Scrapie

An overview of the disease and the National Scrapie Eradication Program

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This overview provides Federal and State regulatory personnel with a basic introduction to scrapie and the National Scrapie Eradication Program (NSEP). Since the association between BSE in cattle and variant CJD in humans was made in the mid-1990s, a great deal of research on TSE diseases has been done. Recognizing the importance of eliminating TSE diseases from food animal populations, in the past decade many countries around the world – including the United States – have initiated aggressive eradication programs for scrapie.

**How to Use this Document**

This introduction to scrapie briefly describes the disease and outlines the major elements of the National Scrapie Eradication Program (NSEP). This document is part of the National Scrapie Reference Library. The Reference Library is a collection of the major documents and templates relevant to the NSEP.

Whenever a topic that has been summarized has more extensive information available, the relevant documents in the National Scrapie Reference Library are be referenced so the reader can learn more on the subject.

This document has bookmarks for each section and then subsection for easier navigation. If the bookmarks panel is not already activated, click on the bookmarks icon along the upper left-hand side of the screen to open it.

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Learning Objectives

After reviewing the material covered in this document, you should be able to do the tasks listed below.

1. List the clinical signs associated with classical scrapie.
2. Explain the differences between classical scrapie and Nor98-like scrapie.
3. List the targeted selection criteria for animals sampled in the RSSS.
4. List the tissues submitted for scrapie testing.
5. Distinguish between the Accelerated Scrapie Eradication Program (NSEP) and the Scrapie Flock Certification Program (SFCP).
6. List identification requirements for sheep and goats.
7. Educate producers on how they can order free scrapie tags.
8. List the most susceptible, rarely susceptible, and resistant sheep genotypes to classical scrapie.
9. Provide a broad overview of the steps involved in tracing a positive or exposed animal.
10. Describe the principle elements of a genetic based flock plan.
11. Describe the basic elements of disinfection of contaminated premises.
12. Describe the main components of the National Scrapie Database.
Section One: History of Scrapie in the United States

Scrapie is the oldest known Transmissible Spongiform Encephalopathy (TSE) disease. It was first described in sheep in the United Kingdom in 1732 and in Germany in 1750. Today the disease is widely distributed; it is endemic in many countries in Europe as well as Canada, the United States, Brazil and Japan. There have also been outbreaks of the scrapie in various other countries in Asia, the Middle East, Africa, South America, and in Australia and New Zealand. As of 2012, only Australia and New Zealand are internationally recognized as free of classical scrapie.

Classical scrapie was not always present in the United States. The disease was first observed in a sheep flock in Michigan in 1947. It is believed that the flock became infected through the import of British origin sheep via Canada. In 1952 classical scrapie was identified in two additional States. The U.S. Livestock Sanitary Association (now known as the U.S. Animal Health Association) passed a resolution calling on the Secretary of Agriculture to declare an emergency. As a result, on October 31, 1952 the USDA initiated a national scrapie eradication program. Since that time there have been on-going efforts to control/eliminate the disease from the U.S. sheep and goat populations.

The first case of scrapie in a goat in the United States was reported in 1969, in a goat that had transferred from its herd of origin in Missouri to the scrapie experimental station in Mission, Texas. Although the goat was diagnosed with scrapie in Mission, epidemiological analysis suggested that the animal became infected around the time of its birth in its herd of origin.

In 1992, the scrapie regulations in 9 CFR part 54 and part 79 were revised through a collaborative effort with States, Tribal Nations and allied industry. The changes reinstated a limited indemnity program for producers with infected and source flock, and they also established a voluntary Scrapie Flock Certification Program (SFCP).

In 2000 the USDA, as part of the Lamb 201 industry initiative, made a commitment to eradicate scrapie from the United States based on a number of different factors: one, concerns raised by BSE in cattle; two, the emergence of variant CJD (vCJD) in humans; and three, to address trade disparities with Australia and New Zealand. The Accelerated Scrapie Eradication Program was established through an emergency declaration by the Secretary of Agriculture. The accelerated program, more commonly called the National Scrapie Eradication Program (NSEP), was put into regulation in September 2001. The NSEP retained the SFCP, updated the interstate movement requirements, established rules for addressing infected, source and exposed flocks, established an ongoing indemnity program based on current market prices, and implemented official mandatory individual animal identification for most sheep and some goats moving in interstate commerce. The NSEP also established the Regulatory Scrapie Slaughter Surveillance (RSSS) program, which began in April 2003.

By the conclusion of Fiscal Year (FY) 2011, the NSEP had made significant progress toward eradicating classical scrapie from the United States. The estimated national prevalence had decreased 85 percent, from 0.2% in 2002-3 to 0.03%; furthermore, since FY 2005 there had been an approximately 33% average annual decrease in the number of newly detected scrapie infected and source flocks (179 new infected/source flocks in FY 2005, down to 15 new infected/source flocks in FY 2011).
Section Two: Basics of Scrapie Disease

Species Affected
Under natural conditions, scrapie affects sheep and goats.

The disease has been identified in mouflon sheep, so it is believed that wild species of sheep and goats can also be infected with scrapie. For regulatory purposes, then, USDA considers all species of captive or domestic sheep and goats in the genus Ovis or Capra subject to regulation in interstate commerce (see 9 CFR part 54 and part 79 and VS Memo 557.7).

Other animal species have been experimentally infected by inoculation, usually by intracerebral or intraperitoneal injection although some experiments have shown transmission by oral route.

Zoonotic Potential
Currently, there is no epidemiological evidence that scrapie is a zoonotic disease. Consuming or working with sheep or goats or their products does not appear to cause scrapie disease in humans.

Etiologic Agent
The exact nature of the agent underlying TSEs is still under investigation. However, the most widely accepted theory is that the disease is caused by an infectious protein, or prion. According to the prion hypothesis, a prion is an abnormal isoform of the normal cellular prion protein that has the ability to convert the normal protein into the abnormal form.

