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Original Article

Standard Operating Procedure for In-house Preparation of ^{131}I-rituximab for Radioimmunotherapy of Non-Hodgkin's Lymphoma

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Abstract

A Standard Operating Procedure (SOP) has been formulated for in-house preparation, quality control, dispensing and administration of ^{131}I-rituximab appropriate for the safe, effective, radioimmunotherapy of non-Hodgkin lymphoma. A decade of experience of semi-automated radioiodination of rituximab in our hospital radiopharmaceutical laboratory was analysed. The methodology was then refined for safe, practical, affordable application to radioimmunotherapy of lymphoma in departments of nuclear medicine in developing countries. This SOP has the potential to be incorporated into good laboratory practice conditions appropriate for local regulatory agency requirements.

Key words: Iodine-131, standard operating procedures, radioimmunotherapy

Standard Operating Procedure

Standard operating procedure for Iodine-131-rituximab preparation, quality control, and dispensing in Radiopharmaceutical Laboratories in Hospital Departments of Nuclear Medicine is described in this chapter. This comprehensive description would cover the following areas:

1. Apparatus
   - 1.1. Infrastructure requirement
   - 1.2. Specialized apparatus
2. Reagents
3. Method
   - 3.1. Preparation prior to labeling
   - 3.2. Labeling procedure
4. Quality control
   - 4.1. Instant thin-layer chromatography (ITLC)
   - 4.2. High-performance liquid chromatography (HPLC)
5. Dispensing and administration of prescribed activity
   - 5.1. Dispensing of prescribed activity
   - 5.2. Administration of prescribed activity
6. Disposal of radioactive waste
7. Alternative methods of synthesis

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1. Apparatus

1.1. Infrastructure requirement

1.1.1. Basic laboratory equipment includes fume hood, lead brick shielding, including lead glass, a dose calibrator (the wellbeing shielded by a cylinder of lead 25 mm in thickness), and a TLC scanner.

1.2. Specialized apparatus

1.2.1. Module for semi-automated synthesis [Figure 1], with a commercially available sterile single-use kit (Therap-I Mab® kit, Go Medical Industries, Subiaco, Western Australia)[1]

1.2.2. A Lucite column scaffold [Figure 2] custom-designed to hold the purification column, and remote manipulation of the 3-way taps.

1.2.3. Two peristaltic pumps, such as model P-1 (Pharmacia Biotech).

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1.2.4. Fabricated lead shielding for labeling apparatus [Figures 3 and 4].

2. Reagents

2.1. Obtain the following reagents:
15 mg rituximab (Roche)
Chloramine T sodium salt trihydrate (MP Biomedicals)
PD-10 Column (GE Healthcare)
Normal saline
Water for injection

2.2. Prepare the following sterile aqueous solutions:
Phosphate buffer 0.1 M, pH 7.0:
Add 50 mL 0.1 molar potassium phosphate monobasic, reagent grade (Aldrich), 13.61 g/L to 29.1 mL 0.1 molar sodium hydroxide, reagent grade (Aldrich), 4 g/L. Check pH. Filter 10 mL aliquots into sterile vials.
Sodium thiosulphate, reagent grade (Aldrich) solution: 1.5 g/100 mL
Filter using 0.22 um filter into 5 mL aliquots into sterile vials.
Phosphate buffered saline (PBS), 0.15 M, pH 7.2.
Sodium chloride, reagent grade (Aldrich), 8 g
Potassium chloride, reagent grade (Aldrich), 0.1 g
Sodium phosphate dibasic, ACS grade (Aldrich) 1.15 g
Potassium phosphate monobasic, reagent grade (Aldrich), 0.2 g
Dissolve in 1 litre of sterile water for injection, filter using 0.22 μm
filter into 50 mL aliquots into sterile vials.

2.3. Test for sterility before use, using the following method.
2.3.1. Soy-casein digest medium (SCDM). All sterilized reagents are to be tested by being incubated for 14 days at 23°C in SCDM broth.
2.3.2. Fluid thioglycollate medium (FTM). All sterilized reagents are to be tested by being incubated for 14 days at 32°C in FTM broth.

