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# *A Pictorial Guide to Collecting Samples for Scrapie Testing*

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United States  
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National Institute  
for Animal Agriculture



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# Specimen Collection Presentation:

## Necropsy / Sample Specimen Collection

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**NOTE:** The following section is a PDF copy of portions of a USDA computer-based training program. The interactive buttons do not work within this file.

### Safety Precautions

Precautions should be taken to avoid direct contact with samples collected for scrapie diagnostics. The following general safety precautions are recommended:

- o Wear personal protective equipment at all times.
- o Cover cuts, abrasions, and wounds with waterproof dressing if left uncovered after putting on personal protective equipment.
- o Use face and respiratory protection such as a well-fitted face shield or goggles to protect from droplets or tissue particles.
- o Take steps to avoid creating aerosols, splashes, and dust when engaged in activities such as sawing through bones of the skull.
- o Wear gloves while handling samples and formalin.
- o Use formalin in a well-ventilated area.
- o Wash hands and exposed skin after collecting samples.
- o Wash and disinfect protective clothing and instruments thoroughly after use. You may use household bleach as a disinfectant. Add 50 ounces (approximately 6 ¼ cups) of bleach to 78 ounces (approximately 9 ¾ cups) of water to give 1 gallon of solution to use as a disinfectant. Allow for 1 hour of surface contact at room temperature to effectively disinfect.



### Personal Protective Equipment

It is the responsibility of the sample collector to take appropriate safety precautions. Personal protective equipment (PPE) is designed to minimize exposure to the scrapie agent while collecting samples. The following PPE must be worn at all times during the collection of scrapie specimens:

- o Eye protection
- o Skin protection
- o Gloves
- o Boots
- o Face masks or respirators

### Sample Collector Responsibilities

Sample collectors must complete the following activities in order to properly submit the specimens for diagnostics:

- o Collect and preserve the designated tissues as described in the current version of the Scrapie Tissue Collection Protocols on page 6 and 7.
- o Collect and submit all animal identification devices, tattoos, and brands. Include a piece of ear tissue with the animal identification device in the event DNA verification of the animal's identity is needed.
- o Label specimen collection containers.
- o Complete all blocks/fields on the appropriate sample submission form (VS Form 10-4 or an electronic equivalent). Ensure the animal ID number and the sample container number and/or barcode are recorded accurately on the submission form.
- o Maintain control of all samples until custody is transferred to the delivery service.
- o Contact the delivery service. Ensure that packages containing fresh tissue will be delivered overnight.
- o Notify the appropriate laboratory of incoming samples, as requested.
- o Make copies of the completed specimen submission form, when applicable:
  - o One for submitter's files
  - o One for the animal owner or collection site
  - o One for Veterinary Services (VS) Area Office
  - o One submitted with the sample and animal identification device(s)



## Necropsy / Sample Specimen Collection - Overview

### Samples Required for Scrapie Testing

Quality samples must be submitted in order to obtain a successful diagnosis of scrapie. Tissues collected for scrapie diagnostics include:

- o Brainstem (including the obex)
- o Complete brain
- o Retropharyngeal lymph nodes
- o Tonsils

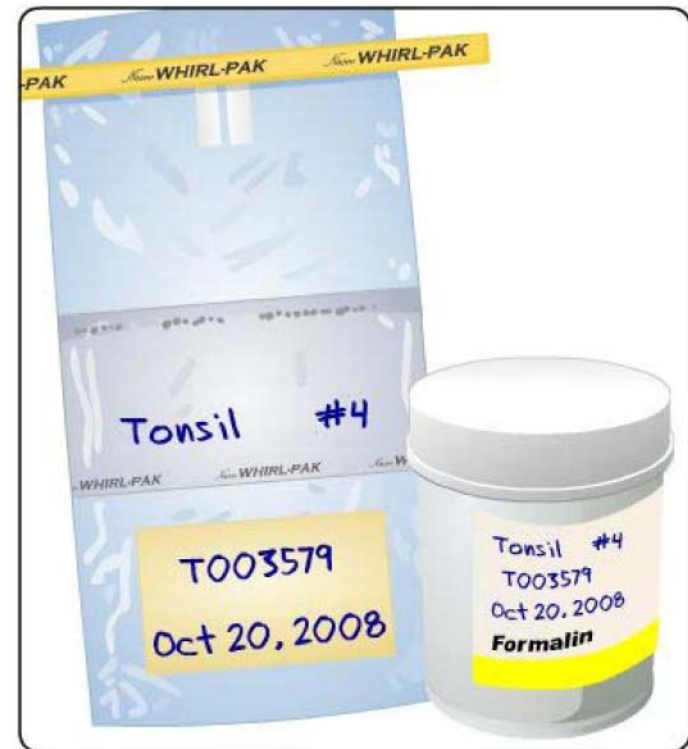
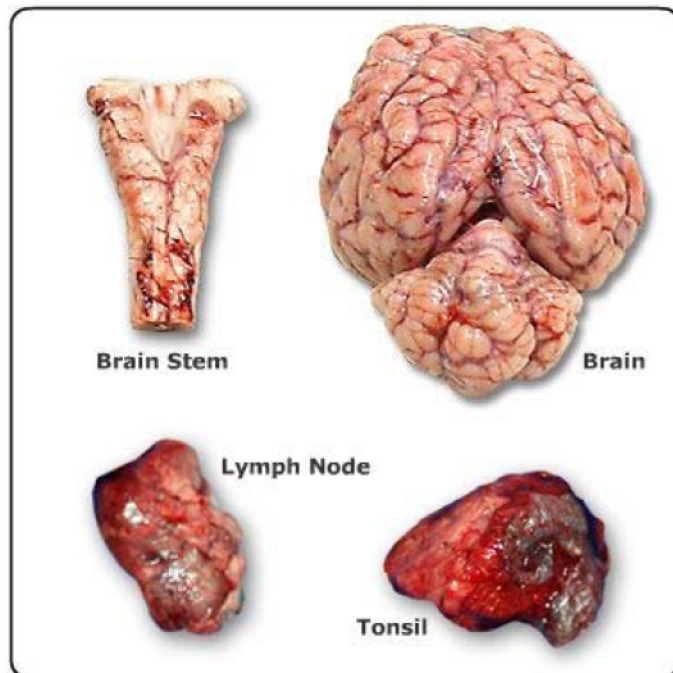
These tissues are either submitted fresh (chilled or, if given prior approval by the regional epidemiologist tissue may be frozen in special circumstances) or fixed in formalin. Refer to the current version of the Scrapie Tissue Collection Protocols chart on page 6 and 7 to determine the appropriate tissues for collection and the preservation method required for each specimen.

### Labeling Sample Containers

Specimen collection containers must be properly labeled. The information on the label should match the information from the completed VS Form 10-4 that must accompany the submission of the specimens. The label includes:

- o Type of specimen
- o Animal identification number
- o Sample identification number (the number assigned to this sample on the VS Form 10-4)
- o Date of collection
- o Barcode sticker, if required

**NOTE:** Use a permanent marker to label the specimen collection containers.





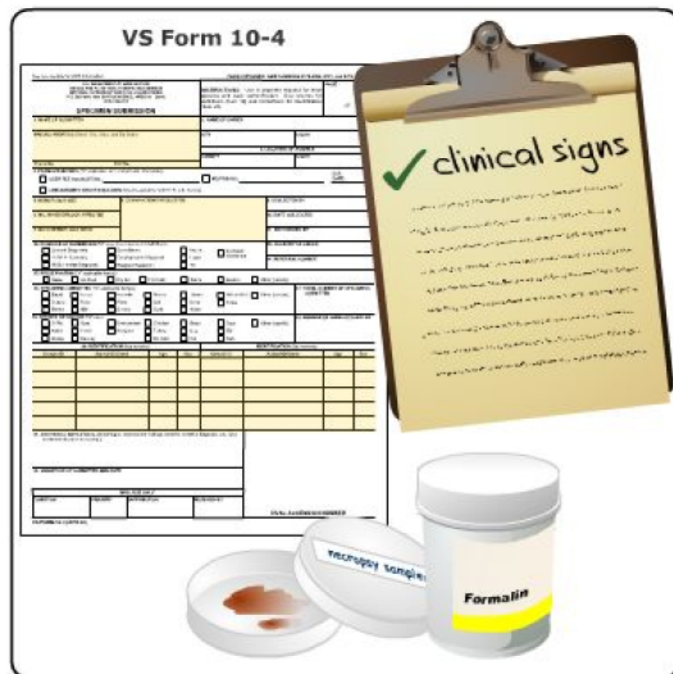
## Necropsy / Sample Specimen Collection - Overview

### Tissue Collection Protocol: Forms

When submitting necropsy/slaughter samples for diagnostics:

- o Submit samples with a VS Form 10-4 or an electronic equivalent ( VSLs).
- o Complete all blocks/fields on the submission form.
- o List any clinical signs the animal is exhibiting, when appropriate.
- o Also, refer to the document “Instructions for Completion of VS Form 10-4” on the current version of the National Scrapie Reference CD for additional details.