All mammals have a normal version of the cellular prion protein, PrPC. The function of the protein is still being characterized, though it may play multiple supporting cellular roles including repair and signaling. According to the prion hypothesis, when the infectious prion, PrPd, is ingested or is otherwise introduced into the body it interacts with the PrPC host’s proteins, changing their three dimensional structure to match its own.

PrPC is normally digested and recycled by the cell. PrPd is resistant to proteinase, so it cannot be broken down by normal cellular processes. When PrPC is converted to PrPd, it becomes capable of converting additional PrPC. As a result, PrPd accumulates in the cell, eventually leading to cell death. In addition to being resistant to cellular degradation processes, prions are also resistant to other host defenses. Prions are extremely resistant to degradation in the environment and can withstand exposure to extreme temperatures, mild to moderate acidic and basic environments, and radiation, presenting a challenge to environmental decontamination.

Transmission
The most common natural route of transmission is believed to be through oral ingestion of the agent, though it can enter the body through other means, such as ocular exposure or contact with abraded skin or mucous membranes. The scrapie agent is thought to spread primarily from infected ewes/does to their offspring and other lambs/kids, and less frequently to adult sheep/goats, through contact with the placenta, birth fluids, and/or contaminated lambing/kidding areas at or shortly after parturition.

1 In addition to mammals, the prion protein has been found in various species in all other vertebrate classes and various species of fungi.
In the U.S. sheep population, black-faced meat breeds such as Suffolk, Hampshire and Shropshire have historically had a significantly higher prevalence of classical scrapie than white-faced wool breeds. White-face meat breeds such as Southdown, Babydoll Southdown, and Montadale may also have a higher prevalence. This breed predilection may be attributed to differences in management practices between these breeds (intensive lambing in small farm flocks verses pasture lambing in range flocks), and not genetic susceptibility.

The average age of onset of clinical signs of scrapie is 40-44 months. It is an insidious disease with a 2 to 5 year incubation period, during which time an infected ewe may be shedding the scrapie agent before showing clinical signs. The disease can thus spread to other animals and other flocks before clinical signs are observed in the first infected animal.

Genetics and age at exposure also play a role in susceptibility to infection and the incubation period. Animals infected by classical scrapie at or near birth typically die by 72 months of age and usually between 36 and 48 months of age. With the exception of valine-dependent type classical scrapie, animals that are infected after weaning typically die at more than 72 months of age.

Both male and female sheep and goats can be infected with scrapie. Currently, though, there is little evidence that male sheep or goats or spayed females transmit scrapie to other animals. However, rams contribute to the susceptibility or resistance to classical scrapie in their offspring, based on their genetics.

Section Three covers the genetics of scrapie in detail, as it has a significant impact on susceptibility to the disease, NSEP flock investigation and cleanup strategies, and movement restrictions on exposed animals.

**Clinical Signs**

- Weakness of any kind, not including those with visible traumatic injuries, and no other sign of scrapie. Signs of weakness may include:
  - Stumbling
  - Falling down
  - Difficulty rising
- Significant weight loss, despite retention of appetite, in an animal with adequate dentition
- Increased sensitivity to noise and sudden movement
- Tremors
- Star gazing
- Head pressing
- Bilateral gait abnormalities, not including abnormalities involving only one leg or one front and one back leg. Signs of gait abnormalities may include:
  - Incoordination (ataxia)
  - High stepping gait of forelimbs
  - Bunny-hop movement of rear legs
  - Swaying of hindquarters
- Repeated intense rubbing accompanied by bare areas or damaged wool in similar locations on both sides of the animal’s body or, if on the head, both sides of the poll
• Abraded, rough, thickened, or hyperpigmented areas of skin in areas of wool/hair loss in similar locations on both sides of the animal’s body or, if on the head, both sides of the poll
• Other central nervous system (CNS) signs
• Death

**Note:** Not all scrapie-affected animals show all clinical signs. In addition, animals in the early stages of the disease may not show any clinical signs.

**Differential Diagnoses**
Other diseases to rule-out include abscesses or other masses in the central nervous system (CNS), viral and bacterial CNS infections such as rabies and *Listeria monocytogenes*, thiamine deficiency, excessive grain intake, lead or other poisoning, and pregnancy toxemia. Any sheep or goat over 12 months of age that died after displaying neurological signs should have tissues submitted for scrapie testing.

**Note:** any time an animal is examined for CNS abnormalities, ensure that special precautions are taken to avoid exposure to rabies.

**Diagnosis**
Historically, a suspect scrapie case was confirmed by suggestive – but not pathognomonic – changes seen on histology of brain and brainstem tissues. The current confirmatory test for scrapie is IHC on CNS and lymphoid tissue. At present, there are no other reliable methods for detecting scrapie in the preclinical animal; however, researchers are investigating the possibility of testing preclinical sheep and goats for scrapie using blood, nasal swabs, urine, and retina analysis.

Testing can be performed on both live and dead animals.

Central nervous system tissues have the highest level of infectivity. Tissues with a moderate level of infectivity include lymphoid tissues (including the spleen), peripheral nerves and the placenta. Those with a low level of infectivity include blood, milk, muscle, liver, nasal mucosa and salivary gland. Low levels of the agent have also been detected in urine, feces, semen and saliva. The presence of inflammation may increase infectivity of certain tissues and fluids, such as milk (mastitis) and urine (nephritis).

**Progression and Treatment**
Once clinical signs manifest, the animal worsens and succumbs within 2 weeks to 6 months, rarely longer. **Note:** some animals die suddenly before the onset of clinical signs.

Like all other TSEs, scrapie is always fatal; there is no treatment or vaccine.

Although scrapie is not considered a zoonotic disease, the carcass of a scrapie infected animal cannot enter any food chain, human or animal. Occasionally, infected animals and their offspring are purchased and moved under permit to a research facility for study. Section Six has additional details about flock cleanup plans and post exposure management and monitoring plans for scrapie infected and source flocks.

