<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-001</td>
<td>SCOPE AND AUTHORITY</td>
<td>1</td>
</tr>
<tr>
<td>5-002</td>
<td>DEFINITIONS</td>
<td>1</td>
</tr>
<tr>
<td>5-003</td>
<td>SPECIES OF RABID ANIMALS</td>
<td>2</td>
</tr>
<tr>
<td>5-004</td>
<td>VACCINATION AGAINST RABIES; REQUIRED</td>
<td>3</td>
</tr>
<tr>
<td>5-005</td>
<td>RABIES CONTROL AUTHORITY; SEIZURE; REQUIREMENTS OF RABIES TESTING; POUNDS; IMPOUNDMENT; NOTICE; RELEASE; FEE; DOMESTIC ANIMALS BITTEN BY A RABID ANIMAL; DISPOSITION</td>
<td>3</td>
</tr>
<tr>
<td>5-006</td>
<td>APPROVED TESTS FOR RABIES DETECTION BY LABORATORY EXAMINATION</td>
<td>5</td>
</tr>
<tr>
<td>5-007</td>
<td>PREPARATION OF SPECIMENS; TRANSPORTATION</td>
<td>7</td>
</tr>
<tr>
<td>5-008</td>
<td>REPORT OF TESTS FOR IDENTIFYING ANIMALS AFFLICTED WITH RABIES</td>
<td>7</td>
</tr>
</tbody>
</table>
5-001  **SCOPE AND AUTHORITY:** These regulations are intended to implement the law governing the control of rabies, Neb. Rev. Stat. sections 71-4401 to 71-4412.

5-002  **DEFINITIONS**

**Department** means the Department of Health and Human Services Regulation and Licensure.

**Domestic animal** means any dog or cat, and cat means a cat which is a household pet.

**Own, unless otherwise specified,** means to possess, keep, harbor, or have control of, charge of, or custody of a domestic animal. This term does not apply to domestic animals owned by other persons which are temporarily maintained on the premises of a veterinarian or kennel operator for a period of not more than 30 days.

**Owner** means any person possessing, keeping, harboring or having charge or control of any domestic animal or permitting any domestic animal to habitually be or remain on, be lodged, or fed within the person's house, yard or premises. This term does not apply to veterinarians or kennel operators temporarily maintaining on their premises domestic animals, owned by other persons, for a period of not more than 30 days.

**Rabies control authority** means county, township, city, or village health departments and law enforcement officials who must enforce Neb. Rev. Stat. sections 71-4401 to 71-4412 relating to the vaccination and impoundment of domestic animals. These public officials are not responsible for any accident or disease of a domestic animal resulting from the enforcement of these sections.

**Vaccination against rabies** means the inoculation of a domestic animal with a rabies vaccine as set forth in 173 NAC 5-004. Vaccinations must be performed by a veterinarian duly licensed to practice veterinary medicine in the State of Nebraska.
5-003 SPECIES OF RABID ANIMALS: The following are species of animals capable of harboring and spreading the rabies virus.

5-003.01 Species amenable to rabies protection by immunization:

1. Dogs;
2. Cats;
3. Ferrets;
4. Cattle;
5. Horses; and

5-003.02 Species not amenable to rabies protection by immunization:

1. Carnivorous:
   a. Skunks;
   b. Raccoons;
   c. Foxes;
   d. Coyotes;
   e. Bobcats;
   f. Bats; and
   g. Hybrids (offspring of wild species bred with domestic dogs or cats).

2. Non-carnivorous: Regard these animals as rabid unless proven negative by the Direct Fluorescent Antibody laboratory test. This category includes but is not limited to the following species of animals:
   a. Civet cats;
   b. Deer;
   c. Groundhogs;
   d. Beavers;
   e. Opossums; and

5-003.03 Other Species: Call the Department’s Rabies Control Program for further information.

5-003.03A Livestock, which includes, but is not limited to, mules, goats and swine.

5-003.03B Rodents and lagomorphs, which includes, but is not limited to, rabbits, hares, squirrels and mice.

5-004 VACCINATION AGAINST RABIES: REQUIRED

5-004.01 Every domestic animal in the State of Nebraska must be vaccinated against rabies with a licensed vaccine. A list of licensed vaccines is available from the Department’s Rabies Control Program.
5-004.02 All domestic animals must be vaccinated against rabies commencing at three months of age, one year and three months, and a booster triennially.

5-004.03 Unvaccinated domestic animals acquired or moved into the State of Nebraska must be vaccinated within 30 days after purchase or arrival unless under the age for initial vaccination.

5-004.04 The provisions of Neb. Rev. Stat. sections 71-4401 to 71-4412, with respect to vaccination, do not apply to any domestic animal owned by a person temporarily remaining within the State of Nebraska for less than 30 days; to any domestic animal brought into the State of Nebraska for field trial or show purposes; or to any domestic animal brought into the State for hunting purposes for a period of less than 30 days. Such domestic animals must be kept under strict supervision of the owner. It is unlawful to bring any domestic animal into the State of Nebraska which does not comply with the animal health laws and import rules and regulations of the State of Nebraska which are applicable to domestic animals.