**NOTE:** Submit necropsy samples from clinical suspects and animals that were test-positive on a live animal test as separate submissions from other samples collected at the same time so that testing of these animals can be prioritized by the National Veterinary Services Laboratories (NVSL).



### Tissue Collection Protocol: Identification

Almost all samples submitted for diagnostics must be traceable to the animal from which they were collected.

- o To ensure positive identification, collect and submit all identification devices, tattoos, and brands on the animal in the formalin jar containing the tissues.
- o Include a penny-sized piece of ear tissue with the animal's identification device -- preferably the official eartag -- in the event DNA verification of the animal's identity is needed.
- o If the tag is so large that placing it in the formalin jar with the specimens will compromise the integrity of the tissue, use a separate container to submit the device(s).
- o Be sure to clearly identify this container and apply a matching barcode so the container with the ID devices can be matched to the container with the appropriate tissue specimens.

**NOTE:** Ear tissue does not need to be collected if the animal is untagged, (e.g., an unidentified black-faced animal collected for RSSS).





## ***Necropsy / Sample Specimen Collection - Overview***

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### **Tissue Collection Protocol: Fresh and Formalin-Fixed Tissues**

When both fresh and formalin-fixed tissues are required, the submission must be sent to NVSL. These include animals that are:

- o Clinical suspects and animals that were suspect or test positive on live animal scrapie tests
- o Collected on-farm with less specific clinical signs
- o Exposed animals

Exceptions to this protocol are only with prior approval by the Regional Epidemiologist.

### **Tissue Collection Protocols: Field/On-Farm Specimen Collections (Including SFCP testing)**

Animals that die or are euthanized and subsequently sampled for scrapie testing should be classified as one of the following designations:

<b>Designation</b>	<b>Animal Description</b>
<b>POS</b>	Positive/suspect result on a live-animal test for scrapie
<b>SUS</b>	A clinical suspect
<b>EXP</b>	An animal exposed to a scrapie-infected animal
<b>ME</b>	An animal that does not fit the designations above, but is tested as part of an exposed flock or missing ewe investigation
<b>NA</b>	All other animals that are tested but do not fit any of the designations above

The designation code for each animal should be reported on the VS Form 10-4 that accompanies the shipment of specimens. The required specimens for each designation are described in the Scrapie Tissue Collection Protocols chart on page 6 and 7.



## Necropsy / Sample Specimen Collection - Overview

### Tissue Collection Protocols: Specimen Collection and Preservation

The specific tissues to be collected and submitted for scrapie diagnostic testing and the method of preservation for these tissues is determined by the physical location at which the sampling occurs and the reason that the animal is being tested. These requirements are described in the current version of the Scrapie Specimen Tissue Collection Protocols chart (see below).

Scrapie Tissue Collection Protocols (April 2008)													
		Formalin						Fresh Tissue: Chilled or Frozen					
	Animal ID <sup>1</sup>	Entire Brainstem (including obex)	RLN	Cerebellum	Rt Half Brain	Tonsil	Obex Only	Entire Brainstem (including obex)	RLN	Cerebellum	Lft Half Brain	Tonsil	Brainstem (obex removed)
Field/On-Farm (Including SFCP testing)													
Non-exposed animals without clinical signs	X	X	X	X									
Exposed animals or animals with less specific signs <sup>2</sup>	X		X			X	X		X	X		X	X
Suspect and test positive animals <sup>3</sup>	X		X	X Rt	X	X	X		X	X Lft	X	X	X
RSSS sites													
Non Clinical	X	(X)	(X)					(X)	(X)				
Less specific signs <sup>2</sup>	X	(X)	(X)	(X)				(X)	(X)	(X)			
Suspect and test positive animals <sup>3</sup>	X		X	X Rt	X	X	X		X	X Lft	X	X	X
Known exposed <sup>4</sup>	X		X			X	X		X	X		X	X
SFCP <sup>5</sup>	X	(X)	(X)	(X)				(X)	(X)	(X)			
Directed by Regional Epi <sup>6</sup>	X	(X)	(X)	(X)				(X)	(X)	(X)			
See reverse side for explanation of superscripts (definitions, preservation methods, and submission information).													



<b>Explanation of superscripts (definitions, preservation methods, and submission information).</b>
RLN = Retropharyngeal lymph node; Rt = Right Half; Lft = Left Half
(X) = Tissue preservation method for these RSSS samples is EITHER Formalin OR Fresh Chilled Tissue as determined by the diagnostic test used in the assigned NAHLN contract laboratory.
Before euthanizing animals contact your Regional Epidemiologist to determine if they are needed for a research or developmental projects.
All samples must be submitted with a completed VS Form 10-4 or an electronic equivalent (VSLS or OTLS). All blocks on the submission form must be completed. See instruction sheet on reference CD.
When both fresh/frozen and formalin tissues are required (known exposed or positive animals and clinical suspects), in most cases the tissues MUST be sent to NVSL. Exceptions to this protocol are only with prior approval by the Regional Epidemiologist. In these exceptions, tissues from EXPOSED ANIMALS ONLY can be tested at an IHC contract laboratory, if the laboratory has the capacity to store the fresh or frozen tissues. Individually bag and identify each fresh tissue. Place the re-sealable bags with tonsil, retropharyngeal lymph node (RLN), brainstem and when required brain into a larger labeled re-sealable bag, i.e. keep cerebellum separate from other fresh tissue.
1 Official eartag(s) and all other forms of identification are to be collected and included in submission. Use a separate container if needed for large tags, trimmed tattoos, and brands. If using barcodes, apply a barcode label to this container to match those of the specimen container. For all submissions, include a penny-sized (any shape) piece of ear tissue around the eartag (preferably an official eartag if one exists, otherwise collect around an unofficial tag). No ear tissue needs to be collected if the animal is untagged, e.g. an unidentified black-faced animal collected for RSSS.
2 Animals with "less specific signs" include those that are: Nonambulatory, Die before slaughter, Unthrifty (if also < 5 years of age for RSSS), or Exhibit wool/hair loss suggestive of rubbing, biting at the legs or side, lip smacking, or intense rubbing without bare areas (if also < 5 years of age for RSSS).
3 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.
4 If collection is limited by the operations at a specific RSSS collection site, discuss tissue collection alternatives with your regional epidemiologist.
5 If more than one designation applies to an SFCP animal that presents at an RSSS site, collect tissues for the first designation to appear in the following list: Suspect > Known Exposed > Less specific clinical signs > Directed by Reg Epi > SFCP > Non-Clinical.
6 Additional tissues may also be requested at the discretion of the regional epidemiologist
Apr-08



### OVERVIEW

#### Overview

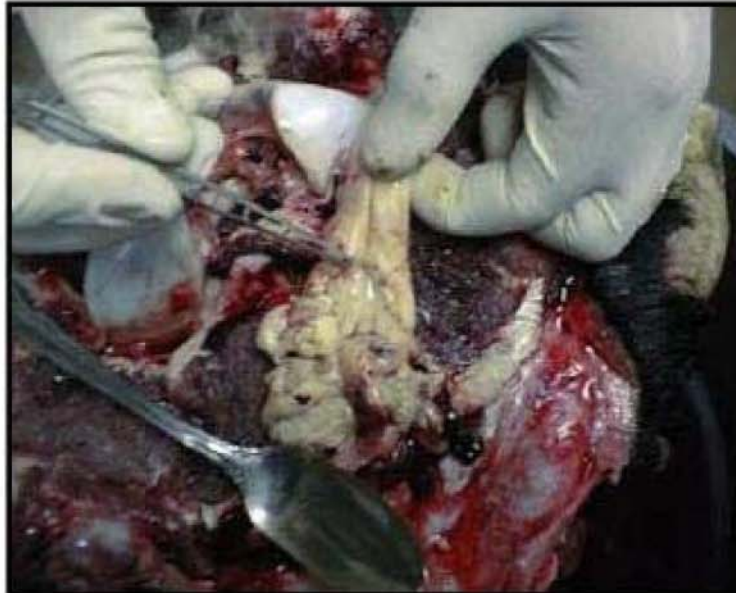
#### Tools



#### Collection Procedure



#### Sample Preservation



#### Overview

If the carcass is reasonably fresh, you may remove the brainstem through the foramen magnum. Whole brain collection should be used if the animal is a clinical suspect for scrapie, or if the brainstem is significantly autolyzed, or if removal of the obex using the spoon method is unsuccessful.

