COLLEGE of AMERICAN PATHOLOGISTS

Master

Cytogenetics Checklist

CAP Accreditation Program

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Cytogenetics Checklist

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Participants of the CAP accreditation programs may download the checklists by logging into cap.org and going to e-LAB Solutions Suite - Accreditation Checklists. They are available in different checklist types and formatting options, including:

- Master — contains ALL of the requirements and instructions available in PDF, Word/XML or Excel formats
- Custom — customized based on the laboratory's activity (test) menu; available in PDF, Word/XML or Excel formats
- Changes Only — contains only those requirements with significant changes since the previous checklist edition in a track changes format to show the differences; in PDF version only. Requirements that have been moved or merged appear in a table at the end of the file.

CHECKLIST ACCREDITATION RESOURCES

CAP accredited laboratories have access to additional checklist accreditation tools and resources found on the CAP website (cap.org) by logging into e-LAB Solutions Suite - Accreditation Resources. Content found in Accreditation Resources includes:

- A library of past Focus on Compliance webinars and laboratory inspection preparation videos
- Answers to the most common checklist questions
- Customizable templates and forms (eg, competency assessment, personnel, validation/verification, quality management)
- Proficiency testing (PT) frequently asked questions, forms, and troubleshooting guides
- IQCP eligibility, frequently asked questions, forms, templates, and examples
- Laboratory director education and resources
- Quality management resources
- Inspector training and inspection tip sheets
- Self and post inspection toolbox

SUMMARY OF CHECKLIST EDITION CHANGES
Cytogenetics Checklist
12/26/2024 Edition

The information below includes a listing of checklist requirements with significant changes in the current edition and previous edition of this checklist. The list is separated into three categories:

1. New
2. Revised:
   - Modifications that may require a change in policy, procedure, or process for continued compliance; or
   - A change to the Phase
3. Deleted/Moved/Merged**:**
   - Deleted
   - Moved — Relocation of a requirement into a different checklist (requirements that have been resequenced within the same checklist are not listed)
   - Merged — The combining of similar requirements

NOTE: The requirements listed below are from the Master version of the checklist. The customized checklist version created for inspections and self-evaluations may not list all of these requirements.

Previously Cited Checklist Requirements

- The **inspector's version** of the checklist contains a listing of previously cited checklist requirements. Specific information on those citations, including the inspection date and inspector comments, is included following each related requirement within the checklist.
- Laboratories can access data on previously cited deficiencies by logging into e-LAB Solutions Suite on cap.org and going to Accreditation Reports - Inspection Summation Report.

NEW Checklist Requirements
None

REVISED Checklist Requirements

Requirement	Effective Date
CYG.32700	12/26/2024
CYG.47880	08/24/2023
CYG.48399	12/26/2024
CYG.49585	12/26/2024
CYG.50000	12/26/2024
CYG.50180	12/26/2024

DELETED/MOVED/MERGED Checklist Requirements

Requirement	Effective Date
CYG.33900	08/23/2023
CYG.47885	12/25/2024

INTRODUCTION

This checklist is used in conjunction with the All Common (COM) and Laboratory General Checklists to inspect a cytogenetics laboratory section or department.

Cytogenetics inspectors should be pathologists, cytogeneticists or cytogenetic technologists who are actively involved with or have extensive experience in the practice of cytogenetics, are knowledgeable about current CAP Checklist and CLIA requirements, and have completed CAP Inspector Training. Inspectors should, to the greatest extent possible, be peers of the laboratory being inspected.

Policy/Procedure icon - The placement of this icon next to a checklist requirement indicates that a written policy or procedure is required to demonstrate compliance with the requirement. The icon is not intended to imply that a separate policy or procedure is required to address individual requirements. A single policy or procedure may cover multiple checklist requirements.

Laboratories not subject to US regulations: *Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist. When the phrase "FDA-cleared/approved test (or assay)" is used within the checklist, it also applies to tests approved by an internationally recognized regulatory authority (eg, CE-marking).*

LABORATORY SAFETY

The inspector should review relevant requirements from the Safety section of the Laboratory General checklist, to assure that the cytogenetics laboratory is in compliance. Please elaborate upon the location and the details of each deficiency in the Inspector's Summation Report.

QUALITY MANAGEMENT

Inspector Instructions:

	• Sampling of quality monitoring records
	• Review records of culture and hybridization failures, and sub-optimal analyses for trends. Determine if the procedures and processes produce a thorough investigation with appropriate corrective action taken.

CYG.20200 Quality Indicators **Phase I**

The laboratory monitors and evaluates key quality indicators, such as the following:

1. **Control of pre-analytic variables (specimen collection and delivery)**
2. **Cytogenetic, in situ hybridization, and chromosomal microarray analysis test ordering practices**

3. Provision of sufficient clinical information to ensure that the proper choice of growth medium, probe sets, and analytic techniques are made

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Developing and Using Quality Indicators for Laboratory Improvement.* 2nd ed. CLSI guideline QMS12. Clinical and Laboratory Standards Institute, Wayne, PA; 2019.

CYG.20800 Procedure Failures **Phase II**

The number or frequency of culture failures, hybridization failures, and/or suboptimal analyses is recorded, and there are records of corrective action when adverse trends occur.

QUALITY CONTROL (QC)

SUPERVISION OF QUALITY CONTROL

Inspector Instructions:

	• Sampling of QC policies and procedures • Sampling of QC records • Records of final report error investigation
	• How do you determine when QC is unacceptable and when corrective actions are needed?
	• Select several occurrences in which QC is out of range and determine whether the steps taken follow the laboratory procedure for corrective action.

CYG.30066 Monthly QC Review **Phase II**

The laboratory director or designee reviews and assesses quality control data at least monthly.

NOTE: QC data may include specimen handling, culture failures, new media QC, new reagent lot verification, etc. The reviewer must record follow-up for outliers, trends, or omissions that were not previously addressed.

The QC data for tests performed less frequently than once per month may be reviewed when the tests are performed.

Evidence of Compliance:
- ✓ Records of QC review **AND**
- ✓ Records of corrective action taken when acceptability criteria are not met

CYG.30325 Reporting Error Investigation **Phase II**

All errors that are identified in the final report are thoroughly investigated, and the results of such investigations are recorded.

NOTE: The results of such investigations must be recorded and reviewed as part of the ongoing laboratory QM process.

CYG.30350 Specimen Handling — Phase II

Records indicate the media used, culture conditions, probes used, and incubation times for all preparations.

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Clinical laboratory improvement amendments of 1988; final rule. Fed Register. 2003(Jan 24): [42CFR493.1276(b)(1)]

CYG.30360 QC Handling — Phase II

The laboratory tests control specimens in the same manner and by the same personnel as patient/client samples.

