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ON-LINE CHECKLIST AVAILABILITY

Participants of the CAP accreditation programs may download the checklists from the CAP website (www.cap.org) by logging into e-LAB Solutions. 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.

SUMMARY OF CHECKLIST EDITION CHANGES
Cytogenetics Checklist
07/28/2015 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 listing of requirements below is from the Master version of the checklist. The customized checklist version created for on-site inspections and self-evaluations may not list all of these requirements.

NEW Checklist Requirements
None

REVISED Checklist Requirements

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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.

Note for non-US laboratories: Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist.

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

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

1. Control of pre-analytic variables (specimen collection and delivery)
2. Cytogenetic, FISH, and cytogenomic 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


CYG.20800 Culture 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 are noted.

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.

**REVISED** 04/21/2014

CYG.30066 Monthly QC Review

Quality control data are reviewed and assessed at least monthly by the laboratory director or designee.

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

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

Evidence of Compliance:

✓ Records of QC review including follow-up for outliers, trends, or omissions

CYG.30325 Reporting Error Investigation

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

REFERENCES

CYG.30550 QC Confirmation of Acceptability Phase II

Results of controls are reviewed for acceptability before reporting of patient results.

**NOTE:** Controls must be reviewed before reporting patient results. It is implicit in quality control that patient test results will not be reported when controls are unacceptable.

**Evidence of Compliance:**
✓ Written policy statement that controls are reviewed and acceptable prior to reporting patient results AND
✓ Evidence of corrective action taken when QC results are not acceptable

REFERENCES

REPORTS

Inspector Instructions:

- **READ**
  - Sampling of reporting policies and procedures
  - Sampling of patient preliminary and final reports for completeness, appropriate use of current ISCN edition and recommendations for genetic consultation or additional studies
  - Sampling of TAT statistics

- **ASK**
  - How does your laboratory maintain records of verbal/telephone preliminary reports?
  - What is your course of action when turnaround times exceed limits?

- **DISCOVER**
  - 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
11. Band resolution (required only for constitutional cases)
12. Banding methods
13. Comment on adequacy of specimen, if indicated
14. Signature of a qualified cytogeneticist as defined in CYG.50000

REFERENCES

CYG.31903 Chromosomal Analysis - Reports Phase II

Reports of cytogenetic tests are available within the time limits listed below in at least 90% of cases (the term "days" as used below means calendar days):
1. Preliminary report (verbal or written), STAT chromosomal analyses: three days
2. Final report, STAT chromosomal analyses: seven days
3. Final report, amniotic fluid and chorionic villi analyses: 14 days
4. Final report, non-neoplastic blood analyses: 28 days
5. Final report, neoplastic blood and bone marrow analyses: 21 days
6. Final report, non-neoplastic fibroblast analyses: six weeks

NOTE: The definition of a STAT test must be defined in the laboratory policy. In consultation with the requesting physician, the patient's specimen may be removed from STAT classification. The turnaround time (TAT) expectation for that specimen would then revert to "routine" for the specimen type.

Evidence of Compliance:
✓ Written policy for TAT of preliminary and final reports on STAT chromosomal analyses AND
✓ Record of TAT statistics

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

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 http://www.abmg.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

**REVISED** 04/21/2014

CYG.32700 Material Retention Phase II

Materials are retained in compliance with applicable laws and regulations and as follows:

