Standard Operating Procedure (SOP) for the Analytical Phase of Generating Results for 1,25-Dihydroxyvitamin D, Serum

1. PURPOSE

The purpose of this procedure is to outline the steps required for the analysis and generation of results for 1,25-Dihydroxyvitamin D in serum samples. This SOP ensures accurate and reliable results following specific protocols and guidelines.

Responsibility:

- It is the responsibility of designated laboratory personnel to follow the steps outlined in this procedure to ensure accuracy and compliance with laboratory standards.
- All staff must be diligent in identifying any issues that may affect test results and must report these to a supervisor immediately.

1. SPECIMEN REQUIREMENTS AND STABILITY

Preferred Specimen:

- 2.0 mL of serum, obtained from blood collected in a plain red-top or serum separator tube (SST).

Unacceptable Specimens:

- Plasma samples are not acceptable.
- Hemolyzed, lipemic, or grossly contaminated samples should be rejected.

Storage:

- Specimens should be stored at 2-8°C and analyzed within 7 days if kept refrigerated.
- If storage exceeds 7 days, specimens should be frozen at -20°C for up to 6 months.
- Repeated freeze-thaw cycles should be avoided.

1. EQUIPMENT, REAGENTS, AND SUPPLIES

- Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) or similar analytical instrument.
- Calibration standards and quality control materials specific for 1,25-Dihydroxyvitamin D.
- Mobile phase solvents and reagents (e.g., methanol, acetonitrile, water, formic acid).
- Sample preparation materials: solid-phase extraction (SPE) cartridges, centrifuge tubes, pipettes.
- Personal protective equipment (PPE).

1. PROCEDURE

A. Sample Preparation:

1. Allow serum samples to reach room temperature if previously refrigerated or frozen.
2. Vortex each sample briefly to ensure homogeneity.
3. Perform protein precipitation by adding an appropriate solvent (e.g., methanol) in a specific ratio to the serum sample.
4. Centrifuge at 10,000 rpm for 10 minutes to pellet precipitated proteins.
5. Carefully transfer the supernatant to a new centrifuge tube.
6. Perform solid-phase extraction (SPE) using cartridges to clean and concentrate the 1,25-Dihydroxyvitamin D from the serum. Follow manufacturer instructions for SPE protocol.
7. Elute the analyte with an appropriate solvent into vials suitable for LC-MS/MS analysis.

B. Instrument Calibration:

1. Prepare calibration standards covering the expected range of 1,25-Dihydroxyvitamin D concentrations.
2. Run calibration standards on the LC-MS/MS according to instrument-specific protocols.
3. Establish a calibration curve by plotting the response of the instrument against the known concentrations of the standards.

C. Quality Control:

1. Run quality control samples alongside patient samples at both low and high concentration ranges to ensure the accuracy of the analysis.
2. Verify that QC results fall within the acceptable range before proceeding with patient sample analysis.
3. Document all QC results and any corrective actions taken if QC results fall outside the acceptable range.

D. Sample Analysis:

1. Inject prepared serum sample aliquots into the LC-MS/MS instrument.
2. Ensure proper sequence and inclusion of blank samples, calibration standards, and quality controls within the sample run.
3. Analyze the chromatograms and identify peaks corresponding to 1,25-Dihydroxyvitamin D.
4. Quantify the concentration of 1,25-Dihydroxyvitamin D in serum samples using the established calibration curve.
5. REPORTING RESULTS

- Results are automatically transmitted to the Laboratory Information System (LIS) for review.
- Technologists must review and verify the results before final reporting.

- Document any issues related to sample integrity or analysis, and take appropriate corrective actions as needed.
- Report results according to site-specific guidelines, including appropriate reference intervals and conditions for specific patient populations.

1. REFERENCES

- Manufacturer's operator manual for LC-MS/MS
- Relevant scientific literature on 1,25-Dihydroxyvitamin D analysis
- Laboratory specific quality control and reporting procedures

This SOP must be reviewed and updated periodically to incorporate changes in laboratory protocols, equipment, regulations, and advances in testing methodologies.

Approved by: _____ Date: _____

This document is for internal use only and must be adhered to by trained laboratory personnel.