SOP: PP004

Gamma-irradiation of *M. tuberculosis*

Materials and Reagents:
1. *M. tuberculosis* cells, live
2. Freezer, -80^{o}C
3. Tape
4. Alamar Blue SOP

Protocol:
1. _____ Remove cells from BHRB and bring to Microbiology building (note 1).

2. _____ Place in –80^{o}C freezer overnight (note 2).

3. _____ Take package to MRB building, room 447.

4. _____ Place in –20^{o}C freezer on west wall of lab.

5. _____ Write a note on the dry erase board by the lab entrance stating that cells are to be irradiated (note 3)

6. _____ Remove irradiated package from MRB –20^{o}C freezer and take to BHRB (note 4).

7. _____ Perform Alamar Blue Test (see SOP SP034).

8. _____ Place cells in –80^{o}C freezer in BHRB room 101.

9. _____ If the Alamar Blue assay is positive, repeat steps 1 to 8.

10. _____ If the Alamar Blue assay is negative, remove cells from BHRB and bring to Microbiology building.

Notes:
1. Follow directions in large scale growth of *M. tuberculosis* SOP PP003 for packaging and removal of biohazardous materials from BHRB. Also, the space in the irradiator is small, and can fit a 225 ml Falcon conical centrifuge tube, or four 50 ml Falcon conical centrifuge tubes, or eight 15 ml Falcon conical centrifuge tubes.
2. Irradiation takes place at room temperature and allows the cells to thaw. It is best to freeze cells as completely as possible prior to irradiation.
3. It is important to leave a note for the graduate student in charge of irradiation that cells are ready to be irradiated; otherwise it is unknown to Dr. Bedford's laboratory that live *M. tuberculosis* is being stored in their freezer. As of November 2004, Wendy is in charge of irradiation, and may be reached at 1-5066.
4. The cells are irradiated in a JL Shepherd 31-14 machine using a 6000 Ci ^{137}Cs source for 1620 minutes (27 hours) at 1543 rads/minute for a total dosage of 2.5 megarads. A 10µl glass capillary pipet will have been taped to the package. The glass pipet provides visual proof that irradiation took place. Irradiation turns glass from clear to brown, and to a lesser degree, turns clear autoclave bags brown.
5. See attached dosimetry documentation for further information.

Irradiation of Bacteria in Relation to the Activity of Biological Molecules

The relationship between the reduction of enzyme activity per unit(rad) of gamma or x-ray radiation is given by the term Do, where the D_O is the dose of radiation necessary to reduce the activity of the enzyme to 1/e, or roughly 37% of the initial activity.

D_O can be calculated as follows:

For molecules of 8,000 daltons or less:

$$D_O = \frac{0.24 \times 10^{12}}{\text{molecular weight}}$$

For molecules greater than 8,000 daltons:

$$D_O = \frac{0.96 \times 10^{12}}{\text{molecular weight}}$$

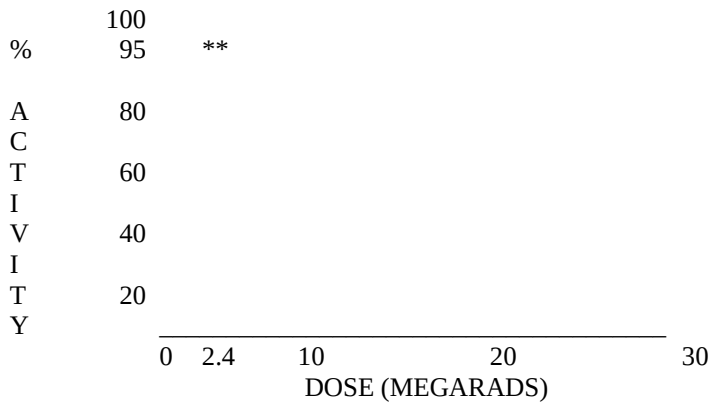
For example, if the enzyme of interest had a molecular weight of 30,000 daltons, the rads necessary to reduce the activity of the molecule to 37% of the initial activity would be:

$$D_O = \frac{0.96 \times 10^{12}}{3 \times 10^4}$$

$D_O = 3.2 * 10^7$ rads or 32 megarads, roughly.

The dose necessary to kill *Mycobacterium tuberculosis* to a 10^{20} degree of certainty is 2.4 megarads.

This dose plotted on a graph of initial activity versus dose of radiation, where 100% is the activity at 0 rads and 37% is the activity at 32 megarads, guarantees that most (i.e., 93-95 %) of the biological activity of this enzyme will be retained. (See graph below.)



Reference:

"Target Theory and Radiation Effects on Biological Molecules," Hutchinson, F., and Pollard, E. In: *Mechanisms in Radiobiology I* (1961). Errera, M., and Forssberg, A., eds. Academic Press, New York. pp. 71-92.