HSC.35292 Loading Analytical Gels **Phase II**

Standard amounts of nucleic acid are loaded on analytical gels, when possible.

HSC.35479 Gel Images **Phase II**

Gel images are of sufficient resolution and quality (low background, clear signal, absence of bubbles, etc.) to permit the reported interpretation.

HSC.35666 Specimen Handling **Phase II**

The laboratory uses appropriate processes to prevent specimen loss, alteration, or contamination.

HSC.36040 Carryover - Enzymatic Amplification **Phase II**

Enzymatic 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; manipulations must minimize aerosolization; following complete reagent addition to the reaction tubes, the patient samples should be added one at a time. The best way to avoid cross-contamination is to use the following order of preparation within an amplification run: actual samples, followed by positive controls, followed by negative controls.

REFERENCES
1) Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989;339:237-238
2) Clinical and Laboratory Standards Institute. *Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline.* CLSI document MM19-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.

HSC.36414 Electrophoretic Gel Interpretation **Phase II**

Electrophoretic gels are interpreted independently by at least two qualified readers using an objective method.

Evidence of Compliance:
- ✓ Patient testing records or worksheets

HSC.36601 End-Point Amplification QC **Phase II**

For end-point amplification assays such as sequence-specific priming, adequate internal controls are used, and criteria defined for a positive reaction.

HSC.36788 Daily Controls **Phase II**

For qualitative and quantitative tests, positive and negative controls are included for each assay, where appropriate, in every run, and as specified in the manufacturer's instructions (as applicable) and laboratory procedure.

Evidence of Compliance:
- ✓ Records of QC results, including external and internal control processes **AND**
- ✓ Manufacturer's product insert or manual, as applicable

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline.* CLSI document MM19-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
2) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR1256(d)(3)(i) and (ii)].

HSC.36795 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 laboratory 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.

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

REFERENCES
1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24): [42CFR493.1256(d)(3)(iv)(v)].

HSC.36975 DNA Contamination — Phase II

There is a process to detect and control for DNA contamination.

NOTE: Contamination must be monitored in different areas by wipe tests using the regular detection for testing. There are records of the results of monitoring and corrective action taken when contamination is detected.

HSC.37162 Molecular Weight Markers — Phase II

Known molecular weight markers that span the range of expected bands are used for each electrophoretic run.

Evidence of Compliance:
- ✓ Records of appropriate markers with each run

HSC.37349 Amplification Quality — Phase II

For hybridization techniques, there are records that the different components and steps are monitored and acceptable, including the amount and integrity of amplified product and the signal intensity produced by each probe.

REFERENCES
1) Clark JR, Scott SD, Jack AL, et al. Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of short tandem repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *Br J Haematol.* 2015;168(1):26-37.

HSC.37536 Pre-Analytic Testing Requirements — Phase II

The conditions (temperature, salt concentration, probe concentration, etc.) for pre-hybridization, hybridization, and solid-phase support systems are optimized to consistently produce accurate results.

HSC.37723 Probe Labeling Validation — Phase II