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2 The agent has been detected at low levels in these using sPMCA technology and transgenic mice bioassay.
Prevention

The most effective method of preventing scrapie from being introduced into a flock is to maintain a closed ewe flock.

If a flock’s management strategy requires ewes to be brought in, the flock owner can reduce the chances of introducing scrapie into the flock through one of these two following methods:

1. Buy ewes of known background where the producer can be confident that the flock is free of scrapie, such as Export Certified flocks from the SFCP (or equivalent if from a foreign country); or
2. Buy ewes of resistant genotypes.
Section Three: Genetics of Scrapie

The cellular prion protein is encoded by the prion protein gene (PRNP). It is made up of 256 codons that instruct cells to build a prion protein, PrP\textsuperscript{C}, which is comprised of 256 amino acids. The specific sequence of these 256 amino acids ultimately determines the three-dimensional structure of PrP\textsuperscript{C}. The genotype inherited can affect susceptibility to scrapie. In sheep, various point mutations in the PRNP gene can increase or decrease the likelihood that the host's PrP\textsuperscript{C} will be converted to PrP\textsuperscript{D} if the host is exposed to the scrapie agent.

**Major codons associated with scrapie in sheep**

**Codon 171** is the major determinant of classical scrapie susceptibility in the United States. It plays a role in sheep susceptibility to major type of classical scrapie found in the U.S., valine-independent scrapie.
- It codes for the amino acids Glutamine (Q), Arginine (R), Histidine (H) or, rarely, Lysine (K).
- Q is associated with increased susceptibility to classical scrapie, while R is associated with increased resistance.
  - H is considered to have the same susceptibility as Q, and the relative susceptibility of K is still under investigation. Therefore both H and K are reported as Q by most laboratories and are treated as Q for regulatory purposes.
  - Over 99 percent of the classical scrapie cases in the U.S. that have been genotyped were QQ.

**Codon 136** plays a role in sheep susceptibility to the less common type of classical scrapie found in the U.S., valine-dependent scrapie.
- It codes for the amino acid Alanine (A), Valine (V) or, rarely, Threonine (T).
- V is associated with increased susceptibility to classical scrapie.
- The occurrence of T is extremely rare and as of September 2012 has never been reported in the U.S. Because its role in susceptibility is unknown, if it were identified in U.S. sheep it would be reported by most laboratories as V and treated as V for regulatory purposes.

**Codon 154** plays a minor role in classical scrapie and is not often used in the United States as part of a breeding or regulatory strategy. It is, however, associated with increased susceptibility to Nor98-like scrapie (see Section Four).

**Codon 141** is associated with increased susceptibility to Nor98-like scrapie (see Section Four). Its role in classical scrapie, if any, is unknown.

**Codon 112** also plays a minor role in classical scrapie, although its association with classical scrapie resistance is still under investigation.

Each parent contributes one copy of their PRNP gene to the lamb. The contributed gene can be any of these three 136/171 combinations: AQ; VQ; and AR.\textsuperscript{3} Each lamb thus inherits two copies of PRNP, and the possible combinations are: AA QQ; AA QR; AA RR; AV QQ; AV QR; and VV QQ. Table 1 illustrates the susceptibility of these genotypes.

\textsuperscript{3} Q can be Q, H or K; and 136 T and VR are not included because they are extremely rare have not been reported in the United States.
### Table 1: Offspring susceptibility to classical scrapie

<table>
<thead>
<tr>
<th>Ewe (136/171)</th>
<th>Ram (136/171)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AQ</td>
<td>VQ</td>
</tr>
<tr>
<td>AQ</td>
<td>AAQQ</td>
<td>AVQQ</td>
</tr>
<tr>
<td>VQ</td>
<td>AVQQ</td>
<td>VVQQ</td>
</tr>
<tr>
<td>AR</td>
<td>AAQR</td>
<td>AVQR</td>
</tr>
</tbody>
</table>

[Highly Susceptible] [Rarely Susceptible] [Resistant]

In the U.S., susceptibility to most strains of classical scrapie is determined by codon 171, with the QQ genotype being most susceptible and the RR genotype being resistant. As of FY 2011, the following can be summarized about classical scrapie cases and genotypes in the U.S.:

- Highly susceptible genotypes have made up over 99 percent of U.S. classical scrapie cases.
- Rarely susceptible genotypes have made up less than 1 percent of U.S. classical scrapie cases.
- There have been no cases of classical scrapie in resistant genotype sheep in the U.S.

### Role of genetics in sheep exposed to different scrapie types

**Valine Independent Scrapie**

This is the most common scrapie type in the U.S. It is mediated by codon 171.

- AAQQ, AVQQ, VVQQ sheep are highly susceptible.
- AAQR and AVQR sheep are rarely susceptible.
- AARR sheep are nearly always resistant to the most common U.S. scrapie type

**Valine Dependent Scrapie**

This is an uncommon type of scrapie in the U.S. It is mediated by codon 136.

- AVQQ, VVQQ, AVQR sheep are susceptible.
- AAQR, AARR, AAQQ sheep are believed to be resistant.

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4 As of September, 2012, only 2 confirmed cases of classical scrapie in genetically resistant sheep have been identified in the world, both in Europe; 1 unconfirmed case has been reported in Japan.
**Ewe genotypes and placenta genotypes:** The genotype of the placenta affects whether or not an infected ewe can transmit the disease via the placenta/birth fluids.

- QQ placentas from infected QQ ewes have been positive for scrapie.
- No AAQR placentas (pregnancy with only AAQR offspring) from infected QQ ewes have been positive for scrapie.

**Genotyping**

Genotyping is the determination of the genetic composition of an individual. In the context of scrapie, it is a test used to identify which codons are in a sheep’s prion gene. The test is used to determine the animal’s susceptibility to classical scrapie, confirm the animal’s identity, or both.