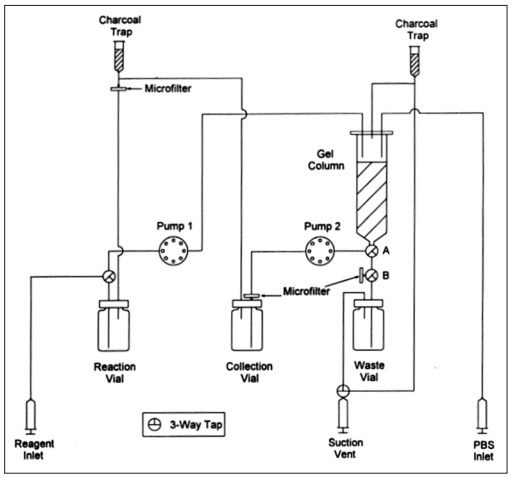

Figure 1: Schematic of semi-automated ^{131}I-rituximab synthesis

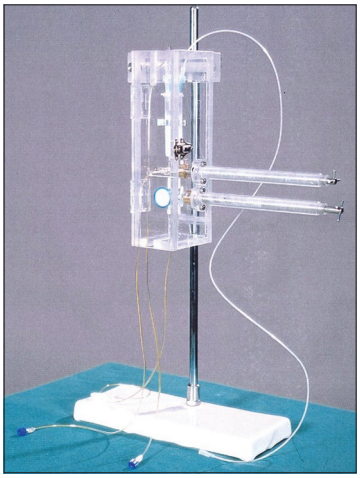

Figure 2: Lucite column scaffold with 3-way tap remote manipulators, showing inlet and outlet tubing

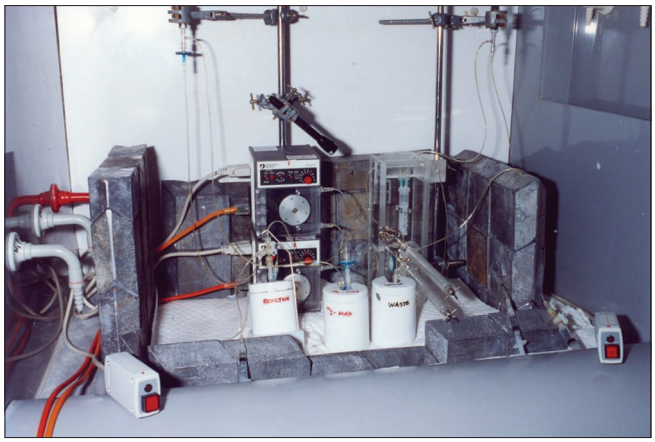

Figure 3: Construction of lead shielding. Note peristaltic pumps (centre) with remote switches outside box and Lucite column scaffold with three-way taps (right). Standard lead bricks are used to construct the walls, the shielded apparatus is housed in a fume hood

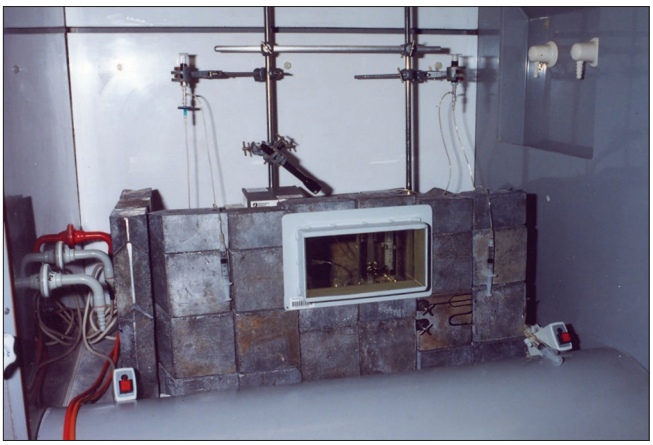

Figure 4: Completed shielding. Note: lead glass window. (*A commercially available hot cell may be used as an alternative to this apparatus*)

2.3.3. Stasis test. This test is to be performed on both media at the end of the expiration period. SCDM should show visible growth of C. albicans within 72 hours; FTM should show visible growth of C. sporogenes within 72 hours.

2.3.4. Pyrogen testing is not routinely performed.

2.4. Unstabilized [131I] sodium iodide, eg., ANSTO (catalogue no: 10016). Stabilized I-131 is unacceptable, as it contains additives that will interfere with labeling. For one therapy administration, 6,000 MBq is required. Specify that the material must be supplied in a 10 mL vial, in less than 500 µL. Note: Unstabilized I-131 is customarily supplied in a small volume V-vial, which cannot be used as the reaction vial in this SOP.

