




Inspector Instructions:

	<ul style="list-style-type: none"> • 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
	<ul style="list-style-type: none"> • 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?
	<ul style="list-style-type: none"> • 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, Viviana 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.