Genotyping can be performed on live animals using whole blood in purple top tubes or on FTA cards, or on tissue from an ear punch. To qualify as an official genotype test, the following requirements must be met:

1. The sample must be obtained by either an accredited veterinarian or a State or Federal animal health official;
2. The test must be conducted by a laboratory approved by APHIS to conduct scrapie genetic susceptibility testing;
3. The animal must be officially identified; and
4. The sample must be submitted on using VS Form 5-29 or the electronic equivalent.

Genotyping can also be performed on dead animals, usually using fresh or formalin fixed tissue.

**Goats and scrapie susceptibility/resistance**

At this time, no resistant genotypes have been sufficiently documented in goats to either be useful to producers or used for regulatory purposes. However, in recent years numerous polymorphisms have been found in goats, and evidence is accumulating that some of these polymorphisms may either confer genetic resistance or prolong the incubation time.
Section Four: Classical vs. Nor98-like Scrapie

In 1998, Norway detected cases of scrapie with clinical and histopathologic characteristics that did not match previously identified cases. These cases were eventually given their own disease name, Nor98 scrapie. Since 1998, almost every country in the European Union, as well as the Falkland Islands, New Zealand, Canada, Australia and the United States have found similar cases. They have been referred to as “atypical scrapie”, “Nor98 scrapie”, “Nor98-like scrapie”, and “nonclassical scrapie” in the literature. In the United States, the preferred name is Nor98-like scrapie. The first cases in the United States were identified in 2007. Between 2007 and September, 2012, there have been a total of 13 cases identified in the U.S. Only two of these cases have had clinical signs.

Nor98-like scrapie and classical scrapie are separate diseases with distinct features.

Table 2: Distinct features of classical scrapie and Nor98-like scrapie

<table>
<thead>
<tr>
<th>Feature</th>
<th>Classical Scrapie</th>
<th>Nor98-like Scrapie</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transmission</strong></td>
<td>Transmitted to other sheep and goats under natural conditions</td>
<td>May be a sporadic disease of sheep and goats that is either not transmitted or transmitted at levels too low to sustain infection under natural conditions</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td>• Not reported in all countries where TSE surveillance is done</td>
<td>• Small number of cases found in most countries where TSE surveillance is done</td>
</tr>
<tr>
<td></td>
<td>• Cases often occur in clusters</td>
<td>• Cases widely distributed and proportionate with sheep and goat populations</td>
</tr>
<tr>
<td><strong>Number of affected animals in the flock or herd</strong></td>
<td>Usually infects more than one animal in a flock or herd(^5)</td>
<td>Rarely found in more than one animal in a flock or herd, and when an additional animal is found the flock size is usually 500 or more sheep</td>
</tr>
<tr>
<td><strong>Average age of onset of clinical signs</strong></td>
<td>3-5 years of age</td>
<td>Greater than 5 years of age</td>
</tr>
<tr>
<td><strong>Influence of genotype sheep</strong></td>
<td>Mediated by codons 136 and 171: AARR are resistant; AAQR and AVQR are less susceptible; and AAQQ, AVQQ and VVQQ are highly susceptible</td>
<td>Mediated by codons 141 and 154: F at 141 and/or H at 154 appear to be more susceptible; and RR at 171 does not convey resistance</td>
</tr>
<tr>
<td><strong>Clinical signs</strong></td>
<td>Clinical signs commonly, though not always, observed</td>
<td>• Clinical signs rarely observed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• When observed, almost indistinguishable from those in classical scrapie – exception is an absence of intense rubbing</td>
</tr>
<tr>
<td><strong>Laboratory findings</strong></td>
<td>Western blot and IHC readily distinguish classical and Nor98-like scrapie</td>
<td></td>
</tr>
</tbody>
</table>

\(^5\) In the U.S., approximately 6-7% of the mature, genetically susceptible sheep in an infected/source flock are infected.
In 2009, the World Animal Health Organization (OIE) recognized Nor98-like scrapie as a separate disease from classical scrapie because of differences in laboratory findings, transmissibility, and distribution. This determination means that Nor98-like scrapie is not a reportable disease to OIE, and should be of no trade concern.

**What is APHIS’ policy regarding Nor98-like scrapie?**
APHIS does not require the depopulation or movement restriction of Nor98-like scrapie exposed sheep and goats. APHIS will propose changes to the Code of Federal Regulations (CFR) in FY 2013 to allow the APHIS Administrator to eliminate or reduce post exposure requirements for certain types of scrapie—such as Nor98-like scrapie—and that are determined to pose minimal risk of lateral transmission under natural conditions.

In the meantime, APHIS is conducting a national scrapie control pilot project for Nor98-like scrapie. The project is for flocks and herds in which Nor98-like scrapie-positive animals were born, lambed, or kidded.

**What does the pilot project do for producers?**
The pilot project allows producers who have sheep and goats exposed to Nor98-like scrapie to retain, sell, exhibit or move them for any purpose.

For specifics on how APHIS currently manages flocks that have sheep and goats exposed to Nor98-like scrapie, refer to the following documents on the National Scrapie Reference Library:
1. VS Memo 557.21
2. Nor98-like (Nonclassical) Scrapie Flock Plan and Post Exposure Monitoring and Management Plan Agreement template
Section Five: Diagnosing Scrapie

Detecting scrapie infection can be a challenge due to the following aspects of the disease:
- Scrapie has a prolonged incubation period;
- Infected animals rarely display clinical signs during the early stages; and
- The only diagnostic tests currently available require brain or lymphoid tissue.

Testing can be done on both live or dead sheep and goats. In dead animals, brain tissue and lymphoid tissue can be examined. In live animals, lymphoid tissue from the rectum, third eyelid, lymph nodes and tonsil can be examined. Currently, the preferred and most common scrapie live animal test used in the U.S. is the rectal biopsy.

Diagnostic tests on tissues include histological evaluation (brain), immunohistochemistry (IHC), Western Blot and ELISA.

