

Specimen Collection and Submission for Scrapie Testing

Accredited Veterinarians

Accredited veterinarians are encouraged to submit tissues or whole heads from sheep or/goats over 18 months of age that die on-farm or are euthanized for scrapie surveillance. Note: In addition to collecting routine surveillance samples, if a sheep or goat is showing or showed clinical signs compatible with scrapie prior to death, the animal must be reported to **State** and/or **Federal** animal health officials.

APHIS provides collection and shipping supplies including waybills for tissue and whole head submissions at no cost. For information on how to get collection, labeling and shipping supplies, contact the **VS Area Office** for your State.

This document covers the following topics:

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General Information

The collector must include the following items with each diagnostic submission.

1. A completed VS Form 10-4, "Specimen Submission", which can be obtained at http://www.aphis.usda.gov/library/forms/pdf/VS_Form10_4.pdf. Hard copies are available from the **VS Area Office** for your State.
Note: the VS Form 10-4 is only for use by an APHIS representative, a State animal health employee, or an accredited veterinarian.
2. All ID devices (with a piece of tissue the size of a U.S. quarter attached), tattoos, and brands on the animal either fresh chilled or in formalin.
3. Age of the animal based on dental examination and owner records.
4. Flock ID if known, species, breed, and sex of animal.
5. Brain and other tissues collected and packaged as described below.
6. Additionally, veterinarians or producers can submit whole heads for scrapie testing.

>>Learn more information about Whole Head Submission

When collecting specimens, the collector must ensure that each of the actions listed below is completed.

Note: If a complete differential diagnosis is necessary or if rabies must be ruled out, please contact the public health or diagnostic laboratory that will be involved for direction on sample collection and submission.

1. Follow the laboratory's procedure for notifying the laboratory of incoming specimens.
2. Contact the delivery service. Ensure the package containing fresh tissues will be delivered overnight.
3. Properly complete the specimen submission form, VS Form 10-4 or electronic 10-4. Be sure to indicate whether the animal was an **exposed animal** or an animal with no known exposure. Also, indicate whether the animal exhibited **clinical signs of scrapie**. If clinical signs were observed, list them in "Additional Data" on the VS form 10-4.
4. Make 4 copies of the completed VS Form 10-4.
 - a. Maintain 1 in the collector's files (submitter's copy).
 - b. Provide 1 to the animal owner or collection site.
 - c. Submit 1 to the VS Area Office.
 - d. Submit 1 with the specimen.
5. Correctly label all specimen collection containers.
6. Properly collect obex, tonsil, cerebellum, and retropharyngeal lymph nodes (RLN). For scrapie suspects, the remainder of the brain must also be collected*.

*Any animal that exhibits CNS signs should be evaluated by for potential rabies infection and handled extremely carefully.

Sample Quality

1. Tissues with no autolysis (deterioration) should be collected and may be submitted to an APHIS - contract laboratory. For more information on contract laboratories for sample submission, contact your [Area Office](#).
2. Samples with mild or moderate autolysis may be collected only if they meet the criteria listed below and may only be submitted to NVSL.
 - a. Brain samples with mild or moderate autolysis may be collected if the obex can be identified.
 - b. Lymph nodes with mild or moderate autolysis should be collected as long as the capsule (outer membrane) is still intact.
3. Regardless of the quality of the sample, if the animal exhibited clinical signs associated with scrapie samples must be submitted. These samples should be submitted to NVSL. Autolyzed samples may only be submitted to NVSL.

Traceability and Identification

Specimens submitted to NVSL or contract laboratories must be traceable to the source animal and farm. To ensure this, the collector must accurately complete the specimen collection and submission process.

Safety Precautions

The collector is responsible for taking appropriate safety precautions. You must take measures to avoid contact with specimens and adhere to the following safety precautions to minimize your risk of exposure to pathogens.

1. Wear personal protective equipment at all times.

2. Cover cuts, abrasions, and wounds with waterproof dressing if left uncovered by personal protective equipment.
3. Take steps to avoid creating aerosols, splashes and dusts.
4. Wash hands and exposed skin following collection procedures.
5. Wash and disinfect protective clothing and instruments thoroughly after use. Use 50 ounces (6¼ cups) bleach to 78 ounces water (9 ¾ cups) to make 1 gallon of solution at room temperature (at least 65 °F) for 1 hour. Thoroughly rinse clothing and equipment after disinfection to minimize corrosion.

