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Toxicity Testing of Compounds in *Galleria mellonella* Larvae

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Juan Jose Quispe Haro[1]

[1]University of Helsinki

Galleria protocols

Juan Jose Quispe Haro
University of Helsinki

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Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

Abstract

This protocol describes a method for toxicity screening of chemical compounds, nanoparticles, or microbial metabolites using *Galleria mellonella* larvae. The procedure utilizes 7th instar larvae injected with test compounds via the last proleg, followed by survival monitoring over 72 hours. With a sample size of 10+ larvae per treatment group, this assay allows for statistically strong, rapid, low-cost preliminary toxicity screening in an invertebrate model that shares key physiological similarities with mammals.

Guidelines

- **Dose volume:** Do not exceed 10–20 µl per larva to avoid mechanical damage.
- **Vehicle toxicity:** Always include vehicle control; DMSO >10% can be toxic.
- **Larvae quality:** Use only healthy, active larvae of similar size.
- **Sterility:** Work aseptically to avoid infection-related death.
- **Replicates:** Repeat experiment at least 3 times independently for statistical power (total n=30 per group across replicates).

Materials

Galleria mellonella larvae: 7th instar larvae (≈300 mg each, cream-colored, active). Minimum 10 larvae per treatment group + 10% extra for selection.
Hamilton syringe (10 µl) with removable needle.
6-well cell culture plates (sterile, non-treated).
Optional: Dissecting microscope or magnifying lamp.

Safety warnings

Follow institutional guidelines for invertebrate use. Euthanize larvae by freezing at -20°C for 2 h after experiment.

Before starting

1 Acclimate larvae at experimental temperature for 24 h before injection.
30 °C or experimental temp.

2 Prepare compound solutions freshly on the day of injection. Filter-sterilize if possible (0.22 µm).

3 Plan groups (n=10-16 larvae per group):

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Group 0: Optional handling control (to test if larvae survive
handling/physical trauma).
Group 1: Vehicle control (same solvent used to prepare the
compound solutions).
Group 2: Positive control (substance known to be toxic with
similar mode of action of test compound)
Groups 3-X: Test compounds at desired concentrations (e.g., low,
medium, high dose, or serial dilutions).
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4 Assign random 7th instar larvae to each group to avoid health/size bias.

Injection Procedure

5 Surface sterilize each larvae before injection by wiping each larva gently with 70% ethanol and allowing to dry on filter paper.

6 Prepare a Hamilton syringe with 10 µl of the desired solution.

7 Grab a larva by its sides with the thumb and the index fingers and flip it upside down to expose its abdomen.

8 Localize the hind-left proleg of the larva and carefully insert the needle of the syringe about 5 mm into the body of the larva.

9 Inject the contents of the syringe slowly to prevent any leaking, and remove the syringe.

10 Place each individual larva in a well of a 6 or 12-well dish and repeat for all the larvae and conditions.

Incubation and Monitoring

11 Transfer the dishes to an incubator and incubate the larvae for 72+ hours at desired temperature with 12-12 hours illumination cycle. Do not provide food during experiment.

12 Monitor survival at 0, 2, 4, 6, 8, 12, 24, 48, 72 hours post-injection (adjust based on kinetics).

13 Monitor health using the Health Score Index points system every 24 hours.
Modified from DOI: 10.1080/21505594.2021.1950269

	A	B	C
	Category	Description	Score
	Activity	no movement	0
		movement only after stimulation	1
		letargic movement	2
		whole body movement	3
	Cocoon formation	no cocoon	0
		partial cocoon	1
		full cocoon	2
	Melanizatio n	black larvae	0
		black spots on brown larvae	1
		≥3 spots on beige larvae	2

	A	B	C
		<3 spots on beige larvae	3
		no melanization	4

Data analysis

14 Calculate percent survival per group over time and plot Kaplan–Meier survival curves. This can be done easily in GraphPad Prism.

15 Compare groups using log-rank test or Cox proportional hazards (if multiple doses).

16 Plot the average health score vs time. Analyse the data by performing a Repeated Measures ANOVA, and post-hoc tests (Bonferroni or Tukey's HSD) to determine if the differences are significant.

17 Determine the LD_{50} using the following formula:
$$LD_{50} = Log_{10}(x_k) + (Log_{10}(d) * (0.5 - \Sigma p_i))$$
where:
x_k = Lowest concentration that produces 100% mortality
d = Log-interval between doses (10-fold dilutions)
Σp_i = Sum of the proportions of mortality for all doses up to but not including x_k

Protocol references

Larvae Health Index Scoring System, modified from **Establishing an invertebrate *Galleria mellonella* greater wax moth larval model of *Neisseria gonorrhoeae* infection,** *Virulence*, 2021 Jul 25;12(1):1900–1920. doi: 10.1080/21505594.2021.1950269