3) Vermeesch JR, Fiegler H, de Leeuw N, Szuhai K, Schoumans J, Ciccone R, Speleman F, Rauch A, Clayton-Smith J, Van Ravenswaaij C, Sanlaville D, Patsalis PC, Firth H, Devriendt K, Zuffardi O. Guidelines for molecular karyotyping in constitutional genetic diagnosis. *Eur J Hum Genet.* 2007 Nov;15(11):1105-14
4) Clinical and Laboratory Standards Institute. *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI Document MM19-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
5) Clinical and Laboratory Standards Institute. *Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications. 1st ed*. CLSI guideline MM21-ED1. Clinical and Laboratory Standards Institute, Wayne, PA, 2015.

CYG.49535 Nucleic Acid Quantity and Quality Determination — Phase II

The quantity and quality of nucleic acids are determined, when appropriate.

NOTE: The quantity and quality of nucleic acids (DNA or RNA) must be measured prior to use in a procedure whose success depends on accurately determining the quantity, concentration, integrity, and/or purity of the nucleic acids. Techniques commonly used to assess nucleic acid quantity and/or quality include electrophoresis, UV/VIS spectrophotometry, and fluorescence spectroscopy.

Evidence of Compliance:

- ✓ Records of nucleic acid quantity and/or quality determination

REFERENCES

1) Clinical and Laboratory Standards Institute. *Diagnostic Nucleic Acid Microarrays; Approved Guideline;* CLSI Document MM12-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2006.
2) Tsui NBY, Ng EKO, Lo YMD. Stability of Endogenous and Added RNA in Blood Specimens, Serum and Plasma. *Clin Chem.* 48:1647-1653, 2002.
3) Farrell R. Gel electrophoresis based assessment of cellular RNA quality may also be used (RNA Isolation Strategies). In: RNA Methodologies: A Laboratory Guide for Isolation and Characterization. Academic Press, 1998.

CYG.49540 Extracted Nucleic Acid Specimens — Phase II

If extracted nucleic acid is accepted as a specimen type, the laboratory ensures that isolation of nucleic acids for clinical testing occurs in a CLIA-certified laboratory or a laboratory meeting equivalent requirements as determined by the CAP and/or the CMS. This policy is clearly displayed to ordering clients.

NOTE: All clinical testing must be performed in CLIA-certified laboratories or laboratories meeting equivalent requirements (refer to GEN.41350). This includes all components of testing that may impact the quality of the test result, including isolation or extraction of nucleic acids. Laboratories may choose to have referring clients formally attest that extracted nucleic acid submitted for testing has been isolated or extracted in an appropriately qualified laboratory.

Evidence of Compliance:

- ✓ Written statement on the test requisition, test catalog, or policy available to referring clients stating that the laboratory only accepts isolated or extracted nucleic acids for which extraction or isolation is performed in an appropriately qualified laboratory

CYG.49545 Validation Studies for DNA-Based Copy Number Array - Specimen Types — Phase II

Validation studies for DNA-based copy number arrays are performed for each specimen type that can be affected by different preanalytic variables, that requires different processes for DNA extraction, and for those specimens with potentially interfering substances (eg, FFPE tissue, decalcified tissue, tissue containing melanin or mucin).

NOTE: A number of preanalytic and analytic processing variables can significantly influence the quality and integrity of nucleic acids extracted from a specimen. Commonly tested tissue sources must be included in the validation, but it is not expected for the laboratory to include every tissue source that could be examined by the assay.

It is the responsibility of the laboratory director or designee meeting CAP director qualifications to determine when a separate validation is needed versus a limited study to demonstrate that the DNA obtained from the specimen performs the same. For example, an array platform that has

been originally validated to detect constitutional copy number abnormalities from peripheral blood will require a separate full validation to detect somatic alterations but may only require a more limited study to allow for a different specimen such as saliva to be used.

If an array has been validated for constitutional copy number alterations from fresh/frozen tissue, every potential tissue source (lung, liver, kidney, etc.) does not require separate validation, unless they potentially include interfering substances (eg, mucin).

Validations can be augmented by, but not supplanted with, additional reference materials (eg, characterized cell lines, cell lines with spiked in nucleic acids). Matrix-appropriate samples must be included.

Evidence of Compliance:
- ✓ Records of validation studies

CYG.49575 Assay Performance Monitoring **Phase I**

Assay performance is monitored for each run and quality metrics are verified prior to reporting results.

NOTE: The monitoring of assay performance includes the review and recording of the quality metrics of each run. This may include:
- *DNA labeling verification (using detection of label, purification and quantitation of labeled DNA fragments, or electrophoretic techniques)*
- *Review of DLRs (Derivative Log Ratio)*
- *Genotyping performance (SNP arrays only)*
- *Number of suboptimal samples*
- *Monitoring the number of copy number alterations per sample*
- *Other quality metrics provided by the array software*

Criteria for acceptable performance must be defined. This includes hardware and analytical software.

Evidence of Compliance:
- ✓ Records of verification

REFERENCES

1) South ST, Lee C, Lamb AN, Higgins AW, Kearney HM, Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee. ACMG standards and guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genet Med.* 2013; 15(11):901-9.
2) Shao L, Akkari Y, Cooley LD, et al. Chromosomal microarray analysis, including constitutional and neoplastic disease applications, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med.* 2021;23(10):1818-1829.
3) Vermeesch JR, Fiegler H, de Leeuw N, Szuhai K, Schoumans J, Ciccone R, Speleman F, Rauch A, Clayton-Smith J, Van Ravenswaaij C, Sanlaville D, Patsalis PC, Firth H, Devriendt K, Zuffardi O. Guidelines for molecular karyotyping in constitutional genetic diagnosis. *Eur J Hum Genet.* 2007 Nov;15(11):1105-14
4) Clinical and Laboratory Standards Institute. *Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications. 1st ed.* CLSI guideline MM21-Ed1. Clinical and Laboratory Standards Institute, Wayne, PA; 2015.

CYG.49580 Array Analytical Wet Bench **Phase II**

The laboratory follows a defined process for performing the array analytical wet bench.

NOTE: The procedure must include:
- *A description of the analytical target regions (eg, targeted or genome-wide)*
- *A description of acceptable sample types (see CYG.49545)*
- *Methods and reagents used for isolating, labeling, and hybridization of nucleic acids, as applicable*
- *Controls (including in silico)*
- *Instrument software and version*
- *Acceptance and rejection criteria for the results generated by the wet bench. These should include criteria for determining when the wet bench process has failed or is suboptimal.*