SOP: PP004.1
Modified: 5-7-15 AS

Gamma-irradiation of *M. tuberculosis*

Materials and Reagents:
1. *M. tuberculosis* cells
2. Freezer, -80ºC
3. Autoclave tape/label
4. SP034.1 Alamar Blue SOP

Protocol:

1._____ Get the required training and access for the BSL3. Bio unit 1 and 2 training, Bio Unit: Bio BSC Training, Bio Unit: Bio Mock BSL3 Unit, Bio Unit 7-6.2 and 9 Dangerous Goods IATA and DOT Training, Bio Unit 4 –Mtb Users, and final training and approval of the Dobos BSL3 trainer.
https://wsnet.colostate.edu/cwis86/WTrainReg/ClassSignUp.aspx?TabID=Biosafety
https://wsnet.colostate.edu/cwis86/WTrainReg/ClassSignUp.aspx?TabID=IDRC

2._____ Must be trained in this unit to transport live Mtb cells: Bio Unit 7 Title: 6.2 and 9 Dangerous Goods IATA and DOT Training.
https://wsnet.colostate.edu/cwis86/WTrainReg/ClassSignUp.aspx?TabID=Biosafety

3._____ Fill out and print the following form. Email the current operator that there is a sample to irradiate.
http://www.cvmbs.colostate.edu/erhs/erhs_docs/erhs-irradiation-request-form.pdf.
Make a copy for your notebook.

4._____ Follow the unit 7 instructions.

5._____ Package your sample in 3 layers of biohazard bags with Lysol spray or a squirt of 2.5% Vesphene between layers 1 and 2 and 2 and 3. Use autoclave tape to seal each bag and label with Organism, Your Name, Date, Dobos, and any other useful information.

6._____ The space in the irradiator is small (about 3x4x6in). It can fit, for example, a 230mL Falcon conical centrifuge tube, 4x50ml Falcon conical centrifuge tubes, 6x15ml Falcon conical centrifuge tubes, or 1x96 well plate. Conicals must have parafilmed caps. Plates must be in a ziploc bag.

7._____ Spray or wipe sample with Lysol spray or 2.5% Vesphene. Place the properly packaged sample on the material transfer cart.

8._____ Leave the barrier and place sample in a transport box containing the proper paperwork. Follow the unit 7 instructions for transport to Irradiation Services sample storage location.

9._____ Leave the sample in the sample storage location and the irradiation request form on the freezer door.

10._____ 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.

11._____ The cells are irradiated for a total dosage of 2.4 MRads. For some small samples, a 1.2 MRad dose is sufficient. A glass capillary exposure test will have been taped to the sample. The glass tube provides visual proof that irradiation took place. Operator initials and date irradiated should also be on the sample.

12._____ Pick up the sample from Irradiation Services sample storage location and transport back to the BRB in transport box with proper paperwork for confirmation of cell inactivation using SP034.

Notes: 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:

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

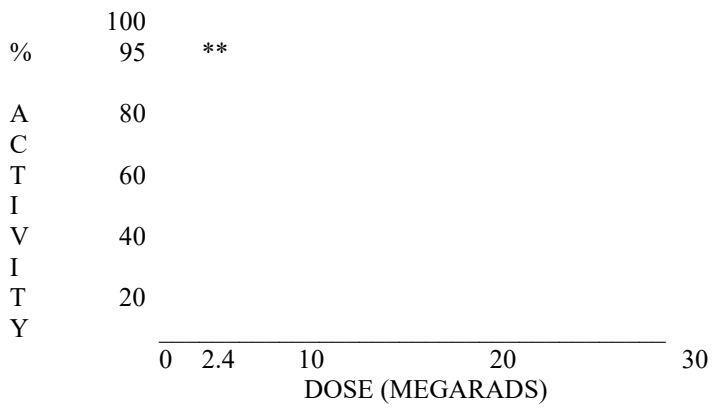
For example, if the enzyme of interest had a molecular weight of 30,000 daltons, the rads necessary to reduce the activity of the molecule to 37% of the initial activity would be:

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

$D_O = 3.2 * 10^{7}$ 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 MRads, guarantees that most (i.e., 93-95 %) of the biological activity of this enzyme will be retained. (See graph below.)



References:

"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.

SOP: PP004 Irradiator: JL Shepherd 31-14 machine using a 6000 Ci ^{137}Cs source