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*A Guide to the
National Scrapie Eradication Program
for Veterinarians*

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This information provided to you by:



National Institute
for Animal Agriculture



Cindy Wolf, DVM
Eileen Kuhlmann



Forward

Dear Veterinarian:

This guide is designed to provide you with information you can use with your sheep and goat clients to bring them up to date regarding scrapie and the U.S. Department of Agriculture's (USDA) National Scrapie Eradication Program (NSEP). Much has been written about the eradication program since the Animal and Plant Health Inspection Services-Veterinary Services (APHIS/VS) program was accelerated in 2001. This guide contains 10 sections. The first five sections of the material focuses on information veterinarians can provide to producers in two main areas: how producers can use genetics to protect their sheep flocks (not goats) from classical scrapie and how genotyping is being used in the eradication program. Sections 6-10 are information and resources for veterinarians. When using the CD you may click on a section below to jump to that section.

Section 1 outlines the basics of classical scrapie genetics. You will find this a handy tool in explaining this complicated subject to your clients including how genetics affect the transmission of the disease.

Section 2 covers general procedures for genotyping for classical scrapie as well as how to conduct official, APHIS-recognized genotype tests when desired by the client or required by APHIS. It includes contact information for approved genotype laboratories as well as an explanation of how to complete the required official VS Form 5-29. This section also covers the use of genetics/genotyping as a flock management tool including the role of scrapie resistant (RR or QR) breeding stock.

Section 3 describes the USDA Genetics Based Flock Clean-up and Monitoring Plans for classical scrapie.

Section 4 contains basic information in a question and answer format about the NSEP program if classical scrapie is found in a flock as well as on the use of genetics as a production management tool.

Section 5 covers one of the most frequently asked questions about the NSEP—"Which categories of sheep need official identification?" There are easy to read flow charts for the national program. Please note that some states have additional ID requirements. It is highly recommended that you check with the State Veterinarian's office for individual state requirements. We also recommend that your clients check with show officials for any additional requirements they might have.

Also, in this section you will find references for what constitutes official identification, required record keeping and specific information on show and exhibition requirements as part of the NSEP.

Section 6 provides guidelines for completing interstate Certificates of Veterinary Inspection (CVIs) for movement of sheep and goats.

Section 7 has Nor98-like scrapie information and references.

Section 8 lists in detail (text) the officially recommended procedures for specimen collection. A pictorial tissue collection guide is included on the CD.

Section 9 describes how to disinfect Scrapie-contaminated areas.

Section 10 lists resources you can use to get more information about scrapie and the eradication program. Also, in this section we have included a directory of whom you should contact for comments, suggestions and/or questions on the NSEP.

Regarding Goats

The occurrence of scrapie in goats is uncommon. In the United States an average of one to two goat herds with positive goat(s) are identified each year. In most cases these goats are or have been commingled with sheep. Each year a few flocks with positive sheep cases also have goats that are exposed. The disease can spread from sheep to goats and on to other goats. Therefore, goats are included in the eradication program. APHIS conducted a prevalence study in 2007 and 2008 to determine the extent of scrapie in goats in the United States. Out of 3,032 goats sampled, the study found no positive goats. Therefore, we can conclude that the scrapie prevalence in goats is greater than 0 and less than 0.1. In addition, little is known about the genetic susceptibility and resistance to scrapie in goats. Researchers have identified a few polymorphisms that may provide resistance and are currently studying whether these will be useful. However, at this time, no resistant genotypes have been sufficiently documented in goats to be useful to producers; therefore, the genetic and genotyping information in this guide applies only to sheep.

Section 1:

Genetic Details

General Information

- Sheep have **one pair** of genes that affects classical scrapie susceptibility.
- This pair of genes is known as **PRNP** genes (**PRioN Protein** genes).
- Each sheep has one pair of the **PRNP**—one copy from each parent.
- In sheep **PRNP** produces a normal cellular prion protein molecule **PrP^c**.
- In infected sheep **PrP^c** is converted to **PrP^{sc}**, scrapie prion protein, the abnormal infectious form of the prion protein molecule.
- PrP^c** exists in all animals with small differences between species.
- PrP^{sc}** makes more of itself by causing misfolding of normal cellular prion protein.
- All** genes, including PRNP, are made up of **codons**.
- Each codon instructs cells to put a *specific amino acid* at a *particular location* when building a protein molecule.
- Prion normal protein PrP^c (produced by the PRNP gene) has **254** amino acids.
- The locations of the 254 amino acids are numbered **1 to 254**.
- In the PRNP gene, **three** codons affect classical scrapie susceptibility:
 - Codon **171**
 - Codon **154**
 - Codon **136**

Genetics and Susceptibility to Classical Scrapie in the U.S.


- Codon 171** – Is a major determinant of scrapie susceptibility.
 - Codon 171 programs for the amino acids **Glutamine (Q)**, **Arginine (R)** (reported as Q and R respectively), **Histidine (H)**, or **Lysine (K)**: H is considered to have the same susceptibility as Q. The susceptibility of K is unknown and is being investigated. Both H and K are reported as Q by most labs and are treated as Q for regulatory purposes; therefore where Q is referred to in the remainder of this document it includes H or K. Over 99 percent of US scrapie cases that have been genotyped were QQ.
- Codon 154** – Plays a minor role and is NOT OFTEN used in the United States.
- Codon 136** – Affects susceptibility in sheep exposed to some scrapie types.
 - Codon 136 programs for the amino acid **Valine (V)** or **Alanine (A)** and rarely **Threonine (T)**
- The genotypes of sheep in the U.S. are primarily referred to in **two ways**:
 - Using the letters of the amino acids: AA QR, AV QR, etc. (the letter placement is in numerical order codon 136/ codon 171), **OR**
 - Codon number followed by the corresponding amino acids: 171QR, 171RR, etc.

NOTE: In the scientific literature each allele is often listed separated by a slash so a 136AA, 154RR, 171QR sheep would be listed as ARQ/ARR.


- Each parent contributes one copy of their gene to the lamb. The contributed gene can be any of these three 136/171 combinations: AQ, VQ, AR (the combination VR is extraordinarily rare and has not been reported in the U.S.).
- Each lamb thus inherits two copies of this gene. The possible combinations are: AA QQ, AA QR, AA RR, AV QQ, AV QR, VV QQ.

Offspring Susceptibility Table


Ewe (136/171)	Ram (136/171)		
	AQ	VQ	AR
AQ	AAQQ (Highly Susceptible)	AVQQ (Highly Susceptible)	AAQR (Rarely Susceptible)
VQ	AVQQ (Highly Susceptible)	VVQQ (Highly Susceptible)	AVQR (Rarely Susceptible)
AR	AAQR (Rarely Susceptible)	AVQR (Rarely Susceptible)	AARR (Resistant)



Highly Susceptible



Rarely Susceptible



Resistant

NOTE: (Data as of February 2009)

- Highly susceptible genotypes make up over 99 percent of U.S. classical scrapie cases
- Rarely susceptible genotypes make up less than 1 percent of U.S. classical scrapie cases
- Resistant genotype - no U.S. classical cases

Section 2:

Genotyping

At this time, no resistant genotypes have been sufficiently documented in goats to be useful to producers; therefore, the genetic and genotyping information in this guide applies only to sheep. Researchers have identified a few polymorphisms in goats that may provide resistance and are currently studying whether these will be useful.

Genotype testing is used to determine whether a sheep is susceptible to contracting classical scrapie if exposed to the disease. The test determines the sheep's genotype at codon 171, 136, and/or 154. Only test results at 171 and 136 are considered by APHIS in determining whether scrapie testing will be required for sheep in an exposed flock. In most situations 171 is sufficient, but in a few cases 136 would also be needed to avoid further testing where the exposed sheep came from a flock with a positive AV QR, VV QQ or AV QQ. Approximately 6 percent of scrapie positive sheep in the U.S. were one of these genotypes. Genotype test results can be official or unofficial.

What makes a genotype test an official APHIS recognized test?

- A. The test conforms to the following procedures:
 - The blood is drawn by an accredited veterinarian
 - The sheep is officially identified (See Section 5)
 - The sample is submitted with a **VS Form 5-29**
 - The lab has been approved by APHIS

Note: The VS Form 5-29 **MUST** be an original with an APHIS serial number. Also, if testing is required at codon 136, you must note that in handwriting on the form otherwise the lab will only test 171. This form is available from the APHIS Area Veterinarian-in-Charge (AVIC) in your state, see Contact List (Section 10). Please allow ample time for the forms to be sent to your office.

