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.*

- *Written procedure for any portion of the wet bench process performed by a referral laboratory, if applicable.*

***REVISED** 12/26/2024*

CYG.49585 Array Analytical Bioinformatics **Phase II**

The laboratory defines the steps in the bioinformatics process (also termed pipeline) used to analyze, interpret, and report array findings.

NOTE: A bioinformatics pipeline includes all algorithms, software, scripts, parameters, reference sequences, and databases, whether in-house, vendor-developed, or open source.

The written procedure must describe the bioinformatics process(es) including, where applicable:

- *Individual software applications (open source, proprietary, and custom scripts) and versioning*
- *Description of input and output data files for each step of the pipeline, including in silico control files or sources*
- *Annotations and their sources (eg, public or private databases, with versions used)*
- *Criteria and thresholds for detection of array findings (eg, minimum number of probes or genomic size for copy number variants)*
- *Determination of the limits of detection*
- *Additional scripts or steps used to connect discrete applications in the pipeline*
- *Quality control metrics, including batch or sample-specific metrics and acceptance and rejection criteria for the results generated by the analytical bioinformatics process. Criteria must be based on metrics and quality control parameters established during test optimization and utilized during validation*
- *Required corrective actions when results fail to meet the laboratory's acceptance criteria*
- *Limitations in the test methodology*
- *Written procedures for any portion of the array bioinformatics process performed by a referral laboratory or a commercial service provider, if applicable. This should include a written description of how the security of identifiable patient information (eg, HIPAA compliance) is ensured during transmission and storage of data by the referral laboratory or commercial service provider.*

CYG.49590 Interpretation and Reporting of Array Findings **Phase I**

The laboratory follows defined criteria for classification, interpretation, and reporting of array findings.

NOTE: The laboratory must have a written algorithm for classifying and interpreting the clinical significance of identified findings. The ACMG guidelines can be used for classification and interpretation of copy number variants in inherited disorders.

Genome-wide array analysis may yield genetic findings unrelated to the clinical presentation for which the patient is undergoing testing. The laboratory policy must describe which, if any, and for what reasons, findings unrelated to the clinical purpose for testing are reported and the method of communication to the ordering physicians and patients, as applicable.

The written policy must include indications for confirmatory testing. The laboratory must determine by confirmation studies during validation if and when confirmatory testing of identified findings should be performed.

Evidence of Compliance:

- ✓ Records of compliance with procedure for classification, interpretation, and reporting of findings **AND**
- ✓ Laboratory database of findings identified and/or reported

REFERENCES