1. Original specimens and cultures - until release of the final report
2. Processed specimens or cell pellets - two weeks after release of the final report
3. Slides
   - Permanently stained slides - three years
   - Slides stained with fluorochromes - retention time as defined in laboratory policy
   - Cytogenomic array slides - retention time as defined in laboratory policy
4. Images - maintained in hard copy (negatives or prints) and/or in retrievable digitized formats as described below
   - FISH for neoplastic disorders - 10 years. For an assay with a normal result, retain an image of at least one interphase or metaphase cell illustrating the normal probe signal pattern. For an assay with an abnormal result, retain images of at least two interphase or metaphase cells illustrating each abnormal clone.
   - FISH for non-neoplastic disorders - 20 years. For an assay with a normal result, retain an image of at least one interphase or metaphase cell illustrating the normal probe signal pattern. For an assay with an abnormal result, retain images of at least two interphase or metaphase cells illustrating each abnormal probe signal pattern.
   - All other light and non-FISH fluorescence microscopy - 20 years
5. Cytogenomic array data - retain the original scan for at least two weeks after the report is completed. Retain sufficient original data to support the final report (e.g. the feature extracted data file) for 20 years
6. Final reports for neoplastic conditions for at least 10 years and for constitutional conditions for at least 20 years. Electronic versions are acceptable.

NOTE 1: The intent is to maintain 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 continues to change, access to some digitally archived material may be lost. However, reasonable due diligence should be exercised to maintain access during the retention guidelines described above.

Evidence of Compliance:
✓ Written record and specimen retention policies

REFERENCES

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:
✓ Written media QC procedure AND
✓ 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:

- Sampling of gas monitoring records for gas-dependent equipment
- Records of biological safety cabinet certification

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

All gas-dependent equipment (e.g. 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 (e.g. 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.33900 Biological Safety Cabinet**  Phase II

The biological safety cabinet (sterile hood) is certified annually to ensure that filters are functioning properly and that airflow meets specifications.

NOTE: All cell cultures must be manipulated under conditions that ensure sterility and that protect the technologists. A sterile, biologic containment hood whose function is certified annually is required.

Evidence of Compliance:
- Maintenance schedule of BSC function checks AND
- Records of testing and certification
PROCEDURES AND TESTS

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 Specimen Handling - Cultures 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.

Evidence of Compliance:
✓ Written procedure for incubation of independent cultures using separate incubators with separate back-up systems/sources OR procedure describing an alternative method to ensure protection from power failures

CYG.40100 Specimen Handling - Cultures 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:
✓ Written procedure for the preparation of back-up cultures for all specimens

CYG.40200 Specimen Handling - Harvesting 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:
✓ Written procedure for harvesting of amniotic fluid and chorionic villus flasks/dishes

DEFINITIONS

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 cytogenic 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.

NUMBER OF CELLS COUNTED

Inspector Instructions:

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

CYG.40500 Stimulated Blood Samples

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

NOTE: 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:
✓ Written policy defining number of cells to be counted for each cytogenetic sample type AND
✓ Patient records/worksheets

REFERENCES

CYG.40600 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:
✓ Written policy defining number of cells to be counted for each cytogenetic sample type AND
✓ Patient records/worksheets

REFERENCES

CYG.40700 Amniotic Fluid or Chorionic Villus Culture (Non-in-situ) Phase II

For any non-in-situ amniotic fluid cell or chorionic villus culture (i.e. 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:
✓ Written policy defining number of cells to be counted for each cytogenetic sample type AND
✓ Patient records/worksheets

REFERENCES

CYG.40900 Solid Tissue Samples Phase II

For solid tissue samples (non-neoplastic), at least 20 cells are counted with the exception of abbreviated studies.
NOTE: Under specific clinical circumstances, fewer than 20 cells may be counted (e.g. 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:
✓ Written policy defining number of cells to be counted for each cytogenetic sample type AND
✓ Patient records/worksheets

REFERENCES

NUMBER OF CELLS ANALYZED

Inspector Instructions:
- 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

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

NOTE: 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 cytogenomic 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.

Evidence of Compliance:
✓ Written policy defining number of cells to be counted and analyzed for each cytogenetic sample type AND
✓ Patient records/worksheets

CYG.41500 Analysis - Neoplastic Disorders

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:
✓ Written policy defining number of cells to be analyzed for each cytogenetic sample type AND
CYG.41550  Culture - Neoplastic Disorders  

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:
✓ Written policy defining number of culture conditions to be analyzed for each cytogenetic sample type AND  
✓ Patient records/worksheets

REFERENCES

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:
✓ Written policy defining number of karyograms per case for each specimen type

REFERENCES
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:
✓ Written policy defining number of karyograms for neoplastic disorder studies in bone marrow/blood/solid tissue

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

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:
✓ Written policy on band resolution

REFERENCES
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

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 mental retardation, dysmorphology and birth defects.