Select one of the numbered buttons below, or select the forward button to advance.

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### TOOLS

#### Overview

##### Tools



##### Collection Procedure



##### Sample Preservation



#### Tools

Instruments and supplies needed for brainstem collection via the foramen magnum include: a brain spoon or brainstem scoop, rat tooth forceps, scissors or curved blunt scissors, forceps, a jar of formalin, and resealable Ziploc-type plastic bag.

Biopsy instruments (forceps and scissors) are single-use, meaning one set is used for each animal, then disposed of properly.

Select one of the numbered buttons below, or select the forward button to advance.


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### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 1  
Place the head upside down in front of you so that you are looking directly at the foramen magnum.

Select one of the numbered buttons below, or select the forward button to advance.

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Intro





### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 2  
With forceps and scissors remove the collar of dense dura mater that surrounds the foramen magnum and spinal cord.

Select one of the numbered buttons below, or select the forward button to advance.

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Intro


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### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 3**  
Gently grasp the end of the protruding spinal cord with forceps and move the spinal cord laterally to expose the cauda cranial nerves.

Cut the cranial nerves with scissors taking care to prevent damage to the brainstem. This is best accomplished with curved, blunt scissors directing the tip of the scissors laterally.

Repeat this procedure on the other side of the brainstem.

Cut on the sides - do not cut into the spinal cord!

Failure to sever cranial nerves is a common cause of damaged samples.

Select one of the numbered buttons below, or select the forward button to advance.

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Intro


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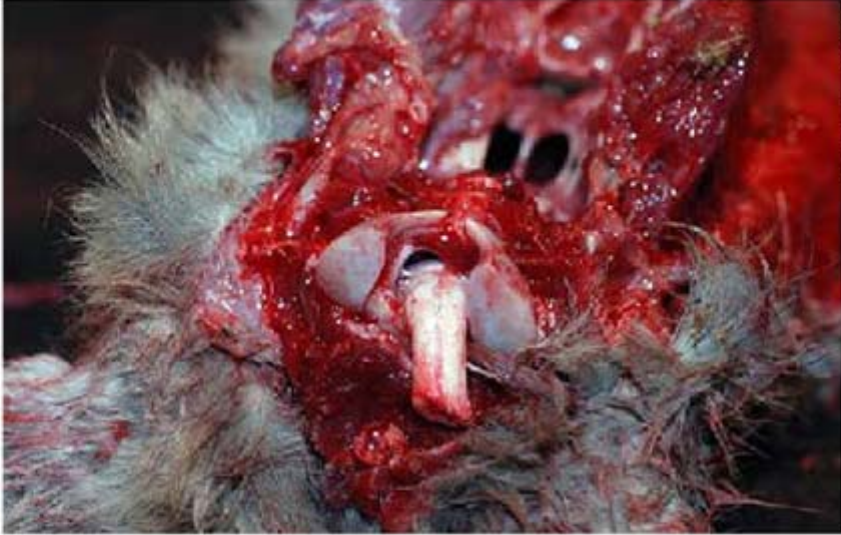
### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 4**  
Once the cranial nerves have been severed, the caudal brainstem will be easier to manipulate within the foramen magnum.

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Intro





### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 5  
With light pressure, use forceps to move the spinal cord ventrally.

Select one of the numbered buttons below, or select the forward button to advance.

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Intro




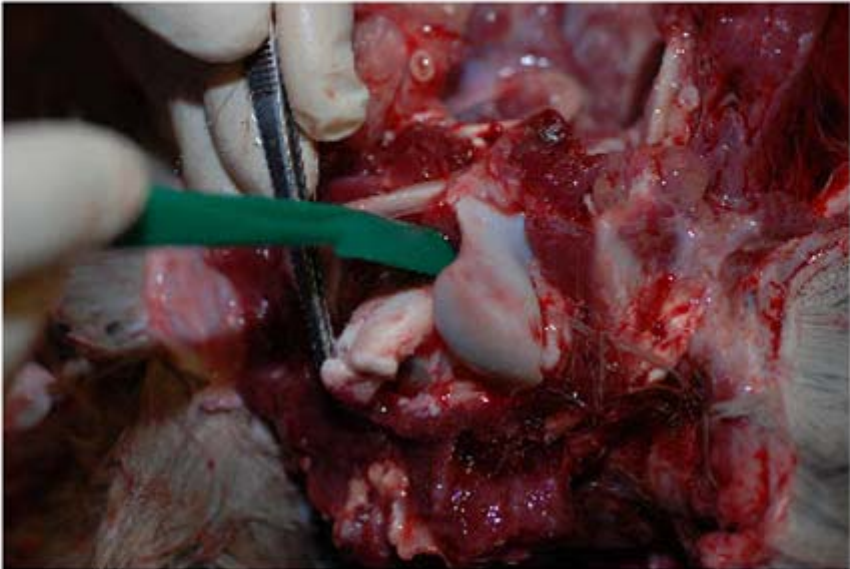
### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 6  
Insert the spoon into the dorsal aspect of the foramen magnum between the brainstem and the dorsal bony calvarium.

Select one of the numbered buttons below, or select the forward button to advance.

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Intro




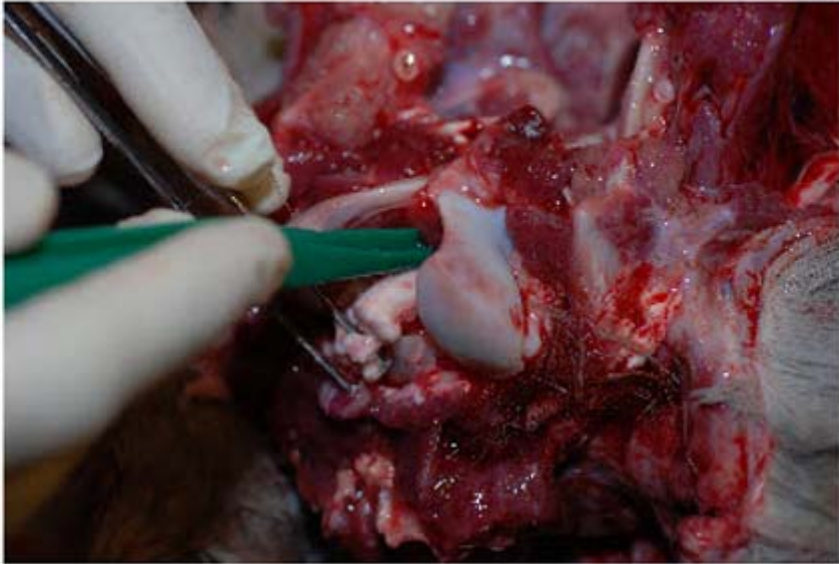
### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 7**  
Advance the spoon cranially 2-3 inches until you feel the leading edge of the spoon hit bone. Sever the cerebellum, and then remove the spoon.

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Intro




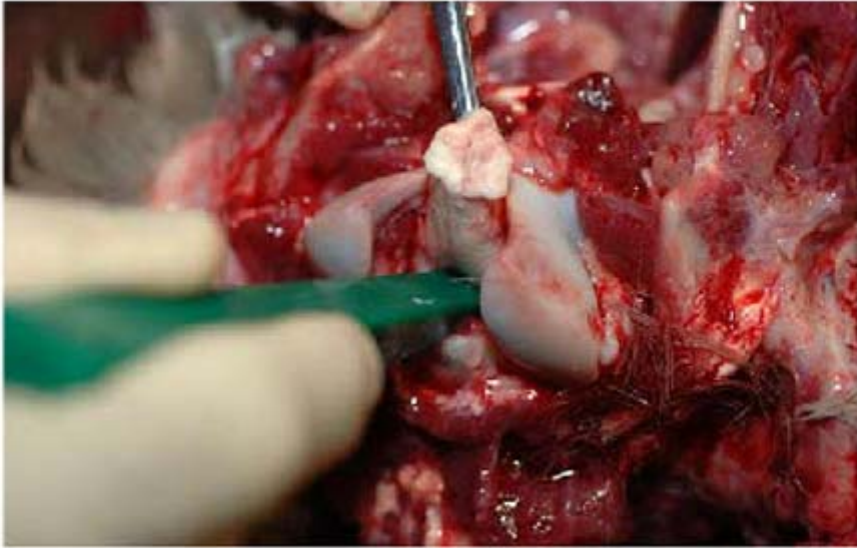
### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 8**  
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. Advance the spoon cranially 2-3 inches until you feel the leading edge of the spoon hit bone. Sever the cerebellum, and then remove the spoon.

Select one of the numbered buttons below, or select the forward button to advance.

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9

10

11


>


Intro





### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 9**

The brainstem should easily be extracted by pulling the spoon toward you while maintaining gentle traction on the spinal cord with the forceps.