Note: Bleach is caustic and can be hazardous if swallowed, gets in the eyes, is breathed in, or is left on the skin. Bleach will discolor and damage some fabrics and materials. Further, care must be taken to prevent contamination of water from run off and to comply with any environmental regulations for use of this product. Read the material safety data sheet prior to use and use appropriate personal protective equipment.

Personal Protective Equipment

Personal protective equipment (PPE) minimizes exposure to pathogens while collecting samples. The Occupational Safety and Health Administration defines PPE as “specialized clothing or equipment worn by employees for protection against health and safety hazards. PPE is designed to protect many parts of the body, i.e., eyes, head, face, hands, feet, and ears.”

PPE is selected based on the environment, the physical hazards, and the ability to complete the task. PPE balances protection and comfort. PPE should protect you from the physical hazards of the collection environment, while allowing you to comfortably collect specimens. Even though the environment specimens are collected differ, the following PPE must be worn at all times during scrapie specimen collections.

Skin Protection

Protect your skin from contact with fluids during specimen collection. Wear waterproof coveralls, preferably disposable, or coveralls with a waterproof apron and forearm protectors.

Eye and Face Protection

Protect your eyes and face from any aerosols, splashes, or dusts that may be created while collecting specimens. Eye protection includes safety glasses, safety goggles, or a face shield.

Hand Protection: Gloves

1. Wear cut resistant metal or mesh gloves. Always wear the cut resistant glove on your off hand (left hand for right-handed person and right hand for a left-handed person). Find a cut resistant glove that fits against your skin and wear a rubber glove over it.
2. Wear latex or nitrile examination gloves or thick rubber gloves that extend halfway up the forearm. Many people prefer long, thick rubber gloves for added protection.

Foot Protection

Protect your feet from injuries that could result from spills, splashes, impact, compression, or exposure. Wear steel-toed rubber boots when collecting specimens. If steel-toed boots are unavailable, then pullover rubber boots are acceptable.

Respiratory Protection

Face masks are recommended. Though scrapie is not known to be transmissible to humans, other zoonotic diseases such as rabies, Q fever, or Listeria may be present and could be transmitted during scrapie sample collection.

Labeling Sample Containers

1. The specimen collection containers must be properly labeled. The information on the label provides detailed information to the laboratory regarding the specimens. The sample number or sample barcode on the sample container must be the same as on the completed VS Form 10-4.
2. Both the top and the side of the sample container must be clearly labeled using the provided bar code sticker. If a bar code sticker is unavailable, the sample may be identified by either typing the information or writing it with a permanent marker. Verify the sample numbers appearing on the top and side of the sample container and the completed VS Form 10-4 are identical.
3. The side label must include the following information:
 - a. Type of specimen;
 - b. Animal ID number; and
 - c. Sample ID number (the number assigned to this sample on VS Form 10-4).

Samples and Sample Packaging

You must properly preserve scrapie specimens to ensure accurate test results. Scrapie diagnosis may require the submission of fresh and fixed specimens.

Fresh tissue specimens. *Fresh tissue specimens must be kept chilled or frozen.* Dry ice may be used, though shipping the chilled or frozen tissues overnight on icepacks is usually best.

Formalin-fixed specimens are used for immunohistochemistry testing, histopathology, and DNA comparison. The specimen must be submerged in 10% buffered formalin (follow the guideline-10 parts formalin per 1 part specimen). *Do not allow the formalin-fixed specimens to freeze.*

Specimens to Collect

Use the following 3 tables as a guide for the proper tissue specimens that must be collected for an animal, based on its situation.

Note: Ensure the sample container correctly lists all included specimens.

Table 1. Tissue specimens for non-exposed animals without clinical signs (routine submission).

Formalin: single container for each animal	Fresh: None required for these animals
1 Retropharyngeal Lymph Node (RLN)	
Entire brainstem (including obex)	
Cerebellum (Collect a minimum of 2 g, removed via the foramen magnum when possible)	
Animal ID device(s). (Collect all animal ID devices with a quarter-sized piece of tissue attached to each device and the skin containing any official tattoo or brand. This will allow DNA verification if necessary.)	