Why would a producer use an official genotyping test?

- A. The value of an official genotype test is that APHIS will recognize the results of the test at any point in time. This means that scrapie-exposed animals that cannot be retested because they are dead or untraceable will be presumed to be the previously recorded official genotype. This is very important to purchasers of sheep that have resistant genotypes that are later determined to be scrapie-exposed. In these instances APHIS will not designate such flocks exposed and will not require testing in those flocks if they can document that the only exposed sheep they received were of resistant genotypes.

- B. If a flock is determined to be an infected flock, a previous official genotype result may be used in lieu of a subsequent official test when determining which sheep need to be removed from the flock.
- C. Owners and buyers can have confidence because official tests are run at USDA approved laboratories and samples are collected by accredited veterinarians from officially identified sheep.

What is an unofficial genotyping test?

- A. A test that does not conform to all of the stipulations above.

Why would producers use an unofficial genotype test?

- A. To reduce the cost of the test by collecting the blood himself/herself in cases where the information is desired strictly to make management decisions.

What are the suggested genotyping procedures?

- A. The blood needs to be drawn into purple top tubes or applied to an FTA card using a new sterile needle for each sheep.
- B. Blood submitted for official genotyping needs to be accompanied by an original VS Form 5-29.
- C. Recommendations regarding the temperature at which the blood sample should be stored and shipped can be found at each lab's website. Note that these recommendations differ by laboratory due to different techniques used for genotyping. Some labs prefer the samples to be cooled immediately and shipped within 72 hours of blood collection, others do not require this type of handling. FTA cards should be allowed to dry before being closed and may be stored and shipped at room temperature.

NOTE: Many producers confuse the fact that genotyping is a test for scrapie susceptibility only and not a test for the disease itself.

To get a current list of APHIS approved labs for official scrapie genotyping go to:
www.aphis.usda.gov/animal_health/animal_diseases/scrapie/

Section 2: **Genotyping**

What is the importance of placental genotype in classical scrapie transmission?

- A. Research has found:
 - All QR placentas that have been examined from infected QQ ewes have been negative for scrapie whereas QQ placentas from infected QQ ewes have been positive.
 - Therefore, QR placenta/birth fluids from infected ewes are unlikely to transmit scrapie to nearby susceptible sheep.
- B. Lambs have a combination of genes from the ewe and from the ram:
 - A QQ ewe bred to an RR ram will produce a QR lamb with QR placenta/birth fluids.
 - A QQ ewe bred to a QQ ram will produce a QQ lamb with QQ placenta/birth fluids.
 - If both ewe and ram are RR, then they will produce an RR lamb with RR placenta/birth fluids.
 - When a QQ ewe is bred to a QR ram, on average, 50 percent of the lambs will be QR and 50 percent will be QQ and will have corresponding placenta/birth fluids.
 - When an RR ewe is bred to a QR ram, on average, 50 percent of the offspring will be QR and 50 percent of the offspring will be RR.
 - If both the ewe and the ram are QR, then on average 25 percent of the offspring will be RR, 25 percent will be QQ, and 50 percent of the offspring will be QR.

What is the role of the ram in classical scrapie transmission?

- A. Rams do get scrapie.
- B. Infected rams are not known to transmit scrapie.
- C. Ram genetics will contribute to scrapie susceptibility or resistance of their offspring.

How can your clients use genotyping as a tool in preventing scrapie?

- A. Genotyping should be considered just one tool, not the only tool.
 - The most effective method of preventing scrapie from being introduced is to maintain a closed ewe flock.
 - If the flock management system requires the producer to bring in ewes, they should either buy ewes of known background where they can be confident that the flock is free of scrapie such as Certified Flocks and/or buy ewes of resistant genotypes.
- B. If the flock type is such that ewes have been purchased from multiple unknown status sources over the years, particularly ewes from high prevalence breeds, then your clients should consider:
 - Starting a proactive genetic selection program to enhance the resistance of their existing flock genotypes.
 - Buying ewes of resistant genotypes.
 - Reducing the risk of transmission from undiagnosed ewes in the flock by using 171 RR rams.
- C. By selecting rams which are RR or QR:
 - RR rams will always sire RR or QR lambs (resistant).
 - QR rams will give an R to 50 percent of their offspring and a Q to the other 50 percent, which results in some lambs being susceptible depending upon the ewe's genotype.
- D. By selecting ewes which are RR or QR:
 - A ewe will pass on her resistance/susceptibility traits only to her lambs, thus affecting far fewer lambs than a ram; therefore, ewe genotyping is far less cost-effective than genotyping rams.
 - Genotyping is practical for only the most important foundation ewes or for ewes the producer is considering introducing into the flock.

171QQ Lamb + Scrapie-infected Placenta = High Probability of a SCRAPIE-POSITIVE Lamb

Section 3:

USDA Genetics Based Flock Clean-up and Monitoring Plans

The National Genetics Based Flock Clean-up Plan

- This plan applies only to producers who have classical scrapie confirmed in their flock (Infected or Source Flocks).
- Flocks exposed to classical scrapie will be placed directly on a Post-Exposure Management and Monitoring Plan (PEMMP). This plan includes testing of exposed sheep and goats and sometimes other flock members to determine the status of the flock. Should scrapie be found the flock would begin the genetics based clean-up plan.
- As of April 2009, if Nor98-like scrapie is found in a flock APHIS typically offers either whole flock depopulation or an exposure-based flock plan. As new scientific information becomes available, this approach may change.

How the Plan Works

A. There are Three Basic Steps in the Plan:

- When an infected flock has been identified, the sheep are genotyped. A sheep's genotype determines its risk for classical scrapie infection.
- Sheep of susceptible genotypes are either removed or their movement restricted.
- The flock is placed under surveillance for five years.

B. Benefits of the Plan:

- In most cases, producers will be able to keep many more of their sheep with a genetic-based plan.
- This plan allows owners to retain or sell without restrictions nearly all of their sheep that are AA RR, AA QR¹ and most of AV QR² from infected or source flocks once owners have met certain conditions.
- It is estimated that, on average, 60 percent of a flock can be preserved when using a genetics-based plan compared to 25 percent when using an exposure-based depopulation plan.

C. Requirements of the Plan:

- All exposed QQ ewes, exposed female goats, and female offspring of scrapie positive ewes will be removed or will be placed under movement restrictions.
- AV QR ewes may be required to be removed or restricted in flocks where positive VV QQ, AV QQ, or AV QR sheep have been identified. Positive animals, with a V at codon 136, in a flock indicate that exposed AV QR ewes from that flock may be susceptible to the type of scrapie that is in the flock.

- All animals in the flock must be officially identified and entered in the USDA's Scrapie National Database by federal and/or state personnel.
- Owners must have a post-exposure management and monitoring plan that includes:
 1. Official identification of sexually intact animals that are sold or acquired and records of such transactions including basic information of buyer/seller.
 2. Reporting of deaths of any mature animals and submission of animals showing signs of scrapie for diagnostic testing.
 3. Annual inspections by state and/or federal officials.
 4. Owners who elect to retain restricted female animals will have to meet additional requirements including testing and restrictions on some offspring.
- D. Other Aspects of the Plan:
 - Owners whose animals must be removed from the flock will receive indemnification from the federal government based on commercial market prices reported by the Agricultural Marketing Service. An additional premium will be paid for registered animals and may be paid for animals for which the owner can document a higher market value such as some club lamb flocks.
 - Further, the federal government will provide testing and assistance with disposal costs. The producer is responsible for gathering and handling the sheep, applying identification, providing adequate handling facilities, cleaning and disinfecting, reporting suspect animals, and maintaining records such as sheep sales, purchases and lambing.

Footnotes:

1. These sheep, AA QR, are restricted only in rare cases when the animal is (a) the female offspring of a female positive animal, (b) a clinical suspect, (c) from a flock with unusually high prevalence, (d) from a flock that has a history of recurrence, or (e) from a flock that included a positive sheep of a resistant genotype.
2. Any AV QR that is likely to have been exposed to a strain to which it is susceptible is restricted.

