# 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*