NOTE: Personnel who routinely perform patient testing must analyze QC specimens; however, this does not imply that each operator must perform QC daily. Personnel must participate in QC on a regular basis. To the extent possible, all steps of the testing process must be controlled.

Evidence of Compliance:

- ✓ Records reflecting that QC is run by the same personnel performing patient testing

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7166 [42CFR493.1256(d)(8)]; 2) *ibid,* 2003(Jan 24):3708 [42CFR493 1256(d)(7-8)].

CYG.30550 QC Confirmation of Acceptability — Phase II

Personnel review control results for acceptability before reporting patient/client results.

Evidence of Compliance:

- ✓ Records of control result approval

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7166 [42CFR493.1256(f)]

CYG.30600 Alternative Control Procedures — Phase II

If the laboratory performs test procedures for which control materials are not commercially available, the laboratory performs and records alternative control procedures to detect immediate errors and monitor test system performance over time.

NOTE: "Performance" includes elements of accuracy, precision, and clinical discriminating power. The following are examples of alternative procedures: split sample testing with another method or with another laboratory, the testing of previously tested patient specimens in duplicate, testing of patient specimens in duplicate, or other defined processes approved by the laboratory director.

Specific examples for cytogenetics include:

- *Confirming the presence of similar karyotypic changes in two independently established cultures analyzed by two different technologists*
- *For SNP array, correlating the results from the SNP and copy number data*
- *Correlating the results obtained by one method with another when a combination of methods are performed (eg, correlating G-banded chromosome analysis with FISH results or genomic array)*

- *Refer to CYG.43200 for in situ hybridization*

Evidence of Compliance:

✓ Records of alternative control procedures

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1256(h)].

REPORTS

Reporting requirements for use of analyte-specific reagents and other reagents used in laboratory-developed tests are included in the All Common Checklist (COM.40850).

Inspector Instructions:

	• Sampling of reporting policies and procedures • Sampling of patient preliminary and final reports for completeness, appropriate use of ISCN edition and recommendations for genetic consultation or additional studies • Sampling of TAT statistics
	• How does your laboratory maintain records of verbal/telephone preliminary reports? • What is your course of action when turnaround times exceed limits?
	• Search for reporting errors. Determine whether the investigation was thorough and appropriate corrective action was taken.

CYG.31825 Preliminary Reports **Phase I**

Provision of preliminary reports (especially verbal, telephone reports) is recorded on the final report.

CYG.31875 Final Report Elements **Phase II**

The final reports contain all of the following required elements:

1. **Name and address of testing laboratory**
2. **Patient name**
3. **Unique identifying number**
4. **Patient date of birth**
5. **Name of physician, or authorized person ordering test**
6. **Specimen source**
7. **Date specimen received in the laboratory**
8. **Date of report**
9. **Clinical indication(s) for the test**
10. **Number of cells counted, analyzed, and karyograms prepared, as applicable**
11. **Band resolution (required only for constitutional cases), as applicable**
12. **Banding methods, as applicable**
13. **Comment on adequacy of specimen, if indicated**

NOTE: Items 10, 11, and 12 above apply to conventional cytogenetics (G-banded) analyses.

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):1043-1044[42CFR493.1276], 1047-1048 [42CFR493.1291]

CYG.31880 Report Review **Phase II**

The final report for conventional cytogenetics (G-banding) and metaphase FISH analyses are reviewed and signed by the cytogenetics section director (or qualified cytogeneticist designated by the section director).

NOTE: A qualified designee must be: 1) a doctor of medicine or doctor of osteopathy licensed to practice medicine in the state in which the laboratory is located; or 2) hold an earned doctoral degree in a biological science or clinical laboratory science from an accredited institution.

In addition, each qualified cytogeneticist must have either a) successfully completed an accredited fellowship with an emphasis on clinical cytogenetics, or b) in the absence of fellowship training, have four years of training or experience or both in human medical genetics or pathology, two of which have been in clinical cytogenetics.

CYG.31903 Turnaround Time **Phase II**

The laboratory has defined sample turnaround times that are appropriate for the intended purpose of the test and performs ongoing monitoring for compliance.

NOTE: Appropriate turnaround times will vary by test type and clinical application. There are certain clinical situations in which rapid completion is essential. For example, inappropriate delays in completing a prenatal diagnosis test can cause unacceptable emotional stress for the parents, make ultimate pregnancy termination (if chosen) much more difficult, or even render the results of the test unusable.

Evidence of Compliance:

✓ Records showing that defined turnaround times are routinely met

REFERENCES
1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
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.

CYG.32071 Final Report Contents **Phase II**

The final report includes a summary of the results and an interpretation that includes correlation of the cytogenetic results with clinical information and previous studies, when appropriate.

NOTE: The interpretation must be written to facilitate understanding by a non-geneticist.

CYG.32100 Nomenclature **Phase II**

For conventional cytogenetic studies, the current International System for Human Cytogenetic Nomenclature (ISCN) is used correctly in the final report.

NOTE: The purpose is to provide universal interpretation of cytogenetic results without pictures of the karyogram.

REFERENCES
1) McGowan-Jordan J, Hastings R, Moore S, eds; International Standing Committee on Human Cytogenomic Nomenclature. *ISCN: An International System for Human Cytogenomic Nomenclature (2020).* Basel, New York: Karger; 2020.

CYG.32250 Recommendations in Final Report **Phase I**

The final report contains recommendations for genetic consultation or additional studies, when appropriate.

REFERENCES
1) American Board of Medical Genetics and Genomics http://www.abmgg.org/
2) National Society of Genetic Counselors http://www.nsgc.org/
3) American Board of Genetic Counseling http://www.abgc.net/

RECORDS

Inspector Instructions:

- Record/specimen retention policy
- Sampling of patient records and materials

CYG.32500 Laboratory Record Information **Phase II**

The laboratory record includes the number of cells counted, analyzed microscopically and the number from which photographic or digitized karyograms were prepared.

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Clinical laboratory improvement amendments of 1988; final rule. Fed Register. 2003(Jan 24): [42CFR493.1276(b)(1)]

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

CYG.32700 Record and Material Retention - Cytogenetics **Phase II**

Records and materials are retained in compliance with applicable national, federal, state (or provincial), and local laws and regulations and as defined in the table below:

Type of Record/Material	Retention Period
Original specimen and cultures	Until release of the final report
Processed specimens or cell pellets	2 weeks after final report
Permanently stained slides	3 years
Fluorochrome-stained slides	At the discretion of the laboratory director
Chromosomal microarray slides	At the discretion of the laboratory director
Images of ISH and non-ISH (eg, G-banded) studies - hard copy (negatives or prints) and/or in retrievable digitized formats	10 years for neoplastic disorders 20 years for non-neoplastic/constitutional disorders (see NOTE 3 below)
Chromosomal microarray data	At least two weeks after the final report is released for the original scan At least two years of sufficient original data to support primary results generation and re-analysis
Final Report (electronic versions are acceptable)	10 years for neoplastic disorders 20 years for constitutional conditions

NOTE 1: The intent is to retain evidence of case results for any future need, such as further family studies, monitoring disease, legal issues, etc.

