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.