

SVH AEC SOP 35

GLOMERULAR FILTRATION RATES/ MEASUREMENTS IN RATS

BACKGROUND

Glomerular Filtration Rates (GFR) are performed in rats to measure kidney function. Rats are intravenously injected with a radioactive isotope, bled 43 minutes after injection and their plasma radioactivity is counted and compared to the counts of the standard.

The radioactive isotope used is ⁹⁹technetium-diethylene triamine penta-acetic acid (⁹⁹Tc-DTPA). The DTPA is prepared at a rate of 37 MBq/ml (1mCi/ml), and 0.26ml of this solution is injected into each rat.

This procedure is usually performed in groups, up to a maximum of 15 rats per session.

MATERIALS

The following are needed:

- DTPA in a vial and contained in a lead cylinder
- 2 labelled eppendorf tubes per rat – one for whole blood and one for plasma. (Plasma eppendorf tube with 2 drops of heparin)
- 100ml Florence Flask
- 100ml Distilled Water
- Centrifuge
- 2 Assay Tubes for each rat
- 2 Assay Tubes for Blanks
- 2 Assay Tubes for Standards
- Pipette
- Gauze swabs – 2 per animal
- Alcohol swabs – 2 per animal
- Towel
- 1ml insulin syringes – 1 per animal
- Bleeding Syringes (26G needled and 1ml Syringe) – 1 per animal
- Ice (2 scoops) and esky
- Heat Lamp
- Rat Cage and Lid (for warming)
- Sharps Container
- Timer/Stopwatch
- Gloves
- Yellow Infectious Waste Bin
- Lead Lined Boxes for Radioactive Waste and Rats

- Radioactive Badge
- Lead Lined Syringe Shield
- Geiger Counter
- GFR Recording Sheet
- Balance

PROCEDURES

Injection (pre-bleeding)

- Weigh each rat and record on GFR sheet.
- Turn on heat lamp on bench.
- Put on a pair of gloves and attach radioactive badge
- Collect DTPA from Nuclear Medicine and put the vial of radioactive DTPA in the lead cylinder.
- Collect esky with ice.
- Aspirate 0.26mls of radioactive DTPA into 1ml insulin syringes with 29 Gauge needle attached. The vial of DTPA should remain in the lead cylinder when aspirating the DTPA and the insulin syringes should be inside the lead syringe shield. Ensure you inject the needles once only through the rubber of the vial otherwise they become blunt and cause pain when injecting into the rats tails. After aspirating the DTPA into the syringes, recap them carefully and place them into a small lead lined box. Place the lead lined box with the DTPA syringes at the furthest end of the bench ie as far away as possible but within reach. This should provide sufficient protection from the radioactive DTPA.
- Prepare enough syringes to have one for each rat, one for the standard and extras for any mistakes that may occur.
- At the end of aspirating, place a rat under the heat lamp and start the timer/stopwatch.
- Place one rat under the heat lamp every three minutes (which is how often they will be injected) so they are all warm enough by the time they need to be injected.
- Whilst the rats are being warmed, organise the following
 - Heparin in tubes and the tubes on ice.
 - GFR record sheet written up and ready to record on.
 - 1ml syringes and needles set up for bleeding
- When the first rat is ready to inject, remove from under the heat lamp and wrap it in a towel.
- Reset the stopwatch/timer.
- Insert the DTPA syringe into the lead shield. Inject the DTPA and start the stopwatch when the last of the DTPA has gone into the tail vein.
- Unwrap the rat and place it back in the box.
- Place all radioactive sharps and waste material in sharps bin contained in lead lined box.
- Take the second rat out at 2:00 mins and wrap it in the towel. Allow one minute to wrap the rat before injection or bleeding.

- If a rat is due at 3:00 mins, the DTPA needs to be injected by 3:00 mins. If the rat does not get injected until 3:17 for any reason, then the time to bleed that rat will be 46.17 mins, not 46:00 mins.
- When the second rat is injected, record the time of injection and time to bleed which will be 43 minutes after the injection time.
- When taking each rat out of the heat lamp to inject, continue to put rats under the heat lamp, ready for injection.
- Continue to inject all the rats and record the times they were injected and the times they are due to be bled.
- As the last of the rats are being injected, place the first rat under the heat lamp again so they are ready to be bled at the appropriate times.

