis will inevitably reveal common benign and rare constitutional CNVs. Rare constitutional CNVs should be noted and investigated for clinical significance, e.g., cancer-predisposing gene aberration and/or deletion or duplication associated with a known constitutional syndrome. Evaluation and reporting of constitutional CNVs should follow the guidelines set forth in the American College of Medical Genetics and Genomics Standards and Guidelines for interpretation and reporting of constitutional CNVs.[30]

Uncertain clinical significance

This category may include CNVs that are not known to be associated with disease but meet the reporting criteria established by the laboratory. A CNV in this category is not clearly pathogenic, and there is insufficient evidence for an unequivocal determination of clinical significance. The laboratory should interpret novel CNVs in light of the available clinical and/or pathological information and current literature. Reporting CNVs of uncertain significance is at the discretion of the laboratory. If reported, they may be categorized as follows:

Uncertain clinical significance, acquired, likely pathogenic. Many neoplastic disorders have well-recognized and/or well-characterized aberrations. However, microarray resolution may reveal uncharacterized CNVs. If reported, the discussion should avoid speculation as to the pathogenicity or clinical significance of the CNV without supporting evidence.

Examples: (i) A CNV described in a single case report of a similar neoplasm. (ii) A CNV with a gene in the interval that has potential or relevant function as an oncogene or tumor suppressor gene or that belongs to another known gene family that has an association with neoplastic processes but not the neoplastic process being studied. (iii) A CNV that appears related to the clonal neoplastic process being studied by having a similar $\log_2$ ratio as the clonal process being studied but is not a recognized aberration. Evolution and heterogeneity of a clonal neoplastic process is common. Microarray is likely to reveal new, but uncharacterized, aberrations that may be reported in this category.

Collection of the data of CNVs of uncertain significance is encouraged to build a database for intralaboratory reference, for correlation with clinical parameters, and for sharing in publications. The eventual understanding of the clinical significance will depend on accumulation of sufficient information and correlation with clinical features.

Uncertain clinical significance, likely constitutional. Refer to the American College of Medical Genetics and Genomics Standards and Guidelines for interpretation and reporting of constitutional CNVs.[30]

Uncertain clinical significance, not otherwise specified. A CNV that meets the laboratory parameters for reporting but has no features to categorize it further.

Benign. Reporting of benign CNVs is at the discretion of the laboratory. The laboratory should be familiar with common benign CNVs, stay current with the literature, and interpret results with this knowledge. This category will include: (i) CNVs reported in multiple peer-reviewed publications or curated databases as a benign variant and (ii) CNVs without relevant genetic content that meet criteria for reporting. It should be recognized, however, that cancer-associated anomalies that occur in known variant regions might not be benign.

REPORTING GUIDELINES FOR MICROARRAY ANALYSIS OF NEOPLASTIC DISORDERS

The following guidelines describe the elements of the clinical report that are necessary to communicate clearly and completely the clinical significance of microarray analysis results.

Reporting criteria

Microarray reports should be written so the result is understandable to a nongeneticist health-care provider and so that the clinical significance of the result for patient management is clear.

Care and special consideration should be given to reporting of certain results in children, e.g., disease-predisposing genes and adult-onset disease–associated genes.

To the extent possible, the current International System for Human Cytogenetic Nomenclature should be used to describe known recurrent, disease-associated, or clinically significant aberrations. FISH and chromosome studies used for confirmation analysis should be described using this nomenclature, which provides a format to report microarray results with the nucleotide boundaries for copy-number gains or losses. Breakpoints should be given to the extent possible given the employed technology.

The laboratory may choose to list relevant genes within the altered region. The specific genome-build nucleotide numbering should be specified, e.g., February 2009 assembly, NCBI37/hg19.

Verbal discussion of microarray results with the health-care provider is encouraged to facilitate communication and understanding of microarray results and clinical significance.