Note that excessive caudal traction on the spinal cord may result in a mutilated non-diagnostic sample.

If the brainstem is not readily removed by this method, stop, and repeat the above steps starting with verifying that the cranial nerves are severed. Failure to sever cranial nerves is a common cause of damaged samples.

Select one of the numbered buttons below, or select the forward button to advance.

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11


Intro


>





### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 10  
The sample extracted with this method is usually 3 to 4 centimeters long with the obex in the center and a portion of the cerebellum attached.

Select one of the numbered buttons below, or select the forward button to advance.

1 2 3 4 5 6 7 8 9 10 11


Intro


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### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 11  
Submit the brainstem as directed in the  
Scrapie Tissue Collection Protocols Chart.

Select one of the numbered buttons below, or select the forward button to advance.

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7

8

9

10

11

>


Intro




## SPECIMEN PRESERVATION PROCEDURES

**Overview**


**Tools**



**Collection Procedure**



**Sample Preservation**



**Scrapie Tissue Collection Protocols (April 2008)**

	Animal ID <sup>1</sup>	Preservation				Preservation (Collection) Protocols							
		Formalin (10%)	Formalin (20%)	Formalin (30%)	Formalin (40%)	Formalin (50%)	Formalin (60%)	Formalin (70%)	Formalin (80%)	Formalin (90%)	Formalin (100%)	Formalin (110%)	Formalin (120%)
<b>Brainstem (including SCN)</b>													
Brainstem (including SCN)	X	X	X	X									
Brainstem (including SCN) (Suspect and final)	X	X	X	X									
Brainstem (including SCN) (Suspect and final) (Formalin-fixed)	X	X	X	X									
<b>Brainstem (including SCN) (Formalin-fixed)</b>													
Brainstem (including SCN) (Formalin-fixed)	X	X	X	X									
Brainstem (including SCN) (Formalin-fixed) (Suspect and final)	X	X	X	X									
Brainstem (including SCN) (Formalin-fixed) (Suspect and final) (Formalin-fixed)	X	X	X	X									
<b>Brainstem (including SCN) (Formalin-fixed) (Suspect and final) (Formalin-fixed)</b>													
Brainstem (including SCN) (Formalin-fixed) (Suspect and final) (Formalin-fixed)	X	X	X	X									
Brainstem (including SCN) (Formalin-fixed) (Suspect and final) (Formalin-fixed) (Formalin-fixed)	X	X	X	X									
Brainstem (including SCN) (Formalin-fixed) (Suspect and final) (Formalin-fixed) (Formalin-fixed) (Formalin-fixed)	X	X	X	X									

See reverse side for explanation of superscripts (definitions, preservation methods, and submission information).

**Step 1**

After collecting the brainstem, refer to the Scrapie Specimen Tissue Collection Protocols chart to determine which specimens to submit and the preservation method for submission to the designated laboratory - formalin-fixed, fresh, or both.

Select one of the numbered buttons below, or select the forward button to advance.

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Refer to the current version of the Scrapie Tissue Collection Protocols chart on page 6 and 7





### SPECIMEN PRESERVATION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 2**  
At this stage the brainstem derived from the whole brain and the brainstem derived with the brainstem collection via the foramen magnum should be similar.

Select one of the numbered buttons below, or select the forward button to advance.

**1** **2** **3** **4** **>**



### SPECIMEN PRESERVATION PROCEDURES

#### Overview

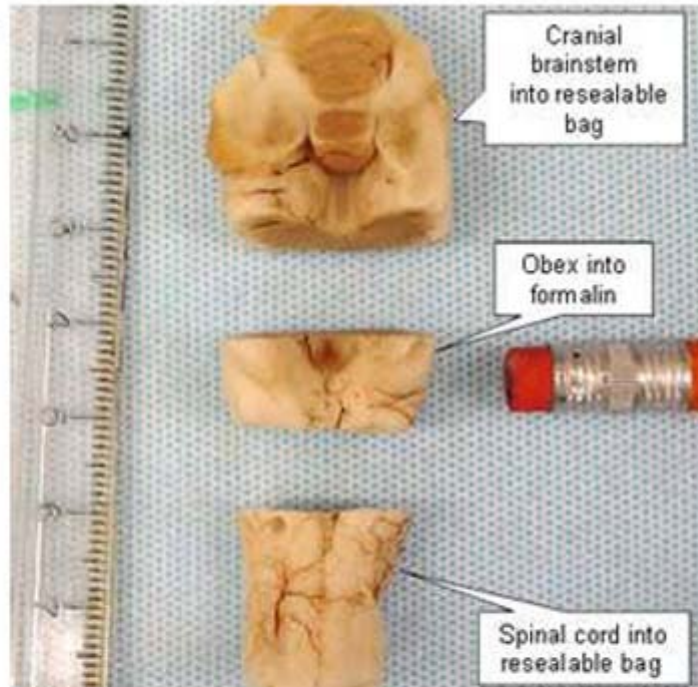
##### Tools



##### Collection Procedure



##### Sample Preservation



Select one of the numbered buttons below, or select the forward button to advance.



#### Step 3

If sectioning of the brainstem is required, remove both ends of the brainstem leaving a 1 to 1 1/2 cm long section of brainstem with the obex centered in the middle. As a guide, you can place a pencil across the brainstem, directly on top of the point of the V, and then cut on either side of the pencil.



### SPECIMEN PRESERVATION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 4  
Preserve the specimens to be submitted in formalin or place in a labeled, resealable plastic bag and refrigerate or chill with ice packs until shipment.

Select one of the numbered buttons below, or select the forward button to advance.

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### OVERVIEW

Overview


**Tools**




**Collection Procedure**



**Sample Preservation**





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Overview

Complete brain removal is required for scrapie clinical suspects. Also, there will be many times when the brainstem is too autolytic to be removed from the foramen magnum, or attempted removal by the spoon method is unsuccessful and removal of the top of the calvarium is your best chance for success. With carcasses or heads that have undergone moderate autolysis, or for which the status is questionable, it is best to remove the top of the skull to expose the entire brain.

There are times when the carcass or head is so autolytic that one must exercise extreme care to remove the brain without the complete disruption of architecture leaving an amorphous brain

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There are times when the carcass or head is so autolytic that one must exercise extreme care to remove the brain without the complete disruption of architecture leaving an amorphous brain paste.

The following process on collecting a complete brain is of an elk head. The process for collecting a complete brain from a sheep is comparable.



### TOOLS

#### Overview

##### Tools



##### Collection Procedure



##### Sample Preservation



#### Tools

The instruments and supplies needed for sampling the complete brain removal include: a bone saw, a wood chisel or wide-tipped large screwdriver, scissors, forceps, a scalpel, a jar of formalin, and resealable Ziploc-type plastic bags

Biopsy instruments such as forceps and scissors are single-use, meaning one set is used for each animal, then disposed of properly.

Select one of the numbered buttons below, or select the forward button to advance.


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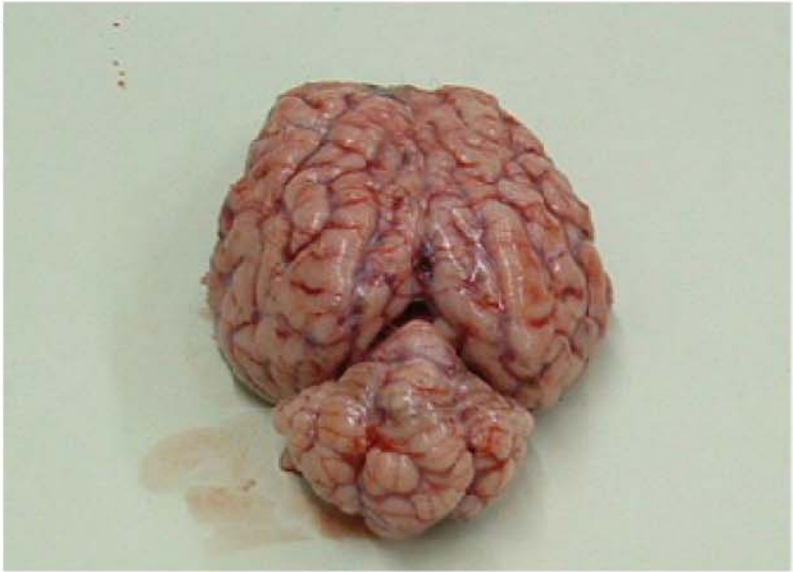
### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Use the following steps to collect the complete brain.

Select one of the numbered buttons below, or select the forward button to advance.

123456


Intro


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### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 1  
Skin the head.