NOTE 2: Because information technology software and hardware continue to change, access to some digitally archived material may be lost. However, reasonable due diligence should be exercised to maintain access for the full retention period.

NOTE 3: There is no retention requirement for retaining images of slide preparations when the source slides remain readable for the required retention period. If slides are expected to become unreadable before the end of the required retention periods (for example, FISH slides), then images that adequately represent findings on the slides must be retained.

If representative images of chromosome ISH slides are retained, those with a normal result must include an image of at least one cell illustrating the normal probe signal pattern, and those with an abnormal result must include images of at least two cells illustrating each relevant abnormal probe signal pattern.

REFERENCES
1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) 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.
3) 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.

REAGENTS

Inspector Instructions:

- Sampling of records of media checks

Additional requirements are in the REAGENTS section of the All Common Checklist.

CYG.33300 Media QC **Phase II**

Each lot of culture medium is checked onsite for sterility and the ability to support growth.

NOTE: Each laboratory must perform its own QC on new lots of culture media. It is not acceptable practice to rely on QC testing performed at another site.

Evidence of Compliance:

✓ Records of media checks and actions taken when media is unsuitable

INSTRUMENTS AND EQUIPMENT

The checklist requirements in this section should be used in conjunction with the requirements in the All Common Checklist relating to instruments and equipment.

Inspector Instructions:

READ

- Sampling of gas monitoring records for gas-dependent equipment

CYG.33700 Gas-Dependent Equipment **Phase II**

All gas-dependent equipment (eg, incubators) is monitored and the gas concentration is recorded each day of use, with records of corrective action when values fall outside the acceptable range.

NOTE: Gas concentrations in equipment using modified atmospheres must be monitored and recorded on each day of use. External methods of monitoring (eg, Fyrite) must be performed monthly and recorded.

The two acceptable ways of recording gas levels are: 1) recording the numerical value, or 2) placing a mark on a graph that corresponds to a numerical value (either manually, or using a graphical recording device). The identity of the individual recording the gas levels must be documented (recording the initials of the individual is adequate).

The use of automated (including remote) gas monitoring systems is acceptable, providing that laboratory personnel have immediate access to the monitoring data, so that appropriate corrective action can be taken if the recorded values are out of the acceptable range. There must be records showing daily functionality of the system.

CYG.33950 ISH Slide Processing System Temperature Checks **Phase II**

Individual slide slots (or a representative sample thereof) of in situ hybridization (ISH) temperature controlled slide processing systems are checked for temperature accuracy before being placed in service and at least annually thereafter.

Evidence of Compliance:

✓ Records of equipment verification

PROCEDURES AND TESTS

DEFINITION OF TERMS

The following definitions of terms are offered as a guide to inspectors and laboratories:

ANALYZED CELLS: banded metaphase cells in which the individual chromosomes are counted and evaluated in their entirety, either at the microscope or from intact digitized images or photographic prints.

COUNTED CELLS: the number of metaphase cells evaluated for chromosome number.

KARYOGRAM: the cutout and paired chromosomes from a photograph or the arranged computer-generated image.

SCORED CELLS: cells assessed for the presence or absence of a specific cytogenetic feature, usually indicated either by a particular clinical history or by the finding of one or two abnormal cells during the course

of a study. Numbers of cells to be scored is to be defined in the laboratory policy, in compliance with specific checklist requirements.

CELL LINE/CLONE: a population of cells with the same chromosome complement. Chromosome gain and structural aberrations are clonal when the gain or structural aberration is present in two or more cells. Chromosome loss is clonal when it is present in three or more cells. (ISCN).

STEMLINE CLONE: The stemline is the most basic clone of a tumor cell population.

SIDELINE CLONE (SUBCLONE): a population of cells with one or more of the same chromosome abnormalities seen in the stemline clone, but which has additional abnormalities not found in the stemline clone.

COLONY: a discrete focus of cells that is harvested and stained while attached to the cell culture growth substrate.

Inspector Instructions:

	• Sampling of test procedures for specimen handling
	• Observe how incubator/alarm systems are connected to power and compressed gas containers • Confirm that prenatal cultures are split between at least two incubator systems

CYG.40000 Culture - Amniotic Fluid and Chorionic Villus **Phase II**

Amniotic fluid and chorionic villus cultures are split between two incubators with independent electrical circuits or emergency power systems, backup gas sources, and emergency alarms.

NOTE: If such arrangements are not feasible, a written protocol must ensure necessary growth requirements for all cultures and protection from power failures.

CYG.40100 Culture - All Specimen Types **Phase II**

Duplicate or independently established cultures are prepared for all specimen types, whenever possible.

NOTE: The intent is to provide backup cultures in the event of failures due to contamination, technical error and other problems, as well as providing the best opportunity to verify true mosaicism and maternal cell contamination.

In cancer studies, the clonal abnormality may be identified in only one culture system. The procedure manual should specify a prioritization scheme for what culture systems shall be set up when the sample volume or cellularity is insufficient to set up all cultures according to the laboratory's routine.

Evidence of Compliance:
✓ Patient records/worksheets

CYG.40200 Harvesting - Amniotic Fluid and Chorionic Villus **Phase II**

Duplicate amniotic fluid and chorionic villus flasks or dishes are harvested independently.

NOTE: To prevent failures due to contamination or technical error, all cultures from a patient should not be harvested in the same batch.

Evidence of Compliance:
- ✓ Patient records/worksheets

NUMBER OF CELLS COUNTED

Inspector Instructions:

READ

- Sampling of test procedures for cells counted
- Sampling of patient records/worksheets

CYG.40500 Counting - Stimulated Blood Samples Phase II

For stimulated blood samples (non-neoplastic disorders), at least 20 cells are counted with the exception of abbreviated studies.

NOTE 1: Under special clinical circumstances (eg, rule out sex chromosome mosaicism or mosaicism of another numerical or structural abnormality), additional cells may need to be counted. The laboratory must have written criteria for these circumstances in which additional cells should be counted.

NOTE 2: Under other special clinical circumstances, fewer than 20 cells may be counted. The laboratory must have written criteria for the circumstances under which an abbreviated study can be performed. Criteria should address the rationale for such studies, the clinical reason for referral, and the minimum number of cells counted.

Evidence of Compliance:
- ✓ Patient records/worksheets

REFERENCES

1) Wiktor AE, Bender G, Van Dyke DL. Identification of sex chromosome mosaicism: Is analysis of 20 metaphase cells sufficient? *Am J Med Genet* Part A 2009; 149A:257-259.
2) Wolff DJ, Van Dyke DL. Laboratory guideline for Turner syndrome. *Genet Med* 2010:12(1):52-55.
3) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition, Section E5.1.2.