Tables 3 through 5 below indicate which tissue samples to collect from sheep and goats, depending on the animal’s status at the time of collection.

| Table 3. 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. This will allow DNA verification if necessary.) |  |

| Table 4. Tissue specimens for exposed animals or animals with less specific signs6 |
|---|---|
| **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. This will allow DNA verification if necessary.) | Cerebellum (in its own re-sealable bag labeled with the animal’s official identification and “Genotype testing”) |

6 *Animals with "less specific signs"* include those that are: nonambulatory, ante-mortem condemned, die before slaughter, unthrifty, or exhibit wool/hair loss suggestive of rubbing, biting at the legs or side, lip smacking, or intense rubbing without bare areas.
Table 5. Tissue specimens for suspect animals and test positive animals

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

*Place the 3 re-sealable 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 6 below summarizes the different tests for scrapie used in the United States, including the tissue used for the test, whether the tissue should be formalin fixed or fresh, the advantage of the test, and any additional notes about the test.

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Suspect animals are highly suspicious for scrapie because they are exhibiting CNS signs and/or 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 required, 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.
### Table 6. Diagnostic tests for scrapie

<table>
<thead>
<tr>
<th>Test</th>
<th>Tissue*</th>
<th>Fresh or Formalin Fixed</th>
<th>Advantage</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>Brain and Lymphoid Tissues</td>
<td>Formalin Fixed</td>
<td>Definitive test. Very specific for abnormal prion protein. Can differentiate between classical and Nor98-like scrapie.</td>
<td><strong>Most common ante mortem scrapie test used in the U.S.!</strong> The IHC test must have fixed tissue that is not autolysed so the obex can be identified.</td>
</tr>
<tr>
<td>Western Blot</td>
<td>Brain and Lymphoid Tissues</td>
<td>Fresh</td>
<td>Definitive Test. Can also differentiate between classical and Nor98-like scrapie.</td>
<td>Labor and costs are higher than IHC. Autolysis is acceptable as long as sample contains the obex.</td>
</tr>
<tr>
<td>Histology</td>
<td>Brain</td>
<td>Formalin-Fixed</td>
<td>When lesions are present, easily located and mapped.</td>
<td>Stain not specific for abnormal prions, lesions do not always occur, and the presence of lesions is not pathognomonic for a TSE.</td>
</tr>
<tr>
<td>ELISA</td>
<td>Brain and Lymphoid Tissues</td>
<td>Fresh</td>
<td></td>
<td>Rarely used for scrapie diagnosis. Suitable for high throughput screening, but not considered optimal for definitive diagnosis.</td>
</tr>
</tbody>
</table>

* Note: Brain, as used in this table, includes the brainstem (obex).
Section Six: National Scrapie Eradication Program

The purpose of the National Scrapie Eradication Program (NSEP) is to:

1. Eradicate classical scrapie from the sheep and goat population of the United States; then
2. Document the eradication of classical scrapie; and
3. Achieve scrapie-free status for the United States, as describe by the World Organization for Animal Health (OIE).8

The NSEP has two major components: a mandatory eradication program called the Accelerated Scrapie Eradication Program (ASEP), and a voluntary certification program called the Scrapie Flock Certification Program (SFCP).

Accelerated Scrapie Eradication Program

The Accelerated Scrapie Eradication Program (ASEP) is the main element of the NSEP. It is mandatory for all sheep and goat producers in the United States, and it has reduced classical scrapie prevalence in the U.S. by over 85 percent since FY 2003.

Animal Identification

In September 2001, the scrapie regulations were revised to require the official identification of sheep and goats not in slaughter channels (except low-risk commercial goats) and any sheep over 18 months of age in interstate commerce – with some exceptions. In addition, the revision required States to implement and enforce official identification of most sheep and goats on change of ownership intrastate in order to move sheep and goats interstate with minimal restrictions.

To assist with identification requirement compliance, APHIS provides free official ID tags to producers. Producers can call 1-800-USDA-TAG (1-800-873-2824); they will be directed to their VS Area Office, and the office will order the tags for them. Note: producers can also choose to purchase a different kind of eartag from any of the APHIS approved tag companies listed at http://www.aphis.usda.gov/animal_health/animal_diseases/scrapie/approved-tag-co.shtml.

Appendix One has two charts showing what types of sheep and goats require official ID under the current Code of Federal Regulations (CFR).

For more information about the animal ID requirements in the scrapie program, refer to the following documents in the National Scrapie Reference Library.

1. 9 CFR 79.2 and 79.3
3. Market Rule: Interstate Movement of Sheep and Goats
4. Official sheep_goat ID Feb2010

8 To meet OIE requirements to be considered free of scrapie, the U.S. must not have a case in a sheep or goat for seven continuous years, and during that time a sufficient number of sheep and goats must be tested to be able to detect scrapie if it exists at a prevalence of 0.1 percent with 95% confidence.
Surveillance
Infected sheep and goats are identified through active slaughter surveillance, reporting of suspect animals by producers and accredited veterinarians, testing of mature sheep or goats that die on farm or at other locations, and live-animal testing of higher-risk animals.

The active surveillance component of the ASEP started in April, 2003, with the implementation of the Regulatory Scrapie Slaughter Surveillance (RSSS) program. RSSS consists of sample collection from mature sheep and goats, meeting targeted selection criteria, sent to participating slaughter facilities. The program also samples dead, disabled, or suspect animals found at concentration points for mature sheep or goats, including markets and cull ewe feedlots.

The RSSS targets mature sheep and goats that meet specific criteria based on age, face color and/or clinical signs at slaughter. Appendix Two lists the targeted selection criteria for RSSS.

The ASEP obtains samples from additional surveillance streams. Currently, nonslaughter surveillance primarily includes the following:
- Exposed and potentially exposed animals identified through trace investigations from infected/source flocks;
- Purchased sheep or goats in source/infected flocks that are depopulated;
- Clinical suspects;
- Mature animals submitted for necropsy to diagnostic laboratories;
- Animals submitted to public health laboratories that test negative for rabies;
- Animals tested for scrapie as part of the SFCP (see below); and
- Samples collected through voluntary on-farm surveillance of animals that die on farm or QQ sheep in flocks with risk factors for scrapie (ante mortem testing).