Written report

The written report should include the following:

1. Case identification with at least two unique patient identifiers: patient name, date of birth, or other unique identifier, e.g., medical record number.
2. Laboratory accession number(s), date of collection and/or receipt of specimen, specimen type, and name(s) of physician(s) or authorized persons to whom the report is to be provided.
3. Indication for the study, e.g., clinical information or diagnosis and/or pathological diagnosis.
4. List of specific CNVs with the following information when relevant:
   - Chromosome location (chromosome number and band designation),
   - LOH and CNVs with linear coordinates and genome build,
   - Genes of potential significance within interval(s), when indicated,
   - Dosage (copy-number loss, gain, amplification with confirmed ploidy/normalization), and
   - Clonality or ploidy, if applicable.
5. Confirmation testing method(s) and results, when applicable, and a statement of additional analyses performed to resolve questions of clonality, as appropriate.

6. Narrative interpretation to correlate the microarray result with patient-specific clinical or laboratory information, e.g., histopathology, immunophenotype, and/or flow cytometric data. The discussion should include the clinical significance of the results for the diagnosis, prognosis, and/or therapeutic management of the patient with reference to current literature. A note/disclaimer should be included to encourage clinicians to consider the results/data along with other laboratory tests, clinical findings, and recent literature.
7. Clinically significant constitutional CNVs should be discussed with recommendations for further testing as appropriate.
8. If a CNV of uncertain clinical significance is reported, a discussion of the possible relationship or significance to the diagnosis with supporting literature should be provided.
9. References as appropriate for the interpretation and that provide helpful information for the health-care provider.
10. Documentation of date of verbal communication of preliminary or final results to health-care provider(s) with notes regarding discussion of acquired and/or constitutional CNVs or abnormalities and the clinical significance, as appropriate.
11. Recommendation(s) for additional testing as appropriate.
12. Recommendation(s) for genetic counseling as appropriate.
13. Technical information for the testing platform and software, e.g., commercial source, coverage, version, and National Center for Biotechnology Information (NCBI) build used for data analysis. Limitations of the testing platform, e.g., detection of LOH, balanced rearrangements, ploidy, and/or low-level clonality. Biases and limitations of whole-genome amplification when appropriate. Methods summary including criteria for calls, e.g., minimum number of consecutive probes and/or length of area of LOH.
14. Qualified individuals must sign all final reports. Password-protected electronic signatures can be used fulfill this requirement.
15. Date of final report.
16. Disclaimers as appropriate, e.g., when and what investigational procedures are employed. Disclaimers as required.

CONCLUSIONS

Each new technological development in the field of genetics brings with it the desire to apply the technology to improve medical care. The transition of a new technology from the research bench into the clinical realm of diagnostic testing must be accompanied by extensive clinical validation to ensure the results reported to the health-care provider are accurate and reliable for use in patient-care decision making. The validation involves extensive comparison to the existing trusted methodologies to demonstrate that the new method has reliable and consistent results and interpretation. Sufficient comparative data must be accumulated and evaluated before the new method becomes a first-tier method. When the new technology provides additional information that is unattainable by the existing method, data accumulation and correlation with clinical parameters can expand the benefit provided by the new technology.

Microarray technologies provide a high-resolution view of the whole genome, which may yield massive amounts of new information. Medical laboratory professionals must be prepared to identify, interpret, and report results with clinical relevance while being mindful of the social, ethical, and legal responsibilities of reporting genetic information. Interpretation of the data from microarrays into clinically relevant information is a difficult and complex undertaking and is the practice of medicine. No algorithm for CNV interpretation can substitute for adequate training and knowledge in the fields of oncology, pathology, and medical genetics. Individuals with appropriate professional training and board certification, i.e., American Board of Medical Genetics clinical cytogenetics, clinical molecular genetics, or molecular genetic pathology should provide the interpretation of genomic microarrays for the clinical investigation of neoplastic disorders.

DISCLOSURE

All authors direct clinical testing laboratories that use the technologies and/or perform tests related to those described in this guideline.

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