

Chorioallantoic Membrane Vascular Assay (CAMVA)

Theory: Vascular damage (i.e. ghost vessels, capillary injection, or hemorrhaging) of the chorioallantoic membrane (CAM) of a 10 day old fertilized chick egg following exposure of a test chemical is used as an endpoint to determine potential conjunctival irritancy.

Applications and Use

Investigation of Vascular Effects such as neovascularization, angiogenesis, and anti-angiogenesis, which are of particular interest to pharmaceuticals development.

Experimental Procedure

Egg Preparation

- Fertilized chick eggs (Day 0) are received: eggs are checked for damage, cleaned with 70% EtOH and are placed in humidified atmosphere ($37\pm 1^{\circ}\text{C}$).
- Day 3, eggs are turned parallel with the rack
- Day 4, eggs are candled for existence and position of embryo. Three ml of albumen are removed from eggs with embryos and a window is cut in the egg and sealed with transparent tape.
- Egg viability is checked each working day.
- Day 10, a teflon ring is placed on the CAM of each egg to serve as a reservoir for the diluted test sample.

Sample and Positive Control Preparation

- Samples are suspended in either H_2O or vegetable oil or other appropriate solvent.
- Suspend test sample in solvent at highest desired concentration. Dilutions are prepared from this sample.
- Sodium lauryl sulfate (SLS) is used as the positive control and is diluted in H_2O to 0.01M
- 10 eggs are left uncut and checked daily for viability to serve as a control group to monitor environmental conditions.

Assay Procedure

- On day 10, 40 μl of test sample dilution, SLS, or solvent are added to the ring inside each egg in a group of eight to ten eggs.
- Eggs are placed back in humidified atmosphere for 30 ± 5 min.
- Using a flashlight, the area inside the ring is visually assessed for vascular damage, using the area outside the ring as a guide.
- All observed effects of each egg in each group are recorded.

Data Evaluation

- Calculate RC_{50} (the concentration of test article resulting in 50% of the eggs showing a positive response) using probit analysis.

SCORING OF EGGS

After 30 minutes \pm 5 minutes, the appropriate eggs to be scored were removed from the incubator, the tape was removed and the egg shell peeled away to increase the viewing area of the CAM. The CAM within the ring was compared with the surrounding CAM with the aid of a flashlight. The vascular effects according to the following criteria will be reported:

Vascular Effect	Score	Description of Observation
0	0	No reaction observed, CAM normal.
Ghost Vessels	1	Vessels devoid of blood flow, appear clear.
Capillary Injection	2	Hyperemia, or increased blood flow of the small CAM blood vessels.
Minimal Hemorrhaging	3	Petechial hemorrhaging covering not more than 25% of the ring area.
Slight Hemorrhaging	4	Blood droplets covering 25%-50% of the area within the ring.
Moderate Hemorrhaging	5	Larger blood droplets or small pools of blood covering 50%-75% of the area within the ring.
Severe Hemorrhaging	6	>75% of the area within the ring is covered by pools of blood; dark scab-like areas may also be formed.

If any vascular effect was observed, the egg was considered positive. Recording of positive or negative responses will be all that is required for determining an RC_{50} value. Recording of severity of vascular effects will be optional at the study director's discretion. Scoring for all eggs was written on the egg scoring sheets and simultaneously entered into the IIVS Laboratory Information Management System.