**This information comes from a brochure for producers, "The ABCs of Genetics Based Flock Clean-up and Monitoring Plans."
This and other scrapie-related information is available at the website, www.eradicatescrapie.org .**

Section 4:

Frequently Asked Questions

Question: With only a few hundred reported classical scrapie cases in the U.S. each year, why is there so much emphasis on eradication?

Answer:

- Classical scrapie costs American sheep producers an estimated \$10 million to \$20 million per year principally in reduced markets for byproducts, lost sales abroad and increased production costs.
- Classical scrapie is a transmissible spongiform encephalopathy (TSE). The eradication of all TSEs is expected by the public and is a responsibility of industry.
- Many cases of scrapie go undetected. The NSEP is proving to be an effective means of controlling this disease.

Question: Do I need to be concerned with the Genetics Based Flock Clean-up Plan?

Answer:

- No, unless a client's flock is categorized as "Infected", "Exposed" or "Source".
 - An infected flock is any flock in which a state or APHIS representative has determined that a scrapie-positive female animal has resided in unless an epidemiologic investigation conducted by a state or APHIS representative shows that the animal did not lamb or abort in the flock. A flock will no longer be considered an infected flock after it has completed the requirements of a flock plan.
 - An exposed flock is any flock in which a scrapie-positive or suspect animal was born or lambed, as well as any flock containing a female high-risk or suspect animal or that once contained such an animal that lambed in the flock. The status may be released by APHIS based on either testing or completion of a monitoring plan.
 - A source flock is a flock in which a state or APHIS representative has determined that at least one animal was born that was diagnosed as scrapie-positive at an age of 72 months or less, or in which a scrapie-positive animal has resided throughout its life.

Question: What management tools should be used on previously exposed flocks that are currently participating in Post-Exposure Management and Monitoring Plans (PEMMP)?

Answer:

- Lambing management - do not house or lamb sick ewes in the lambing barn/area, and provide rapid disposal of placenta and soiled bedding after lambing.
- Select for and use scrapie resistant breeding stock.
- Avoid having goats in the lambing area with sheep.
- Report suspect sheep so that they can be tested for scrapie.

Question: Why should my clients officially genotype their sheep?

Answer:

- To boost customer confidence in the test results.
- APHIS will recognize the results of the test. This means that scrapie-exposed animals that can't be retested will be presumed to be the recorded genotype, and if the exposed sheep is of a resistant genotype, the flock to which it was sold will not be considered an exposed flock and no additional testing will be required by that flock. On the other hand, if a ewe of unknown or susceptible genotype is introduced into a flock, and that ewe is later determined to have been an exposed animal but is no longer available for testing, then all lambs born into that flock from the time that ewe first lambed will require testing. This means that they will be genotyped and those that are QQ will in many cases be third eyelid or rectal biopsied to test for classical scrapie. Further, in some cases euthanasia and testing on necropsy samples will be done including sheep that have insufficient lymph node tissue in their third eyelids for a valid test. Sheep that are euthanized for testing are eligible for indemnity payment. The third eyelid/rectal biopsy testing requirement will limit the producers' ability to market these sheep because the sheep must be held until they are old enough to test (14 months). Also, retesting by third eyelid, rectal biopsy or necropsy is typically required for between 10 percent to 40 percent of sheep tested.

Section 4: ***Frequently Asked Questions***

Question: If a client's sheep do not have classical scrapie, when should I recommend using scrapie genetics as a management tool?

Answer:

- If the flock has a high potential for scrapie because:
 - It is a breed in which scrapie is prevalent and ewes have been purchased of unknown scrapie status.
 - Ewes have been purchased from an infected flock.
 - Signs of scrapie have been detected in the flock in the past.
- Or, their customers request breeding stock to be scrapie resistant.

Question: If a client's flock has potential exposure to classical scrapie through the purchase of ewes, what should I recommend?

Answer:

- Use RR breeding rams to increase the percentage of genetically more resistant sheep in the flock and to prevent shedding of the agent in the birth fluids and placenta of potentially infected ewes.

Question: If Nor98-like scrapie is detected in one of my client's flock, what will happen?

Answer:

- APHIS officials will work with the flock owner to determine the best course of action, which takes into account current regulations and research findings.

Section 5:

Identification Requirements

Official Identification

Is defined as identification approved by APHIS for use in the national scrapie eradication program. Detailed information on official identification including what should be on the tag (flock/herd ID numbers), tag options, use of tattoos, and paint brands, can be found at www.eradicatescrapie.org and click on Educational Resources, then What You as a Producer Need to Know and see Step #2 and #3. For record keeping see Step #4. At the same website a handy, pocket-sized record keeping book titled, National Scrapie Eradication Program Record Guide for Sheep and Goats is available to order or can be downloaded. Your clients may order tags or specific questions may be answered by calling 1-866-USDA-Tag (1-866-873-2824), your AVIC office.

Please note that some states have additional ID requirements. Some states have set more stringent standards for movement of livestock into the state, as well as regulate intrastate movement of livestock for sale or attending shows. It is highly recommended that you check with the State Veterinarian's office for individual state requirements. Contact information for each State Veterinarian's office can be found in Section 10. We also recommend that your clients check with show officials for any additional requirements they might have.

NOTE: Regarding Electronic Implantable Devices (EIDs): EIDs are also known as microchips. Sheep or goats registered with national associations that allow the use of electronic implants for official registry identification may use EIDs as official identification in the Scrapie Eradication Program. Please note that the following conditions must be met:

- The animals are registered with a national registry association;
- The electronic implant number is recorded by the registry on the registration certificate accompanying the animal, and the animal is accompanied by an implant reader that can read the implant in the animal;
- At exhibitions, an implant reader that can read the implant in the animal is available for use by the APHIS or State authorities;
- For an EID to be considered official identification, a reader must be available and if sold registration papers must be transferred to new owner's name; and
- Any standards set by the National Animal Identification System for EID devices and scanners, shall also apply to any device implanted after implementation of those standards. Such animals moved in interstate commerce to a market or are for sale without registration papers, must be identified with visible official identification, such as an official eartag or tattoo.