3. Method

3.1. Preparation prior to labeling

3.1.1. In a sterile laminar flow unit, equilibrate a PD-10 column in advance, using gloves and sterile technique:
Remove excess liquid on the column, remove the bottom cap and cut off the blind tip of the column.
Support the column over a waste receptacle and equilibrate the gel bed with 25 mL of sterile PBS.
Re-cap the ends.

3.1.2. Assemble the semi-automated apparatus as shown in the enclosed schematic diagram, using a sterile Therap-I Mab® kit and two peristaltic pumps. Note: The tubing kit is designed for use with Pharmacia P-1 peristaltic pumps. If other types of pump are substituted, they should be tested for compatibility with the system prior to radiolabelling.[2]
It is recommended that a rigid Lucite scaffold be used to support the gel column, and that remote manipulators are built into the box to control the two 3-way taps under the column.
All three vials should be shielded in lead pots of appropriate thickness, such as those in which the 131I is shipped. Cut 12 mm wide slots in the pot lids to accommodate inlet/outlet tubing, and house the whole in a suitably shielded fume hood.

3.1.3. Test the peristaltic pumps for correct operation.

3.1.4. Rotate the two 3-way taps below the gel column to this configuration: Top tap ┣ bottom tap ┣. Add 1.5-2 mL PBS to the top of the column via the PBS Inlet, using a 1.5 mL air flush, and allow some PBS to flow into the Waste Vial.

3.1.5. Rotate the top 3-way tap to this configuration: ┳ to stop the flow. Leave a small amount of PBS on top of the column for storage.

3.2. Labeling procedure

3.2.1. Obtain 15 mg of rituximab in 1.5 mL sterile solution, reserved from the stock of unlabeled antibody that will be administered to the patient. (NB: Inform the manufacturing pharmacist before the rituximab is diluted, since pure rituximab is required for labeling).

3.2.2. Ensure that power to the peristaltic pumps is on. Test the remote controls and check for speed and direction of flow. Obtain a laboratory timer.

3.2.3. Prepare fresh chloramine-T solution:
Tare a sterile plastic or glass 10 mL container on the balance and weigh out 0.01g chloramine-T (Allow the chloramine-T to reach room temperature, as condensation formation will compromise the reagent). Add 10 mL 0.1 M phosphate buffer, pH 7.0 from a sterile syringe and dissolve the reagent.

3.2.4. Dispense each of the following sterile reagents into separate syringes:
1.5 mL rituximab
0.3 mL chloramine-T solution
0.2 mL sodium thiosulphate solution

3.2.5. Measure the activity of the I-131 in the 10 mL-shipping vial. The shipping vial will be the reaction vial: Swab the septum with alcohol and place the vial in an appropriate lead pot in the labeling apparatus.

3.2.6. Penetrate the septum of the Reaction Vial, first with the vent needle, then the long spinal needle.

3.2.7. Open the Reagent inlet port into the Reaction Vial so that the 3-way tap has this configuration: ┳. Add 1.5 mL (15 mg) rituximab to the vial through the Reagent Inlet port, flushing the tubing with air. Add 0.3 mL chloramine-T solution, flushing the tubing with air. Agitate and allow the solution to react for 5 minutes.

3.2.8. Add 0.2 mL sodium thiosulfate with an air flush, to quench the reaction. Incubate a further 5 minutes.

3.2.9. In the interim, drain excess PBS from the top of the gel column by opening the two 3-way taps below the column so they both are in this configuration ┣.

3.2.10. Ensure that the 3-way tap on the Suction Vent syringe is set so that the Waste Vial is

open to the charcoal trap, and the PBS will drain by gravity to waste. If flow does not start, close the 3-way tap on the Suction Vent line of the Waste Vial and gently draw some air into the attached syringe. When flow starts, open the tap again to vent the vial. Flow will stop when the solution drains completely into the gel.

3.2.11. Load the contents of the Reaction Vial onto the column as follows: Rotate the Reaction Vial 3-way tap so its configuration is thus: ├. Rotate the 3-way tap directly beneath the gel column to this configuration: ┬.

3.2.12. Run Pump 1 and watch until fluid flow starts, then wait until it is all transferred to the top of the column. Stop the pump. Open the 3-way taps below the column so they both have this configuration:?. 2 mL PBS from the column will drain by gravity to waste (If flow does not start, use the Suction Vent line as in Step 15).

3.2.12.1 If the volume of the received unstabilized 1-131 was less than 50 microlitres, add 0.5 mL PBS to the column, otherwise reduce the volume of PBS by the amount of the volume in the delivered vial, e.g. If the delivered vial of I-131 was 150 microlitres, add 500-150 = 350 microlitres = 0.35 mL of PBS. Use an air flush, and allow the PBS to drain to waste.

