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

control(s). For FDA-cleared or approved ISH assays, biorepositories 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.

*****REVISED** 12/26/2024***

BAP.06780 Image and Slide Retention - ISH **Phase II**

Photographic or digitized images or permanent slides are retained of all in situ hybridization (ISH) assays for an appropriate period.

NOTE: Images or permanent slides of ISH assays for neoplastic disorders must be retained for 10 years; images or permanent slides of ISH assays for constitutional disorders must be retained for 20 years. For an ISH assay with a normal result, retain an image of at least one cell illustrating the normal probe signal pattern. For an ISH assay with an abnormal result, retain images of at least two cells illustrating each relevant abnormal probe signal pattern.

There is no retention requirement for retaining images of slide preparations when the source slides remain readable for the required retention period. If slides are expected to become unreadable before the end of the required retention period (for example, FISH slides), then images of the slides must be retained.

If representative images of chromosome ISH slides are retained, those with a normal result must include an image of at least one cell illustrating the normal probe signal pattern, and those with an abnormal result must include images of at least two cells illustrating each relevant abnormal probe signal pattern.

REFERENCES

1) American College of Medical Genetics, Standards and guidelines for clinical genetics laboratories, 2021 edition.

BAP.06790 ISH Interpretation **Phase II**

If an in situ hybridization (ISH) study requires consultation with a qualified pathologist and/or a cytogeneticist for an accurate interpretation, the appropriate expert is consulted and their involvement is recorded.

REFERENCES

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

INSTRUMENTS AND EQUIPMENT

A variety of instruments and equipment are used to support the biorepository. All instruments and equipment should be properly operated, maintained, serviced, and monitored to ensure proper performance. The procedures and schedules for instrument maintenance and function checks must be as thorough and as frequent as specified by the manufacturer. Examples of equipment include, but are not limited to centrifuges, microscopes, incubators, heat blocks, microwaves, etc.

The checklist requirements in this section should be used in conjunction with the requirements in the All Common Checklist relating to instruments and equipment.