*Table 2. Tissue specimens for exposed animals or animals with less specific signs.**

Formalin: single container for each animal	Fresh: ice packs or frozen
Obex – 1-2 cm of brainstem that includes the apex of the V at the obex.	Remainder of brainstem in its own labeled re-sealable bag**
1 tonsil	1 tonsil in its own labeled re-sealable bag**
1 RLN	1 RLN in its own labeled re-sealable bag**
Animal ID device(s). (Collect all animal ID devices with a quarter-sized piece of tissue attached to each device and the skin containing any official tattoo or brand. This will allow DNA verification if necessary.)	Cerebellum (in its own re-sealable bag labeled with the animal's official identification and "Genotype testing")

* Animals with "less specific signs" include those that are: nonambulatory, unthrifty, or exhibit wool/hair loss suggestive of rubbing, biting at the legs or side, lip smacking, or intense rubbing without bare areas.

** Place the 3 resealable bags with tonsil, retropharyngeal lymph node (RLN), and brainstem into a larger labeled re-sealable bag (i.e., keep cerebellum separate from other fresh tissue).

Table 3. Tissue specimens for suspect animals* and test positive animals.

Formalin: single container for each animal	Fresh: ice packs or frozen
1 tonsil	1 tonsil in its own labeled re-sealable bag**
1 RNL	1 RLN in its own labeled re-sealable bag**
Right Half of the brain (cerebrum, midbrain, cerebellum)	Left half of the brain (cerebrum, midbrain, remaining brainstem) in its own labeled re-sealable bag**
Obex – 1-2 cm of brainstem that includes the apex of the V at the obex.	Left half of the cerebellum (in its own re-sealable bag labeled with the animal’s official identification and “Genotype Testing”)
Animal ID device(s). (Collect all animal ID devices with a quarter-sized piece of tissue attached to each device and the skin containing any official tattoo or brand. This will allow DNA verification if necessary.)	

***Suspect animals** are highly suspicious for scrapie because they exhibit CNS signs, have a chronic wasting condition, and/or intense repeat rubbing or abrasions with bare areas. Complete brain removal is required for all clinical suspects. Suspect and test positive animals should be submitted on a separate VS Form 10-4 and shipped separately to allow NVSL to prioritize testing these cases. **Note:** If rabies testing is indicated, submit entire brain to the rabies laboratory unless arrangements have been made in advance with the rabies lab to collect and place the obex in formalin. After rabies testing is completed, proceed with scrapie sampling on rabies negative brains.

**Place the 3 resealable bags with tonsil, retropharyngeal lymph node (RLN), and left-brain into a larger labeled re-sealable bag (i.e., keep cerebellum separate from other fresh tissue).

Collection Procedures

The collection of the obex, tonsils, and retropharyngeal lymph nodes (RLN) may be completed using several methods. These collection procedures describe the preferred collection methods to prevent inadvertent damage to the tissues during collection. Other methods may be used. Contact an experienced professional for more information regarding alternative collection methods. A [pictorial guide](#) of the procedures outlined in this section is available.

The equipment listed below will help to ensure proper specimen collection.

1. Sharp boning knives.
2. Disposable scalpel blades, disposable scalpels, or a large scalpel blade is acceptable.
3. Aggressively toothed forceps (rat tooth).
4. Meat cutting bone saw, hacksaw, or electric saw when brain removal is required.
5. Disposable cutting surfaces such as cardboard, plastic, or Styrofoam.
6. Small hand nippers may be used on the hyoid bones, or you may cut through at the joint using a knife.
7. Sharp stainless steel scissors.
8. European brain spoon, grapefruit knife, or other brainstem scoop.

Obex Collection Procedures

General Guidelines: Two methods are available to collect the obex: via the foramen magnum and whole brain collection.

1. Via the foramen magnum.
 - a. Collection of the obex via the foramen magnum is the preferred method for routine surveillance collections (i.e., the animal is *not* a scrapie suspect).
 - b. Collect the obex via the foramen magnum when the carcass is reasonably fresh
2. Whole brain collection. This procedure should be used in the following circumstances:
 - a. The animal is a scrapie suspect;
 - b. The brain is too autolysed; and/or
 - c. Removal by the spoon method is unsuccessful.

Obex collection via the foramen magnum

Tools

1. Aggressively toothed forceps (rat tooth).
2. European brain spoon, grapefruit knife, or other brainstem scoop.
3. Curved blunt scissors.