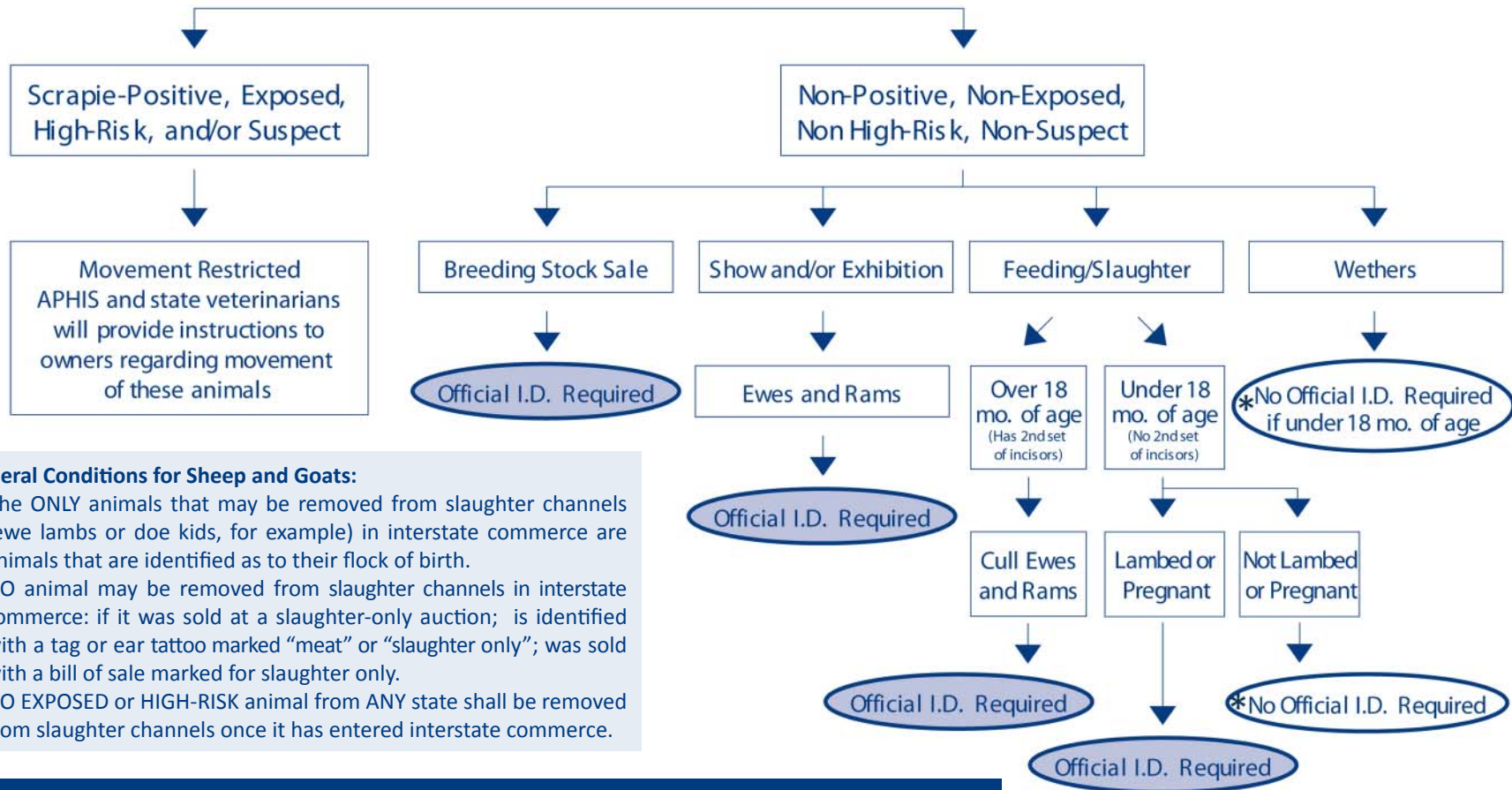
EID/Microchip Placement

The recommended placement of EIDs (microchips) in sheep and goats is on the top of the ear between the skin and the cartilage near where the ear meets the head. FDA considers EIDs to be unapproved food additives. If an animal that has been implanted is sent to slaughter, the Food Safety Inspection Service inspector should be notified of the presence and location of the EID (microchip) device prior to slaughter so that it can be removed from the carcass after slaughter to prevent adulteration of the carcass.

Section 5: **Identification Requirements**

The following charts are a quick reference for official identification of sheep and goats in the National Scrapie Eradication Program:

SCRAPIE ERADICATION PROGRAM SHEEP IDENTIFICATION REQUIREMENTS



General Conditions for Sheep and Goats:

1. The ONLY animals that may be removed from slaughter channels (ewe 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; is identified with a tag or ear tattoo marked "meat" or "slaughter only"; 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.

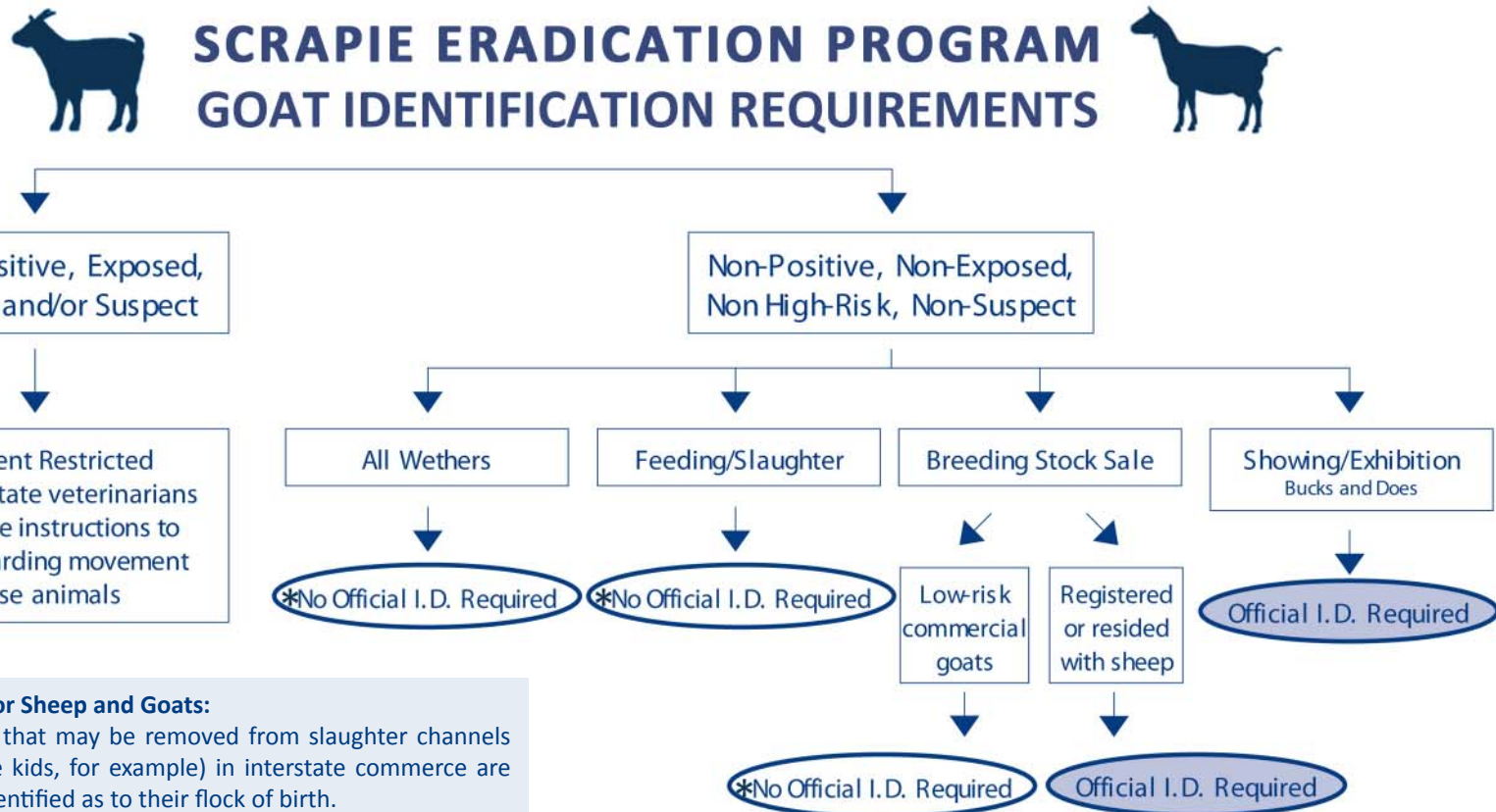
These are regulations according to the Federal Guidelines. Please contact your state veterinarian's office (see Section 10, Contact Information) for more information on regulations in your state as many states have additional requirements above and beyond federal NSEP ID requirements.

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.

* States may have additional ID requirements so check with your state officials and also with officials of states to which you may be shipping your animals for their regulations.

Section 5: **Identification Requirements**

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Section 6:

Certificates of Veterinary Inspection (CVIs) for Sheep and Goats in Interstate Commerce

State importation requirements may vary, so you should check each state's regulations prior to sending the animal.

CVIs are required by USDA for breeding¹ sheep and goats that cross state lines except the following:

- A. Sheep and goats being moved for grazing without change of ownership.
- B. Low-risk commercial sheep that require an owner and veterinarian's statement instead of a CVI.
- C. Low-risk commercial breeding goats.
NOTE: An owner and veterinarian's statement is **not** required for low-risk commercial goats.
- D. CVIs will be considered to meet the intent of the federal regulation if they include:
 - Either the animal's official individual identification number(s) or the flock of origin's scrapie flock number.
 - The number of animals covered by the CVI.
 - The purpose of the movement.
 - The points of origin and destination.
 - The name and address of the consignor and consignee.
 - A statement by the issuing accredited, state, or federal veterinarian that the animals were not exhibiting clinical signs associated with scrapie at the time of examination. CVIs that have certification statements indicating that "the animals have no history of clinical signs or exposure to contagious or infectious diseases," or words to that effect, will suffice in lieu of the specific scrapie statement.

NOTE:

The scrapie eradication UM&R is the official guidance for interpreting the federal regulation. It can be found at www.aphis.usda.gov/animal_health/animal_diseases/scrapie/downloads/umr_scrapie.pdf or you may request a hard copy from your local APHIS, VS Area Office.

