POLICY NUMBER:  A-010

TITLE: Mouse Tail Snip Policy for the Purpose of Genotyping

1.0 PURPOSE

This policy describes age and anesthesia requirements and surgical or non-surgical classification for tail biopsies (tail snips) performed when genotyping mice.

2.0 REVISION HISTORY

<table>
<thead>
<tr>
<th>R&amp;D Approval Date</th>
<th>Revision #</th>
<th>Change</th>
<th>Reference Section(s)</th>
<th>Effective Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 25, 2017</td>
<td>1.1</td>
<td>None</td>
<td>N/A</td>
<td>July 28, 2017</td>
</tr>
<tr>
<td>June 14, 2016</td>
<td>1.1</td>
<td>None</td>
<td>N/A</td>
<td>June 17, 2016</td>
</tr>
<tr>
<td>June 23, 2015</td>
<td>1.1</td>
<td>Clarifying acronym; formatting and numbering</td>
<td>Section 3.0; Sections 6.0 and 7.0</td>
<td>June 26, 2015</td>
</tr>
<tr>
<td>July 8, 2014</td>
<td>N/A</td>
<td>N/A</td>
<td>New</td>
<td>July 11, 2014</td>
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3.0 SCOPE

This policy applies to all VA Pittsburgh Healthcare System (VAPHS) and Veterans Research Foundation employees (including those with without compensation [WOC] appointments) working with mice within the VAPHS Animal Research Facility (ARF) and VAPHS laboratories as specified in an IACUC-approved Animal Component of Research Protocol (ACORP).

4.0 BACKGROUND

Transgenic animal colonies require accurate genotyping of litters to achieve research goals and reduce the overall use of animals. The most commonly performed methods used in determination of genotype are polymerase chain reaction (PCR) or Southern Blotting.

DNA used in genotyping can be obtained from blood, tissues removed during animal identification (ear punches or toe clips), hair samples, stool, oral swabs or tail snips. Tail biopsy (2 - 5 mm at the distal end of the tail) that severs distal coccygeal vertebrae prior to completion of mineralization (at or before 3 weeks of age) causes only minimal pain.

5.0 POLICY

Mouse tail biopsy may be performed in variously aged animals provided that the procedures outlined in Sections 6.0 and 7.0 are followed.

6.0 GUIDELINES

6.1 Determination of the DNA source should include consideration of:

6.1.1 the least painful or stressful method of collecting the sample from the animal

6.1.2 the amount of DNA required
6.1.3 the ability to isolate an unambiguous DNA source from an individual animal. For example, although minimally stressful, stool samples obtained from socially housed mice would not allow assignment of genotype to an individual animal.

6.2 When tail snips are used as a source of DNA, care should be taken to minimize pain and distress to the mouse.

6.3 Hemostasis should be assured prior to returning animals to the holding colony. Common methods include direct digital pressure at the tip of the tail, medical grade styptic powder, or cautery. The veterinarians should be consulted if problems are encountered or when working with mutant mice having clotting disorders.

6.4 Procedures used to obtain samples for DNA extraction must be described within the IACUC-approved ACORP.

7.0 PROCEDURES

Mice undergoing tail snips are separated into the following categories:

<table>
<thead>
<tr>
<th>Age at Sampling</th>
<th>Tail sample length (millimeters)</th>
<th>Anesthesia Required?</th>
<th>Surgical description required in ACORP?</th>
<th>Procedure Card Required?</th>
<th>USDA pain category</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 21 days</td>
<td>&lt; 5 mm</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>C</td>
</tr>
<tr>
<td>22-28 days</td>
<td>&lt; 5 mm</td>
<td>Yes</td>
<td>Yes – Must be described in the ACORP and Appendix 5</td>
<td>No</td>
<td>D</td>
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<tr>
<td>&gt;28 days</td>
<td>&lt; 5 mm</td>
<td>Yes*</td>
<td>Yes – Must be described in the ACORP and Appendix 5</td>
<td>Yes</td>
<td>D</td>
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<tr>
<td>Repeat tail biopsy</td>
<td>&lt; 5 mm</td>
<td>Yes*</td>
<td>Yes – Must be described in the ACORP and Appendix 5</td>
<td>Yes</td>
<td>D</td>
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<tr>
<td>All ages</td>
<td>&gt; 5 mm</td>
<td>NA</td>
<td>Not permitted for the purposes of genotyping</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Additional requirements may be requested by the Veterinarian(s).

The following procedures must be followed:

7.1 All tail snips must be performed in a room designated for procedures (not in the animal holding room) regardless of anesthetic requirement.

7.2 Only the minimum amount of tissue required for accurate genotyping may be taken. In most cases, this can be achieved with 2-3 mm of sample. Under no circumstances should more than 5 mm be excised.

7.3 Isoflurane is typically administered using the “drop method” and must be performed in a currently certified hood. This procedure is described in the VAPHS ARF Standard Operating Procedures (SOP).

7.4 Animals undergoing isoflurane anesthesia must be monitored from induction of anesthesia through recovery.
7.5 All personnel using isoflurane must have undergone Waste Anesthetic Gas training and comply with the Waste Anesthetic Gas procedures outlined in the VAPHS ARF SOP.
7.6 Use of aseptic technique is required.
7.7 Ages and procedures must be specified in the ACORP and be approved prior to implementation.
7.8 Should the need to repeat a biopsy arise, IACUC approval is required (in the original ACORP or through an amendment to the ACORP).
7.9 Exceptions to this policy must be approved by the IACUC.

8.0 REFERENCES

- VAPHS ARF SOP

//signed copy on file //

Gretchen L. Haas, PhD
Research and Development Committee Chair

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Steven H. Graham, MD, PhD
Associate Chief of Staff for Research and Development