Standard Operating Procedure (SOP) for the Analytical Phase of Generating Results for 2,3-Dinor 11 Beta-Prostaglandin F2 Alpha, 24 Hour, Urine

1. PURPOSE

To outline the procedure for the accurate and precise analysis of 2,3-Dinor 11 Beta-Prostaglandin F2 Alpha (2,3-dinor-11β-PGF2α) in 24-hour urine samples. This SOP ensures that the analysis is standardized and the results are reliable.

Responsibility:

It is the responsibility of designated laboratory personnel to follow this procedure for the analysis of 2,3-dinor-11β-PGF2α in urine samples. It is the responsibility of the supervisor to ensure that staff are trained and compliant with this SOP.

1. SCOPE

This procedure applies to the analysis of 2,3-dinor-11β-PGF2α in 24-hour urine specimens received in the clinical laboratory.

1. SAFETY

All personnel must adhere to laboratory safety guidelines, including the use of personal protective equipment (PPE) such as lab coats, gloves, safety goggles, and appropriate footwear. Follow all waste disposal regulations for chemical and biological materials.

1. MATERIALS

A) Equipment:

- High-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS)
- Centrifuge
- Volumetric flasks
- Pipettes and pipette tips
- Vortex mixer
- pH meter
- Analytical balance
- Refrigerated storage

B) Reagents and Supplies:

- Calibration standards for 2,3-dinor-11β-PGF2α
- Internal standards if applicable
- Mobile phase solvents (e.g., methanol, water, acetonitrile)
- Solid-phase extraction (SPE) cartridges and reagents
- 24-hour urine collection containers
- LC-MS grade water

- LC-MS grade acetonitrile
- Calibration and control material

1. SPECIMEN REQUIREMENTS

A) Collection:

- 24-hour urine: Instruct the patient to collect all urine over a 24-hour period in a provided container.
- Ensure the container is kept refrigerated during the collection period.
- Record the total volume of the 24-hour collection.

B) Transport and Storage:

- Transport specimens to the laboratory as soon as possible after the collection.
- Store urine samples at 2-8°C if not immediately processed.
- If long-term storage is required, freeze at -20°C or lower.

1. SAMPLE PREPARATION

A) Pre-treatment:

- Thaw the urine sample if frozen.
- Mix the urine sample thoroughly.
- Measure and record the total urine volume.
- Aliquot a suitable volume (e.g., 4 mL) of urine into a centrifuge tube.
- Add internal standard to the urine sample if applicable.

B) Extraction:

- Dilute the urine sample with LC-MS grade water.
- Adjust the pH of the sample if necessary, using a pH meter.
- Load the sample onto a pre-conditioned SPE cartridge following the manufacturer's instructions.
- Wash the cartridge with appropriate wash solutions to remove impurities.
- Elute 2,3-dinor-11β-PGF2α from the cartridge using an appropriate solvent.
- Evaporate the elution solvent under nitrogen gas or in a vacuum concentrator.

C) Reconstitution:

- Reconstitute the dried residue in a suitable volume (e.g., 100 µL) of mobile phase.
- Vortex mix the sample and transfer it to an HPLC vial for analysis.

1. ANALYTICAL PROCEDURE

A) Instrument Calibration:

- Calibrate the HPLC-MS/MS instrument using calibration standards for 2,3-dinor-11β-PGF2α.
- Prepare a series of calibration standards to generate a calibration curve.
- Run the calibration standards and create a calibration curve based on the response ratios.

B) Quality Control:

- Run quality control samples at the beginning, middle, and end of each analytical batch.
- Ensure quality control samples fall within established acceptable ranges.

C) Sample Analysis:

- Load the HPLC-MS/MS with the reconstituted urine samples, calibration standards, and quality control samples.
- Set and verify the HPLC and mass spectrometry parameters according to the instrument's user manual.
- Inject and analyze the urine samples.
- Monitor the chromatograms and ion ratios for consistent and accurate peak identification.

1. REPORTING RESULTS

- Review and validate the data to ensure the accuracy of the results.
- Calculate the concentration of 2,3-dinor-11β-PGF2α using the calibration curve.
- Report results in ng/mL or appropriate units.
- Include any relevant comments or observations (e.g., hemolysis, turbidity) in the report.

1. QUALITY CONTROL AND ASSURANCE

- Perform routine instrument maintenance and calibration.
- Participate in external proficiency testing (EPT) programs if available.
- Follow internal and external quality control requirements and document all quality control measures.

1. REFERENCES

- Manufacturer’s instructions for SPE cartridges and HPLC-MS/MS.
- Relevant clinical laboratory guidelines and regulations regarding urine analysis.

1. END OF PROCEDURE

Review and update this SOP as needed to reflect new methods, technologies, or changes in laboratory policies.