should not be more than 10% smaller than the target amplicon and the use of a smaller internal control should be justified.

BAP.06630 Melting Temperature **Phase I**

For tests that generate a result based on a T_m, appropriately narrow temperature ranges (+/- 2.5°C) are defined and recorded each day of use.

IN SITU HYBRIDIZATION (ISH)

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 in 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
	• How are ISH cut-off values established? • How does your laboratory validate/verify assay performance prior to test implementation? • What is your course of action when a probe does not produce an internal control signal?

BAP.06710 ISH Probe Validation/Verification **Phase II**

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

NOTE: 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.

BAP.06720 Interphase ISH - Cut-off Value **Phase II**

For interphase in situ hybridization (ISH), the biorepository 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/verification 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.

BAP.06740 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

BAP.06750 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 may not be 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

BAP.06760 ISH Scoring **Phase II**

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

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.

BAP.06770 ISH Controls **Phase II**

The biorepository 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