For more information about surveillance, refer to the following documents in the National Scrapie Reference Library.
1. National Scrapie Surveillance Plan, September 2010
2. Scrapie UM&R, Part VI "Monitoring and Surveillance"
3. VS Memo 557.11
4. Surveillance Toolbox Folder
Tracing Positive and Exposed Animals
When a positive sheep or goat is identified through slaughter or other surveillance streams, trace investigations (forward and backward) are initiated to identify additional infected and source flocks. These investigations involve identifying the flock(s) that may have been exposed to an infected animal or were the source of the infection for that animal.
Mandatory identification requirements have been very successful in sheep; since 2003, approximately 85% of animals that test positive have been successfully traced back to their flocks of origin/flocks of birth.

Flock Cleanup
When a trace identifies a new infected or source flock, the flock is placed under movement restrictions (quarantine) and the State’s Designated Scrapie Epidemiologist (DSE) works with the flock owner to develop a flock cleanup plan. The cleanup plans most commonly used are the genetic flock plans. All plans have the key requirements listed below.

- Official individual identification of all sheep and goats within the flock using official eartags.
- Establishment and maintenance of records according to specified guidelines.
- Genotyping of all sheep in the flock by an APHIS or State representative.
- Provide State or APHIS representatives access to the animals and necessary records to complete tracing of exposed animals.
- Removal and destruction of high-risk, suspect and scrapie-positive sheep and exposed goats in the flock.9
- Cleaning and disinfection of the premises.

When an infected or source flocks completes all the requirements, the flock is placed on a Post Exposure Management and Monitoring Plan (PEMMP) for at least five years. During this period, flock owners follow a specified set of requirements including the following: completing an annual inspection; officially individually identifying all animals in the flock; maintaining records on all animals, including new additions; reporting deaths and/or suspect animals; and moving restricted animals only under conditions specified in the PEMMP.

9 The animals removed can vary. Genetic plans are available that allow for retention of some genetically susceptible sheep or goats, with additional movement restrictions placed on the flock.
APHIS provides the following assistance to owners of exposed and infected/source flocks or herds that participate in cleanup plans, including owners of exposed animals that have been sold out of infected and source flocks or herds:

- Indemnity for high-risk, suspect, and scrapie-positive animals and exposed goats that owners agree to destroy;
- Genetic testing of exposed and potentially exposed sheep; and
- Scrapie testing on live or dead animals.

A flock in which a confirmed positive scrapie animal either lambed or was born is designated as either infected or source and placed under a flock clean-up plan. There are three basic steps in the plan:

1. All sheep in the flock are genotyped to determine the genetic susceptibility to classical scrapie (See Section Three);
2. Exposed genetically susceptible sheep and all exposed goats are either removed and properly disposed of or their movement is restricted; and
3. The flock is placed under surveillance for at least five years.

Once all suspect and test positive animals and, depending on the flock plan, the genetically susceptible exposed ewes and all exposed female goats have been removed, the premises must be cleaned and disinfected to complete the flock clean-up plan. All contaminated and potentially contaminated areas and materials on the affected premises should be decontaminated using the following recommendations.

- **Pastures**
  - If practical, till soil under or do not use the area to graze susceptible animals.
  - If this is not practical, do not use the pasture until the animal waste has decomposed and the weather has had an opportunity to dilute infectivity.

- **Dry lots**
  - Remove the manure and bedding and, when practical, the top 1 to 2 inches of soil to reduce contamination.
  - Bury or till under the removed material; or, compost the removed material in areas not accessed by domestic or wild ruminants until it can be buried or tilled under.

- **Earth surfaces inside structures or used for confined lambing pens**
  - Remove the organic material and, when practical, the top 1 to 2 inches of soil to reduce contamination.
  - Bury or till under the removed material; or, compost the removed material in areas not accessed by domestic or wild ruminants until it can be buried or tilled under.

- **Non-earth surfaces**
  - These include cement, wood, metal, tools, equipment, instruments, feed, hay, bedding, and other materials.
  - Remove all organic material. Bury, incinerate, or compost the removed material in areas not accessed by domestic or wild ruminants and then till under, bury or incinerate.
  - When practical for other items, bury or incinerate by high-temperature incineration methods.
  - Clean and wash surfaces and remaining items using hot water and detergent. Allow all surfaces, tools, and equipment to dry completely before disinfecting and sanitizing using the following suggested methods.
Autoclave instruments, small tools, and other items at 136 °C (277 °F) for 1 hour. (This method is more effective when preceded by the treatment described in the following two points.)

To clean dry surfaces, apply a 2 percent available chlorine solution (equivalent to 20,000 p/m available chlorine):
- Add 50 ounces (6 ¼ cups) 5.25% bleach to 78 ounces water (9 ¾ cups) to give 1 gallon of solution; and
- Apply the solution at room temperature (at least 18.3 °C [65 °F]) for 1 hour.

To clean dry surfaces, apply 1-molar solution of sodium hydroxide:\n- Make an approximately 4-percent solution [5 ounces sodium hydroxide dissolved in 1 gallon water]]; and
- Apply the solution at room temperature (at least 18.3 °C [65 °F]) for 1 hour.

Note: bleach is caustic and can be hazardous if swallowed, gets in the eyes, is breathed in, or is left on the skin. Additionally, 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 or hire trained personnel to do the work.

For more information about flock cleanup and indemnity, refer to the following documents in the National Scrapie Reference Library.
1. 9 CFR 54.3-54.8, Scrapie Indemnification Program
2. Scrapie UM&R, Part VIII “Flock Cleanup Plans”
3. VS Memo 534.1, “Compensation: Procedures, Appraisal and Indemnity Claim”
5. Scrapie Disinfection Guidelines, June 2007

Education and Prevention
The NSEP staff, in collaboration with field personnel and State and industry partners, provides education on the disease, prevention, and the eradication program. The APHIS website is a resource for program documents. APHIS partners with the National Institutes of Animal Agriculture and the American Sheep Industry Association to create, update, and disseminate flyers, brochures, and guidebooks on scrapie and the NSEP to producers and private veterinarians, in both English and Spanish. VS field personnel provide direct education to producers as well as sheep and goat markets, dealers, slaughter plants and fairs. VS is also represented at sheep and goat industry meetings throughout the year, providing program and research updates.

