

Delayed Hypersensitivity Test

Equipment: - 'Quickmini' thickness gage (Mitutoyo Canada, Mississauga, Ontario)

Supplies:

- 4-ethoxymethylene – 2- phenyl – 2-oxazolin-5-one (**oxazolone** sensitizing agent, Sigma-Aldrich Canada Ltd., Oakville, Ontario)
- ethanol
- acetone
- olive oil
- 5 mL polypropylene tubes
- 1.5 mL microtube
- 100µL pipette and tips
- shaver

General Information:

The delayed hypersensitivity test (DHT) is a screen for the T-cell mediated response to a sensitizing chemical, **oxazolone**. Mice are primed twice with oxazolone solutions by applying it to their abdomen. A subsequent application to the surface of the ear elicits an inflammatory reaction. This can be monitored by measuring the change in ear thickness from the baseline.

Procedure:

For the first priming, a 5% w/v oxazolone solution in ethanol/acetone (3:1 v/v) is freshly prepared. The mouse is scruffed at the back of the neck and the fur on the abdomen is shaved off. 100µL of the 5% solution is pipetted onto the exposed abdomen and gently massaged into the skin with the side of the tip.

After at least 4 days, mice are primed a second time using freshly prepared 1% w/v oxazolone solution in ethanol/acetone (3:1 v/v). Again, 100µL of the 1% solution is pipetted onto the shaved abdomen and gently massaged into the skin.

On the first test day, a solution of 1% w/v oxazolone solution in olive oil/acetone (3:1 v/v) is prepared for application to the ear. The baseline ear thickness (Day 0) is measured with the Quickmini thickness gage. The instrument contains a spring giving constant but not excessive pressure against the ear. The mouse is scruffed at the neck to expose the left ear. Note that only the right ear is used for ear punch identification. The thickness is taken by placing the gage around the middle of the lateral surface of the left ear. Care must be taken to avoid catching fur, which would significantly alter the measurement. Three readings from this area are taken and averaged. Following baseline reading, 10µL of the 1% solution is pipetted onto each surface of the left ear (20µL total) and gently massaged with the tip. After 24 +/- 1 hours, the Day 1 measurement is determined. The thickness is taken as above, with 3 readings averaged. The gage is moved along the edge of the ear slightly so that the same spot of the usually swollen ear is not always depressed. Thickness is again measured on Day 7.

Reported Results:

For each mouse, the changes from the baseline in left ear thickness at Day 1 and Day 7 are reported.

Acknowledgements:

The CMHD requests that the users of our screening service acknowledge the technical assistance of our facility in any presentations or publications that report results generated by our services. A suitable acknowledgement for publications is as follows: "The authors would like to acknowledge the Samuel Lunenfeld Research Institute's CMHD Mouse Physiology Facility for their technical screening services (www.cmhd.ca)."

Additionally, please send reprints or information on such publications or presentations when they are submitted or available. Such acknowledgements will help promote the use of our service and assist us in obtaining continued financial support to help defray service fees.