CYG.40600 Counting - Amniotic Fluid or Chorionic Villus (In Situ) Samples Phase II

For amniotic fluid or chorionic villus (in situ) samples, at least 15 cells from 15 different colonies are counted, with cells from at least two cultures, with the exception of abbreviated studies.

NOTE: The number of cells counted should be distributed as equally as possible between independently established cultures. Under special circumstances, fewer than 15 cells may be counted. The laboratory must have written criteria for the circumstances under which an abbreviated study can be performed. Criteria should address the rationale for such studies, the clinical reason for referral, and the minimum number of cells counted.

Evidence of Compliance:
- ✓ Patient records/worksheets

REFERENCES

1) Claussen U, *et al.* Exclusion of chromosomal mosaicism in perinatal diagnosis. *Human Genetics.* 1984;67:23-28
2) Hsu LYF, *et al.* Proposed guidelines for diagnosis of chromosome mosaicism in amniocytes based on data derived from chromosome mosaicism and pseudomosaicism studies. *Prenatal Diag*. 1992;12:555-573
3) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
4) Ing PS, *et al*. Detection of mosaicism in amniotic fluid cultures: a CYTO2000 collaborative study. *Genet Med.* 1999 Mar-Apr;1(3):94-7

CYG.40700 Counting - Amniotic Fluid or Chorionic Villus Culture (Non-In Situ) Phase II

For any non-in situ amniotic fluid cell or chorionic villus culture (ie, trypsinized culture), at least 20 cells are counted, with cells from at least two cultures (this may include any combination of in situ and non-in situ cultures), with the exception of abbreviated studies.

NOTE: The number of cells counted should be distributed as equally as possible between independently established cultures. Under special clinical circumstances, fewer than 20 cells may be counted. The laboratory must have written criteria for the circumstances under which an abbreviated study can be performed. Criteria should address the rationale for such studies, the clinical reason for referral, and the minimum number of cells counted.

Evidence of Compliance:
- ✓ Patient records/worksheets

REFERENCES
1) Claussen U, *et al.* Exclusion of chromosomal mosaicism in perinatal diagnosis. *Human Genetics.* 1984;67:23-28
2) Hsu LYF, *et al.* Proposed guidelines for diagnosis of chromosome mosaicism in amniocytes based on data derived from chromosome mosaicism and pseudomosaicism studies. *Prenatal Diag.* 1992;12:555-573
3) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.

CYG.40900 Counting - Non-Neoplastic Solid Tissue Samples Phase II

For non-neoplastic solid tissue samples, at least 20 cells are counted with the exception of abbreviated studies.

NOTE: Under specific clinical circumstances, fewer than 20 cells may be counted (eg, for confirmation of an abnormal prenatal chromosome result). The laboratory must have written criteria for the circumstances under which an abbreviated study can be performed. Criteria should address the rationale for such studies, the clinical reason, and the minimum number of cells counted.

Evidence of Compliance:
- ✓ Patient records/worksheets

REFERENCES
1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.

NUMBER OF CELLS ANALYZED

Inspector Instructions:

READ

- Policy for number of cells analyzed
- Sampling of patient records/worksheets

Analyses should be performed from two independent cultures, if possible.

CYG.41100 Analysis - Non-neoplastic Samples Phase II

A minimum of five cells, with the exception of abbreviated studies, are analyzed.

NOTE 1: Under special clinical circumstances, fewer than five cells may be analyzed. Examples of such circumstances are confirmation of an abnormal prenatal chromosome result, in conjunction with chromosomal microarray analysis, or peripheral blood chromosome studies on family members to exclude a previously identified chromosome rearrangement. The laboratory must have written criteria for the circumstances under which an abbreviated study can be performed. Criteria should address the rationale for such studies, the clinical reason for referral, and the minimum number of cells analyzed.

NOTE 2: Under other special clinical circumstances (eg, rule out mosaicism of a numerical or structural abnormality, including sex chromosome mosaicism, which may involve both numerical and structural abnormalities), additional cells may need to be analyzed. The laboratory must have written criteria for the circumstances in which additional cells should be analyzed.

Evidence of Compliance:

✓ Patient records/worksheets

REFERENCES

1) Wiktor AE, Bender G, Van Dyke DL. Identification of sex chromosome mosaicism: Is analysis of 20 metaphase cells sufficient? *Am J Med Genet* Part A 2009; 149A:257-259.
2) Wolff DJ, Van Dyke DL. Laboratory guideline for Turner syndrome. *Genet Med* 2010:12(1):52-55.
3) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition, Section E5.1.2.

CYG.41500 Analysis - Neoplastic Samples — Phase II

For neoplastic disorders studied in marrow, blood or solid tumor specimens, at least 20 cells are analyzed, if possible.

NOTE: Under special clinical circumstances, fewer than 20 cells may be analyzed in lymphomas, solid tumors, and metastatic neoplasms with complex karyotypes. A sufficient number of metaphase cells (generally at least 10) should be analyzed to permit characterization of the abnormal clone(s). The circumstances under which abbreviated studies may be performed must be stated in the laboratory procedure.

Evidence of Compliance:

✓ Patient records/worksheets

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.

CYG.41550 Analysis - Neoplastic Samples — Phase I

For neoplastic bone marrow/blood/solid tumor specimens, two or more cultures are analyzed, when possible.

NOTE: For neoplastic bone marrow/blood/solid tumor specimens, cells from two or more culture conditions are analyzed, when possible.

Evidence of Compliance:

✓ Patient records/worksheets

REFERENCES

1) Lebeau M. A.C.T. cytogenetic analysis of hematological malignant diseases, In Cytogenetics laboratory manual, MJ Barch (ed), 2nd ed. Raven Press, 1991:chap 9

NUMBER OF KARYOGRAMS

Inspector Instructions:

- Sampling of test procedures for number of karyograms
- Sampling of patient records/worksheets

CYG.41600 Karyograms per Case **Phase II**

There is a minimum of two karyograms per case, with at least one karyogram per cell line, for the following specimen types.

1. **PHA-stimulated blood cells**
2. **Amniotic fluid (in situ or flasks)**
3. **Chorionic villus**
4. **Solid tissue (non-neoplastic)**

NOTE: For abbreviated studies, a minimum of one karyogram is required. Examples of such circumstances are confirmation of an abnormal prenatal chromosome result, or peripheral blood chromosome studies on family members to exclude a previously identified chromosome rearrangement. The laboratory must have written criteria for the circumstances under which an abbreviated study can be performed. Criteria should address the rationale for such studies, the clinical reason for referral, and the minimum number of karyograms.