Procedure

- Place the head upside down in front of you so you are looking directly at the foramen magnum.
- With forceps and scissors, remove the collar of dense dura mater surrounding the foramen magnum and spinal cord.
- Then gently grasp the end of the protruding spinal cord with forceps and move the spinal cord laterally to expose the caudal cranial nerves.
- Cut the cranial nerves with scissors, taking care to prevent damage to the brainstem. This is best accomplished with curved blunt scissors directing the tip of the scissors laterally. Repeat this procedure on the other side of the brainstem.

- Once the cranial nerves have been severed, the caudal brainstem will be easier to manipulate within the foramen magnum.
- With light pressure, use forceps to move the spinal cord to the ventral part of the foramen magnum.
- Insert the spoon into the dorsal aspect of the foramen magnum between the brainstem and the dorsal bony calvarium.
- Sever the cerebellum by advancing the spoon cranially 2 to 3 inches until you feel the leading edge of the spoon hit bone.
- Remove the spoon.
- With the forceps, lift the spinal cord dorsally and reinsert the spoon into the ventral aspect of the foramen magnum between the brainstem and the ventral bony calvarium. Sever the brain stem by advancing the handle of the spoon until the leading edge of the spoon touches bone.
- Pull the spoon toward you with gentle traction on the spinal cord with the rat-toothed forceps.
- If the brainstem is not readily removed by this method, stop. Reexamine the brainstem and sever any remaining cranial nerves or connections to the dura. Use caution, as excessive caudal traction on the spinal cord may result in a mutilated, non-diagnostic sample.
- After cutting any remaining cranial nerves and repeating the spoon technique to completely sever any residual attachments of the caudal brainstem from the mid brain, the brainstem should easily be extracted by caudal movement of the spoon cradling the brainstem and caudal pressure on the spinal cord with forceps.
- The sample extracted with this method is usually 3 to 4 centimeters long with the obex in the center. Trim out the central 1/3 containing the obex and place in formalin. Place the caudle piece (spinal cord) and cranial piece (cranial brainstem) into a plastic bag for chilling or freezing.

Obex collection by complete brain collection (required for clinical suspects)

Tools

1. Meat-cutting bone saw, hacksaw, or electric necropsy saw.
2. Wood chisel or large wide-tipped screwdriver.
3. Aggressively toothed forceps (rat tooth).
4. European brain spoon, grapefruit knife, or other brainstem scoop.
5. Curved blunt scissors.
6. Scalpel.

Procedure

1. Skin the head.
2. Use a bone saw to remove the top and back of the skull. This requires three cuts:
 - a. The first cut is directed from the medial aspect of the occipital condyle dorsally to the top of the skull and then cranially to a transverse line 1 cm caudal to the lateral canthus of the eye;
 - b. Repeat this cut on the other side starting at the medial aspect of the other occipital condyle; and
 - c. The final cut is a transverse cut connecting the cranial aspects of the two longitudinal cuts approximately 1 cm caudal to the lateral canthi of the eyes.
3. Pry off the skullcap by inserting a wood chisel or a large wide-tipped screwdriver at the level of the transverse cut and hinge the skullcap caudally.
4. If the top of the calvarium is not readily removed, review the procedure and verify that cuts are through the bone. If the cuts are placed too far laterally or cranially, the sinuses will be entered and additional sawing will be necessary to free up the top and back of the calvarium. *Note: If the sides or front of the cerebrum has been inadvertently damaged during the previous steps of the procedure, the samples will not be compromised.*

5. Open the dense, fibrous dura mater covering the sides and top of the brain with scissors and forceps by making a midline longitudinal cut from the cranial aspect of the cerebrum to the spinal cord. Ensure that you completely incise the extra tough section of the dura mater, known as the tentorium cerebelli, which lies between the cerebrum and cerebellum.
6. Once the entire brain is exposed, direct the nose dorsally, resting the occipital condyles on a flat surface, such as a table or floor, and sever the cranial nerves starting with the olfactory nerves and proceed caudally cutting the cranial nerves and allow gravity to assist removal of the brain from the cranial vault.
7. For scrapie diagnosis, separate the brainstem from the fore brain by a transverse cut between the cerebrum and cerebellum.
8. Remove the cerebellum from the brainstem at the level of the peduncles. At this stage, the brainstem derived from the whole brain and the brainstem derived with the spoon method should be similar.
9. Remove obex by placing a pencil such that it just covers the apex of the V and slicing on either side to give an 8-10 mm cross section.
10. Place the obex into formalin.
11. Place the remaining brainstem tissues including the spinal cord and brain stem into a plastic bag.
12. Then divide the cerebrum, midbrain, and cerebellum longitudinally into left and right halves. Put the right half in formalin. Put the left cerebellum in its own bag and label genotyping and the left midbrain and cerebrum in another bag and seal.
13. Place each of the remaining fresh tissue samples into their own sample bags and seal.