Footnote:

1. Breeding sheep and goats are sexually intact animals that are not moving directly to slaughter, through slaughter channels to slaughter, or to a feedlot prior to slaughter.

Section 7:

Nor98-like Scrapie

Regarding Nor98-like Scrapie

In February 2007 APHIS, USDA, officially announced the discovery of the first Nor98-like scrapie case in a ewe from a flock in Wyoming. The following gives background on Nor98 and what we know about it. It also describes the difference between it and classical scrapie.

The Science Behind Nor98

Nor98 is a prion disease or transmissible spongiform encephalopathy of sheep and goats. This disorder was first described in sheep in 1998 in Norway, although a retrospective study has revealed a case in England in 1989. Cases have since been found in other countries as a result of improved methods for diagnostic testing and the increased slaughter surveillance initiated in 2002 in many European countries. Most cases are identified in clinically normal sheep tested during routine slaughter surveillance. The disease is experimentally transmissible to sheep and genetically altered mice by inoculation into their brains but no data are yet available on whether the disease is transmitted between sheep in an affected flock. The low-within-flock prevalence, the wide geographical distribution, and similar prevalence between countries using similar surveillance methods suggests that this disorder is sporadic and either not transmissible or poorly transmissible under natural conditions.

Clinical Signs

Most cases have been discovered in clinically normal sheep and goats tested at slaughter. Of the few clinical cases, a common sign is progressive incoordination (ataxia), occurring most likely because the abnormal prions accumulate in the cerebellum which normally integrates information coming in from the senses and then sends nerve impulses going to the muscles.

Diagnosis

Both classical scrapie and Nor98 scrapie are characterized by accumulation of abnormal prion proteins. However, the distribution of the prion proteins differs. In classical scrapie, prions are usually found earliest in the lymph nodes and later in the region of the brain associated with innervations of the gut. As discussed above, abnormal prions are found in different areas of the brain in cases of Nor98. Specifically, a diagnosis of Nor98 scrapie can be made when the Western blot shows a recognizable protein band with molecular mass of less than 15kDa or when immunohistochemistry of the obex demonstrates PrP^{Sc} immunostaining in the nucleus of the trigeminal nerve but not in the dorsal motor nucleus of the vagal nerve as is seen in classical scrapie. Nor98, Nor98-like, nonclassical and atypical scrapie are different terms used

in the scientific literature to describe scrapie cases that meet these diagnostic criteria. Further, prion proteins are not found in the lymph nodes of sheep with Nor98 so the current live-animal tests based on lymphoid tissues are not suitable for detection of Nor98-like scrapie cases. Nor98 is a challenging diagnosis but skilled pathologists utilizing immunohistochemistry and/or western blot can accurately diagnose the disease in the brain of affected sheep.

Genetics

Susceptibility to classical scrapie is associated with naturally occurring differences in the gene for the prion protein, particularly differences at position 136 and 171. Each sheep has two copies of this gene and commercially available genotype tests show the differences at those positions. Sheep with the genotypes 136AA 171QQ, 136AV 171QQ and 136VV 171QQ are very susceptible to classical scrapie strains. Sheep with the 136AA 171QQ genotype are susceptible to the most common classical scrapie strain in the United States and also represent the most common genotype found in U.S. scrapie cases. Sheep with at least one copy of the gene 136A 171R are generally resistant to the more common type of classical scrapie.

Although no genotype is considered to be resistant to Nor98 scrapie, the disorder is found most frequently in sheep with changes in positions 141 and/or 154. The allele AFRQ indicates a sheep with 136A, 154R and 171Q with the additional change to “F” at 141. The allele AHQ indicates a sheep with 136A and 171Q with a change to “H” at position 154. A large survey of 4,000 sheep in Europe and numerous reports on smaller study populations has demonstrated that sheep with either the AFRQ or the AHQ allele were eight to 15 times more likely to be diagnosed with Nor98 scrapie than were sheep with the most common allele ARQ. Sheep with both changes (AFRQ/AHQ) were more than 20 times more likely to be diagnosed with Nor98 scrapie. Sheep with the 171R form of the gene are generally resistant to classical scrapie but are susceptible to Nor98 scrapie, particularly in 171QR sheep that have an AFRQ allele.

Epidemiology

Classical scrapie is usually found in more than one sheep in a flock with prevalence as high as 30 percent. Usually no additional cases are found in flocks where a case of Nor98 scrapie has occurred. In the case of large flocks with 500 or more sheep there is a greater likelihood of finding a second case. This finding is consistent with the reported prevalence of the disorder within the cull population as a whole. The prevalence of Nor98 scrapie has been reported

to be as low as 0.1 percent and as high as 1.4 percent in the cull slaughter populations of various European countries. In addition to genotype, age appears to represent a significant risk factor for Nor98 scrapie. In the large European study, 80 percent of the cases of classical scrapie were found in sheep ages 3-5 years, a finding similar to that reported in the United States. In contrast, more than 60 percent of the sheep with Nor98 scrapie were older than five years and more than 25 percent were more than 10 years old. Nor98 is found in most countries performing large-scale surveillance.

The low prevalence of Nor98 within affected flocks, the wide geographic distribution of the disorder, the older typical age of onset or diagnosis, suggest that Nor98 may be a sporadic disease of sheep, appearing primarily but not exclusively in older sheep. Additional findings from experimental studies and large scale surveillance using improved diagnostic methods will be useful in understanding this prion disease of sheep.

References

- Benestad SL, Arzac JN, Goldmann W, and Nöremark M.** 2008. Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. *Vet Res.* 2008 Jul-Aug;39(4):19.
- Lühken G, Buschmann A, Brandt H, Eiden M, Groschup MH, and Erhardt G.** 2007. Epidemiological and genetical differences between classical and atypical scrapie cases. *Vet Res.* Jan-Feb;38(1):65-80.
- Arsac JN, Andreoletti O, Bilheude JM, Lacroux C, Benestad SL, and Baron T.** 2007. Similar Biochemical Signatures and Prion Protein Genotypes in Atypical Scrapie and Nor98 Cases, France and Norway. *Emerg Infect Dis* 13, no. 1: 58-65.
- Simmons HA, Simmons MM, Spencer YI, Chaplin MJ, Povey G, Davis A, Ortiz-Pelaez A,** Atypical scrapie in sheep from a UK research flock which is free from classical scrapie.
- Hunter N, Matthews D, Wrathall AE.** *BMC Vet Res.* 2009 Feb 10;5:8. <http://www.biomedcentral.com/1746-6148/5/8>
- Colussi S, Vaccari G, Maurella C, Bona C, Lorenzetti R, Troiano P, Casalnuovo F, Di Sarno A, Maniaci MG, Zuccon F, Nonno R, Casalone C, Mazza M, Ru G, Caramelli M, Agrimi U, Acutis PL.** Histidine at codon 154 of the prion protein gene is a risk factor for Nor98 scrapie in goats. *J Gen Virol.* 2008 Dec;89(Pt 12):3173-6.
- Fediaevsky A, Tongue SC, Nöremark M, Calavas D, Ru G, Hopp P.** A descriptive study of the prevalence of atypical and classical scrapie in sheep in 20 European countries. *BMC Vet Res.* 2008 Jun 10;4:19.
- Del Rio Vilas VJ, Böhning D, Kuhnert R.** A comparison of the active surveillance of scrapie in the European Union. *Vet Res.* 2008 May-Jun;39(3):37. Epub 2008 Feb 29.
- Green DM, Del Rio Vilas VJ, Birch CP, Johnson J, Kiss IZ, McCarthy ND, Kao RR.** Demographic risk factors for classical and atypical scrapie in Great Britain. *J Gen Virol.* 2007 Dec;88(Pt 12):3486-92.

Section 8:

Specimen Collection and Submission

Introduction

- A. Safety Precautions
- B. Personal Protective Equipment (PPE)
- C. Instructions for Veterinarians and Animal Health Technicians
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 - 5. Obex Collection
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 - 7. Complete Brain Removal - Required for Clinical Suspects
 - 8. Tonsil Collection
 - 9. Retropharyngeal Lymph Node (RPLN) Collection
- D. Alternate Collection Procedure That May be Used by Owners if Desired

When scrapie is suspected in a live or dead animal the owner should contact an APHIS representative, a State animal health official, or an accredited veterinarian so that the animal can be evaluated and or tested. Participants in the Scrapie Flock Certification Program are also required to report mature animals that die on premises. Owners are to be encouraged to have samples submitted from sheep and goats over 14 months of age that die on farm other than from routine slaughter for scrapie surveillance. This is particularly important if they have higher prevalence breeds. The following section describes the process for sample collection and submission. A pictorial collection guide is included on the CD.