See the “Additional Resources” section for these and other educational resources available to USDA personnel, State partners, and the public.

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\(^{10}\) Synonyms for sodium hydroxide are caustic soda, soda lye, and sodium hydrate.
Scrapie Free Flock Certification Program (SFCP)

The SFCP is a voluntary program that was started in 1992 to identify flocks that have been free of clinical evidence of scrapie over specified time periods, with the ultimate objective of a participating flock to become certified as having negligible risk for scrapie. Any flock owner may apply to enter the SFCP. Participants in the program follow specified reporting, record keeping, access, and sampling requirements.

The SFCP has three categories: Export, Complete and Select. The Export category has two statuses (Export Monitored and Export Certified). The Complete category also has two statuses (Completed Monitored and Certified). The Select category has one status (Select Monitored).

Export and Complete Categories
When accepted into the Complete or Export categories, flocks are assigned an enrollment date and a status date. Initially, the flock’s status date is the same as the enrollment date and will be maintained if the flock continues to meet all program requirements. The older the status date, the longer the flock has been meeting program standards.

When a Complete Monitored flock has met SFCP standards for 5 consecutive years, it is eligible to apply for advancement to Certified status. Sheep or goats from Certified flocks are considered less likely to be infected with scrapie.\(^{11}\) When an Export Monitored flock has met SFCP standards for 7 consecutive years, including meeting sampling requirements specific for this category, it is eligible to apply for advancement to Export Certified status. Sheep from Export Certified flocks are considered to have a negligible risk of scrapie infection, and because they have met certification standards recommended by OIE they are eligible for export to a greater number of countries.

Selective Category
This category has one status, Selective Monitored. It is open to any flock, but it was designed for slaughter lamb producers to allow for scrapie surveillance in large production flocks. Selective Monitored flocks do not advance to a certified status.

The most current version of the SFCP standards was published in 2007.

Note: The SFCP will be revised in FY 2012 – FY 2013. An updated version of the standards, reflecting the revisions, is due to be published in late fall/early winter, 2012/2013.

For more information about the SFCP, refer to the following documents in the National Scrapie Reference Library.
1. SFCP Standards, June 2007
2. VS Memo 557.21, “Revisions to the SFCP and Export Monitored category” July 2009
3. SFCP Basics Jun2010
4. SFCP Q&A Final 4-09

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\(^{11}\) Scrapie has been identified in 0.5% of Certified flocks after their designation as Certified.
Section 7: Information Collection and Reporting in the NSEP

NSEP stores program information in the National Scrapie Database (NSD). The NSD is a web-based interface for both inputs and outputs, and includes the Animal Health and Surveillance Management (AHSM) system the Animal Identification Management System (AIMS). Access to these systems is limited to approved personnel and external partners. Please contact your Designated Scrapie Epidemiologist and/or Regional Scrapie Epidemiologist if your work assignments require you to access any of these systems.

Animal Health and Surveillance Management (AHSM)

Within AHSM are two scrapie applications: the National Scrapie Generic Database (SNGD), and Veterinary Services Laboratory Services (VSLS).

SNGD

The SNGD stores premises and flock data (including SFCP flock data), information about scrapie disease investigations, and flock statuses. Administrative personnel in the Area Offices and State offices, AHTs and VMOs enter this information.

Users can also query the database for information and generate reports.

VSLS

VSLS stores information and results for scrapie laboratory submissions in three separate modules:
- Slaughter surveillance (RSSS);
- On-Farm surveillance; and
- Scrapie susceptibility genotyping.

State and federal employees and employees of partner laboratories approved to run surveillance and genotyping tests use this system to submit, track and report scrapie tests and genotyping tests.

Animal Identification Management System (AIMS)

The scrapie program tag module of AIMS is used to order scrapie tags; it also stores official identification (eartag) distribution records.

VS personnel enter tag orders into the system. Tag manufactures enter order fulfillment information into the system. Users can also query the database for information and generate reports.

Note: In FY 2013, the SNGD is scheduled to be replaced by the scrapie module in the new SCS (Surveillance Collaboration System).
Section 8: Additional Information

In addition to the National Scrapie Reference Library, additional information about scrapie and the National Scrapie Eradication Program (NSEP) can be obtained at the following sites:

  - The APHIS, VS scrapie webpage provides online access to program documents in addition to program reports, fact sheets, and program contacts.
    - Scrapie Final Rule, 9 CFR part 54 and part 79
    - National Scrapie Eradication Program standards
    - National Scrapie Surveillance Plan
    - Scrapie Free Flock Certification Program (SFCP) standards
    - Monthly and annual program progress reports
    - Flock status reports by breed, status and State
    - Links to sheep and goat industry and health studies

http://animalhealth/scrapie/default.aspx
  - The VS Scrapie SharePoint site provides reference materials on the scrapie program for VS employees.
    - The Scrapie National Reference Library
    - Indemnity Calculators
    - Scrapie bimonthly webinar presentations and files
    - Scrapie scientific articles and reports
    - **Note:** at this time, the scrapie SharePoint site is only available to APHIS employees

http://www.eradicatescrapie.org/
  - The “Eradicate Scrapie” website, a joint project of the USDA and the National Institute of Animal Agriculture, provides educational resources for producers and private veterinarians.
    - Summary of State sheep and goat ID requirements
    - State and Federal contacts
    - Tag companies
    - Educational documents and CDs, some in both English and Spanish
    - Newsroom

http://sheepindustrynews.org/scrapie_guide/
  - Dynamic web presentation of the American Sheep Institute’s “A Producer’s Guide to Scrapie.”

