BAP.04800 Nucleic Acid Quantity and Quality Determination **Phase II**

The quantity and quality of nucleic acids are determined, when appropriate.

NOTE: The quantity and quality of nucleic acids (DNA or RNA) must be measured prior to use in a procedure whose success depends on accurately determining the quantity, concentration, integrity, and/or purity of the nucleic acids. Techniques commonly used to assess nucleic acid quantity and/or quality include electrophoresis, UV/VIS spectrophotometry, and fluorescence spectroscopy.

Standard measure for DNA purity is A260/280 ratio of 1.6 to 2.0. Values less than 1.6 are indicative of protein contamination and values of >2.0 are indicative of RNA contamination, RNA should have A260/280 ratio of greater than 2.0. Analytical measures of nucleic acids include, but are not limited to: A260/280 spectrophotometric ratio, RNA-specific measures, double-stranded DNA (dsDNA), or integrity by agarose gel electrophoresis. RNA integrity assessments should be determined if such a quality indicator would exclude samples from specific downstream methodologies.

RNA in specimens is highly labile because RNase is ubiquitous and difficult to inhibit. For human RNA targets, RNA quality must be assessed. However, depending on the target, it may not be necessary for all specimens to be assessed for RNA quality. RNA quality is not assessed, for example, for many types of viral RNA targets; however, the false negative rate must be recorded.

Evidence of Compliance:

✓ Records of nucleic acid quantity and/or quality determination

REFERENCES

1) Tsui NBY, Ng EKO, Lo YMD. Stability of Endogenous and Added RNA in Blood Specimens, Serum and Plasma. *Clin Chem* 48:1647-1653, 2002
2) Farrell R. Gel electrophoresis based assessment of cellular RNA quality may also be used (RNA Isolation Strategies). In: RNA Methodologies: A Laboratory Guide for Isolation and Characterization. Academic Press, 1998
3) Clinical and Laboratory Standards Institute. *Diagnostic Nucleic Acid Microarrays: Approved Guideline*; CLSI Document MM12-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2006.

BAP.04900 Human/Non-Human DNA **Phase I**

When the downstream application requires an estimation of the ratio of human versus non-human genomic DNA in the specimen, the human/non-human DNA quantity is measured.

BAP.05100 Neoplastic Cell Content **Phase II**

For paraffin-embedded tumor specimens from which DNA or RNA is extracted for analysis (eg, microsatellite instability, *KRAS* or *KIT* analysis), there is a record of histological assessment of neoplastic cell content.

NOTE: In addition to confirming the presence or absence of neoplastic cells by a qualified pathologist, it may be necessary for some assays to estimate and consider neoplastic cellularity in relation to the lower limit of detection of the assay.

A corresponding H&E section from the same tissue block used for nucleic acid extraction may be used to assess sample adequacy. Alternatively, a stain such as toluidine blue may be used to stain the slide that is being used for nucleic acid extraction. When assessment of sample adequacy is performed outside of the testing laboratory, a record of such assessment must accompany the sample.

This requirement is applicable to all molecular methods for the detection of sequence variants (eg, Sanger sequencing, NGS, PCR).

BAP.05125 Ribonuclease-Free Conditions **Phase I**

Ribonuclease-free conditions are maintained for all assays that detect RNA or use an RNA probe.

NOTE: RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA or RNA probes, special precautions are needed.

Evidence of Compliance:

- ✓ Records that RNase-free conditions are maintained (ie, wipe test in event of contamination incident) with corrective action if conditions are not met

REFERENCES
1) Gulley ML, *et al*. Guidelines for interpreting EBER *in situ* hybridization and LMPI immunohistochemical tests for detecting Epstein-Barr virus in Hodgkin lymphoma. *Am J Clin Pathol. 2002*;117:259-267

BAP.05200 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 special precautions are taken. 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) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A. Clinical and Laboratory Standards Institute, Wayne, PA, 2011.

BAP.05300 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 facility 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.

Evidence of Compliance:

- ✓ Records of assay validation and monitoring statistics for test result trends

CELL FRACTIONATION

The purpose of cell fractionation is to obtain a pure sample of part of the original whole, such as mitochondria, plasma membranes, DNA, RNA, soluble proteins or even specific macromolecules. There are many procedures defined for each target material, such as tissue, plant cells, animal cells, cell membranes and molecular