Bleeding

- Bleed at exactly 43 minutes after the injection
- Swab tail with alcohol.
- Begin bleeding the rat with a 1ml syringe and 26 Gauge needle attached. When 0.5mls of blood is aspirated, note the time and continue to bleed to until 1ml of blood is collected. The time bled is the time at 0.5ml. The rate of the blood collection must remain constant throughout bleeding, with the time point recorded at 0.5ml.
- Use gauze swab at veinpuncture site and apply pressure to control bleeding.
- Discard the needle and place the blood in the eppendorf tube, cap it and invert the tube to mix the blood with the heparin. Place the tube back on ice and unwrap the rat. Record the time bled on GFR Recording sheet.
- Continue this procedure until all rats have been bled.

Post-Procedure Clean Up

- After bleeding place all radioactive equipment in lead lined boxes. Note that after seven days, any radioactive contamination will have decayed and the equipment can be discarded or reused.
- Monitor bench top and environment with Geiger counter to check that area is not contaminated. Should contamination be present it can usually be removed using soap and water.
- Wash hands thoroughly after the procedure has been completed.

Radiation Safety

- For further advice on de-contamination and other radiation safety procedures see the Hospital Radiation Safety Manual on the Intranet.

SOP.35 Glomerular Filtration Rates

Approved: 7 Feb 2007 Revised: 5 June 2007 Re-approved: 14 August 2007 Revised: 13 October 2010

Author: Ms Jemma Court



7th October, 2010

Dear Researcher,

Re: SOP.35: GLOMERULAR FILTRATION RATES/ MEASUREMENTS IN RATS

The protocol is designed to measure Glomerular Filtration Rates (GFR) in rats using an intravenously injection of radioactive ^{99m}technetium-diethylene triamine penta-acetic acid (^{99m}Tc-DTPA). The DTPA is prepared at a rate of 37 MBq/ml (1mCi/ml), and 0.26ml of this solution is injected into each rat. This implies each rat should receive less than 10 MBq.

The use of the specified quantity of the radioisotope ^{99m}Tc is noted and is permitted under the hospital's radiation management licence (Licence No. 300042061).

From a radiation safety perspective this study does not present any significant issues provided sensible safe working practices are adhered to. It is noted that the research protocol does specifically outline suitable practices regarding the storage of all potentially radioactive sharps and waste materials.

For completeness additional specific recommendations for the staff handling the animals are:

- When working with radioactive material in the Biological Research Centre (BRC) facilities signage is required and as such any work areas should have the standard "trefoil" sign affixed to them. This should include any lead screens and any cages housing the radioactive animals.
- Always wear gloves when handling radioisotopes. Be prepared to change them frequently.
- When infusing animals and when taking blood samples work do so in a contained area. Note that during the time of infusions and subsequent blood taking any animal excreta must be treated as potentially radioactive. It is important that the animals be confined during this time frame in order to avoid the spread of potentially contaminated material. A large stainless steel tray lined with "blueys" is ideal.
- Use a decontamination monitor to confirm the work area is not contaminated. If contamination is detected scrub area thoroughly with soap and/or a decontamination agent such as "DECON".
- Wash hands thoroughly after procedure completed.
- Check that hands are not contaminated and if they are wash them thoroughly being careful not to abrade skin surfaces.
- Potentially contaminated items such as syringes, swabs, MUST be regarded as solid radioactive waste and disposed of accordingly (see Radiation Safety Manual).
- Sacrificed animal carcasses should be frozen until collected by Chemsal.
- Any water soluble liquid (not counting scintillation fluid) radioactive waste may be flushed down the sewers as the concentration level does not exceed allowable disposal levels for these isotopes. However, the sink used for this

PO Box 2900
Fitzroy Victoria 3065 Australia
Telephone 03 9288 2211
www.svhm.org.au

St. Vincent's Hospital
(Melbourne) Limited
ABN 22 052 110 755
Caritas Christi Hospice Limited
ABN 51 052 110 880
St. George's Health Service Limited
ABN 64 074 683 748
Prague House Limited
ABN 17 066 184 585



St Vincent's

Continuing the Mission of
the Sisters of Charity

purpose should be flushed thoroughly afterwards.

For more complete details of safe practices in laboratories see the revised Hospital Radiation Safety Manual (4th Edition 2009) available on the Hospital Intranet (see Policies & Manuals).

If you have any questions regarding these comments please do not hesitate to contact me.

Yours sincerely,

A handwritten signature in black ink, appearing to read 'L. Wilkinson', written in a cursive style.

Luke Wilkinson, M.Sc.
Medical Physicist & Radiation Safety Officer

Direct (03) 9288 4591

Fax (03) 9288 4347

Email luke.wilkinson@svhm.org.au