Evidence of Compliance:
✓ Written policy on band resolution

REFERENCES
BANDING AND RESOLUTION

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

REFERENCES


2) ISCN: An International System for Human Cytogenetic Nomenclature, Shaffer L.G., Tommerup N. (eds); S. Karger, Basel

FLUORESCENCE IN SITU HYBRIDIZATION

Inspector Instructions:

- Sampling of FISH procedures
- Sampling of probe validation studies
- Sampling of QC records
- Sampling of patient test reports performed with Class I ASRs including appropriate disclaimer

- How are FISH cut-off values established?
- How does your laboratory validate assay performance prior to test implementation?

- Review a sampling of FISH cases and controls. Evaluate signal, background and morphology.

FISH Probe Validation

There are policies, procedures, and records of validation of all FISH (fluorescence in-situ hybridization) probes.

NOTE: Refer to the All Common Checklist for specific test method validation requirements.

REFERENCES


QC - FISH Assay

There is ongoing monitoring of FISH assay performance.

NOTE: Assay performance monitoring should include monitoring hybridization efficiency, probe signal intensity and overall assay results, including controls.
Evidence of Compliance:
✓ Written procedure defining acceptance criteria for FISH assay performance AND
✓ Records of QC monitoring of FISH assay performance at defined frequency

CYG.42775 New Reagent Lot - FISH Probes

Each lot of FISH probe(s) is checked for acceptable performance.

Evidence of Compliance:
✓ Written procedure for the verification of new lot of FISH probes prior to use AND
✓ Records of verification

CYG.42900 Interphase FISH - Cut-off Value

For interphase FISH, the laboratory establishes a normal cut-off value for results for each probe used.

Evidence of Compliance:
✓ Written procedure for establishing normal cut-off values AND
✓ Records from cut-off value studies

REFERENCES

CYG.43000 FISH Scoring

There are written procedures for scoring FISH results, including the number of cells scored, and all analyses are scored according to these procedures.

REFERENCES

**REVISED** 07/28/2015

CYG.43200 FISH Controls

A hybridization control, internal or external, is used for each FISH analysis.

NOTE: When normal chromosome targets are expected to be present within a sample, an internal control for that target should be used during each hybridization (i.e. a locus specific probe at a different site on the same chromosome and/or a normal locus on the abnormal homolog). If a probe is used that does not produce an internal control signal (e.g. a Y chromosome probe in a female), another sample that is known to have the probe target must be run in parallel with the patient sample.

If an internal quality control process (e.g. electronic/procedural/built-in) is used instead of an external control material to meet daily quality control requirements, the laboratory must have an individualized quality control plan (IQCP) approved by the laboratory director to address the use of the alternative control system. Please refer to the Individualized Quality Control Plan section of the All Common Checklist for the eligibility of tests for IQCP and requirements for implementation and ongoing monitoring of an IQCP.

Evidence of Compliance:
✓ Written policy defining use of control loci with each FISH analysis AND
✓ Records of QC results
REFERENCES

CYG.43250 FISH Probe Intended Target

Phase I

There is a system in place to ensure that the FISH 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:
✓ Written policy defining the system for ensuring use of the appropriate FISH probe AND
✓ Records confirming intended target

CYG.43600 ASR Disclaimer

Phase II

If FISH testing is performed using Class I analyte-specific reagents (ASRs) obtained or purchased from an outside vendor, the patient report includes the disclaimer statement required by federal regulations.

NOTE: ASRs are antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens.

An ASR is the active ingredient of a laboratory-developed test system.