http://www.oie.int/
  - This site provides access to the Terrestrial Code and the Terrestrial Manual, which contain extensive details about scrapie including the disease, diagnostic tests and recommendations for international trade in live animals and animal products.
  - This site also provides information on the scrapie status of member countries.
Section 9: Scrapie Contacts

**Designated Scrapie Epidemiologists (DSEs)**

A current list of DSEs can be obtained at the following address:  

**Regional Scrapie Epidemiologists**

**Dr. Chuck Gaiser**  
*Eastern Region Scrapie Epidemiologist*  
Raleigh, NC  
(919) 855-7271  
charles.n.gaiser@aphis.usda.gov

**Dr. Dianne Norden**  
*Western Region Scrapie Epidemiologist*  
Ft. Collins, CO  
(970) 494-7371  
dianne.k.norden@aphis.usda.gov

**Staff**

**Dr. Diane Sutton**  
National Scrapie Program Coordinator  
Riverdale, MD  
(301) 851-3509  
diane.l.sutton@aphis.usda.gov

**Dr. Alan Huddleston**  
Associate National Scrapie Program Coordinator  
Riverdale, MD  
(301) 851-3497  
alan.r.huddleston@aphis.usda.gov

**Dr. Amie Butler**  
Staff Veterinarian  
Helena, MT  
(406) 461-3454  
amie.f.butler@aphis.usda.gov

**Earl Thorpe**  
Veterinary Program Specialist  
Riverdale, MD  
(301) 851-3432  
earl.t.thorpe@aphis.usda.gov
Appendix One: Sheep and Goat Identification Requirements

**SCRAPIE ERADICATION PROGRAM SHEEP IDENTIFICATION REQUIREMENTS**

- **Scrapie-Positive, Exposed, High-Risk, and/or Suspect**
  - Movement Restricted
  - APHIS and state veterinarians will provide instructions to owners regarding movement of these animals.
  - Official I.D. Required

- **Non-Positive, Non-Exposed, Non-High-Risk, Non-Suspect**
  - Breeding Stock Sale
  - Sheep and/or Ewe
  - Fed and/or Labeled
  - Under 18 mos. of age
  - Under 18 mos. of age
  - Under 18 mos. of age
  - Under 18 mos. of age
  - Official I.D. Required
  - Official I.D. Required

**General Conditions for Sheep and Goats**
1. The ONLY animals that may be removed from slaughter channels (ewes lambs or doe kids, for example) in interstate commerce are animals that are identified as to their flock of birth.
2. NO animal may be removed from slaughter channels in interstate commerce if it was sold at a slaughter-only auction. This is identified with a tag or ear tattoo marked “meat” or “slaughter only.” It was sold with a bill of sale marked for slaughter only.
3. NO Exposed or HIGH-Risk animal from ANY state shall be removed from slaughter channels once it has entered interstate commerce.

These are regulations according to the Federal Guidelines. Please contact your state veterinarian’s office (see Section 16). Contact information for more information on regulations in your state is available from the USDA. States may have additional ID requirements to check with your state officials and also with officials of states to which you may be shipping your animals for their regulations.

**SCRAPIE ERADICATION PROGRAM GOAT IDENTIFICATION REQUIREMENTS**

- **Scrapie-Positive, Exposed, High-Risk, and/or Suspect**
  - Movement Restricted
  - APHIS and state veterinarians will provide instructions to owners regarding movement of these animals.
  - Official I.D. Required

- **Non-Positive, Non-Exposed, Non-High-Risk, Non-Suspect**
  - All Goats
  - Feeding/Slaughter
  - Breeding Stock Sale
  - Shearing/Exhibition Buck and Doe
  - Low-risk commercial goats
  - Registered or reared with sheep
  - Official I.D. Required
  - Official I.D. Required

**General Conditions for Sheep and Goats**
1. The ONLY animals that may be removed from slaughter channels (ewes lambs or doe kids, for example) in interstate commerce are animals that are identified as to their flock of birth.
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3. NO Exposed or HIGH-Risk animal from ANY state shall be removed from slaughter channels once it has entered interstate commerce.

These are regulations according to the Federal Guidelines. Please contact your state veterinarian’s office (see Section 16). Contact information for more information on regulations in your state as many states have additional requirements above and beyond federal NSSEP ID requirements.

**NOTE:** Feeding as used in this chart is feeding to enhance the animal’s condition for slaughter. Any animal that may be used for breeding must be identified as required for breeding stock.
Appendix Two: Targeted Selection Criteria for RSSS

Targeted Selection Criteria for RSSS

March 2012

1. Is at least one permanent incisor completely erupted (applies to both sheep and goats)?
   - Yes
   - No

2. Is this a black-faced sheep? Or is it a traceable Southdown or Montadale?
   - Yes
   - No

3. Is the sheep or goat displaying clinical signs that are suspicious for scrapie?
   - Yes
   - No
   - Central nervous system (CNS) signs (incoordination, weakness, abnormal behavior, etc)
   - Intense rubbing, abrasions or rough, thickened and/or hyperpigmented skin with bare areas on both sides of the body and/or poll

4. Did this sheep or goat arrive for slaughter on a VS 1-27 permit?
   - Yes
   - No

5. Did the sheep or goat die, was it nonambulatory prior to slaughter, or ante-mortem condemned?
   - Yes
   - No

6. Is the flock ID associated with this animal on the current “Do Not Collect” list?
   - Yes
   - No

7. Is the animal “traceable”?
   - Yes
   - No
   - “Traceable” means that the animal has an official identification number (ID) or is otherwise traceable to its flock of origin, including single source groups.

8. Does the animal have an SSCP tag?
   - Yes
   - No

9. Is the animal a sheep or goat 2, 3, 4, or 5 years based on dentition*, or as directed by the Regional Epidemiologist?
   - Yes
   - No
   - * Do NOT include those with broken, missing, splayed, or severely worn teeth.

Collect Sample

Do NOT Collect Sample