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MEASUREMENT OF GLOMERULAR FILTRATION RATE (GFR) (by Iohexol plasma level determination)

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ABSTRACT

Glomerular Filtration Rate (GFR) value is widely accepted as the best overall index of kidney function in health and disease. The aim of this standad operative procedure (SOP) is to determine the GFR by utilizing the plasma clearance of iohexol, a non-radioactive contrast agent. Briefly, on the morning of renal function evaluation, 5 ml of iohexol solution (Omnipaque 300, GE Healthcare, Milan, Italy) is injected intravenously over 2 minutes.
According to the expected GFR estimated by the CKD-Epi equation (eGFR), blood samples are taken at 120, 180, 240, 300, 360, 420, and 480 min for patients with eGFR ≤40 mL/min/1.73m^2, and at 120, 150, 180, 210, and 240 min for those with eGFR >40 mL/min/1.73m^2. Iohexol plasma levels are measured by high-performance-liquid chromatography (HPLC).
The clearance of the marker is first calculated according to a one-compartmentmodel (CL1) by the formula: CL1 = injected dose of iohexol/AUC where AUC is the area under the curve of the plasma concentration of iohexol. Then the value obtained is corrected according to Bröchner-Mortensen, in order to estimate the corrected GFR (plasma clearance).

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We use this protocol and it's working

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OBJECTIVE

1 To describe the procedure for plasma collection to ensure sample uniformity, quality and integrity.

SCOPE

2 This SOP applies to all visits where the protocol requires a collection of biological plasma samples.

INTRODUCTION

3 Glomerular filtration rate (GFR) is the standard measure of renal function and is critical for the diagnosis and management of renal diseases.
GFR will be measured using the plasma clearance of the unlabelled exogenous marker iohexol, a gold standard technique to determine true GFR.

PERSONNEL

4 Appropriate staff to undertake the procedures described in this SOP may include:
• Research Nurse/Practitioners
• Laboratory technician
• Members of clinical staff trained to take blood, including doctors and nurses on the Unit

OPERATING INSTRUCTIONS

5 **Material and Labeling**

Decide the blood sample time point according to the expected GFR estimated by the CKD-EPI equation:

- for eGFR > 40 ml/min/1.73m^2 sampling at: **120, 150, 180, 210, 240 min** after Iohexol injection

- for eGFR ≤ 40 ml/min/1.73m^2 sampling at: **120, 180, 240, 300, 360, 420, and 480 min** after Iohexol injection

Before starting the procedure make sure the following items are available:

- Omnipaque 300 mg I/ml (OR 240, 350 mg I/ml)
- BD Venflon^{TM} Pro Safety (or similar catheter)
- Vacuette ® Quickshield Safety Tube Holders (or similar)
- Vacuette ® Luer adapter (or similar)
- BD Connecta^{TM}three way stopcock (or similar)
- 6 or 8 x 3 ml K_2 EDTA BD Vacutainer blood collection tube (lavender cap)
- Saline solution
- Sterile disposable plastic Pasteur pipettes
- 17 syringe (2 – 2.5 ml)
- A -20°C freezer
- 6 or 8 x 2 ml cryo vials
- Cryobox for 2 ml cryo vials
- A centrifuge capable of generating at least 1700 Relative Centrifugal Force (RCF) at the tube bottom.

Follow the instruction below for the labelling:

1. Take the label strip according to the expected GFR.
2. Label properly the vacutainer blood collection tube (lavender cap).
3. Label the cryovials. Pay attentionto apply the label in order to allow the barcode scan.
4. Check to have the same number of vacutainers and cryovials.
5. Before every transfer of plasma pay attention to the correspondence between the vacutainer and cryovials.

5.1 Collection of blood samples

Blood samples will be collected from all study subjects at the designated time-points. Plasma will be obtained after centrifugation (from 3 ml of collected blood will be obtained about 1.5 ml of plasma after centrifugation). Plasma (~ 1.5 ml per sample) will be transferred into labeled cryo vials (2 ml) with the subject's unique study ID number along with the date, visit number, and timepoint and stored on-site at -20 ^{o}C for up to analysis.

5.2 Preparation materials

1. Draw exactly 5 ml of Iohexol regardless of Omnipaque concentration, using the syringe.
2. Prepare a syringe containing 10 ml of saline solution to be used immediately after Iohexol injection.
3. Prepare another syringe containing 10 ml of saline solution applying at the extremity the three way stopcock. This will facilitate the change of the stopcock after Iohexol infusion.

5.3 Procedures

1. Weight the patient and record the exact weight in kilograms on the requisition form.
2. With the patient comfortably reclined, position the BD VenflonTM Pro Safety (or similar catheter) in an antecubital vein and connect the BD ConnectaTM three way stopcock (or similar).
3. Take the Vacuette® Luer adapter (or similar) and connect the Vacuette® Quickshield Safety Tube Holders (or similar).
4. Collect baseline blood sample for measurement of basal iohexol concentration (Blank sample).
5. Wash the catheter with 2 ml of normal saline solution.
6. Inject slowly 5 ml of Iohexol into the catheter in no more than 2 minutes. Ensure that all fluid is injected intravenously and there is no subcutaneous leakage.
7. Wash the catheter with 10 ml of saline solution.
8. Remove immediately the three way stopcock and replace it with another one to avoid any possible contamination during the following blood samplings.
9. Wash the catheter with 10 ml of saline solution.
10. Note the exact time point on the requisition form. (Time 0 = end of Iohexol injection)
11. Collect blood samples at different time point according to the expected GFR. Do not collect the sample before the time point expected.
12. Schedule the time of blood drawing starting from Time 0.