This checklist requirement concerns Class I ASRs. Class I ASRs are not subject to preclearance by the US Food and Drug Administration (FDA) or to special controls by FDA. Most ASRs are Class I. Exceptions include those used by blood banks to screen for infectious diseases (Class II or III), or used to diagnose certain contagious diseases (e.g. HIV infection and tuberculosis) (class III).

If the laboratory performs patient testing using Class I ASRs, federal regulations require that the following disclaimer accompany the test result on the patient report: “This test was developed and its performance characteristics determined by (laboratory name). It has not been cleared or approved by the US Food and Drug Administration.”

The CAP recommends additional language, such as “FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing.”

The disclaimer is not required for tests using reagents that are sold in kit form with other materials or an instrument, nor reagents sold with instructions for use.

The laboratory must establish or verify the performance characteristics of tests using Class I ASRs in accordance with the Method Performance Specifications section of the All Common Checklist.

REFERENCES

**PGD Report**  
Phase I

If FISH testing is performed on cells obtained from embryo biopsy for the purposes of preimplantation genetic diagnosis (PGD), the final report must include 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 FISH 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 FISH 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**

3) Ruangvutilert P, Delhanty JD, Rodeck CH and Harper JC (2000b) Relative efficiency of FISH on metaphase and interphase nuclei from non-mosaic trisomic or triploid fibroblast cultures. Prenat Diagn 20, 159-162. [CrossRef][ISI][Medline]

**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**


**FISH Interpretation**  
Phase II

If a FISH study requires a histopathological/cytological interpretation, there is documentation that a qualified pathologist is involved.

**FISH Interpretation**  
Phase II

If a FISH study requires cytogenetics interpretation, there is documentation that a qualified cytogeneticist is involved.

**HER2 (ERBB2) Assay Validation**  
Phase II

If the laboratory tests for HER2 (ERBB2) gene amplification by fluorescence *in situ* hybridization (e.g. FISH, *CISH, *SISH), the laboratory has records of validation for the assay(s).

**NOTE:** This requirement applies to both new and existing assays. Initial test validation must be performed on a minimum of 20 positive and 20 negative samples for FDA-cleared/approved assays; or 40 positive and 40 negative samples for laboratory-developed tests (LDTs). Equivocal samples need not be used for validation studies. If the initial validation of existing assays...
does not meet the current standard, it must be supplemented and brought into compliance. It is permissible to do this retroactively by review of performance on past proficiency testing challenges or by sending unstained slides from recent cases to a reference laboratory for correlation. If there are no records of the initial validation, the assay must be fully revalidated and records retained.

Validation may be performed by comparing the results of testing with a validated alternative method (i.e. IHC vs. FISH) either in the same laboratory or another laboratory, or with the same validated method performed in another laboratory; validation testing must be done using the same set of cases in both labs. The validation records should identify the comparative test method(s) used.

The validation data should clearly show the degree of concordance between methods, e.g. for IHC: 0, 1+, 3+; for FISH, CISH, SISH: positive, negative, as defined by the cut-offs listed in the latest version of the CAP/ASCO guideline.

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

Samples used for validation must be handled in conformance with the guidelines in this checklist. If specimens are fixed in a medium other than 10% neutral buffered formalin, the validation study must show that results are concordant with results from formalin-fixed tissues.

If significant changes are made in testing methods (e.g. probe, pretreatment protocol), revalidation is required.

This checklist requirement applies to laboratories that perform the technical testing of specimens for HER2 (ERBB2) amplification. Patient specimens should be fixed in the same manner as the specimens used for the validation study(ies).

(*CISH = chromogenic in-situ hybridization; *SISH = silver-enhanced in-situ hybridization)

Evidence of Compliance:
✓ Records of validation data including criteria for concordance

**REVISED** 04/21/2014
CYG.48932 HER2 (ERBB2) by FISH - Fixation Phase I

If the laboratory assesses HER2 (ERBB2) gene amplification by fluorescence in situ hybridization (FISH), there is a written procedure to ensure appropriate specimen fixation time.

