



Veterinary Laboratories Agency

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



Table of Contents

Samples collected from the field:.....	3
TS5200: Bovine Field Case Diagnosis	3
Additional Materials	4
Samples Collected at VLA Weybridge:.....	5
TS5700 Additional Tissues (BSE).....	5
TS5700 Bovine Necropsies – Tissues and Fluid Summaries	6
FT0813 (FAIR-CT98-3651).....	7
FT0813 Bovine Necropsies – Tissues and Fluid Summaries.....	8
Time Course Samples.....	11
TS5700 Bovine Controls – Monthly blood sampling	11
SE1930 Attack Rate	11
SE1736: Bovine BSE Pathogenesis Study (completed February 2006) .	12
SE1736 Bovine Necropsies – Tissues and Fluid Summaries	13



Summary of Bovine Tissue and Fluid Sources

Samples collected from the field:

TS5200: Bovine Field Case Diagnosis

The Archive receives samples collected surplus to diagnostic requirements from BSE suspects identified through passive surveillance and have been euthanized on farms by a Veterinary Officer (VO). Current procedure dictates that the heads are sent to VLA Regional Laboratories (RLs) for brain removal under scrapie sterile conditions¹. The TSE Archive normally receives the left side of the brain (rostral to obex), which would have been frozen at -20°C at the RL before being sent to VLA Weybridge for storage at -80°C in the TSE Archive.

The TSE Archive also stores at -80°C brain stem (rostral to obex) collected between 1996-2002 and whole brains collected between January 2002 and April 2004. All samples collected from April 1997 onwards would have been collected following a scrapie sterile collection protocol.

In addition to brain material, we receive whole blood samples that are collected prior to euthanasia on the farms (clotted, heparinised, and EDTA). These are processed in our Containment Level 3 laboratory (CL3), and stored at -80°C. Blood sample breakdown is as follows:

Clotted	Serum
EDTA and Heparin	Whole blood (prior to spinning)
	Plasma
	Buffy Coat
	Red Blood Cells

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



Additional Materials

CSF

The Archive stores CSF sampled between 1997 to 1998. The CSF was sampled prior to euthanasia on the farms, and submitted frozen to TSE archive.

Cervical spinal cord

In addition to brain stem, the archive received and stored cervical spinal cord between November 1996 and March 1999.

BSE Cohorts (Clinically Normal Controls)

The Archive stores blood sampled between 1997 to 2000 from farms with confirmed incidence of BSE.

As these were collected from live cattle (i.e. not followed by euthanasia), there is no brain material to support confirmation of BSE-free status.



Samples Collected at VLA Weybridge:

TS5700 Additional Tissues (BSE)

In addition to the CNS 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 specified groups:

- Bovine clinically normal controls (CNC) or non-exposed to BSE controls
- BSE clinical bovine suspects – confirmed positive or negative

These necropsies are carried out on an ***occasional*** basis, when cases are acquired (Controls) or are certified fit for transport to VLA Weybridge by a VO (BSE Suspects).

All tissue samples are collected to transmission standards under scrapie sterile conditions. Head tissues are collected in a separate sealed area to body transmission and are sampled using aseptic instruments.

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



TS5700 Bovine Necropsies – Tissues and Fluid Summaries

Blood	Clotted	Serum
	EDTA & Heparin	Whole blood
		Plasma
		Buffy coat
		Red blood cells

All blood is collected prior to euthanasia.

Head	CSF	
	LRS	Lateral retropharyngeal LN
		Submandibular LN
		Medial retropharyngeal LN
		Palatine tonsil
	CNS	Spinal cord C1-2
		Caudal medulla
		Cerebellum
		Rostral medulla
		Brain stem (rostral to obex)
		Cerebral hemisphere (right & left)
	PNS	Nodose ganglion
	Muscular-Skeletal	Masseter
Body	LRS	Superficial cervical LN
		Spleen
		Mesenteric LN
		Tracheobronchial LN
		Bronchomediastinal LN
	PNS	Phrenic nerve
		Stellate ganglion
		Vagus nerve
	Alimentary	Distal ileum (intact)
		Spiral colon
		Jejunum



Body	Alimentary	Duodenum
	Viscera	Liver
	Urine	

FT0813 (FAIR-CT98-3651)

Following an initiative by the EU Commission in 1997, VLA was actively involved in the collection of BSE material for the exclusive use of European Commission funded research. This collection was organised by the EU through project FAIR-CT98-3651 (a Concerted Action which has an almost complete overlap with regards to Neuroprion partners). The project was completed by the end of 2003, and the material (approximately 44,000 samples from 25 animals) stored at VLA. Since the TSE Archive maintains this collection on behalf of the Commission, the set of samples are potentially available to Neuroprion partners without the involvement of the Independent Archive Advisory Group (IAAG). This material can be requested by contacting the TSE Archive. Provision is granted by the Neuroprion Archive Repository Group (NARG).

Tissues were sampled from two specified groups:

- Bovine clinically normal controls (CNC);
- BSE clinical bovine suspects – confirmed positive or negative



FT0813 Bovine Necropsies – Tissues and Fluid Summaries

Blood	Clotted	Serum
	EDTA & Heparin	Whole Blood
		Plasma
		Buffy Coat
		Red Blood Cells

All blood is collected prior to euthanasia.

