



Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of ISH policies and procedures • Sampling of probe validation records • Sampling of QC records • Sampling of patient test reports
	<ul style="list-style-type: none"> • How are ISH cut-off values established? • How does your laboratory validate assay performance prior to test implementation? • What is your course of action when a probe does not produce an internal control signal?

ANP.22956 ISH Probe Validation/Verification

Phase II

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

NOTE: Refer to ANP.22978 for specific validation/verification requirements for tests that provide independent predictive information (eg, HER2 in breast carcinoma). Additional requirements for test method validation/verification are in the All Common Checklist.

Evidence of Compliance:

- ✓ Records of ISH probe validation/verification

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) 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
- 4) 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.
- 5) 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.
- 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; 138(1):47-52.

ANP.22957 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. *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline*. 2nd ed. CLSI Document MM07-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.

ANP.22959 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.