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1 2 3 4 5 6

Intro

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### COLLECTION PROCEDURES

#### Overview

##### Tools



##### Collection Procedure



##### Sample Preservation



#### Step 2

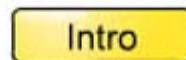
Using a bone saw, remove the top and back of the skull. This requires three (3) cuts.

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.


Select one of the numbered buttons below, or select the forward button to advance.








### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 3  
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.

Select one of the numbered buttons below, or select the forward button to advance.

1 2 3 4 5 6


Intro


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### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 4**

Open the dense, fibrous dura mater covering the sides and top of the brain with scissors and forceps by making a midline longitudinal cut from the cranial aspect of the cerebrum to the spinal cord. Ensure that you completely incise the extra tough section of the dura mater known as the tentorium cerebelli which lies between the cerebrum and cerebellum.

Select one of the numbered buttons below, or select the forward button to advance.

1

2

3

4

5

6


Intro


>





### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 5**  
Once the entire brain is exposed, direct the nose dorsally, resting the occipital condyles on a flat surface such as a table or floor.

Sever the cranial nerves starting with the olfactory nerves.

Proceed caudally cutting the cranial nerves and allow gravity to assist removal of the brain from the cranial vault.

Select one of the numbered buttons below, or select the forward button to advance.

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Intro


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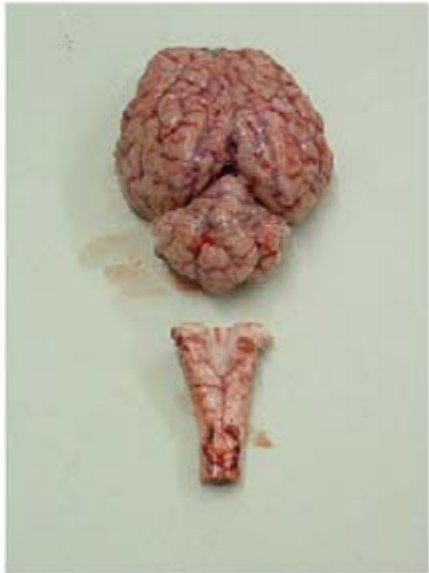
### COLLECTION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 6  
Remove the cerebellum from the brainstem at the level of the peduncles.

Note: The European spoon and saw methods of sample collection have been described. There are many other methods that work well. Learning a method, usually from a more experienced individual, and practicing is the key.

Select one of the numbered buttons below, or select the forward button to advance.

1

2

3

4

5

6

Intro


>




## SPECIMEN PRESERVATION PROCEDURES

**Overview**


**Tools**



**Collection Procedure**



**Sample Preservation**



Scrapie Tissue Collection Protocols (April 2008)											
	Animal ID <sup>1</sup>	Formalin					Fresh Tissue: Chilled or Frozen				
		Online Resources (including others)	MS	Classification	Refract	Other	Online Resources (including others)	MS	Classification	Refract	Other
Highly Susceptible (including SNCP)											
Non-susceptible											
Animal without clinical signs	X	X	X	X							
Exposure to scrapie agent											
Animals with less specific signs <sup>2</sup>											
Suspect and test											
Presumptive scrapie <sup>3</sup>											
Known scrapie <sup>4</sup>											
Non-clinical	X	(2)	(2)				(1)	(2)			
Less specific signs <sup>2</sup>		(2)	(2)	(2)			(1)	(2)	(2)		
Suspect and test											
Presumptive scrapie <sup>3</sup>											
Known scrapie <sup>4</sup>	X		X		X	X		X	X	X	X
SNCP <sup>5</sup>		(2)	(2)	(2)			(1)	(2)	(2)		
Directed by Regional	X	(2)	(2)	(2)			(1)	(2)	(2)		

See reverse side for explanation of superscripts (definitions, preservation methods, and submission information).

Select one of the numbered buttons below, or select the forward button to advance.

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**Step 1**


After collecting the whole brain, refer to the Scrapie Specimen Tissue Collection Protocols chart to determine how to submit the tissue to the designated laboratory - formalin-fixed, fresh, or both.


Refer to the current version of the Scrapie Tissue Collection Protocols chart on page 6 and 7





### SPECIMEN PRESERVATION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 2

At this stage the brainstem derived from the whole brain and the brainstem derived with the brainstem collection via the foramen magnum should be similar.

Select one of the numbered buttons below, or select the forward button to advance.

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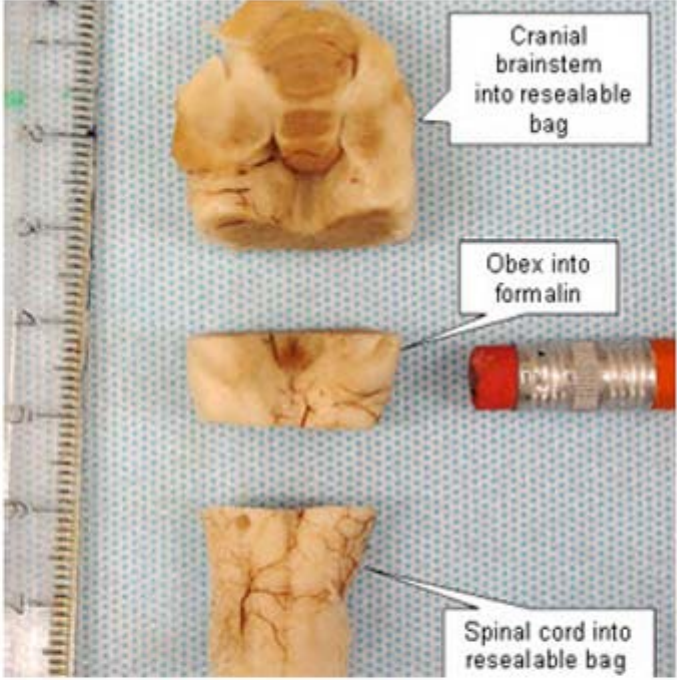
### SPECIMEN PRESERVATION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Cranial brainstem into resealable bag

Obex into formalin

Spinal cord into resealable bag

Step 3

If sectioning of the brainstem is required, remove both ends of the brainstem leaving a 1 to 1 1/2 cm long section of brainstem with the obex centered in the middle. As a guide, you can place a pencil across the brainstem, directly on top of the point of the V, and then cut on either side of the pencil.

Select one of the numbered buttons below, or select the forward button to advance.

**1** **2** **3** **4** **5** **>**



### SPECIMEN PRESERVATION PROCEDURES

#### Overview

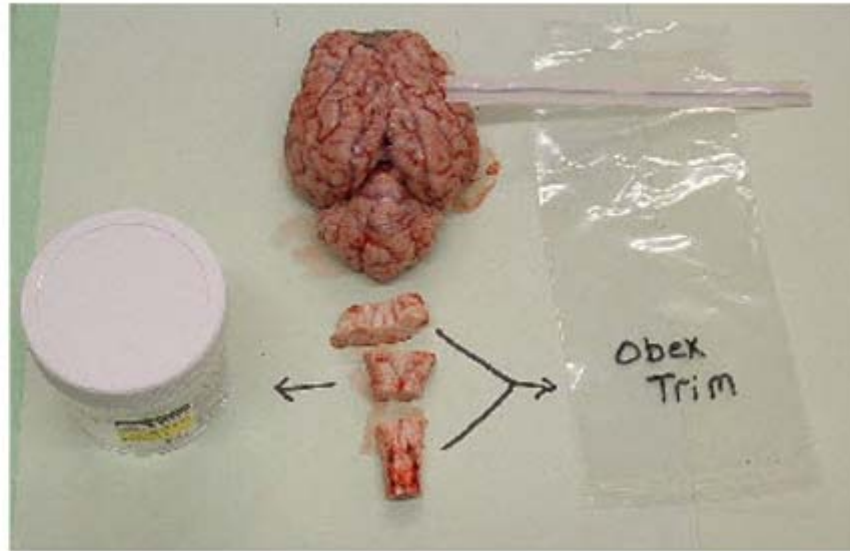
##### Tools



##### Collection Procedure



##### Sample Preservation



#### Step 4

Place the caudal piece (spinal cord) and cranial piece (cranial brainstem) into a plastic bag and refrigerate or chill with ice packs.

Select one of the numbered buttons below, or select the forward button to advance.





