TARGET AMPLIFICATION/POLYMERASE CHAIN REACTION (PCR)

Inspector Instructions:

	• Sampling of amplification/PCR policies and procedures
	• Physical containment practices (frequent glove change, separate manipulation of pre- and post-specimens, dedicated pipettes)
	• How does your laboratory distinguish a true negative from a false negative result?

BAP.06610 Carryover - Nucleic Acid Amplification **Phase II**

Nucleic acid amplification procedures (eg, PCR) use appropriate physical containment and procedural controls to minimize carryover (false positive results).

NOTE: This item is primarily directed at ensuring adequate physical separation of pre- and post-amplification samples to avoid amplicon contamination. The extreme sensitivity of amplification systems requires that the laboratory take special precautions. For example, pre- and post-amplification samples should be manipulated in physically separate areas; gloves must be worn and frequently changed during processing; dedicated pipettes (positive displacement type or with aerosol barrier tips) must be used; and manipulations must minimize aerosolization. Enzymatic destruction of amplification products is often helpful, as is real-time measurement of products to avoid manual manipulation of amplification products.

REFERENCES
1) Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989;339:237-238
2) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

BAP.06620 Internal Controls - Nucleic Acid Amplification **Phase II**

In nucleic acid amplification procedures, internal controls are run to detect a false negative reaction secondary to extraction failure or the presence of an inhibitor, when appropriate.

NOTE: The biorepository should be able to distinguish a true negative result from a false negative due to failure of extraction or amplification. Demonstration that another sequence can be successfully amplified in the same specimen should be sufficient to resolve this issue. For quantitative amplification assays, the effect of partial inhibition must also be addressed.

The internal control should not be smaller than the target amplicon. There are some rare exceptions to this rule due to sequence length and design. In this situation the internal control

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.