

- SOP No:** AHP 54
- SUBJECT** Transplantation of haematopoietic stem cells into lethally-irradiated mice
- REASON FOR USE:** This is a basic technique used to investigate the role of stem cells
- POLICY:** This procedure must be performed by an experienced operator
Operators must have radiation experience and be licensed.
- PRECAUTIONS:** Gloves, mask, long-sleeve gown, closed in shoes.
Laminar flow cabinet
Dispose of waste appropriately
- EQUIPMENT:** anaesthetic of choice
29G needles
1mL syringes
antibiotic cover (oral suspension)
gamma irradiator
- PROCEDURE:**
- 1. Lethal Irradiation of recipient mice (transplant conditioning)**
Irradiate mice with two doses of 5.5 Grays, 3 hours apart (irradiation is split to minimize gut toxicity).
 - a) Capture mice by hand and place into specially designed Perspex boxes (with breathing holes), into the gamma irradiator.
 - b) Expose mice to a 5.5 Gray dose and immediately return to their cages. Give a second irradiation, same dose, 3-4 hours later following the same procedure.
 - c) Transplant of recipient mice is performed at 24-48 hours following the first irradiation.

Any lethally-irradiated mice that have not been transplanted must be sacrificed ([AHT 35 Euthanasia of Mice \(Cervical Dislocation\)](#)).
 - 2. Transplantation of donor cells by retro-orbital injection into recipient mice**
Lightly anaesthetise mice and inject with donor cells. Use retro-orbital injections ([AHT 56 Retro-Orbital Injection in Mice](#)) to reduce discomfort to the host mice

during injection, and provide faster transplant recovery times, resulting in:

- i) lower overall mouse morbidity and mortality, and
- ii) more reliable transplant results (less experimental variability) for the researchers (ultimately meaning that less mice need to be used).

3. Post-transplant monitoring and care

- a) As the mice will be transiently immuno-suppressed following irradiation, antibiotics must be provided in drinking water for the first 10 days following irradiation. For example, children's oral suspension antibiotic formulae (such as 'Bactrim', Roche) can be added to drinking water at 1/100 dilution (resulting in final concentration of 8mg/mL trimethoprim and 4mg/mL sulfamethazole).
- b) Mice must be monitored daily for general condition using the clinical scoring sheet (see below) for the first 10 days. After 10 days, mice usually score 0 (having regained any weight or activity loss). Scoring must continue for any mouse still registering a grade until health resumes.

Criteria	Grade 0.0	Grade 0.5	Grade 1.0	Grade 1.5	Grade 2.0
Weight Loss	<5%	5-10%	10-15%	15-20%	20-25%
Posture	Normal	Slight hunching	Hunching noted only at rest	Constant hunching	Severe hunching impairs movement
Activity	Normal	Slight decrease	Decreased	Significant decrease	Stationary unless stimulated
Fur Texture	Normal	Slight ruffling	Ruffled	Significant ruffling	Severe ruffling/poor grooming

Animals that exceed a single score of 2.0 in any category will be euthanized. Similarly, animals with a cumulative grade of 6.0 will be euthanized.

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REFERENCES

- 1 Purton¹, LE and Scadden DT (2007) Limiting Factors in Murine Hematopoietic Stem Cell Assays. *Cell Stem Cell* 263-270
- 2 Zuber J and Radtke I (2009) Mouse models of human AML accurately predict chemotherapy response. *Genes Dev.* 23: 877-889