### SPECIMEN PRESERVATION PROCEDURES

#### Overview

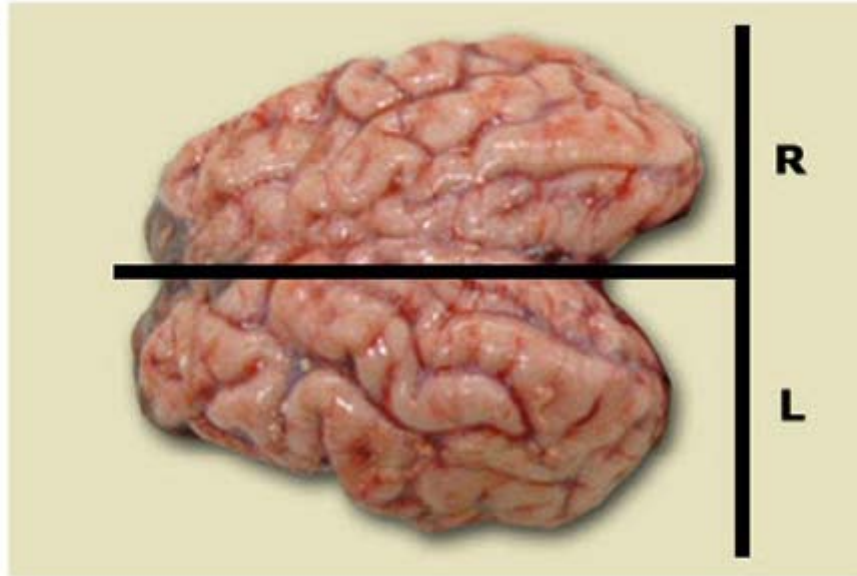
##### Tools



##### Collection Procedure



##### Sample Preservation



#### Step 5

Divide the brain longitudinally into left and right halves. Place the left half of the brain in a labeled, re-sealable plastic bag and refrigerate or chill with ice packs. Place the right half of the brain in formalin.

In some cases, it will be necessary to also section the cerebellum and preserve as for the brain.

Select one of the numbered buttons below, or select the forward button to advance.







### OVERVIEW

Overview


**Tools**




**Collection Procedure**



**Sample Preservation**





Select one of the numbered buttons below, or select the forward button to advance.

1

>

Overview

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. Collecting and submitting the mucosal crypts and leaving the deep tonsillar lymphoid tissue behind is one of the most common scrapie submission errors identified at the laboratory.



### TOOLS

#### Overview

##### Tools



##### Collection Procedure



##### Sample Preservation



#### Tools

The tools required to collect the tonsils include a sharp boning knife, a scalpel, sharp stainless steel scissors, aggressively toothed forceps, a jar of formalin, and resealable plastic bags.

Instruments such as forceps and scissors should be thoroughly rinsed between animals and disinfected after use. Instruments used to collect necropsy tissues should not be used for biopsy specimen collection from live animals.

Select one of the numbered buttons below, or select the forward button to advance.


1


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### COLLECTING TONSILS

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 1**  
Place the head upside down on the table.  
Remove the skin from the ventral surface  
of the mandible.

Select one of the numbered buttons below, or select the forward button to advance.

1

2

3

4

5


Intro


>





### COLLECTING TONSILS

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 2**  
Grab the pharynx with your non-cutting 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.

Select one of the numbered buttons below, or select the forward button to advance.

1

2

3

4

5


Intro


>





### COLLECTING TONSILS

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 3**  
The oropharynx (cranial) and nasopharynx (caudal) will now be exposed. Grab the ventrolateral aspect of the oropharynx with forceps and observe the tonsillar crypts opening into the dorso-lateral aspect of the oropharynx. Identify the tonsillar crypts as a landmark. The submucosal tonsillar lymphoid tissue will always be continuous with the tonsillar crypts. Begin a dissection plane between the pharynx and the lateral pharyngeal muscles. As the dissection is extended dorsally, a bulge of lymphoid tissue, connected to the tonsillar crypts, will be seen protruding from the lateral pharyngeal wall.

Select one of the numbered buttons below, or select the forward button to advance.

12345


Intro


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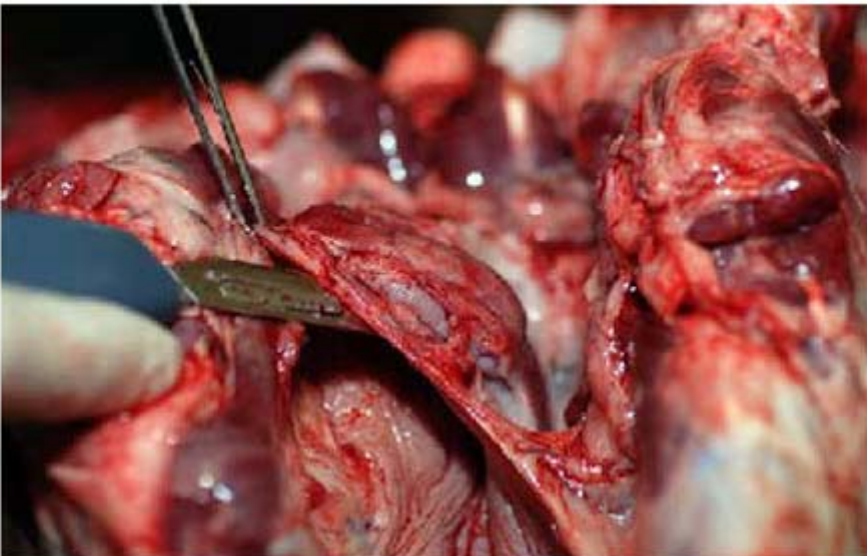
### COLLECTING TONSILS

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 4**

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.

The most common submission error is the collection and submission of the mucosal crypts instead of the tonsillar lymphoid tissue. Ensure that you have collected the deep tonsillar lymphoid tissue. In sheep, the tonsillar lymphoid tissue will vary from 3-7 mm in diameter.

Select one of the numbered buttons below, or select the forward button to advance.

**1** **2** **3** **4** **5**


**Intro**


**>**





### COLLECTING TONSILS

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 5  
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.

Select one of the numbered buttons below, or select the forward button to advance.

1 2 3 4 5

Intro

>

### SPECIMEN PRESERVATION PROCEDURES

Step 1: After collecting the tonsils, refer to the Scrapie Specimen Tissue Collection Protocols chart (on pages 5-6) to determine which specimens to submit and the preservation method for submission to the designated laboratory - formalin-fixed, fresh, or both.


Step 2: If the tonsils are large, divide them in half longitudinally (lengthwise) into two halves. Place half of each tonsil into a jar of formalin. Place the remaining halves into a plastic bag for chilling or freezing. If the tonsils are small, place one in formalin and one in a plastic bag.




### OVERVIEW

Overview


**Tools**




**Collection Procedure**



**Sample Preservation**





Overview

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.

Select one of the numbered buttons below, or select the forward button to advance.

1

>



### TOOLS

#### Overview

##### Tools



##### Collection Procedure



##### Sample Preservation



#### Tools

The instruments and supplies needed for sampling the lymph nodes include: a knife or scalpel, sharp stainless steel scissors, rat-toothed forceps, a jar of formalin, and resealable plastic bags.

Biopsy instruments such as forceps and scissors should be used for biopsy specimen collections from live animals, and should be thoroughly rinsed between animals and disinfected after use.

Select one of the numbered buttons below, or select the forward button to advance.

1

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### COLLECTING MEDIAL RETROPHARYNGEAL LYMPH NODES (RPLN)

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  

**Step 1**  
Place the head upside down on the table. Remove the skin from the ventral surface of the mandible.

Select one of the numbered buttons below, or select the forward button to advance.

1 2 3 4 5


Intro


>





### COLLECTING MEDIAL RETROPHARYNGEAL LYMPH NODES (RPLN)

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 2**  
Grab the pharynx with your non-cutting 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.

Select one of the numbered buttons below, or select the forward button to advance.

**1** **2** **3** **4** **5**


**Intro**


**>**





### COLLECTING MEDIAL RETROPHARYNGEAL LYMPH NODES (RPLN)

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 3**  
The hyoid bones that you encounter will need to be cut with poultry shears or disarticulated at a joint with the knife.

Select one of the numbered buttons below, or select the forward button to advance.

**1** **2** **3** **4** **5**


**Intro**


**>**





### COLLECTING MEDIAL RETROPHARYNGEAL LYMPH NODES (RPLN)

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 4**

The medial and lateral RPLNs are paired tissues located in the head.

The medial RPLNs 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 slaughter processes. They are the largest lymph nodes of the head and neck.

They can be palpated by placing the index finger in the nasopharynx and the thumb caudally on the caudal pharyngeal muscles. When the thumb and index finger are brought together the medial retropharyngeal node can

Select one of the numbered buttons below, or select the forward button to advance.

1 2 3 4 5

Intro

>

#### Slide Text Continued:

be palpated between them. The opposite node will be about one centimeter medial to the first.

The lateral RPLNs are relatively superficial and are found on either side of median line midway between the larynx and the foramen magnum. They will typically be visible if the head is removed at the atlanto-occipital joint.



### COLLECTING MEDIAL RETROPHARYNGEAL LYMPH NODES (RPLN)

#### Overview

##### Tools



##### Collection Procedure



##### Sample Preservation



#### Step 5

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.

