Location: Queen’s University

Responsibility: Principal Investigators (PI), Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the method of oral gavage in mice.

1. Introduction and Definitions: To administer precise amounts of liquid diet, drugs or test compounds orally to mice using the gavage method. To minimize stress to the animal, the person must be skilled in the gavage technique before starting a study.

The choice of whether to use a rigid or flexible gavage needle or to use a straight or curved gavage needle is according to operator preference and the needs of the study. Gavage needles are available in disposable plastic or reusable stainless steel. All gavage needles have a ball or pear-shaped smooth rounded tip to prevent injury to the esophagus and other tissues.

2. Materials:
- Appropriate feeding needles (also known as gavage needles or feeding tubes)
- 1 ml sterile syringe
- Scale
  - The volume to be administered will depend on the body weight of the animal. The volume should not exceed 1% (10ml/kg) of the animals’ body weight (e.g. 20g = 0.2ml).
- Marker
- Isoflurane anesthetic as needed

### Mouse Gavage Needle Sizes

<table>
<thead>
<tr>
<th>Weight range (g)</th>
<th>Gauge</th>
<th>Length (inches)</th>
<th>Ball diameter (mm)</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>to 14g</td>
<td>24</td>
<td>1”</td>
<td>1 ¼ mm</td>
<td>Straight, curved</td>
</tr>
<tr>
<td>15-20g</td>
<td>22</td>
<td>1”, 1 ½”</td>
<td>1 ¼ mm</td>
<td>Straight, curved</td>
</tr>
<tr>
<td>20-25g</td>
<td>20</td>
<td>1”, 1 ½”, 2”</td>
<td>2 ¼ mm</td>
<td>Straight, curved</td>
</tr>
<tr>
<td>25-30g</td>
<td>18</td>
<td>1”, 1 ½”, 2”</td>
<td>2 ¼ mm</td>
<td>Straight, curved</td>
</tr>
<tr>
<td>30-35g</td>
<td>18</td>
<td>2”, 3”</td>
<td>2 ¼ mm</td>
<td>Straight, curved</td>
</tr>
</tbody>
</table>

3. Procedures:
- Set up work surface with the above materials.
- Draw the desired amount of liquid compound into the syringe with the gavage needle in place.
- Identify the animal and pick it up with one hand using the scruffing technique (scruff the fur on the back of the neck, and immobilize the rest of the body by pinning the tail between your little finger and palm of your hand). Ensure your grasp of the scruff is tight enough that it cannot move its head, but not so tightly that its breathing is impaired.
Prior to performing the procedure, measure the distance from the oral cavity to the tip of the xyphoid process. This is where the stomach lies. Mark this distance on the feeding needle. Do not advance the needle further than this point to avoid perforation of the stomach. When gavaging, the tip of the needle is to be positioned just below the stomach’s cardiac sphincter.

With the mouse’s head moderately extended in vertical alignment, gently insert the ball of the needle into the lateral side of the mouth, behind the teeth.

The needle is then advanced gently along the upper palate towards the back of the throat. Slight pivoting of curved needles will help feed the needle past the epiglottis and fall into its correct midline placement (esophagus).

The mouse may exhibit a swallowing reflex at this point.

Once the esophagus is reached, gravity should be used to help guide the needle as it slips down into the esophageal tract.

Forcing the needle can cause damage to the esophageal wall or force the needle into the trachea. If the animal is struggling, it may not be inserted properly and should be carefully removed. Allow the animal to rest before trying the procedure again. No more than three attempts are allowed.

Once in position, inject the fluid slowly to prevent it from coming back up into the oral cavity or rupturing the esophagus. If the animal starts to cough or choke, stop injecting. Remove the needle and allow the animal to recover in its cage. Do not attempt again for a minimum of 24 hours.

Once administered, remove the needle gently, following the same angle as insertion.

Place animal back in cage and monitor for 10 minutes.

**Serial daily gavage may include brief isoflurane anesthesia for the animal prior to needle insertion and compound administration. Animal must have righting and swallowing reflex before return to home cage.**
4. Complications
Improper gavage technique can lead to several complications, acute or delayed. These may include:
- Esophagitis (inflammation of the esophagus)
- Perforation of the esophagus, trachea or lungs
- Damage to the cardiac sphincter (upper stomach sphincter)
- Insertion of needle and solution into the lungs/inadvertent tracheal administration
- Lung perforation
- Damage to the oral cavity
- Aspiration of solution into the lungs from regurgitation (needle is too short)
- Traumatic injuries related to improper restraint
- Gastric rupture
- Esophageal impaction
- Aspiration pneumonia

5. Clinical Signs of Complications
Requiring close monitoring and possible euthanasia if not resolved, or at the recommendation of the Veterinarian team:
- Respiratory distress/dyspnea (increased respiratory rate and effort)
- Blood on the needle
- “Noisy” breathing or clicking when breathing
- Pale or blue extremities
- Hunched appearance
- Squinted eyes
- Piloerection
- Blood at nose or mouth
- Swelling of neck or under front legs due to air or fluid escaping from damaged esophagus)
- Loss of weight due to inability to swallow

References:
2) https://iacuc.wsu.edu/documents/2016/06/WSU_sop_10.pdf/
3) Vol 55, No 6 Journal of the American Association for Laboratory Animal Science November 2016, Carissa P Jones,* Kelli L. Boyd, and Je Anne M. Wallace, Evaluation of Mice Undergoing Serial Oral Gavage While Awake or Anesthetized

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