NOTE: Specimens subject to HER2 (ERBB2) testing should be fixed in 10% neutral buffered formalin for at least six hours and up to 72 hours. The volume of formalin should be at least 10 times the volume of the specimen. Decalcification solutions with strong acids should not be used.

Laboratories must communicate the following fixation guidelines to clinical services:

1. Specimens should be immersed in fixative within one hour of the biopsy or resection
2. If delivery of a resection specimen to the pathology department is delayed (e.g. specimens from remote sites), the tumor should be bisected prior to immersion in fixative. In such cases, it is important that the surgeon ensure that the identity of the resection margins is retained in the bisected specimen; alternatively, the margins may be separately submitted.
3. The time of removal of the tissue and the time of immersion of the tissue in fixative should be recorded and submitted to the laboratory

Communication may be through memoranda, website, phone, face-to-face meetings, or other means. The laboratory should consider monitoring compliance and contacting clients when these guidelines are not met.
If specimens are fixed in a medium 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 should have a policy that addresses time of fixation. Information on time of fixation may be obtained by appropriate requirements on the laboratory’s requisition form.

Reports should qualify any negative results for specimens not meeting the above guidelines.

**REVISED** 07/28/2015  
CYG.49465 HER2 (ERBB2) by FISH/ISH - Scoring  
Phase II

If the laboratory interprets HER2 (ERBB2) gene amplification by in situ hybridization (FISH, CISH, SISH), results are reported using either the ASCO/CAP scoring criteria or the manufacturer’s instructions.

NOTE: The table below contains the ASCO/CAP scoring criteria used to determine HER2 (ERBB2) gene status by in-situ hybridization.

Careful attention should be paid to the recommended exclusion criteria for performing or interpreting in situ hybridization for HER2 (ERBB2) (e.g. signal obscured by background; for FISH, difficulty in defining areas of invasive carcinoma under UV light).

Variable ISH positivity (heterogeneity) must also be considered when analyzing ISH studies. ISH slides are scanned at low power prior to counting to determine if there is a discrete population of amplified cells representing more than 10% of the invasive tumor cells in that area; such cases are reported as HER2 (ERBB2) positive (amplified).

For FDA-cleared/approved test systems that use different scoring criteria, the manufacturer’s instructions may be followed.

<table>
<thead>
<tr>
<th>Method</th>
<th>Result</th>
<th>Ratios of HER2 (ERBB2) to CEP17**</th>
<th>Average HER2 (ERBB2) Copy Number (Signals/Cell)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 (ERBB2) ISH - Test systems with internal control probe</td>
<td>Positive (amplified)</td>
<td>&gt;2.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;2</td>
<td>≥6.0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>&lt;2.0</td>
<td>&lt;4.0</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>&lt;2.0</td>
<td>4.0 and &lt;6.0</td>
</tr>
<tr>
<td>HER2 (ERBB2) ISH - Test systems without an internal control probe</td>
<td>Positive (amplified)</td>
<td>N/A</td>
<td>≥6.0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>N/A</td>
<td>&lt;4.0</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>N/A</td>
<td>4.0 and &lt;6.0</td>
</tr>
</tbody>
</table>

**Criteria in both columns must be met for tests with internal reference probes. For example, for a result to be negative, the ratio must be <2.0 and the average copy number must be <4.0.

REFERENCES
CYTOGENOMIC MICROARRAY ANALYSIS

This technology is used to assess copy number of genomic regions. Regardless of platform used (e.g. 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 maintained for all aspects of analysis.

Inspector Instructions:

- Sampling of microarray procedures
- Sampling of microarray validation studies
- Sampling of QC records
- Sampling of patient test reports

- How does your laboratory validate assay performance prior to test implementation?

- Review records of continuous quality monitoring. If any problems are found during review of quality monitoring, or when asking questions, further evaluate the laboratory’s investigation and resolution.

CYG.49500 Copy Number Phase II

There are policies, procedures, and records of validation of microarray genomic copy number assessment.

