1.0 OBJECTIVE

1.1 The objective of this procedure is to describe the procedures for the use of human cells in experimental animals at the MMRI Animal Research Facility.

2.0 SCOPE

2.1 This procedure applies to animals used for experimentation.

3.0 POLICY

3.1 It is the policy of MMRI to establish written and approved procedures to ensure that the health and well being of employees is protected, and that potentially hazardous procedures are performed in a safe manner.

4.0 RESPONSIBILITIES

4.1 It is the responsibility of Manager of Animal Research or designated alternate to implement this procedure and revise it when necessary.

5.0 REFERENCES

5.1 SOP# ANP005, Euthanasia of Rats and Mice

5.2 MMRI Radiation Safety Manual

5.3 SOP # ANP017, Necropsy procedures.

5.4 SOP # ANP012, Collection of Blood, and Urine and Fecal Samples.

6.0 SAFETY PRECAUTIONS

6.1 It is the responsibility of all personnel to use good judgment and safe practices in the laboratories. Protective clothing (e.g., laboratory coats, coveralls, boots, face masks, aprons, rubber gloves and safety glasses) are provided by the company.

6.2 Disposable rubber gloves, closed toe shoes and safety glasses are worn when handling human cells, as all samples should be treated as though they cointain potentially infectious materials, especially when cells are a primary culture and have not been analysed for the presence of infectious agents.

6.3 Disposable plastics used in the culture and manipulation of human cells are treated with a 10% solution of bleach before disposal.

6.5 All injury accidents are promptly reported to the appropriate Supervisor.

7.0 EQUIPMENT AND MATERIALS

7.1 Growth of Cells

7.1.1 Human cells are grown in disposable plastic tissue culture flasks, with filter-vented caps, when possible, to prevent bacterial or yeast contamination of the cell population.

7.1.2 Cell Growth Media

Specific, commercially available media is required for the growth of cells, the identity of the media being dependant on the cell type. Cells are grown in the appropriate media with the addition of specific growth supplements, specific for the cell type being grown.

7.2 Preparation of cells for injection into experimental animals.

7.2.1 Growth kinetics are established for any given cell line by recording cell density over time. Cells are required to be in a linear growth phase at the time of injection into experimental animals.

Cells are grown in the appropriate media, with supplements specific for a given cell population.

Suspension cells are removed directly from the culture flask and 10 uL of the cell suspension is counted using the hemocytometer.

Cells grown in monolayer are grown in 150 cm2 tissue culture grade dishes, and when at the appropriate density for harvesting, are washed with a single wash of sterile pre-warmed (37^{o}C) phosphate buffered saline (PBS). A volume of 5 mL PBS is then added to the cell monolayer, and cells are detached from the plastic culture flask surface using a sterile tissue culture cell scraper. The approximate 5 mL cell suspension is transferred to a sterile 50 mL polypropylene centrifuge tube.

7.2.2 Cells are to be injected into animals at a concentration of 2×10^{7} cells per 0.1 mL of injection buffer per animal, and to ascertain the cell concentration of a given cell population, cells are counted using the hemocytometer.

Suspension cells are counted and the appropriate volume taken for the number of animals involved in the study. Cells are transferred to either a 15 mL or 50 mL polypropylene centrifuge tube (depending on the volume of cells required), and centrifuged at 800 rpm, for 4 min. The media is removed and stored for treatment with bleach, and the cell pellet is resuspended in the appropriate volume of cold PBS (4^{o}C) appropriate for a cell concentration of 2×10^{7} cells per 0.1 mL. Cells are stored on ice until the time of injection, and the population is mixed before drawing up into the syringe.

From the monolayer cell suspension, 100 uL of cells are removed, and to this 100 uL aliquot, 500 uL of 0.25% trypsin:EDTA is added to disrupt any cell clumps, and the suspension is incubated for 5 min at room temperature. A 10 uL aliquot is then removed for hemocytometer counting. Cells are then re-centrifuged under the same conditions, and the cell pellet is re-suspended in a volume of cold PBS (4^{o}C) appropriate for a cell concentration of 2 x 10^{7} cells per 0.1 mL. Cells are stored on ice until the time of injection, and the population is mixed before drawing up into the syringe.

7.3 Equipment and other supplies

7.3.1 Disposable: syringes, 75 cm2 tissue culture flasks, 5 ml, 10 mL, and 25 mL pipettes.

7.3.2 Growth media and other solutions: growth media is cell specific, as are all supplements required by those cells for normal growth, calcium and magnesium free phosphate buffered saline.

7.3.3 Hemocytometer, bench-top refrigerated centrifuge.

8.0 PROCEDURE

8.1 Injection of human cells

8.1.1 Animals are placed in a cage with a heating pad or with a heat lamp above to dilate the vessels (about 10-15 minutes). A Broome restrainer (Plas-Labs) is used to allow easy access to the tail. A tuberculin syringe with a Gauge 27 needle is used to administer the cells or drug. The needle is inserted in the lateral tail vein and the plunger is pulled back until the needle hub gets filled with blood (this confirms that the needle is inside the vein). The content of the syringe is injected by slowly pushing on the plunger (about 2 mL/minute).

8.1.2 Intracardiac injections. Mice are anesthetized by isoflurane inhalation. The mice are then placed on dorsal recumbency. A tuberculin syringe with a Gauge 27 needle is used to administer the cells or drug. The needle is inserted under the xiphoid cartilage. Holding the syringe at an angle of about 30 degrees to the horizontal, it is pushed slowly forwards. The plunger is pulled back until blood is seen in the needle hub indicating that the heart has been entered. The content of the syringe is injected by slowly pushing on the plunger (about 2 mL/minute).

8.2 Organ collection will be as cited in SOP #ANP017 with the following exceptions.

8.2.1 Bone marrow collection.

After terminating the mice using CO2 asphyxiation, the femur is dissected out. Both ends of the femur are cut using a large pair of scissors or a bone cutter. A gauge 20 needle connected to a tuberculin syringe filled with saline is inserted in one end of the femur and the bone marrow, expelled into a sterile microcentrifuge tube, pushing the plunger with force.

8.2.2 Blood Collection.

Blood is collected from the saphenous vein. The mouse is placed into a Broome restrainer (Plas-Labs) headfirst and the hind leg is extended and fixed by holding the fold of skin between the tail and thigh. The hair is shaved with a clipper and a 23 Gauge needle used to puncture the vein. Approximately 300 uL of blood (depending on the size of the mouse) is collected in a Microvette collection tube (Sarstedt). The Micovette is sealed with a plastic cap and the open end closed. A gauze swab is applied to the puncture until bleeding had stopped.

8.2.2 Splenic cell collection.

After terminating the mice using CO2 asphyxiation, the spleen is collected and placed in a small petri dish. Holes are punched in the spleen using a gauge 23 needle. A needle connected to a tuberculin syringe filled with saline is inserted into one end of the spleen and cells are flushed using gentle pressure.

8.3 SCID animals are housed in autoclaved microisolator cages with filter tops, placed in enclosed racks in which exhausted air is one pass only and HEPA filtered.

8.3 All animal specimens and animal waste will be bagged in appropriately designated biohazard waste containers, placed in a durable, leak proof container, and closed for pick up and transport from the laboratory.

8.4 At the time of sacrifice, animals will be discarded in biohazard bags, stored in a –20° freezer, and will be removed from the building as biohazard waste.

8.5 Cage droppings will be treated and discarded as biohazard waste.

8.6 Cage components will be washed and autoclaved