Bell jar or Open-Drop System

1.0 Purpose:

1.1 This SOP outlines the proper use of a bell jar or equivalent chamber — often referred to as an open drop jar — for delivery of inhalant anesthesia to rats, mice or other small mammals and birds. Other species must be anesthetized with inhalants via a calibrated vaporizer. A bell jar may only be used to deliver inhalant anesthesia for very short-term procedures (i.e., 30-60 seconds). The vaporizer must be used if a calibrated vaporizer is available or if long-duration anesthesia is needed.

2.0 Responsibility:

2.1 This SOP applies to all veterinary or laboratory staff that use a bell jar to deliver inhalant anesthesia to rodents, other small mammals and small birds.

3.0 Materials:

3.1 Fume hood
3.2 Bell jar or other container of known volume with tightly fitting lid (Fig. 1)
3.3 Mesh platform (plastic or woven wire)
3.4 Cotton balls or gauze squares
3.5 Conical tube(s) (Fig. 2)
3.6 Isoflurane
3.7 Propylene glycol (1,2-Propanediol USP grade)
3.8 Recovery cage with supplemental heat source

4.0 Procedures:

4.1 Ensure Safety of Personal Exposure

4.1.1 Bell jar anesthesia contains no provisions for scavenging anesthetic waste gases. Therefore, the bell jar must be used in a fume hood to protect personnel from inhalation exposure to anesthetic gases. If the use of a bell jar is required outside of a fume hood, an isoflurane exposure test must be performed by EH&S to ensure adequate safety exposure levels. Please contact the Office of the Campus Veterinarian at 335-6264 or or.ocv.alert@wsu.edu to set up the testing. Refer to IACUC Policy #31 for further details on Safety Requirements.

4.2 Volume of isoflurane appropriate for different sizes of anesthetic chamber

4.2.1 Volatize the liquid anesthetic (isoflurane) by placing it on the cotton ball or gauze in the bottom of the jar. The following chart illustrates the approximate concentration of anesthetic achieved based on volume of the chamber and volume of isoflurane used.
### Concentration of Isoflurane (%)

<table>
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<tr>
<th>Internal Volume of Anesthetic Chamber</th>
<th>1L</th>
<th>2L</th>
<th>3L</th>
<th>4L</th>
<th>5L</th>
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<tbody>
<tr>
<td>1 way</td>
<td>0.05</td>
<td>0.10</td>
<td>0.15</td>
<td>0.20</td>
<td>0.26</td>
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<tr>
<td>2 way</td>
<td>0.10</td>
<td>0.20</td>
<td>0.31</td>
<td>0.41</td>
<td>0.51</td>
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<tr>
<td>3 way</td>
<td>0.15</td>
<td>0.31</td>
<td>0.46</td>
<td>0.61</td>
<td>0.77</td>
</tr>
<tr>
<td>4 way</td>
<td>0.20</td>
<td>0.41</td>
<td>0.61</td>
<td>0.82</td>
<td>1.02</td>
</tr>
<tr>
<td>5 way</td>
<td>0.26</td>
<td>0.51</td>
<td>0.77</td>
<td>1.02</td>
<td>1.28</td>
</tr>
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</table>

Volumes in the shaded area are in mL and indicate the volume of isoflurane to be applied to a cotton swab or gauze in the bell jar.

From: "Anesthesia and Analgesia in Laboratory Animals" 2nd edition pg. 86

4.3 Alternatively, isoflurane may be diluted in propylene glycol solution before soaking the cotton or gauze. Dilution of isoflurane reduces the vapor in the bell jar and creates a more stable anesthetic.

4.3.1 For mice, use a 20% v/v isoflurane in propylene glycol solution.

4.3.2 For rats, use a 30% v/v isoflurane in propylene glycol solution.

4.3.3 Example:

4.3.3.1 To make 500 mL of 20% v/v isoflurane in propylene glycol: \(500 \times 0.2=100 \text{ mL of isoflurane} + 400 \text{ mL of propylene glycol}\).

4.3.3.2 To make 500 mL of 30% v/v isoflurane in propylene glycol: \(500 \times 0.3=150 \text{ mL isoflurane} + 350 \text{ mL of propylene glycol}\).

4.3.4 Use one mL (1cc) isoflurane/propylene glycol mixture per 500 cc volume of jar.

4.4 Induction

4.4.1 Working inside a fume hood, soak a cotton ball or gauze with the appropriate amount of isoflurane (see Table above).

4.4.2 Cover the isoflurane/propylene glycol-soaked cotton ball (or gauze) with a mesh platform. This is to prevent the rodent from coming in direct contact with the anesthetic agent. Place one animal at a time in the jar and close the lid tightly.

4.4.3 Monitor the animal closely while in the jar, with particular attention to the breathing pattern. Once the animal has lost the righting reflex and breathing has slowed (~50% i.e., 80-100 breaths/min) but is regular, a plane of anesthesia has been reached.

4.4.4 Remove the animal from the bell jar. Check the color of the mucous membranes, rate of respiration, and withdrawal reflexes. The procedure may begin if there are no reflexes but the mucus membranes and respiration...
appear normal.

4.4.5 For short-duration anesthesia, a conical tube (15 mL or 50 mL) can be used for smaller or larger rodents and birds.

4.4.5.1 In the fume hood, wet a cotton ball or gauze with the isoflurane/propylene glycol mixture (0.5-1.0 mL).

4.4.5.2 Place the cotton ball or gauze in the conical tube at the end so that there is no direct contact with the animal.

4.4.5.3 After induction in the bell jar, place the animal in the conical tube. Make sure the nose of the animal remains close to the terminal portion of the conical tube. Do not put the entire face in the nose cone; there should be space for air to move around the animal’s face.

(Figure 2)

4.5 The outlined SOP is only intended for terminal or short procedures (30-60 seconds).

4.6 When the procedure is complete, place the animal in the recovery cage or euthanize as outlined in the ASAF.

4.7 For Euthanasia purpose, the use of 5% isoflurane is recommended to render the animal unconscious quickly.

4.8 Fill out the appropriate surgery or procedure form.

4.9 Clean the bell jar before the next use.

Figure 1.
Figure 2. The conical tube method for short-duration anesthesia using a mixture of isoflurane diluted propylene glycol.

References
https://iacuc.wsu.edu/documents/2016/06/policy_31.pdf/
https://www.buffalo.edu/content/dam/www/research/pdf/laf/sop/2A8.pdf
http://web.jhu.edu/animalcare/rdf/open-drop.html

Revision History:
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