3.2.13. Rotate the 3-way tap directly beneath the column 90 deg.,ees anti-clockwise to this configuration: ┴.

3.2.14. Add 3-4 mL PBS to the top of the column via the PBS inlet, using an air flush. This will elute the labeled antibody from the column.

3.2.15. Run Pump-2. Watch until the fluid has drained completely into the gel bed and stop the pump immediately. All of the labeled material will have flowed into the pump tubing; further drainage of the column would lead to the elution of free iodine, and air may enter and block the column.

3.2.16. To pump the labeled protein to the Collection Vial, arrange the two 3-way taps under the column to this configuration: Top tap ┬, bottom tap ┴. This so that air can be pumped through the microfilter on the bottom tap, and the column will not drain.

3.2.17. Run Pump 2 again until the entire sample has passed through the microfilter attached to the Collection Vial. (Note: Air will not pass easily through a wet microfilter, so stop the pump when fluid has been pushed through the top part of the filter, otherwise excessive air pressure will build up in the tubing).

3.2.18. Remove the inlet tubing and microfilter from the Collection Vial, leaving the spinal needle in place. Cap the spinal needle (2 sterile caps are supplied with the Therap-I Mab® kit) and remove the vent needle from the vial. Measure the activity of the labeled protein. Yield should be 75% or above.

3.2.19. Using sterile technique, place a filtered vent needle from the Accessories Bag of the Therap-I Mab® kit into the septum of the labeled antibody vial, and attach a length of sterile tubing from the kit to the long spinal needle. Add sterile Saline for Injection to the labeled antibody to make a total volume of 10 mL, mix and leave the needles and tubing in place.

4. Quality control

4.1. ITLC-SG

Spot and develop and ITLC SG strip, with 85% methanol/water as the mobile phase. The labeled protein stays at the origin (Rf 0), free iodine-131 will move with the solvent front (Rf 1.0). Radiochemical purity should not be less than 95% for a therapy administration.

4.2. HPLC

HPLC can be performed if desired.
Column: Size exclusion; Macro-sphere GPC 300A 7u, 300 mm × 7.5 mm (Alltech Associates)
Mobile phase: 0.05 M NaH2PO4, pH 7
Flow rate: 1 mL/min.
Program length: 30 minutes.
Retention time of labeled protein: ~12 minutes.

5. Dispensing and administration of prescribed activity

5.1. Dispensing of prescribed activity.

5.1.1. Ascertain the prescribed activity from the physicist and administering physician.

5.1.2. Calculate the fraction of the 10 mL volume of labeled antibody required to deliver the activity needed for the patient. Using a 10 mL syringe and appropriate syringe shield, draw up the required activity. Make up to 8 to 10 ml with sterile normal saline.

5.1.3. Assay the dispensed activity in the dose calibrator in the presence of the administering physician.

5.2. Administration of prescribed activity within one to two hours of labelling.

5.2.1. Attach the shielded syringe to a three-way tap. To this three-way tap affix sufficient sterile minimum volume extension tubing for administration, and a 10 mL syringe

containing sterile normal saline for flushing the shielded syringe ('flush syringe').

5.2.2. Under the direction of the administering physician, administer to the patient at a rate of approximately 1 mL/min using a suitable syringe driver, housed behind appropriate shielding.

5.2.3. Once the shielded syringe plunger is fully depressed, manually flush the shielded syringe using the attached flush syringe, and manually flush the shielded syringe through the extension tubing into the patient. This ensures residual activity in the shielded syringe is not retained.

5.2.4. Receive contaminated cannula, swabs and tubing from physician for disposal.

5.2.5. Measure the activity remaining in the empty syringe and tubing, calculate the net activity administered and record the therapy activity in the patient's documentation.

6. Disposal of radioactive waste

6.1. Any unused ^{131}I-rituximab in the collection vial, as well as the PD-10 column, should be labeled correctly and stored in the designated radioactive storage area in lead pots of sufficient thickness. Tubing from the labelling and administration process should be placed in a sealable bag, properly labeled, and stored in an appropriately shielded waste receptacle.

7. Alternate methods of synthesis

7.1. Given the availability of more sophisticated infrastructure such as a commercially available synthetic module, for example the Synthera® module, the synthesis of 131I-Rituximab may be automated.

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