5-004.05 Domestic animals assigned to a research institution or a similar facility are exempt from Neb. Rev. Stat. sections 71-4401 to 71-4412 and Title 173 NAC 5.

5-005 RABIES CONTROL AUTHORITY; SEIZURE; REQUIREMENTS OF RABIES TESTING; POULDS; IMPOUNDMENT; NOTICE; RELEASE; FEE; DOMESTIC ANIMALS BITTEN BY A RABID ANIMAL; DISPOSITION

5-005.01 Any animal which is owned by a person and has bitten any person or caused an abrasion of the skin of any person must be seized by the rabies control authority for a period of not less than ten days if:

1. The animal is suspected of having rabies, regardless of the species and whether or not the animal has been vaccinated;
2. The animal is not vaccinated and is determined to be a rabid species; or
3. The animal is of a species which has been determined to be a rabid species not amenable to rabies protection by immunization, whether or not the animal has been vaccinated.

5-005.02 If, after observation and examination by a veterinarian, at the end of the ten-day period the animal shows no clinical signs of rabies, the animal may be released to its owner. If federal, state, or local laws prohibit ownership of wild or other animals, release of the animal may be prohibited.

5-005.03 Whenever any person has been bitten or has an abrasion of the skin caused by an animal owned by another person, which animal has been vaccinated in accordance with Neb. Rev. Stat. section 71-4402 and 173 NAC 5-004, or if the injury to a person is caused by an owned animal determined to be a rabid species amenable to rabies protection by immunization which has been vaccinated, the animal must be confined by the owner or other responsible person, as required by the rabies control authority, for a period of at least ten days and must be observed and examined by a veterinarian at the end of the ten-day period. If no clinical signs of rabies are found by the veterinarian, the animal may be released from confinement.

5-005.04 A vaccinated animal owned by a law enforcement or governmental military agency
which bites or causes an abrasion of the skin of any person during training or the performance of the animal's duties may be confined as provided in 173 NAC 5-005.03. The agency must maintain ownership of and must control and supervise the actions of the animal for a period of 15 days following the injury. If during the 15-day period the death of the animal occurs for any reason, a veterinarian must within 24 hours of the death have the tissues of the animal examined for clinical signs of rabies.

5-005.05 Any animal of a rabid species which has bitten a person or caused an abrasion of the skin of a person and is unowned or the ownership of which cannot be determined within 72 hours of the time of the bite or abrasion, must be immediately subject to any tests described in 173 NAC 5-006 to determine whether the animal is afflicted with rabies. The 72-hour period includes holidays and weekends and must not be extended for any reason.

5-005.06 In the case of domestic animals known to have been bitten by a rabid animal, the following rules apply:

5-005.06A If the bitten or exposed domestic animal has not be en vaccinated in accordance with Neb. Rev. Stat. section 71-4402 and 173 NAC 5-004, the bitten or exposed domestic animal must be immediately destroyed unless the owner is willing to place the domestic animal in strict isolation in a kennel under veterinary supervision for a period of not less than six months; and

5-005.06B If the bitten or exposed domestic animal has been vaccinated in accordance with Neb. Rev. Stat. section 71-4402, the domestic animal is subject to the following procedure:

1. The domestic animal must be immediately revaccinated and confined for a period of not less than 30 days following vaccination;
2. If the domestic animal is not immediately revaccinated, it must be confined in strict isolation in a kennel for a period of not less than six months under the supervision of a veterinarian; or
3. The domestic animal must be destroyed if the owner does not comply with either 173 NAC 5-005.06B, item 1 or 2.

5-005.07 The rabies control authority may authorize an animal pound or pounds or may enter into a cooperative agreement with a licensed veterinarian for the establishment and operation of a pound.

5-005.08 Any dog found outside the owner’s premises whose owner does not possess a valid certificate of rabies vaccination and valid rabies vaccination tag for the dog must be impounded. The rabies control authority may require the impoundment of domestic animals other than dogs. All impounded domestic animals must be given proper care, treatment, and maintenance. Each impounded domestic animal must be kept and maintained at the pound for a period of not less than 72 hours unless reclaimed earlier by the owner.

5-005.09 Notice of impoundment of all animals, including any significant marks of identification, must be posted at the pound as public notification of impoundment. Any unvaccinated domestic animal may be reclaimed by its owner during the period of impoundment by payment of
prescribed pound fees and by complying with the rabies vaccination requirement of Neb. Rev. Stat., sections 71-4401 to 71-4412 and 173 NAC 5 within 72 hours of release. Any vaccinated domestic animal may be reclaimed by its owner by furnishing proof of rabies vaccination and payment of all impoundment fees prior to release.

5-005.10 At the expiration of impoundment, a domestic animal may be claimed by payment of established pound fees and by compliance with the rabies vaccination requirement of Neb. Rev. Stat., sections 71-4401 to 71-4412 within 72 hours of release. If the domestic animal is unclaimed at the end of five days, the authorities may dispose of the domestic animal in accordance with applicable laws or rules and regulations.