Completed VS Form 10-4 (**NOTE:** the VS Form 10-4 is ONLY for use by an APHIS representative, a State animal health official, or an accredited veterinarian with approval of the AVIC). If the owner is submitting a whole head the owner needs to provide all the information below so the submitting official can complete the VS Form 10-4:

1. Owner name, address, phone number, and any clinical signs observed;
2. All ID devices, tattoos, and brands on the animal;
3. Age of animal based on dental examination and owner records;
4. Flock ID, species, breed, and sex of animal;
5. Brain and other tissues collected and packaged as described below; and
6. Any additional samples as requested by the AVIC or State Veterinarian, including samples requested for research.

A. Safety Precautions

It is the responsibility of the collector to take appropriate safety precautions. Measures should be taken to avoid contact with specimens. Adhere to the following safety precautions to ensure that you minimize your risk of exposure to pathogens:

1. Wear personal protective equipment (PPE) at all times and ensure persons not wearing protective equipment stand back at least 10 feet to avoid exposure;
2. Cover cuts, abrasions, and wounds with waterproof dressing if left not covered by PPE;
3. Use face and respiratory protection, which includes a well-fitted respiratory mask and face shield or goggles to protect from infective droplets or tissue particles. Wear gloves while handling specimens and formalin;
4. Use formalin in a well ventilated area;
5. Take steps to avoid creating aerosols, splashes, and dusts;
6. Wash hands and exposed skin following collection procedures; and
7. Wash and disinfect protective clothing and instruments thoroughly after use. Use 50 ounces [6 ¼ cups] bleach to enough water (78 ounces or 9 ¾ cups) to give 1 gallon of solution) at room temperature (at least 18.3 degrees C [65 degrees F]) for 1 hour.

B. Personal Protective Equipment (PPE)

PPE is designed to minimize exposure to pathogens while collecting samples. According to Occupational Safety and Health Administration, PPE is defined 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 upon the environment, physical hazards, and ability to complete the task. PPE is a balance between 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 where you are collecting specimens will differ, the following PPE must be worn at all times during collection of scrapie specimens:

1. 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.

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2. **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.

3. **Hand Protection Gloves:**

- a. Wear metal or mesh gloves. Always wear the cut resistant glove (Hantover, Koch, or Packer) on your off hand (left hand for right-handed individual and right hand for left-handed individual.) Find a cut resistant glove that fits against your skin and then wear a rubber glove on top of it.
- b. Wear latex or nitrile examination gloves or thick rubber gloves that extend half way up the forearm. Many people prefer the long thick rubber gloves for the added protection.

4. **Foot Protection:**

Protect your feet from injuries such as spills or splashes, impact, compression or exposure. Wear steel toed rubber boots when collecting specimens. If steel toed boots are not available, pullover rubber boots are acceptable.

5. **Respiratory Protection:**

Face masks/respirators are recommended if the environment includes aerosols, splashing, or flying debris as may be encountered with certain methods of brain removal or tissue handling. Though scrapie is not known to be transmissible through air nor is it known to be transmissible to man, during scrapie specimen collection other zoonotic diseases such as rabies, Q fever, or Listeria may be present.

C. **Instructions for Veterinarians and Animal Health Technicians**

1. **Collector's Responsibility**

It is vital that specimens submitted to NVSL or the contract laboratories are able to be traced to the source animal and farm. As the collector of the specimens, it is vital that you accurately complete the specimen collection and submission process. Failure to accurately collect and submit specimens may result in the erroneous eradication of animals, which is an irretrievable loss to farmers and producers.

When collecting specimens you are responsible for:

- a. Following the laboratory's procedure for notifying the laboratory of incoming specimens;

- b. Contacting the delivery service. Ensure that the package containing fresh tissues will be delivered overnight;
- c. Properly completing 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 was exhibiting clinical signs. If the animal exhibited clinical signs, list the signs in the Additional Data Section of the VS form 10-4;
- d. You will need to make four copies of the completed VS Form 10-4:
 - o One for your files (submitter's copy);
 - o One for the animal owner or collection site;
 - o One submitted to the VS Area Office; and
 - o One submitted with the specimen.
- e. Correctly label specimen collection containers; and
- f. Properly collect obex, tonsil, cerebellum, and retropharyngeal lymph nodes (RPLN). For scrapie suspects, the remainder of the brain must also be collected.

2. **Labeling Sample Containers**

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.

Ensure that you clearly label both the top and the side of the sample container using the provided barcode sticker. If you do not have a barcode sticker, identify the sample by either typing the information or using a permanent marker. Verify that the sample number that appears on the top and side of the sample container and the completed VS Form 10-4 are identical.

The side label must include:

- a. Type of specimen;
- b. Animal ID number; and
- c. Sample ID number (the number assigned to this sample on the VS Form 10-4).

3. **Preserving Specimens**

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

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Fresh specimens that are used for DNA comparison and additional testing must be kept chilled or frozen. While dry ice may be used, it is usually best to ship the chilled or frozen tissues overnight on icepacks.

Fresh specimens for routine submissions (animals that are not scrapie suspects) include:

- a. 2 Retropharyngeal Lymph Nodes (RPLN) (1 medial and 1 lateral);
- b. Brainstem/cord tissue (the cranial and caudal brainstem and spinal cord that remain after the obex section is removed); NOTE: There should be about 1 inch of tissue cranial and caudal to the obex or 5 grams of tissue.
- c. Entire cerebellum; and
- d. Animal ID device (Collect the animal ID device with the ½ inch of adjoining tissue. This will allow DNA verification if necessary.)

Fresh specimens for animals that are scrapie suspects include:

- a. 2 RPLN (1 medial and 1 lateral);
- b. 1 tonsil;
- c. Brainstem/cord tissue (the cranial and caudal brainstem and spinal cord that remain after the obex section is removed); NOTE: There should be about 1 inch of tissue cranial and caudal to the obex or 5 grams of tissue.
- d. ½ cerebellum;
- e. The left half of the cerebrum and midbrain;
- f. Animal ID device. (Collect the animal ID device with the ½ inch of adjoining tissue. This will allow DNA verification if necessary.)

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

Formalin-fixed specimens for routine submissions (animals that are not scrapie suspects) include:

- a. 1 medial RPLN;
- b. 1 tonsil; and
- c. Obex – pencil width slice (1 cm) that includes the apex of the V.

Formalin-fixed specimens for animals that are scrapie suspects include:

- a. 1 medial RPLN;
- b. 1 tonsil;
- c. Obex – pencil width slice (1 cm) that includes the apex of the V;

- d. ½ cerebellum;
- e. The right half of the cerebrum and midbrain; and
- f. Animal ID device (Collect the animal ID device with the ½ inch of adjoining tissue. This will allow DNA verification if necessary.)

NOTE: Place all formalin fixed specimens, the obex, tonsil and RPLN, from a single animal with the animal's ID devices, in the same formalin collection container. Each fresh tissue must be placed in a separate bag. Ensure the sample container correctly lists all specimens included.

4. Collection Procedures

The collection of the obex, tonsils and RPLN can be completed using several methods. However 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.

The following equipment will help to ensure proper specimen collection:

- a. Sharp boning knives;
- b. Disposable scalpel blades, disposable scalpels, or a large scalpel blade is acceptable;
- c. Rat-tooth forceps;
- d. Meat cutting bone saw, hack saw, or electric saw when brain removal is required;
- e. Disposable cutting surfaces such as cardboard, plastic or styrofoam;
- f. Small hand nippers can be used on the hyoid bones or you may cut through at the joint using a knife;
- g. Sharp stainless steel scissor; and
- h. European brain spoon, grapefruit knife, or other brainstem scoop.

5. Obex Collection

There are two methods for collection the obex. Use the following guidelines to determine when to use each method for the collection. Collection of the obex via the foramen magnum is the preferred method for routine surveillance collections. Whole brains should be collected from scrapie suspects.