Evidence of Compliance:

✓ Patient records/worksheets

REFERENCES
1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.

CYG.42000 Karyograms - Neoplastic Disorders **Phase II**

For neoplastic disorders studied in marrow, blood or solid tumor specimens, there are at least two karyograms per stemline, one karyogram from each sideline (subclone) and one karyogram of a normal cell (if observed in the analysis).

Evidence of Compliance:

✓ Patient records/worksheets

BAND RESOLUTION

Inspector Instructions:

- Sampling of test procedures for band resolution

	• Under what circumstances might your laboratory use a lower band resolution?
	• Examine the karyograms from cases. Determine whether the band level is sufficient to provide the rendered interpretation.

CYG.42200 Band Level - Constitutional Cases **Phase II**

The band level for constitutional cases is at least at the 400-band level of resolution.

NOTE: Constitutional cases must be banded at least at the 400-band level (International System for Human Cytogenetic Nomenclature - ISCN).

Evidence of Compliance:

✓ Karyograms with appropriate band resolution

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) McGowan-Jordan J, Hastings R, Moore S, eds; International Standing Committee on Human Cytogenomic Nomenclature. *ISCN: An International System for Human Cytogenomic Nomenclature (2020).* Basel, New York: Karger; 2020.
3) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Clinical laboratory improvement amendments of 1988; final rule. Fed Register. 2003(Jan 24): [42CFR493.1276(b)(2)]

CYG.42300 Band Level - Blood Samples **Phase II**

At least the 550-band level of resolution is achieved in appropriate blood samples.

NOTE: The 550-band level is the minimum goal of all such studies, particularly in cases of developmental delay/intellectual disability, dysmorphology and birth defects.

Evidence of Compliance:

✓ Karyograms with appropriate band resolution

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) McGowan-Jordan J, Hastings R, Moore S, eds; International Standing Committee on Human Cytogenomic Nomenclature. *ISCN: An International System for Human Cytogenomic Nomenclature (2020).* Basel, New York: Karger; 2020.

CYG.42400 Banding and Resolution **Phase II**

The quality of banding and resolution is sufficient to render the reported interpretation.

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Clinical laboratory improvement amendments of 1988; final rule. Fed Register. 2003(Jan 24): [42CFR493.1276(b)(3)]

IN SITU HYBRIDIZATION

The use of the term in situ hybridization (ISH) in this section applies to all ISH methods, including fluorescence (FISH), chromogenic (CISH), silver (SISH), and brightfield (BRISH) in situ hybridization.

Please refer to the Definition of Terms section of the All Common (COM) Checklist for definitions of analytical validation and analytical verification.

Inspector Instructions:

	• Sampling of ISH policies and procedures • Sampling of probe validation/verification records • Sampling of QC records • Sampling of patient test reports • Sampling of predictive marker assay validation, verification, and revalidation/verification studies
	• How are ISH cut-off values established? • How does your laboratory validate/verify assay performance prior to test implementation? • How do you validate/verify the most recently added predictive marker on your test menu? • What is your course of action when a probe does not produce an internal control signal?
	• Review a sampling of ISH cases and controls. Evaluate signal, background and morphology.

CYG.42700 ISH Probe Validation/Verification **Phase II**

All in situ hybridization (ISH) probes are validated/verified.

NOTE: Refer to CYG.48399 for specific validation/verification requirements for tests that provide independent predictive information (eg, HER2 predictive marker testing in breast carcinoma). Additional requirements for test method validation/verification are in the All Common Checklist.

Evidence of Compliance:

✓ Records of validation/verification of ISH probes

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.
3) Wiktor AE, Van Dyke DL, Stupca PJ, *et al*. Preclinical validation of fluorescence in situ hybridization assays for clinical practice. *Genetics in Medicine* 8:16-23, 2006
4) Weremowicz S, Sandstrom DJ, Morton CC, Miron PM. Validation of DNA probes for preimplantation genetic diagnosis (PGD) by fluorescence in situ hybridization (FISH) R1. *Prenat Diagn*. 2006 Nov;26(11):1042-50
5) Lawrence Jennings, Vivianna M. Van Deerlin, Margaret L. Gulley (2009) Recommended Principles and Practices for Validating Clinical Molecular Pathology Tests. *Archives of Pathology & Laboratory Medicine*: Vol. 133, No. 5, pp. 743-755
6) Saxe DF, Persons DL, Wolff DJ, Theil KS; Cytogenetics Resource Committee of the College of American Pathologists. Validation of fluorescence in situ hybridization using an analyte-specific reagent for detection of abnormalities involving the mixed lineage leukemia gene. *Arch Pathol Lab Med.* 2012; 136(1):47-52.

CYG.42750 ISH Assay Performance **Phase I**

There are records of in situ hybridization (ISH) performance for each assay.

NOTE: Assay performance should include monitoring hybridization efficiency, probe signal intensity and overall assay results, including controls, as applicable.

Evidence of Compliance:

✓ Records of QC monitoring of ISH assay performance at defined frequency

CYG.42900 Interphase ISH - Cut-off Value **Phase II**

For interphase in situ hybridization (ISH), the laboratory establishes a normal cut-off value for results for each probe used, when applicable.

NOTE: Refer to the All Common Checklist for specific test method validation requirements. Cut-off values are usually required when ISH testing uses locus-specific probes against nuclear DNA.

Evidence of Compliance:

✓ Records from cut-off value studies

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.

CYG.43000 ISH Scoring — Phase II

Scoring of in situ hybridization (ISH) assays, including the number of cells scored, is performed as defined in written procedure.

NOTE: For predictive marker testing, refer to CYG.47880 for requirements on reporting of the scoring method used.

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.

CYG.43200 ISH Controls — Phase II

The laboratory performs and records controls (internal and/or external) for each in situ hybridization (ISH) analysis.

NOTE: What functions as a control depends on the specific assay, signal pattern present, and sample type. For example, assays designed to detect deletions may use internal controls that include both the probe of interest and a control locus probe, both of which map to the same chromosome. In this situation, there are two internal controls, the signal for the probe of interest on the normal homolog and the control locus signals on both the normal and deleted homolog. For a dual fusion assay, the probe signals on each of the normal homologs function as internal controls. If a probe is used that does not produce an internal control signal (eg, a Y chromosome probe in a female) another sample that is known to have the probe target must be run in parallel as an external control with the patient sample. In addition, many ISH assays use an external control(s). For FDA-cleared or approved ISH assays, laboratories must follow manufacturer's instructions for quality control at minimum.

Evidence of Compliance:

✓ Records of QC results

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.
2) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.
3) Stupca P, Meyer RG, Dewald GW. Using controls for molecular cytogenetic testing in clinical practice. *J Assoc Genet Tech*. 2005;31:4-8.