Select one of the numbered buttons below, or select the forward button to advance.



Intro





## SPECIMEN PRESERVATION PROCEDURES

Overview

Tools

Collection Procedure

Sample Preservation

Scrapie Tissue Collection Protocols (April 2008)

	Animal ID <sup>1</sup>	Orbital Nucleus (including skin)	MS	Formalin				Fresh Tissue: Chilled or Frozen					
				Concentration	Hours	Final	Chase (days)	Orbital Nucleus (including skin)	MS	Concentration	Hours	Final	Chase (days)
Field Defect (including SNCF testing)													
Not exposed animals without clinical signs		X	X	X									
Exposed animals: animals with less specific signs <sup>2</sup>							X		X			X	
Suspect and test positive animals <sup>3</sup>				X	X	X	X		X	X	X	X	X
Known exposed <sup>4</sup>									X	X		X	X
SNCF <sup>5</sup>									X	X	X		
Directed by Region <sup>6</sup>									X	X	X		

Select one of the numbered buttons below, or select the forward button to advance.

1

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Step 1

After collecting the lymph nodes, refer to the Scrapie Specimen Tissue Collection Protocols chart to determine which specimens to submit and the preservation method for submission to the designated laboratory - formalin-fixed, fresh, or both.


Refer to the current version of the Scrapie Tissue Collection Protocols chart on page 6 and 7


53





### SPECIMEN PRESERVATION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 2  
Cut each lymph node lengthwise.

Select one of the numbered buttons below, or select the forward button to advance.

**1** **2** **3** **4** **>**



### SPECIMEN PRESERVATION PROCEDURES

#### Overview

#### Tools



#### Collection Procedure



#### Sample Preservation



#### Step 3

Place half of the left and half of the right lymph node into a jar of formalin.


Select one of the numbered buttons below, or select the forward button to advance.








### SPECIMEN PRESERVATION PROCEDURES

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 4  
Place the remaining halves into a plastic bag and refrigerate or chill with ice packs until shipment.

Select one of the numbered buttons below, or select the forward button to advance.

1234

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### OVERVIEW

The use of rectal biopsy tissue was approved for scrapie testing by APHIS in January 2008. The recto-anal mucosa-associated lymphoid tissue (RAMALT) is a band of lymphoid tissue detectable at the anal mucocutaneous junction and proceeding cranially for approximately 1.5 centimeters. It is comprised of a nearly complete 360 degree ring of lymphoid tissue, with relatively sparse areas of lymphoid follicles at the 12 o'clock and 6 o'clock positions.

Several factors make the RAMALT useful for live animal diagnostic testing for scrapie in sheep and goats. Collecting rectal biopsy samples is faster and technically less demanding than collecting third eyelid biopsy samples. The procedure causes minimal discomfort and has a low rate of surgical complications, and multiple locations can be sampled accommodating serial testing over time. The minimum test-eligible age is 14 months. If testing a flock to determine whether the flock is infected, those ewes potentially exposed as adults must be tested at least 18 months after the last possible lambing of the infected animal. (See Scrapie UM&R for details).

### TOOLS

#### Overview

##### Tools



##### Collection Procedure



##### Sample Preservation



#### Tools

Tools needed to collect a rectal biopsy include a topical analgesic, containing 2% Lidocaine gel, suitable for mucosal membranes, adjustable head light, a speculum designed to expose specific areas of rectal mucosa immediately dorsal to RAMALT, fine dissecting forceps and fine sharp-ended scissors, biopsy sponges and cassettes, and containers with 10% buffered formalin for fixation. Both the forceps and scissors are 115mm (approximately 4 inches in length).

Biopsy instruments (speculum, forceps, and scissors) are single-use, meaning one set is used for each animal, then disposed of properly.

Select one of the numbered buttons below, or select the forward button to advance.


1








### COLLECTION PROCEDURE

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 1**  
Place sheep over the width of a rectangular bale of straw so it is resting with the bale underneath its ventral abdomen with its front legs on one side and back legs on the other. This both restrains the sheep and allows easy placement of the speculum.

Select one of the numbered buttons below, or select the forward button to advance.

123456789


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Intro





### COLLECTION PROCEDURE

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 2**  
Apply topical analgesia cream to the rectal mucosa to a depth of 2.5cm (1") and to the outer surface of the speculum.

Select one of the numbered buttons below, or select the forward button to advance.

1

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5

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7

8

9


>


Intro





### COLLECTION PROCEDURE

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 3**  
Insert the speculum to full the permissible depth with the cut-away portion positioned ventrally. Allow the dorsal part of the rectal sphincter to curl over the dorsal part of the speculum. This, along with the two 'wings,' will result in the speculum being self-retaining.

Select one of the numbered buttons below, or select the forward button to advance.

123456789


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Intro





### COLLECTION PROCEDURE

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 4

Adjust the head light to illuminate the rectal mucosa exposed in the cut-away portion of the speculum. Identify the biopsy site about 1 cm beyond the mucocutaneous boundary.

Select one of the numbered buttons below, or select the forward button to advance.

123456789


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Intro




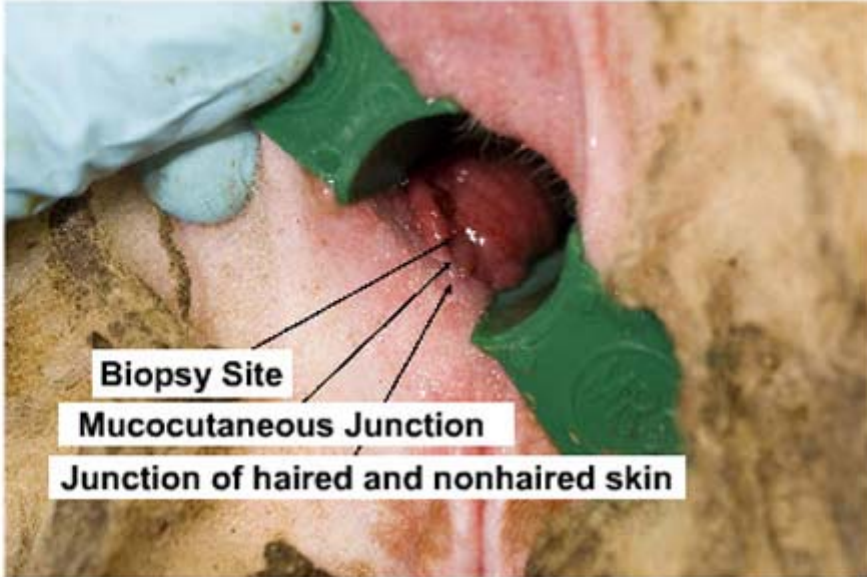
### COLLECTION PROCEDURE

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Biopsy Site**

**Mucocutaneous Junction**

**Junction of haired and nonhaired skin**

**Step 5**  
Place the closed fine dissecting forceps between and to the bottom of two adjacent rectal mucosal folds (ridges).

Select one of the numbered buttons below, or select the forward button to advance.

1 2 3 4 5 6 7 8 9

Intro

>



### COLLECTION PROCEDURE

#### Overview

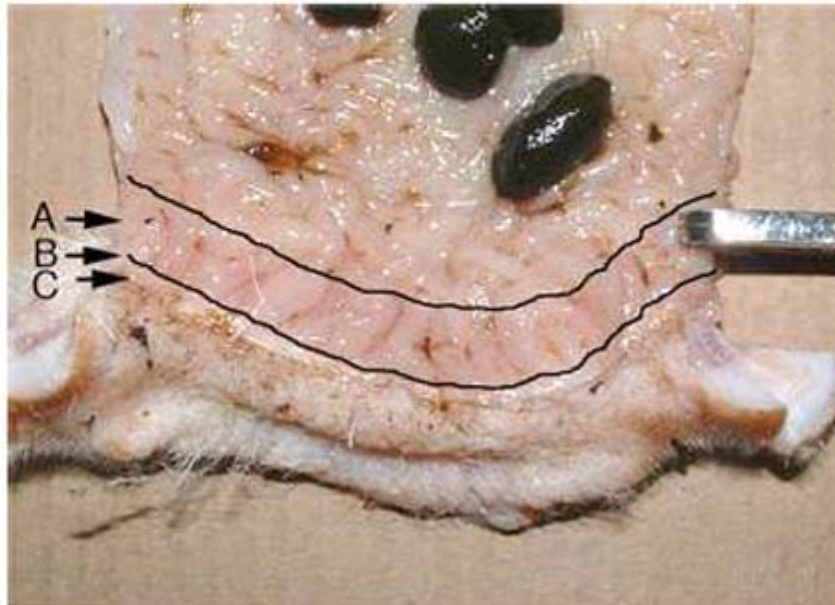
##### Tools



##### Collection Procedure



##### Sample Preservation



#### Step 6

This figure of a dissected rectum illustrates landmarks used to identify the rectal biopsy collection site.