- a. Collect the obex via the foramen magnum when:
 - o The carcass is reasonably fresh.
- b. Collect the obex via a complete brain removal procedure when:
 - o The animal is a scrapie suspect;
 - o The brain stem is too autolyzed; or

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6. Obex via the Foramen Magnum

- a. Tools
 - o Aggressively toothed forceps (rat tooth);
 - o European brain spoon, grapefruit knife, or other brainstem scoop; and
 - o Curved blunt scissors.
- b. Procedures
 - o Place the head upside down in front of you so that you are looking directly at the foramen magnum;
 - o With forceps and scissors remove the collar of dense dura mater that surrounds the foramen magnum and spinal cord;
 - o Then gently grasp the end of the protruding spinal cord with forceps and move the spinal cord laterally to expose the caudal cranial nerves;
 - o 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;
 - o Once the cranial nerves have been severed, the caudal brainstem will be easier to manipulate within the foramen magnum;
 - o With light pressure, use forceps to move the spinal cord to the ventral part of the foramen magnum;
 - o Insert the spoon into the dorsal aspect of the foramen magnum between the brainstem and the dorsal bony calvarium;
 - o Sever the cerebellum by advancing the spoon cranially 2 to 3 inches until you feel the leading edge of the spoon hit bone;
 - o Remove the spoon;
 - o With the forceps, lift the spinal cord dorsally and re-insert 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;
 - o Pull the spoon toward you with gentle traction on the spinal cord with the rat-toothed forceps;
 - o If the brainstem is not readily removed by this method, stop. Re-examine the brainstem and sever any remaining cranial nerves or connections to the dura. Use caution, excessive caudal traction on the spinal cord may result in a mutilated non-diagnostic sample;
 - o 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; and

- o The sample extracted with this method is usually 3 to 4 centimeters long with the obex in the center. Trim out the central $\frac{1}{3}$ containing the obex and place in formalin. Place the caudal piece (spinal cord) and cranial piece (cranial brainstem) into a plastic bag for chilling or freezing.

7. Complete Brain Removal – Required for Clinical Suspects

- a. Tools
 - o Meat cutting bone saw, hack saw, or electric necropsy saw;
 - o Wood chisel or large wide-tipped screwdriver;
 - o Toothed forceps (rat tooth);
 - o European brain spoon, grapefruit knife, or other brainstem scoop;
 - o Curved blunt scissors; and
 - o Scalpel.

Several methods may be used for collection of the obex via complete brain removal.

- b. Procedures
 - o Skin the head;
 - o Using a bone saw, remove the top and back of the skull. This requires three cuts:
 - 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;
 - Repeat this cut on the other side starting at the medial aspect of the other occipital condyle;
 - 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.
 - o Pry off the skullcap by inserting a wood chisel or a wide-tipped large screwdriver at the level of the transverse cut and hinge the skullcap caudally;
 - o 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;

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NOTE: If the sides or front of the cerebrum have been inadvertently damaged during the previous steps of the procedure, the samples will not be compromised.

- o 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, that lies between the cerebrum and cerebellum;
- o 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;
- o For scrapie diagnosis, separate the brainstem from the forebrain by a transverse cut between the cerebrum and cerebellum;

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.

- o 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;
- o 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;
- o Place the obex into formalin;
- o Place the remaining brainstem tissues including the spinal cord and brain stem into a plastic bag;
- o 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;
- o Place all the fresh tissue sample bags except the cerebellum into another bag and seal.

8. Tonsil Collection

There are various successful approaches to collecting 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.

a. Tools

- o Sharp boning knife;
- o Scalpel;
- o Sharp stainless steel scissors; and
- o Rat-toothed forceps.

b. Procedures

- o Place the head upside down on the table;
- o Remove the skin from the ventral surface of the mandible;
- o 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 that you encounter will need to be cut with poultry shears or disarticulated at a joint with the knife;
- o 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;
- o 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 and laterally there is a readily palpable, well circumscribed mass of lymphoid tissue that will feel like a small, round, sometimes relatively flat lymph node;
- o 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 above may be used to locate the tonsils. The crypt is the landmark for the tonsillar lymphoid tissue subjacent (deep or submucosal) to the crypt; and

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- o Place one tonsil into a jar of formalin and the other in a resealable bag and then into the bag with the other fresh tissues from that animal.

9. Retropharyngeal Lymph Node (RPLN) Collection

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 and 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.

a. Tools

- o Sharp boning knife;
- o Scalpel;
- o Sharp stainless steel scissors; and
- o Rat-toothed forceps.

b. Procedures

- o 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;
- o Dissect both medial retropharyngeal nodes from the surrounding pharyngeal muscles with rat-toothed forceps and scissor, scalpel, or knife;
- o Place one medial RPLN into a jar of formalin; and
- o Place two RPLN (one medial and one lateral) into a plastic bag for chilling or freezing.

D. Alternate Collection Procedure that may be Used by Owners if Desired

1. Tools for Head Removal and Whole Head Packaging

- a. Sharp boning knife; and
- b. Two heavy duty plastic bags.

2. Procedures for Head Removal and Whole Head Packaging

- a. If the carcass is intact, remove the head from the carcass. This is done at the atlanto-occipital joint, which is where the skull meets the first cervical vertebrae.
- b. Position the animal in dorsal recumbency (lying on its back).

- c. Remove the head, at the hinge joint where the skull meets the first cervical vertebrae (just behind the ears) using the following steps:
 - o To locate the “hinge” area where the skull meets the first cervical vertebrae, grasp the nose and move the head up and down to locate the joint.
 - o Insert the knife into the neck between the first cervical vertebrae and the throat then cut outward (ventrally) with blade directed away from you through the throat tissue and skin. (Cutting down through the skin readily dulls the blade.)
 - o Cut down (dorsally) to the membrane that covers the spinal cord; cut through the membrane exposing the spinal cord. Then cut the spinal cord as far from the head (caudally) as possible so that it is kept as long as practical.
 - o Cut the lateral ligaments connecting the skull to the vertebra in a ventral to dorsal direction on both sides. This is usually best accomplished with the tip of the knife directed between the skull and vertebra.
 - o Once the lateral ligaments have been severed, cut through the remaining tissue to remove the head from the carcass.
 - o Now move the head with a portion of the spinal cord protruding from the foramen magnum (hole in skull from which spinal cord is protruding) to a comfortable height for sample collection or to package the whole head.
 - o Ideally, specimens should be collected from the head onsite by a veterinarian or animal health technician; however, sometimes it may be necessary to ship the entire head to the laboratory. When this is the case, skin the head leaving the ears with ID in place, place the head in a large heavy-duty plastic bag. If you are presented with a skinned head such as at slaughter plants, place the animal’s ID with at least an inch of ear attached in a separate bag with the bagged head inside the second bag.
 - o Double bag the head.
 - o Secure each bag in a manner that will prevent leakage such as by tying a knot in the bag or using twist ties, string, or cord.
 - o Chill the head prior to placing in the cool box and refrigerate the head until and during shipment to the laboratory in the cool box.

To pack the cool box: Put cool packs in the bottom, insert large plastic bag, insert absorbent material, insert double bagged heads, and seal bag, place cool packs on top of bag and close cooler top. Insert submission form between cooler top and exterior box. Ship overnight. Use at least four chill packs per box and an additional chill pack for each additional head if more than two heads are shipped in the same cool box.

Section 9:

Scrapie Disinfection Guidelines

None of the following suggested disinfection and inactivation procedures will guarantee total and complete elimination and inactivation of the infectious agent. Based on current information on the efficacy under laboratory conditions of the disinfection methods listed, it is likely that they will reduce the amount of infectivity in the environment. Until more specific information becomes available, good sanitary practices are recommended following each lambing. The following methods below should be applied to lambing areas where infected or exposed animals have lambed.

A. Pastures

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

B. Drylots

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

C. Earth Surfaces Inside Structures or Used for Confined Lambing Pens

Remove the organic material and when practical, also remove the top 1 to 2 inches of soil to reduce contamination. Bury, till under, or compost the removed material in areas not accessed by domestic or wild ruminants until it can be buried or tilled under.