CYG.43250 ISH Probe Intended Target — Phase I

A system is used to ensure that the in situ hybridization (ISH) probe used is for the intended target.

NOTE: Examples can include (but are not limited to): 1) concurrent analysis of any available metaphase cells in an interphase cell analysis; 2) inclusion of an internal or external target that results in a positive signal for each hybridization; 3) written protocols that ensure the respective probe is applied to the intended specimen.

Evidence of Compliance:

- ✓ Records confirming intended target

CYG.44666 PGD Report **Phase I**

If in situ hybridization (ISH) testing is performed on cells obtained from embryo biopsy for the purposes of preimplantation genetic diagnosis (PGD), the final report includes an interpretation with information on the limitations of single cell diagnosis in preimplantation embryos.

NOTE: Because only one or two cells may be collected for ISH chromosome analysis using blastomere biopsy, testing that can be conducted is limited and does not allow analysis of all chromosomes for abnormalities. Mosaicism can affect the results of PGD when blastomere biopsy is performed. Also, signal overlap, diffuse hybridization, poor hybridization or poor specimen quality can affect ISH results. Because of the inherent risk of inaccuracy of results, it is important to make patients aware of prenatal follow-up and testing options. The interpretation must be written to facilitate understanding by a non-geneticist.

REFERENCES

1) Munné S and Cohen J (1998) Chromosome abnormalities in human embryos. Hum Reprod Update 4, 842-855.[Abstract/Free Full Text]
2) Ruangvutilert P, Delhanty JDA, Serhal P, Simopoulou M, Rodeck CH and Harper JC. FISH analysis on day 5 post-insemination of human arrested and blastocyst stage embryos. *Prenat Diagn.* 2000; 20(7):552-60.
3) Ruangvutilert P, Delhanty JD, Rodeck CH and Harper JC. Relative efficiency of FISH on metaphase and interphase nuclei from non-mosaic trisomic or triploid fibroblast cultures. *Prenat Diagn.* 2000; 20(2):159-162.
4) Malmgren H, Sahlen S, Inzunza J, Aho M, Rosenlund B, Fridstrom M, Hovatta O, Ahrlund-Richter L, Nordenskjold M, Blennow E. Single cell CGH analysis reveals a high degree of mosaicism in human embryos from patients with balanced structural chromosome aberrations. *Mol Hum Reprod*. 2002 May;8(5):502-10

CYG.46799 Modified FDA-Cleared/Approved Assay **Phase II**

If the laboratory modifies an FDA-cleared/approved assay, the modified procedure has been validated to yield equivalent or superior performance.

Evidence of Compliance:

- ✓ Records of validation studies for modified FDA-cleared/approved assays

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2021 edition.

CYG.47866 ISH Interpretation **Phase II**

If an in situ hybridization (ISH) study requires consultation with a qualified pathologist and/or a cytogeneticist for accurate interpretation, the appropriate expert is consulted and their involvement is recorded.

PREDICTIVE MARKERS

The term predictive marker as used in this section refers to in situ hybridization (ISH) biomarkers used independent of histologic findings to identify individuals who are more likely to experience a favorable or unfavorable effect from a specific (targeted) therapy, compared to individuals with the same diagnosis lacking the biomarker. Rather than confirming a specific diagnosis, these biomarkers predict responsiveness to a specific treatment among cases of the same diagnosis.

The current CAP guidelines (CAP Guidelines) relating to predictive marker testing (eg, ASCO/CAP HER2 in breast cancer) may be found at cap.org in the Protocols and Guidelines section. The guidelines are periodically updated based on new evidence. Laboratories should review updated predictive marker guidelines and promptly implement changes for items relating to requirements in the checklists (eg, validation, fixation, scoring criteria).

If digital image analysis is used, additional requirements in the Digital Image Analysis section also apply.

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CYG.47880 Report Elements **Phase I**

For in situ hybridization (ISH) tests that provide independent predictive information, the patient report includes information on specimen processing, the probe, and the scoring method used.

NOTE: The following information must be included in the patient report:

1. *The type of specimen fixation and processing (eg, formalin-fixed paraffin-embedded sections, air-dried imprints)*
2. *The probe and, if applicable, the detection system used (ie, LSAB, polymer, proprietary kit, vendor name, etc.; information on the type of equipment used is not necessary)*
3. *Criteria used to determine a positive vs. negative result and scoring system (eg, manual or automated)*
4. *Laboratory interpretation of predictive marker testing (ISH) is reported according to the manufacturer's instructions, or when available, following the structure, format, and criteria set forth in the current CAP guidelines relating to predictive marker testing (eg, ASCO/ CAP HER2 testing in breast cancer and CAP/ASCP/ASCO HER2 in gastroesophageal carcinoma).*
5. *Limitations relating to suboptimal preanalytical factors that may impact results, such as prolonged cold ischemia time, unknown ischemia time, or over- or under-fixation.*

Evidence of Compliance:

- ✓ Report template containing all required elements **AND**
- ✓ Copies of patient reports confirming inclusion of the required elements **AND**
- ✓ Established guidelines used by the laboratory

REFERENCES

1) Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA.
2) Bartley AN, Washington MK, Ventura CB, et al. HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline from the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. *Arch Pathol Lab Med.* 2016:140(12):1345-1363.

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

CYG.48399 Validation/Verification - Predictive Marker Testing **Phase II**

Predictive marker testing by in situ hybridization is validated/verified and records of validation/verification are retained.

*NOTE: For validation of **laboratory-developed and modified FDA-cleared/approved HER2 (ERBB2) breast predictive assays**, the validation must be performed on a minimum of 40 cases (20 positive and 20 negative samples).*

*For verification of **unmodified FDA-cleared/approved HER2 (ERBB2) breast predictive assays**, the laboratory must follow the instructions provided by the manufacturer. If the instructions do not list a minimum number of samples for assay verification, the verification must be performed on a minimum of 20 positive and 20 negative tissues.*

*For **other predictive marker assays**, the laboratory director must determine the appropriate number of positive and negative samples to be used to adequately validate/verify the test. In general, laboratories should consider using higher numbers of test cases when assessing laboratory-developed tests or modified FDA-cleared/approved tests than is necessary for unmodified FDA-cleared/approved tests for the same analyte. For genetic abnormalities where positive cases are rare, the laboratory director may determine that fewer validation cases are necessary. However, the rationale for using fewer cases must be recorded.*

The validation/verification data must clearly show the degree of concordance between the assays or methods. Acceptable concordance levels should be defined by the laboratory and follow the current CAP guidelines if available.

The characteristics of the cases used for validation/verification should be similar to those seen in the laboratory's patient population (ie, core biopsy vs. open biopsy, primary vs. metastatic tumor, etc.).

Samples used for validation/verification must be handled in conformance with the guidelines in this checklist. Laboratories should use tissues that have been processed using the same fixative and methods as cases that will be tested clinically.

