THE UNIVERSITY OF SYDNEY

STANDARD OPERATING PROCEDURE

Procedure	Using *Galleria mellonella* Infection Model
School/Department:	School of Molecular Bioscience
SOP prepared by:	Megan Truong, Leona Campbell
Version:	SMB061.1

Section 1 - Personal Protective Equipment (PPE)

1. Gloves
2. Safety glasses/goggles
3. Lab coat
4. Enclosed shoes
5. Hair tied back if long

Section 2 – Potential Hazards

1. Needle stick injuries.
2. Exposure to cell culture aerosols during homogenisation and injection.
3. Exposure to larvae haemocoel when injecting or killing larvae.
4. Neck, back and shoulders strain during infection procedures.
5. Workers with pre-existing medical conditions (e.g. allergy, immunocompromised state, chemical sensitivity) and workers who are pregnant or expecting pregnancy must consult with their supervisor AND medical specialist AND the university's WHS services before performing this procedure. If there are any serious concerns expressed by any of these individuals, this task must not be performed.

Section 3 – Procedure

- All operators must be trained in handling larvae with persons trained in the procedure, minimise risk of needle stick injuries. Protective gloves must be worn.
- All operators must familiarise themselves with the SDS of pathogens/chemicals/drugs to be used and a copy kept readily available.
- If infecting larvae with pathogens/chemicals, ensure all work is done in a Class II Biosafety cabinet.

Infection

- Perform infections in designated PC2 biological cabinet to prevent exposure to cell culture aerosols.
- PRIOR to infection, operators must have had practice injecting phosphate buffered saline into spare larvae to perfect technique and minimise injury to themselves and to the larvae.

NOTE: during needle insertion, larva will wriggle. Restrain the larva, belly up, to expose its pro-legs and wrap the larva around the middle finger restraining the head and tail with the pointer and thumb, respectively. Once the needle has penetrated into the pro-leg holes and the inoculum has been delivered, slightly relax the hold on the larva before withdrawing the syringe, to ensure that the cell inoculum and haemocoel do not leak from the injection point.

Transport

- Ensure that the lids of glass jars are securely tightened.
- Ensure petri dishes are well sealed with four pieces of sticky tape.

Section 4 – Disposal / Spills / Incidents

Disposal

- At the termination of the experiment, freeze dead/alive larvae in an allocated -20°C freezer before disposal in an appropriate biohazard bin.
- Chemically sterilise any remaining uninfected microorganisms with bleach.
- Dispose of used and/or broken syringes in sharps bin.

Spills

- Any biohazard spills must be cleaned up immediately using bleach. See SOP for "Working with risk group 2 microorganisms".

Incidents

- Any injuries, incidents or near misses (hazardous situations not resulting in an incident) must be reported to your supervisor and via the online reporting system.

Section 5 – Repairs / Certification / Validation

- Ensure Class II Biosafety cabinet is certified (this requires manual inspection).

Section 6 – Relevant safety data sheets (to be available and accessible)

- Ensure you are familiar with the types of biohazards present in the School, and have read the risk assessment and SOP for Working with risk group 2 microorganisms, and the risk assessment and SOP for Biohazard Spills.
- Essential: Risk assessments and SOPs for Biosafety II Cabinet (SMB005), Biohazard Spills (SMB004)
- Other (varies depending on work done): Working with Risk Group 2 microorganisms (SMB026), Animals and animal tissues (SMB002)

Section 7 - References

- Check relevant SDS for any chemicals used.
- Risk assessments and SOPs for:
  - Working with Risk group 2 microorganisms (SMB026)
  - Biohazard Spills (SMB004)
- AS/NZS 2243.3:2010 Safety in Laboratories Part 3: Microbiological aspects and containment facilities

SOP Consultation, Training and Approval

Print names and enter signatures and dates to certify that the persons named in this section have been consulted/trained in relation to the development and implementation of this Standard Operating Procedure. WHS Representative (WHS Committee) certifies that consultation has taken place.

Position	Name	Signature	Date

Name Authorising (Printed): *DIANNE FISHER*.....

Signature: .....***Date:*** *15/7/15* .....

WHS Committee Representative Name (Printed): *MARKUS HOFER*.....

Signature: .....***Date:*** *15/7/15* .....