



Veterinary Laboratories Agency

**Summary of frozen tissues and fluids
from ovine sample collection
procedures.**



Table of Contents

Summary of Ovine Tissue and Fluid Sources	3
Samples Collected from the Field:.....	3
TS5300: Scrapie in Sheep and Goats	3
Summary of Tissues and Fluids collected from Scrapie Field Cases	4
Samples Collected at VLA Weybridge.....	5
TS5700 Additional Tissues (Scrapie).....	5
Time Course Samples.....	8
TS5700 Scrapie Fast Track	8
SE1846 – BSE into sheep	8
SE1945 – BSE into sheep.	8
SE1946 – BSE into sheep (breeding flock).....	9
Summary of time course samples for TS5700 Scrapie fast track, SE1846, SE1945 and SE1946	9
TSE Archive Ovine Necropsy Tissues – Project summary	10



Summary of Ovine Tissue and Fluid Sources

Samples Collected from the Field:

TS5300: Scrapie in Sheep

Collection of tissue and blood from suspects identified through passive surveillance on farms. Live cases are examined clinically and bled generally on farm by a Veterinary Officer (VO) following notification and are then submitted to the local VLA Regional Laboratory (RL) alive for diagnosis, unless they are deemed not fit to travel or have died in transit.

Blood samples are normally sent direct to VLA Weybridge by the VO or the RL for processing in our Containment Level 3 Laboratory (CL3). All blood is collected into EDTA.

Tissue samples are removed following Scrapie sterile collection protocol¹. The Archive receives frozen CNS and lymphoreticular (LRS) tissue, which are sampled within one hour of euthanasia if the animal has been submitted to the RL alive. The tissues are sent to VLA Weybridge the next working day after collection.

¹ Brain material is extracted using a fresh set of scrapie-free instruments and fresh disposable gloves for each head.

**Summary of Tissues and Fluids collected from Scrapie Field Cases**

Blood	EDTA	Whole blood
		Plasma
		Buffy coat
		Red blood cells
CNS	Rostral brain stem	
	Left frontal cortex (2g)	
	Left half brain	
LRS	Spleen (5 x 10g)	
	Mesenteric LN (5 x 10g)	
	Prefemoral LN	
	Prescapular LN	
	Retropharyngeal LN	
	Palatine tonsil	
	Rectal tonsil	
Alimentary	Distal ileum	



Samples Collected at VLA Weybridge

TS5700 Additional Tissues (Scrapie)

In addition to the CNS, LRS and blood samples received from the field, additional tissues are collected for storage in the TSE Archive from cases that have been culled at VLA Weybridge. These are taken from two control groups:

- Ovine non-exposed to scrapie controls derived from:
 - Romney controls from the Horkstow sheep unit in Yorkshire (no longer running).
 - New Zealand derived sheep from the Arthur Rickwood Sheep Unit in Cambridgeshire (SE1931 – current)
- Scrapie clinical ovine suspects – these can be from field cases identified through passive surveillance, which have been transported to Weybridge or from the scrapie flock maintained at Ripley.
- Scrapie exposed culls – these are gathered from routine culls transported from the scrapie flock maintained at Ripley.

All tissue samples are collected to transmission standard under scrapie sterile conditions within necropsy facilities. Head tissues are collected in a separate sealed area to body transmission and are sampled using aseptic instruments. All blood sampled is processed in the CL3 within an hour of collection.

Where there are a recognised demand for certain tissues or more blood samples, the Archive will send in a detailed written request to Neuropathology for prospective sampling.



TS5700 Ovine Necropsies – Tissues Summary

Tissue Type		Scrapie Field Cases	Ripley Suspects	Ripley Culls	Ovine Controls	
Blood	Serum	Yes	Yes	Yes	Yes	
	EDTA & Heparin Plasma	Yes	Yes	EDTA and Citrate	Yes	
Head	LRS	Submandibular LN	No	No	Yes	No
		Medial retropharyngeal LN	Yes	Yes	Yes	Yes
		Lateral retropharyngeal LN	No	No	No	Yes
		Palatine tonsil	Yes	Yes	Yes	Yes
		Nictitating membrane	No	No	No	Yes
	CNS	Caudal medulla	No	Yes	Yes	Yes
		Cerebellum	Yes	Yes	Yes	Yes
		Rostral medulla	Yes	Yes	Yes	Yes
		Brain stem tissues (rostral to obex)	Whole brain stem	Thalamus/Midbrain & brain stem / basal nuclei	Thalamus/Midbrain & brain stem / basal nuclei	Thalamus/Midbrain & brain stem/ basal nuclei
		Cerebral hemisphere tissues	In two halves	Frontal, occipital & parietal cortex	Frontal, occipital & parietal cortex	Frontal, occipital & parietal cortex
	Other	Eyes	No	No	No	Yes