If changes are made to the testing methods (eg, probe, pretreatment protocol), the laboratory director is responsible for determining the extent of the performance verification or revalidation needed based on the scope of the changes in the test method.

This requirement is applicable to both new and existing assays. If review of the initial validation/ verification does not meet the current standard, it must be supplemented and brought into compliance. It is possible to do this retroactively by review and documentation of past proficiency testing challenges or by sending unstained slides from recent cases to a referral laboratory for correlation. If no records exist from the initial validation/verification, the assay must be fully revalidated/verified.

This checklist requirement applies to laboratories that perform the technical portion of the testing process.

Evidence of Compliance:

✓ Records of validation/verification data including criteria for concordance

REFERENCES

1) Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA.

CYG.48932 Fixation - HER2 (ERBB2) Breast Predictive Marker Testing Phase I

If the laboratory assesses HER2 (ERBB2) gene amplification by in situ hybridization (ISH) for breast predictive marker testing, the laboratory monitors cold ischemia time (one hour or less) and appropriate specimen fixation time.

NOTE: The CAP strongly recommends that specimens subject to HER2 (ERBB2) testing be placed in fixative within one hour of biopsy or resection (cold ischemia time) and remain in 10% neutral buffered formalin for at least six hours and up to 72 hours (formalin fixation time) at room temperature. Refer to ANP.22983 for ideal fixation parameters.

If specimens are fixed in a solution other than 10% neutral buffered formalin, the laboratory must perform a validation study showing that results are concordant with results from formalin-fixed tissues.

Laboratories testing specimens obtained from another institution must have a policy that addresses cold ischemia time and time of fixation. This information may be obtained by using the laboratory requisition form. Laboratories must communicate with the submitting service to facilitate appropriate specimen handling and proper recording of fixation parameters (refer to ANP.22983 for details).

Evidence of Compliance:

✓ Records of action taken when cold ischemia and fixation times are consistently outside of required parameters or are not available to the laboratory

REFERENCES

1) Wolff AC, Somerfield MR, Dowsett M, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* Published online June 7, 2023. doi: 10.5858/arpa.2023-0905-SA.
2) Compton CC, Robb, JA, Anderson MW, et al. Preanalytics and Precision Pathology: Pathology Practices to Ensure Molecular Integrity of Cancer Patient Biospecimens for Precision Medicine. *Arch Pathol Lab Med.* 2019;143(11):1346-63.

3) Allison KH, Hammond EH, Dowsett M, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch Pathol Lab Med.* 2020-144(5):545-63.

CYG.48950 Predictive Marker Testing - Decalcified Specimens **Phase I**

If the laboratory performs in situ hybridization (ISH) for predictive markers on decalcified specimens, the assay was validated for decalcified specimens or the results include a disclaimer noting that these assays have not been validated on decalcified specimens.

NOTE: Decalcification may adversely affect patient results. If the assay has not been validated for decalcified specimens, a disclaimer must be included in the patient report, such as, "This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the possibility of false negative results on decalcified specimens."

Use of decalcification solutions with strong acids is not recommended.

REFERENCES
1) Darvishian F et al. Impact of decalcification on receptor status in breast cancer. *The Breast Journal* 2011. 17:689-91.
2) Hanna W et al. Testing for HER2 in breast cancer: current pathology challenges faced in Canada. *Curr Oncol* 2012. 19:315-323.
3) Gertych A et al. Effects of tissue decalcification on the quantification of breast cancer biomarkers by digital image analysis. *Diag Pathol* 2014. 9:213.

DIGITAL IMAGE ANALYSIS

This section applies to laboratories using digital image analysis to evaluate specific features in a specimen or tissue section image following enhancement and processing of that image, including but not limited to morphometric analysis, ISH and cytogenetics (evaluation of metaphase chromosomes).

If predictive marker testing is performed, additional requirements in the Predictive Markers section also apply.

Inspector Instructions:

READ	• Sampling of validation and calibration policies and procedures • Sampling of validation/calibration records • Sampling of specimen analysis policies and procedures • Sampling of patient digital image analysis reports for completeness
ASK	• What is your course of action if calibration is unacceptable?
DISCOVER	• Select a representative case and follow the entire process from receipt to final reporting

CYG.49470 Preanalytic Testing Phase Validation **Phase II**

There are records showing that the preanalytic phase of the test system has been validated for each assay, including fixation and processing.

NOTE: Applicable requirements under the "Test Method Validation and Verification-Nonwaived Tests" section of the All Common Checklist must be followed.

REFERENCES
1) Hipp J, Bauer TW, Bui MM, et al. *CAP Pathology Resource Guide: Digital Pathology.* Version 7.0(2). Northfield, IL: College of American Pathologists; 2017.

CYG.49475 Calibration **Phase II**

Each instrument is calibrated in accordance with the specifications of the instrument.

REFERENCES
1) Hipp J, Bauer TW, Bui MM, et al. *CAP Pathology Resource Guide: Digital Pathology.* Version 7.0(2). Northfield, IL: College of American Pathologists; 2017.

CYG.49480 Quality Control - Digital Image Analysis **Phase II**

Control materials are run concurrently with patient specimens to ensure appropriate functionality of the digital image system.

NOTE: Controls are samples that act as surrogates for patient/client specimens. They are periodically processed like a patient/client sample to monitor the ongoing performance of the analytic process. Controls should check test performance at relevant decision points for the digital image analysis system.

For qualitative tests, a positive and a negative control may be sufficient. For quantitative or semiquantitative tests, controls at more than one level should be used.

Evidence of Compliance:
- ✓ Records of QC results

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1256(d)(3)(i)].
2) Clinical and Laboratory Standards Institute. *Statistical Quality Control for Quantitative Measurement Procedures, Principles and Definitions.* 4th ed. CLSI guideline C24. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.

CYG.49485 Area of Analysis **Phase II**

A qualified pathologist selects or confirms the appropriate areas for analysis prior to reporting results, as applicable.

NOTE: Specimens that do not represent "in situ" samples embedded in paraffin may not require pathologist review. Examples include cultured preparations and direct preparations of liquid specimens including blood, urine, pleural fluid, etc.

CYG.49490 Analysis Guidelines **Phase II**

There are written guidelines for identification of appropriate areas and cells for analysis.

NOTE: Evaluation of heterogeneous cell populations requires use of specific guidelines and procedures to ensure analysis of the appropriate areas and/or cells, particularly if there is background or nonspecific staining, or if there is cell debris, endogenous pigment, and/or artifacts of aging, sectioning or preparation.

Test results may be affected by fixation parameters, including time of fixation, type of fixative used, hemorrhage, necrosis, and autolysis of tissue.