Collect the biopsy specimen, the recto-anal mucosa associated lymph tissue (RAMALT), from the area between the two lines marked A in this figure.

Note: B is the mucocutaneous junction, and C is the junction of haired and non-haired skin.

Select one of the numbered buttons below, or select the forward button to advance.




Intro








### COLLECTION PROCEDURE

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 7**  
Open forceps and grasp the mucosa of the crypt over the initial 0.8 - 1.0cm rostral to the rectal muco-cutaneous junction.

Select one of the numbered buttons below, or select the forward button to advance.

123456789


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Intro





### COLLECTION PROCEDURE

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 8**  
Raise forceps to create a "tent" of tissue above the two adjacent mucosal folds. Avoid sampling tissues at the 6 and 12 o'clock positions as these tissues have the least amount of lymphoid tissue.

Select one of the numbered buttons below, or select the forward button to advance.

123456789


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Intro





### COLLECTION PROCEDURE

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




**Step 9**  
Slide the open fine scissors under the fine dissecting forceps to the base of the "tent" of mucosa and cut. Collect a 1 x 1.5 cm strip of tissue.

Select one of the numbered buttons below, or select the forward button to advance.

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Intro



### PRESERVATION

#### Overview

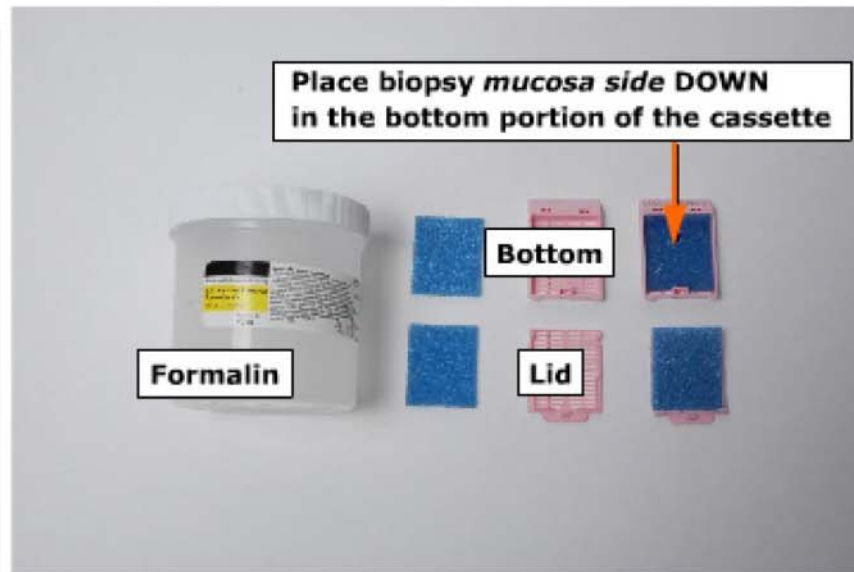
##### Tools



##### Collection Procedure



##### Sample Preservation



#### Step 1

Prepare the biopsy cassette for packaging by placing the gel foam in both the top and bottom compartments of the cassette.


Select one of the numbered buttons below, or select the forward button to advance.








### PRESERVATION

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 2  
Place the specimen into the biopsy cassette mucosal side-down.


Select one of the numbered buttons below, or select the forward button to advance.


1 2 3 4 5 6 >




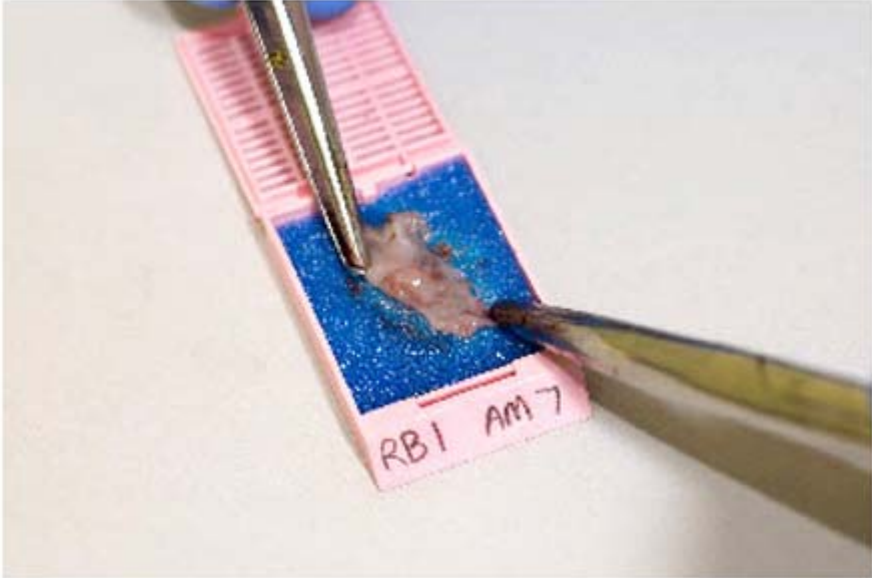
### PRESERVATION

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 3  
Spread the tissue as flat as possible.

Select one of the numbered buttons below, or select the forward button to advance.


1 2 3 4 5 6


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### PRESERVATION

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 4  
Close the cassette.


Select one of the numbered buttons below, or select the forward button to advance.


**1** **2** **3** **4** **5** **6** **>**





### PRESERVATION

**Overview**

**Tools**  


**Collection Procedure**  


**Sample Preservation**  




Step 5  
Record the animal ID number on the cassette.

Select one of the numbered buttons below, or select the forward button to advance.

1

2

3

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6

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### PRESERVATION

#### Overview

#### Tools



#### Collection Procedure



#### Sample Preservation



#### Step 6

Place the filled cassette in 10% formalin.

If rectal biopsy specimens are collected from multiple animals on the same farm, multiple cassettes can be placed in a single formalin jar.

Prior to shipping, tape the formalin jar shut to prevent leakage.

Select one of the numbered buttons below, or select the forward button to advance.





### POTENTIAL COMPLICATIONS AND REMEDIES

Introduction

Follicles

Mucosal Ridges

Speculum

Recovery



Potential complications and remedies include the following:

Select one of the numbered buttons below, or select the forward button to advance.





### POTENTIAL COMPLICATIONS AND REMEDIES

#### Introduction

#### Follicles

#### Mucosal Ridges

#### Speculum

#### Recovery



Sometimes the tissue sample does not contain enough follicles. Ensure you collect biopsies 1 cm inwards from the junction of the non-haired skin of the anus and the rectal mucosa, and use a speculum, headlamp, restraint, and anything else that will help you visualize the collection site.

Select one of the numbered buttons below, or select the forward button to advance.





### POTENTIAL COMPLICATIONS AND REMEDIES

#### Introduction

#### Follicles

#### Mucosal Ridges

#### Speculum

#### Recovery



Ensure the sample is collected from between the mucosal ridges immediately in front of where the whitish skin meets the pink rectal mucosa and not the hairless skin, that is just inside the anus. There are no lymphoid follicles in the skin. However, there are many pain receptors and the animal will react to the biopsy procedure. The lymphoid tissue does not extend up the mucosal ridges, so you must ensure that the forceps grasp the mucosa from the base of the crypt between two adjacent mucosal ridges.

Select one of the numbered buttons below, or select the forward button to advance.





### POTENTIAL COMPLICATIONS AND REMEDIES

#### Introduction

#### Follicles

#### Mucosal Ridges

#### Speculum

#### Recovery



Occasionally, when the speculum is inserted, the mucosa of the rectum is stretched and the mucosal ridges flatten out. If this happens, insert your little finger into the cut-out area of speculum and gently pull the tissue ventrally. This will remove the tension on the portion of rectal mucosa in this part of the speculum allowing the mucosal ridges to reform. Be gentle when you do this or the mucosal surface will hemorrhage, making recognition of the reforming mucosal folds difficult.


Select one of the numbered buttons below, or select the forward button to advance.





### POTENTIAL COMPLICATIONS AND REMEDIES

**Introduction**  
**Follicles**  
**Mucosal Ridges**  
**Speculum**  
**Recovery**



**Always warn owners that some bleeding is normal with rectal biopsy**

Sometimes there is rectal bleeding after sampling. The following may alleviate this:

Keep the animals calm following sample collection.

Stay superficial when you collect biopsies.

The biopsy should consist of mucosa only: there shouldn't be any connective tissue sticking to the underside of the biopsy (if there is, you went too deep).

Select one of the numbered buttons below, or select the forward button to advance.

1 2 3 4 5

>





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