D. Nonearth Surface, Includes Cement, Wood, Metal, Tools, Equipment, Instruments, Feed, Hay, Bedding, and Other Materials

1. 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.
2. When practical for other items bury or incinerate by high temperature incineration methods.
3. 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:
 - a. Autoclave instruments, small tools, and other items at 136 degrees C (277 degrees F) for 1 hour. This method is more effective when preceded by the treatment described in b or c, below.
 - b. To clean dry surfaces, apply a 2 percent available chlorine solution (equivalent to about 20,000 p/m; available chlorine: 50 ounces [6 ¾ cups] bleach to enough water (78 ounces. or 9 ¾ cups) to give 1 gallon of solution) at room temperature (at least 18.3 degrees C [65 degrees F]) for 1 hour.
 - c. Alternative disinfection method when the preceding methods are not available: Expose dry surfaces by applying 1 molar solution of sodium hydroxide (approximately 4 percent solution [5 ounces sodium hydroxide dissolved in 1 gallon water]) at room temperature (at least 18.3 degrees C [65 degrees F]) for at least 1 hour. Synonyms for sodium hydroxide are caustic soda or lye.

NOTE: Bleach and sodium hydroxide are hazardous. Follow precautions on the label and material safety data sheet, wear protective equipment, do not spray above waist level, wear a respirator if using indoors, may damage or discolor surfaces.

Section 10:

List of Resource Materials and Contacts for Questions, Comments and Suggestions

The following materials are available upon request at www.eradicate.scrapie.org or by calling 719-538-8843, extension 10.

- The ABC's of Genetic Based Flock Clean-up and Monitoring Plans
- Goat Identification: Visual and Electronic (PowerPoint Presentation)
- A Guide for Veterinarians
- Identification Requirements of the National Scrapie Eradication Program for Sheep (PowerPoint Presentation)
- Markets and Dealers: Your Role in the New Scrapie Eradication Program (Poster)
- National Scrapie Eradication Program Record Guide
- Requirements for "Going to the Show"
- Sheep Identification Requirements (Poster)
- The Use of Genetics to Control Scrapie (PowerPoint Presentation)
- What You as a Producer Need to Know

For individual state regulations contact your State Veterinarian(SV) or AVIC, listed below.

Alabama

SV 334-240-7253
www.agi.state.al.us
AVIC
334-223-7141

Colorado

SV 303-239-4161
www.ag.state.co.us
AVIC
303-231-5385

Georgia

SV 404-656-3671
www.agr.state.ga.us
AVIC
770-922-7860

Iowa

SV 515-281-5305
www.agriculture.state.ia.us
AVIC
515-284-4140

Maryland

SV 410-841-5810
www.mda.state.md.us
AVIC
410-349-9708

Alaska

SV 907-375-8214
www.state.ak.us
AVIC
360-753-9430

Connecticut

SV 860-713-2505
www.state.ct.us
AVIC
508-865-1421

Hawaii

SV 808-483-7111
www.hawaiiag.org/hdoa
AVIC
360-753-9430

Kansas

SV 785-296-2326
www.accesskansas.org/kda
AVIC
785-270-1300

Massachusetts

SV 617-626-1790
www.state.ma.us
AVIC
508-865-1421

Arizona

SV 602-542-4293
www.agriculture.state.az.us
AVIC
480-491-1002

Delaware

SV 302-698-4560
www.state.de.us/deptagri/
AVIC
410-349-9708

Idaho

SV 208-332-8540
www.agri.idaho.gov
AVIC
208-378-5631

Kentucky

SV 502-564-3956
www.kyagr.com
AVIC
502-227-9651

Michigan

SV 517-373-8118
www.michigan.gov
AVIC
517-324-5290

Arkansas

SV 501-907-2400
www.aripc.org
AVIC
501-224-9515

District of Columbia

AVIC
410-349-9708

Illinois

SV 217-782-4944
www.agr.state.il.us
AVIC
217-862-6689

Louisiana

SV 225-925-3980
www.ldaf.state.la.us
AVIC
225-389-0436

Minnesota

SV 651-201-6825
www.bah.state.mn.us
AVIC
651-290-3691

California

SV 916-654-0881
www.cdfa.ca.gov/ahfss
AVIC
916-854-3950

Florida

SV 850-410-0914
www.doacs.state.fl.us
AVIC
352-333-3120

Indiana

SV 317-227-0300
www.state.in.gov/boah
AVIC
317-290-3300

Maine

SV 207-287-3701
www.state.me.us
AVIC
508-865-1421

Mississippi

SV 601-359-1170
www.mdac.state.ms.us
AVIC
601-965-4307

Section 10: *List of Resources and Contacts*

Missouri

SV 573-751-3377
www.mda.mo.gov
AVIC
573-636-3116

Montana

SV 406-444-0782
www.agr.mt.gov
AVIC
406-449-2220

Nebraska

SV 402-471-6806
www.agr.state.me.us
AVIC
402-434-2300

Nevada

SV 775-688-1182 Ext 232
www.agri.state.nv.us
AVIC
916-854-3950

New Hampshire

SV 603-271-2404
www.agriculture.nh.gov
AVIC
508-865-1421

New Jersey

SV 609-292-3965
www.state.nj.us
AVIC
609-259-8387

New Mexico

SV 505-841-6161
www.nmdaweb.nmsu.edu
AVIC
505-761-3160

New York

SV 518-457-3502
www.agmkt.state.ny.us
AVIC
518-869-9007

North Carolina

SV 919-733-5657
www.ancgr.com
AVIC
919-855-7700

North Dakota

SV 701-328-2655
www.agdepartment.com
AVIC
701-250-4210

Ohio

SV 614-728-6220
www.ohioagriculture.gov
AVIC
614-469-5602

Oklahoma

SV 405-522-6319
www.oda.state.ok.us
AVIC
405-427-9413

Oregon

SV 503-986-4680
www.oregon.gov/ODA/
AVIC
503-399-5871

Pennsylvania

SV 717-772-2852
www.pda.state.pa.us
AVIC
717-782-3442

Puerto Rico

SV 787-766-6050
AVIC
787-766-6050

Rhode Island

SV 401-222-2781 Ext.4503
AVIC
508-865-1421

South Carolina

SV 803-788-2260 Ext 234
www.scda.state.sc.us
AVIC
803-788-1919

South Dakota

SV 605-773-3321
www.state.sd.us
AVIC
605-225-6186

Tennessee

SV 615-837-5120
www.state.tn.us
AVIC
615-781-5310

Texas

SV 512-719-0700
www.tahc.state.tx.us
AVIC
512-383-2400

Utah

SV 801-538-7162
www.utah.gov
AVIC
801-524-5010

Vermont

SV 802-828-2421
www.vermontagriculture.com
AVIC
508-865-1421

Virgin Islands

SV 809-778-0997

Virginia

SV 804-692-0601
www.vdacs.virginia.gov
AVIC
804-343-2560

Washington

SV 360-902-1878
www.agr.wa.gov
AVIC
360-753-9430

West Virginia

SV 304-558-2214
www.wvagriculture.org
AVIC
614-469-5602

Wisconsin

SV 608-224-4880
www.datcp.state.wi.us
AVIC
608-270-4000

Wyoming

SV 307-777-6443
www.wyagric.state.wy.us
AVIC
307-432-7960

For program information and questions, to order official tags/forms, or to report a suspect animal, contact your local APHIS, VS Area Office:

Phone: 1-866-USDA-Tag (1-866-873-2824)

Website: http://www.aphis.usda.gov/animal_health/area_offices/

**Program policy is reviewed annually.
Please send suggestions for changes to:
National Scrapie Program Coordinator: Dr. Diane Sutton
Address: USDA, APHIS, VS, NCAHP
4700 River Road, Unit 43, Riverdale, MD 20737
E-Mail: Scrapie_Program@aphis.usda.gov
Phone: 301-734-6954 • Fax: 301-734-7964**



**United States
Department of Agriculture**

4700 River Road, Unit 43
Riverdale, MD 20737
Ph: 866-873-2824
www.aphis.usda.gov/vs/scrapie



**National Institute
for Animal Agriculture**

13570 Meadowgrass Drive, Suite 201
Colorado Springs, CO 80921
Ph: 719-538-8843
www.animalagriculture.org/scrapie



UNIVERSITY OF MINNESOTA

**University of Minnesota
College of Veterinary Medicine
*Cindy Wolf, DVM
Eileen Kuhlmann***

225 Veterinary Medical Center
1365 Gortner Avenue
Saint Paul, MN 55108