CYG.49495 Final Report Elements - Digital Image Analysis **Phase II**

The final report includes the specimen source, name of the vendor and imaging system used, probe, and the detection method, as well as any limitations of the test result, if applicable.

GENOMIC COPY NUMBER ANALYSIS USING ARRAYS

This technology is used to assess copy number of genomic regions. Regardless of platform used (eg, CGH, SNP), reagents for hybridization and detection, or analytic components for evaluation, the laboratory is responsible for assuring that appropriate controls are performed and records retained for all aspects of analysis. This technology may also include a variety of reverse and forward hybridization formats. Reverse hybridization arrays use multiple unlabeled probes on a solid support to investigate a patient sample that carries a label, either direct (fluorescent or radioactive) or indirect (affinity labels such as biotin, digoxigenin, etc.). Another form of array involves multiple real-time amplification assays to measure multiple targets simultaneously. Controls for arrays monitor those steps carried out by the laboratory (sample preparation and labeling, hybridization and detection) and by the manufacturer (assay preparation, detection and hybridization reagents). Manufacturers also contribute to QC by producing products under good manufacturing practices, providing control material for each analyte, and by providing sequence information or confirmatory tests to resolve ambiguous results.

Inspector Instructions:

READ	• Sampling of array procedures, including nucleic acid extraction and analytical wet bench and bioinformatics processes • Sampling of array validation studies • Sampling of array performance monitoring records • Sampling of patient test reports
ASK	• How does your laboratory validate assay performance prior to test implementation? • What processes are used to monitor ongoing assay performance?
DISCOVER	• Review records of assay performance monitoring. If any problems are found during review of performance monitoring records, or when asking questions, further evaluate the laboratory's investigation and resolution.

CYG.49525 Nucleic Acid Extraction/Isolation/Purification Phase II

Nucleic acids are extracted, isolated, and purified by methods reported in the literature, by an established commercially available kit or instrument, or by a validated method developed by the laboratory.

NOTE: Extraction procedures may combine purification or isolation of nucleic acids according to the level of purity needed for downstream applications.

Evidence of Compliance:

✓ Records to support nucleic acid extraction/isolation/purification is performed by a validated method

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. *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.*

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

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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

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) Rehder CW, David KL, Hirsch B, Toriello HV, et al. American College of Medical Genetics and Genomics: standards and guidelines for documenting suspected consanguinity as an incidental finding of genomic testing. *Genet Med.* 2013; 15(2):150-2.
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.49600 DNA Copy Number Array Reports Elements Phase I

In addition to all relevant items outlined in CYG.31875, reports for DNA copy number analysis using arrays include the following elements:

1. **Platform used**
2. **Genome build used**
3. **Analysis and/or reporting strategy: Detection and/or reporting criteria for copy number and homozygosity (if applicable), including number of probes and/or size limitations**
4. **ISCN-compliant nomenclature for reported findings**
5. **References to any databases used**
6. **A statement on the need for genetic counseling when indicated**
7. **A statement recommending further testing when indicated**
8. **Clinical significance of DNA copy number changes, when applicable**

NOTE: Platform information includes, but is not limited to manufacturer, array version, number of probes, average probe spacing, SNP content, and targeted regions. Analysis strategy includes the copy number controls methodology: comparative/competitive or in silico.

Complex findings should be reported using ISCN-compliant nomenclature (eg, table format, "cth" for chromothripsis), when possible.

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) McGowan-Jordan J, Hastings R, Moore S, eds; International Standing Committee on Human Cytogenomic Nomenclature. *ISCN: An International System for Human Cytogenomic Nomenclature (2020).* Basel, New York: Karger; 2020.
5) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Clinical laboratory improvement amendments of 1988; final rule. Fed Register. 2003(Jan 24):1043-1044 [42CFR493.1276], 1047-1048 [42CFR493.1291]
6) 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.

PERSONNEL

NOTE: For purposes of CAP accreditation, the "laboratory director" is that individual who oversees all sections of the laboratory, and in whose name accreditation is granted. Specific requirements for that person are found in the Director Assessment Checklist. The section director (technical supervisor) refers to the person responsible for the medical, technical and/or scientific oversight of the cytogenetics laboratory section.

Inspector Instructions:

READ

- Records of personnel qualifications

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CYG.50000 Section Director/Technical Supervisor Qualifications - Cytogenetics **Phase II**

The cytogenetics laboratory has a qualified physician or doctoral scientist as section director/technical supervisor.

NOTE: The section director/technical supervisor of the cytogenetics laboratory must 1) be a doctor of medicine, doctor of osteopathy licensed to practice medicine in the state in which the laboratory is located; or 2) hold an earned doctoral degree in a biological science or clinical laboratory science from an accredited institution, or 3) meet the educational requirement found in CLIA regulation 42CFR493.1443(b)(3)(i)(B). More detailed information on the educational requirements can be found in the CAP Personnel Guidance Document located in e-LAB Solutions Suite on cap.org (log-in required) under Accreditation Resources - Accreditation Checklists.

In addition, the section director must have either a) successfully completed an accredited fellowship with an emphasis on clinical cytogenetics and have at least four years of training or experience, or both (including the fellowship), in genetics, two of which in clinical cytogenetics; or b) in the absence of fellowship training, have four years of training or experience, or both, in human medical genetics or pathology, two of which are in clinical cytogenetics.

If more stringent state or local regulations are in place for supervisory qualifications, including requirements for state licensure, they must be followed.

For laboratories not subject to US regulations, the section director may be either a doctor of medicine or have a doctoral degree in an appropriate science. In either case, the individual must have four or more years of fulltime general laboratory training and experience, of which at least two years were in clinical cytogenetics.

Evidence of Compliance:

- ✓ Records of section director/technical supervisor qualifications including diploma, transcript(s), primary source verification report, equivalency evaluation, board certification, or current license (if required) **AND**
- ✓ Records of work history in related field

REFERENCES

1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2023(Dec 28):[42CFR493.1449(i)].

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CYG.50180 General Supervisor Qualifications - Cytogenetics **Phase II**

The cytogenetics general supervisor has at least a bachelor's degree in a chemical, biological, clinical or medical laboratory science, or medical technology with at least two years of experience in clinical cytogenetics under a qualified director (as defined in CYG.50000).

Evidence of Compliance:

- ✓ Records of qualifications including diploma, transcript(s), primary source verification report, equivalency evaluation, board certification, or current license (if required) **AND**
- ✓ Records of work history in related field

PHYSICAL FACILITIES

Utilities should be adequate for the overall workload of the cytogenetics section, and must meet all safety requirements.

Inspector Instructions:

- Temperature and humidity are sufficient

CYG.61400 Climate Control **Phase I**

Ambient temperature and humidity are maintained within a defined and acceptable range to facilitate optimal chromosome spreading.

Evidence of Compliance:

✓ Temperature and humidity records in the slide preparation area