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