NOTE: Refer to the All Common Checklist for specific test method validation requirements.

REFERENCES


CYG.49525 Nucleic Acid Extraction/Purification Phase I

Nucleic acids are extracted and purified by methods reported in the literature, by an established commercially available kit or instrument, or there is validation of a method developed in-house.

Evidence of Compliance:
✓ Records to support nucleic acid extraction/purification is performed by a validated method

REFERENCES

Cytogenetics Checklist
07.28.2015

CYG.49550  QC - Assay Components  Phase II

The quality of critical assay components (i.e. arrays, labeling reagents) is verified prior to reporting results.

Evidence of Compliance:
✓ Written procedure for verifying the quality of critical assay components AND
✓ QC records including actions taken should the component(s) fail to meet quality criteria

REFERENCES

CYG.49575  Assay Performance Monitoring  Phase I

There are records of continuous quality monitoring of assay performance.

NOTE: This includes hardware and analytical software.

REFERENCES

CYG.49600  Reports  Phase I

In addition to all relevant items outlined in CYG.31875, the report includes:

1. Platform used
2. Genome build used
3. Methods
4. Resolution
5. Current ISCN-compliant nomenclature
6. References to any databases used
7. A statement on the need for genetic counseling when indicated
8. A statement recommending further testing when indicated
9. All disclaimers required by federal guidelines
10. Clinical significance of DNA copy number changes

NOTE: Resolution includes but is not limited to: the number of probes on the array, approximate distance between probes and threshold levels for determining a copy number change.

REFERENCES
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 Team Leader Assessment of Director & Quality 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:

- Records of personnel qualifications

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CYG.50000  Section Director/Technical Supervisor Qualifications  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, or doctor of podiatric medicine licensed to practice medicine, osteopathy, or podiatry in the state in which the laboratory is located, and have four years of training or experience, or both, in genetics, two of which have been in clinical cytogenetics; or 2) hold an earned doctoral degree in a biological science, including biochemistry, or clinical laboratory science from an accredited institution, and have four years of training or experience, or both, in genetics, two of which have been in clinical cytogenetics.

For laboratories not subject to US regulations, the person in charge of technical operations 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 degree or transcript, current license (if required) and work history in related field

REFERENCES

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**REVISED** 04/21/2014

CYG.50180  Supervisor Qualifications  Phase II

The cytogenetics laboratory supervisor in charge of bench testing has at least a bachelor’s degree in a chemical, physical, biological, or clinical laboratory science or medical technology and at least two years experience in clinical cytogenetics under a qualified director.

Evidence of Compliance:  
✓ Records of laboratory supervisor qualifications including degree or transcript and at least two years experience in clinical cytogenetics
Personnel performing the technical work of clinical cytogenetics have at least one year of training and/or experience in clinical cytogenetics and qualify as high complexity testing personnel with a minimum of the following:

1. Bachelor’s degree in a chemical, physical, biological or clinical laboratory science or medical technology; or
2. Associate degree in a chemical or biological science or medical laboratory technology from an accredited institution, or equivalent laboratory training and experience meeting the requirements defined in the CLIA regulation 42CFR493.1489. The qualifications to perform high complexity testing can be assessed using the following link: CAP Personnel Requirements by Testing Complexity.

**NOTE:** Technologists must work under the supervision of a qualified section director and/or supervisor and have training and/or experience in cytogenetic procedures. Persons gaining experience in the field must work under the direct supervision of persons who are qualified.

**Evidence of Compliance:**
✓ Records of qualifications including degree or transcript, current laboratory personnel license (if required), and work history in related field

**REFERENCES**
2) *Fed Register*, 10/1/03, section 493.1489, p.1070-1071

**PHYSICAL FACILITIES**

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

**Inspector Instructions:**

<table>
<thead>
<tr>
<th>OBSERVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Temperature and humidity are sufficient</td>
</tr>
</tbody>
</table>

**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