Tonsil Collection Procedures

Various successful approaches are available to collect the tonsils. The tonsillar crypts on the dorso-lateral aspect of the oropharynx are useful landmarks. Keep in mind that the actual tonsillar lymphoid tissue is located deep to the superficial mucosal crypts in the submucosa. The tonsillar lymphoid tissue is readily palpable and visible when adequately exposed. Ensure that you have collected the deep tonsillar lymphoid tissue. The most common scrapie submission error is the collection and submission of the mucosal crypts instead of the tonsillar lymphoid tissue.

Tools

1. Sharp boning knife
2. Scalpel
3. Sharp stainless steel scissors
4. Aggressively toothed forceps (rat tooth)

Procedure

1. Place the head upside down on the table.
2. Remove the skin from the ventral surface of the mandible.
3. Grab the pharynx with your noncutting hand and pull it toward you (stretching out the pharynx), place the knife on the mandibular symphysis and cut caudally with the blade touching the ventral aspect of the mandible. As you cut caudally, follow the angle of the mandible dorsally as you approach the rami of the mandible. The hyoid bones you encounter will need to be cut with poultry shears or disarticulated at a joint with the knife.
4. The oropharynx (cranial) and nasopharynx (caudal) will now be exposed. Grab the ventrolateral aspect of the oropharynx with rat tooth forceps and observe the tonsillar crypts opening into the dorso-lateral aspect of the oropharynx. Begin a dissection plane between the pharynx and the lateral pharyngeal muscles. As the dissection is extended dorsally, a bulge of lymphoid tissue will be seen protruding from the lateral pharyngeal wall. Use the tonsillar crypt as a landmark. The lymphoid tissue is always connected to the tonsillar crypt. Be sure to collect the lymphoid tissue in addition to the crypt.
5. Once the bulge of tonsillar lymphoid tissue is identified, remove it with scissors or a scalpel and forceps. The tonsil with associated lymphoid tissue will contain medial crypts; laterally it will have a readily

palpable, well circumscribed mass of lymphoid tissue that will feel like a small, round, sometimes relatively flat lymph node.

6. Alternatively, the tongue can be loosened cranially and laterally at the mandibular symphysis and retracted caudally until the crypts are visible and a similar dissection as described in 5 may be used to locate the tonsils. The crypt is the landmark for the tonsillar lymphoid tissue subjacent (deep or submucosal) to the crypt.
7. Place one tonsil into a jar of formalin; place the other in a resealable bag, and then into the bag with the other fresh tissues from that animal.

Retropharyngeal Lymph Node (RLN) Collection Procedures

The medial retropharyngeal nodes are medial to the stylohyoid bones on the dorsolateral surface of the pharyngeal muscles and dorsal to the carotid artery. They are medial, deep, and rarely removed by normal processing procedures. The lateral retropharyngeal nodes are found on either side of median line midway between the larynx and the foramen magnum. They are generally smaller than the medial nodes and sometimes remain with the neck.

Tools

1. Sharp boning knife
2. Scalpel
3. Sharp stainless steel scissors
4. Aggressively toothed forceps (rat tooth)
5. Flock owners: Have your veterinarian contact your inspector or the VS Area Office for collection kits and shipping containers.

Procedure

1. The medial retropharyngeal nodes are caudal to the nasopharynx. Place your index finger and thumb in the nasopharynx and the thumb caudally on the caudal pharyngeal muscles to feel the nasopharynx. The opposite node will be about 1 centimeter medial to the first.
2. Dissect both medial retropharyngeal nodes from the surrounding pharyngeal muscles with rat-toothed forceps and scissor, scalpel, or knife.
3. Place 1 medial RPLN into a jar of formalin.
4. Place 2 RPLN (1 medial and 1 lateral) into a plastic bag for chilling or freezing.

Submitting Samples

All **tissue samples** must be submitted to NVSL or an APHIS-contract laboratory. Contact your **Area Office** for the contract laboratory designated for samples from your state.