5-006 APPROVED TESTS FOR RABIES DETECTION BY LABORATORY EXAMINATION

5-006.01 The Direct Fluorescent Antibody (DFA) test is the only test approved as a non-bioassay method, and must be performed in a manner to include at least the following techniques:

5-006.01A Tissue preparations must include microscope slide brain tissue impressions that are made from the brain stem, cerebellum, hippocampus and cerebral hemisphere. Two methods can be used to collect the tissue samples from the brain, including:

1. The cranium is opened, the brain is dissected and a small piece of each of the tissues identified above is removed and impressed onto a clean glass microscope slide.

2. A sampling tube (clear drinking straw or a clear plastic pipette) is introduced through the occipital foramen and pushed toward one eye. The tube is pulled out containing a sample of the brain cylinder. The brain cylinder will contain material from the brain stem, cerebellum, hippocampus and cortex and medulla oblongata.

5-006.01B The microscope slide tissue impressions from a suspected rabid animal must be examined using a fluorescence microscope. In addition, the examination must include tissue impressions with their respective determinations accomplished as prescribed. No DFA test result is considered valid unless the determinations are accomplished as prescribed below:

5-006.01B1 Determining test specificity must be performed using rabid mouse brain suspension-adsorbed conjugate on any positive brain tissue impressions. Subsequent examination under the fluorescence microscope must result in the confirmation of the absence of fluorescence.

5-006.01B2 Determining test method sensitivity must be performed using positive rabies tissue impressions on a microscope slide for a positive control. Normal mouse brain-adsorbed conjugate must be used. Subsequent examination under the fluorescence microscope must result in the confirmation of the presence of fluorescence.
5-006.01B3 Determining test method true negative reactivity for rabies must be performed using known negative animal impressions on a microscope slide. Subsequent examination under the fluorescence microscope must reveal the absence of fluorescence.

5-006.01C A positive DFA test for rabies is the appearance of specific rabies antibody fluorescence in the brain tissue upon excitation of ultraviolet illumination using a fluorescence microscope.

5-006.01D If the DFA test is inconclusive, additional tests must be performed and must include at least a virus isolation test.

5-006.02 The virus isolation test is approved as a bioassay method and must be performed in a manner to include at least the following techniques:

5-006.02A Pieces of tissue from the brain stem, hippocampus, cerebellum, and cerebral hemispheres taken from both sides of the suspected animal brain, if available, are weighted together. A 20% (w/v) suspension of each brain specimen for inoculation is made in growth medium (EMEM) and is centrifuged at 500 g for 10 minutes. The supernatant is filtered through a 0.2 membrane filter and 0.5 ml is added to $6 \times 10^6$ cells suspended in a conical tube.

5-006.02B Cells and virus are incubated for 1 hour at 37 degrees C. mixing every 15 minutes to adsorb virus onto cells in suspension. Cells and virus mixture are centrifuged at 500 g for 5 minutes. Supernatant is discarded and cells are resuspended in 6 ml of growth medium.

5-006.02C Cells are seeded onto flasks and Labtek slides. Approximately $3 \times 10^6$ cells are used to seed 3 Labtek slides and $3 \times 10^5$ are used to seed 1 T25 tissue culture flask. One of the 3 Labtek slides is fixed and stained with FITC-labeled anti-rabies antibody at 24, 48 and 72 hours.

5-006.02D If one of the initial Labtek slides is positive, the T25 flask can be autoclaved and discarded. If initial slides are negative, the T25 flask is pulled on day 3 and the growth medium discarded and replaced with viral medium. The flask is incubated for 3-4 days. Medium is discarded, cells are trypsinized and cells are harvested into 6 ml of EMEM. All cells are seeded into Labtek slides as described above. If the DFA is negative with the second set of Labtek slides, the specimen is considered to be negative for rabies virus.

5-007 PREPARATION OF SPECIMENS; TRANSPORTATION: The rabies control authority must assure arrangements for preparation and transportation of specimens for testing are made according to the following procedures:

5-007.01 Preparation of animal for identifying affliction with rabies:

1. Animals larger than one pound weight must be decapitated prior to transport to a testing laboratory.
2. Animal brain tissue for the Direct Fluorescent Antibody test must not be frozen or preserved with formalin or alcohol.

5-007.02 Transportation of potential rabid animal tissue must include the use of approved packaging. Approved packaging consists of the following:

1. Place the specimen in several layers of plastic bags and tie securely to prevent leakage.
2. Place the specimen enclosed in plastic bags into a leak proof container; i.e., Styrofoam.
3. Place frozen gel packs around the specimen. Do not use loose ice as it may leak and cause contamination.
4. Seal the container.
5. Place the leak proof container into another box and seal the box.

5-008 REPORT OF TESTS FOR IDENTIFYING ANIMALS AFFLICTED WITH RABIES: The laboratory must report the results of the test to the rabies control authority which submitted the specimen for testing.