TS5700 Ovine Necropsies – Tissues Summary

Tissue Type		Scrapie Field Cases	Ripley Suspects	Ripley Culls	New Zealand Controls	
Body	LRS	Prescapular LN	Yes	No	No	No
		Prefemoral LN	Yes	No	No	No
		Mesenteric LN	Yes	Yes	Yes	Yes
		Spleen	Yes	Yes	Yes	Yes
		Rectal tonsil	Yes	Yes	Yes	No
	CNS	Spinal cord	Caudal, thoracic and lumbar sections	Caudal, thoracic and lumbar sections	No	Caudal spinal cord only
	Viscera	Liver	No	No	No	Yes
		Trachea	No	No	No	Yes
	Alimentary	Distal ileum (intact)	Yes	Yes	Yes	Yes
		Caecum	No	No	No	Yes
		Jejunum	No	No	No	Yes
		Duodenum	No	No	No	Yes
	Urine		Yes	No	No	No
	Faeces		No	No	No	Yes
	Other	Triceps brachii	No	No	No	Yes



Time Course Samples

TS5700 Scrapie Fast Track

The Scrapie Fast Track project was set up to collect monthly time course blood samples through to clinical end point from 16 ARQ/VRQ [confirm] sheep selected from the Ripley flock and dosed subcutaneously with a known titre of scrapie (5ml).

Necropsy tissues are also held by Archive.

SE1846 – BSE into sheep

Time course samples are collected at 6 week intervals through to clinical end point from two groups of 20 ARQ/ARQ sheep on this project and stored by Archive. Each group is split according to breed (Suffolk or Romney) and both have 10 x sheep orally dosed with 5g BSE and 10 x unchallenged controls kept in separate pens.

Necropsy tissues are held by Project Store.

SE1945 – BSE into sheep.

Time course samples are collected at 6 week intervals through to clinical end point from two groups of 10 ARQ/ARQ sheep on this project and stored by Archive. Each group is split according to breed (Suffolk or Romney) and both have 5 x sheep orally dosed with 5g BSE and 5 x unchallenged controls kept in separate pens.

Necropsy tissues are held by Project Store.

**SE1946 – BSE into sheep (breeding flock)**

The Archive stores time course samples collected at 6 week intervals through to clinical end point from this project, which is a study of BSE oral challenge into a breeding flock. Samples have been sourced from the following:

- 30 x ARQ/ARQ ewes dosed orally with 5g BSE at the outset of the project. Necropsy tissues for these sheep are held by Archive. Four remain alive;
- 20 x ARQ/ARQ undosed, in contact ewes introduced into the flock at the beginning of the project;
- To date, 70 x ARQ/ARQ undosed, in contact offspring lambs bred in subsequent years, (2003, 2004 and 2006).

A further 8 x ARQ/ARQ lambs were dosed orally with 1g BSE and kept separate from the main project. Time course blood samples and necropsy tissues are both stored by Archive.

Summary of time course samples for TS5700 Scrapie fast track, SE1846, SE1945 and SE1946

Clotted	Serum
EDTA , Fluor Oxalate and Heparin	Whole Blood (SE1846 & SE1945 EDTA only)
	Plasma (SE1846 & SE1945 FI Ox and Heparin only)
	Buffy coat (SE1846 & SE1945 Heparin only)
	Red blood cells (TS5700 only)



TSE Archive Ovine Necropsy Tissues – Project summary

Tissue Type			TS5700 Scrapie Fast Track	SE1946
Blood	Serum		Yes	Yes
	EDTA Heparin	& Plasma	Yes	Yes
Head	CSF			
	LRS	Submandibular LN	No	Yes
		Medial retropharyngeal LN	Yes	Yes
		Palatine tonsil	Yes	Yes
		Nictitating membrane	No	Yes
	CNS	Caudal medulla	No	Yes
		Cerebellum	Yes	Yes
		Rostral medulla	Yes	Yes
		Brain stem tissues (rostral to obex)	In two halves	Thalamus/Midbrain & brain stem / basal nuclei
		Cerebral hemisphere tissues	In two halves	Frontal, parietal and occipital cortex.
	PNS	Trigeminal ganglia	No	Yes
	Other	Eyes	No	No

Body	LRS	Cervical thymus	No	Yes
		Prescapular LN	Yes	No
		Prefemoral LN	Yes	No
		Mesenteric LN	Yes	Yes
		Spleen	Yes	Yes
		Rectal tonsil	Yes	Yes
	CNS	Spinal cord	Caudal, thoracic and lumbar sections	Caudal, thoracic and lumbar sections
	PNS	Celiac Mesenteric Plexus	No	Yes
		Vagus nerve	No	Yes
		Sympathetic chain	No	Yes



Body	Viscera	Liver	No	Yes
		Trachea	No	No
		Kidney	No	Yes
	Alimentary	Distal ileum (intact)	Yes	Yes
		Caecum	No	No
		Jejunum (intact)	No	Yes
		Duodenum	No	No
	Reproductive	Caruncle	No	Yes
		Cotyledons	No	Yes
	Urine		Yes	No
	Faeces		No	No
	Other	Triceps brachii	No	No