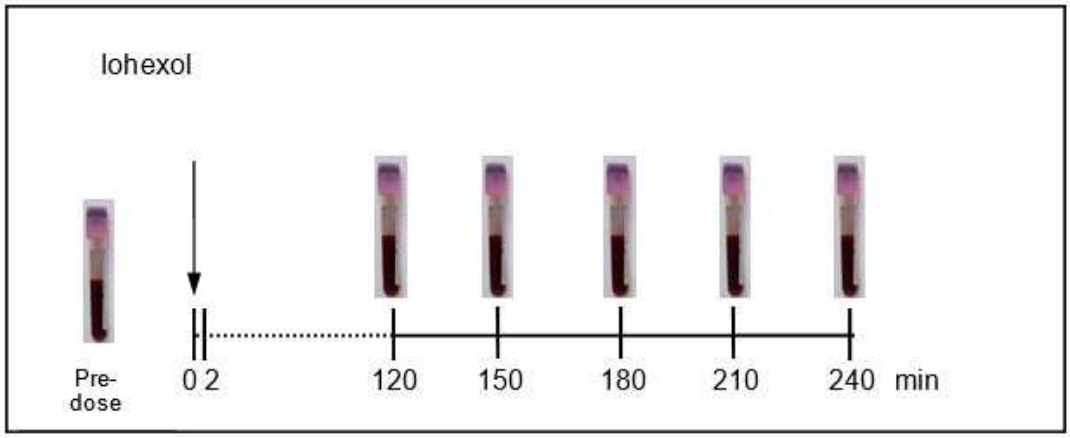
***Note**: before any blood sampling, discharge a blood volume at least equivalent to the BD Venflon^{TM} Pro Safety (or similar) volume (1.5 ml) in order to avoid blood dilution with saline. Similarly, at the end of blood collection fill the entire catheter with saline solution (2 ml).*

5.4 Sample processing

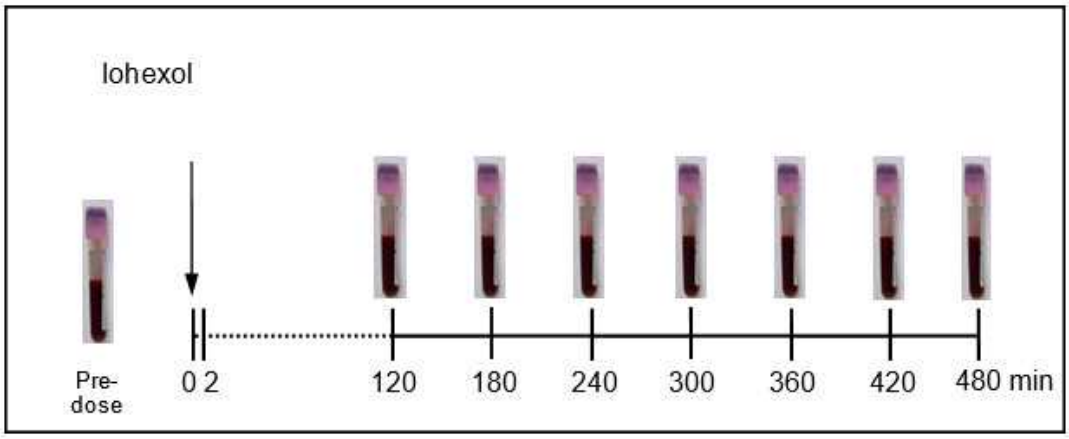
Blood samples can remain at room temperature until the end of the procedure before plasma separation.

1. Centrifuge blood sample (3 ml, K2 EDTA blood tube) at room temperature 1700 RCF rpm for 10 minutes.
2. Use a pipette and transfer at least 500 microliters of plasma into the corresponding labeled cryo vial.
3. Store the cryo vials in the cryobox at -20°C until the shipment to laboratory for the assay.

For an expected eGFR > 40 mL/min/1.73m²



For an expected eGFR ≤ 40 mL/min/1.73m²



SAMPLE ANALYSIS AND REPORT

6 Iohexol plasma levels for GFR evaluation are measured by high performance liquid chromatography (HPLC).
Iohexol clearance is first calculated according to a one-compartment model (CL_1) by the formula:

$$CL_1 = \text{injected dose of iohexol/AUC}$$

where AUC is the area under the curve of the plasma concentration of iohexol. Then the value obtained is corrected according to Bröchner-Mortensen, in order to estimate the corrected GFR (plasma clearance) by using the formula:

$$GFR = (0.990778 \times CL_1) - (0.001218 \times CL_1^2)$$

IMPORTANT INFORMATION

7 Critical points

The accuracy of this test depends on correct and complete injection of test substance and blood collection at the proper times.

- Inject the exact dose of Iohexol (5 ml independently of Iohexol concentration). If a volume otherwise is injected (5 ml), the test may proceed but it is important to note the exact amount that is injected on the requisition form (ml and Iohexol concentration).
- Note the exact times at which blood samples are taken, even if they deviate from the planned times.
- All blood samples apart from the baseline blood sample should be drawn relative to Time 0, which is the end of the Iohexol injection.
- Ensure that all fluid is injected intravenously and there is no subcutaneous leakage. If subcutaneously leakage occurs, the procedures must be stopped and the patient should return at least 4 - 7 days later to have the test repeated.
- Avoid saline dilution in the blood samples by drawing at least to 1.5 ml from the catheter prior the collection time.
- At the end of blood collection wash the catheter with 2 ml of normal saline solution.

7.1 Additional information

- Following the Iohexol injection the patient may move about. A light breakfast is allowed.
- Document all deviations that may have occurred during any part of the procedure.