Head	CSF	
	LRS	Lateral retropharyngeal LN
		Submandibular LN
		Parotid LN
		Medial retropharyngeal LN
		Palatine tonsil
		Nictitating membrane
	CNS	Caudal medulla
		Cerebellum
		Cortex (frontal, occipital & parietal)
		Basal nuclei
		Thalamus
		Midbrain & brain stem (rostral medulla)
	PNS	Facial nerve
		Retina
		Optic nerve
		Trigeminal ganglion
	Muscular-Skeletal	Masseter
		Tongue
	Other	Submandibular salivary gland
		Parotid salivary gland
		Conjunctiva
		Dura
		Pituitary
Respiratory epithelium		



Body	LRS	Superficial inguinal LN
		Spleen
		Renal LN
		Hepatic LN
		Lateral & medial iliac LN
		Mesenteric LN
		Tracheobronchial LN
		Bronchomediastinal LN
		Cervical thymus
		Popliteal LN
		Prefemoral LN
		Prescapular LN
		PNS
	Coeliaco mesenteric ganglion	
	Phrenic nerve	
	Stellate ganglion	
	Femoral nerve	
	Thoracic ganglion	
	Cervical ganglion	
	Vagus nerve	
	Sciatic nerve	
	Radial nerve	
	Alimentary	Duodenum
		Distal ileum (intact)
		Distal ileum Peyer's patches (separate from ileum)
		Distal ileum (excluding Peyer's patches).
		Jejunum
		Jejunum Peyer's patches (separate from ileum)
Spiral colon		
Caecum		



Body	Alimentary	Rumen
		Omasum
		Abomasum (pyloric)
		Rectum
		Cranial oesophagus
	Viscera	Liver
		Kidney
		Gall bladder
		Aorta
		Lung left caudal
		Pericardium
		Heart – left ventricle
		Heart valve (mitral)
		Trachea
	Muscular-skeletal	Diaphragm
		Sternocephalicus
		Triceps
		Bone marrow (sternum)
		Bone (femur)
	Other	Pancreas
		Adrenals
		Faeces
		Skin (from back)
Urine		



Time Course Samples

TS5700 Bovine Controls – Monthly blood sampling

From April 2007, blood has been sampled monthly from 6 cattle kept under controlled conditions on farms at VLA Weybridge, for the purpose of ensuring that there is a source of control time course samples for the purpose of future requests.

Clotted	Serum
Citrate	Whole blood
	Plasma
	Buffy coat
EDTA	Whole blood
	Plasma
	Buffy coat

SE1930 Attack Rate

The Archive has stored blood collected at 3 month intervals from this project since it was started in February 1998. Cattle on this project (60 in total) were all orally infected with a known titre of BSE ranging from 0.005g to 1g and are kept in mixed pens. Samples from unchallenged controls kept with the dosed animals are also collected.

Clotted	Serum
Heparin	Plasma
	Buffy coat
	Red blood cells

***SE1736: Bovine BSE Pathogenesis Study (completed February 2006)***

SE1736 was set up to establish a bank of sequentially harvested tissues and body fluids from cattle of known BSE incubation or clinical status, inoculated with a known infectious titre of BSE and from age-matched controls, for present and future research studies.

Samples collected from the study were broken down thus:

- Blood, Urine, CSF, Saliva and Nasal secretions, sampled at timed intervals between 1998 (pre and post challenge) to March 2005 from an initial group of 90 x unchallenged, 90 x 1g BSE Oral Dose and 90 x 100g BSE Oral Dose male castrates.
- Milk, cream and colostrums sampled at timed intervals between 2000 to June 2005 and placenta at every calving from an initial group of 10 unchallenged, 10 x 1g BSE Oral Dose and 10 x 100g BSE Oral Dose cows.
- A wide range of tissues including CNS, LRS, Blood, CSF, Urine, Viscera and other from timed culls at Weybridge, which commenced in November 1998 at intervals of 3 months (later 6 months). Last male castrate culled March 2005. The last female was culled in February 2006.



SE1736 Bovine Necropsies – Tissues and Fluid Summaries

Blood	Clotted	Serum
	EDTA & Heparin Citrate (last culls)	Whole Blood
		Plasma
		Buffy Coat
		Red Blood Cells

All blood is collected prior to euthanasia.

Head	CSF	
	LRS	Submandibular LN
		Medial retropharyngeal LN
		Palatine tonsil
		Nictitating membrane
	CNS	Medulla (caudal & rostral)
		Cerebellum
		Cortex (frontal, occipital & parietal)
		Basal nuclei
		Thalamus
		Midbrain & brain stem
	PNS	Facial nerve
		Retina
		Optic nerve
		Trigeminal ganglion
		Cranial cervical ganglion
	Muscular-Skeletal	Masseter
		Tongue
	Other	Submandibular salivary gland
		Parotid salivary gland
		Oral mucosa
		Pharynx
		Conjunctiva
Dura		
Pituitary		
Respiratory epithelium		



Body	LRS	Spleen
		Hepatic LN
		Mesenteric LN
		Bronchomediastinal LN
		Cervical thymus
		Popliteal LN
		Prefemoral LN
		Prescapular LN
	PNS	Phrenic nerve
		Stellate ganglion
		Radial nerve
		Vagus nerve (later culls)
		Sciatic nerve
		Dorsal root ganglia (mid-cervical)
		Dorsal root ganglia (mid thoracic)
	CNS	Spinal cord (cervical, thoracic and lumbar sections)
		Cauda equina
	Alimentary	Duodenum
		Distal ileum (intact)
		Distal ileum Peyer's patches (separate from ileum)
		Distal ileum (excluding Peyer's patches).
		Jejunum
		Jejunum Peyer's patches (separate from ileum)
		Spiral colon
		Rumen
		Omasum
		Abomasum (pyloric)
Rectum		
Cranial oesophagus		
Viscera	Liver	



Body	Viscera	Kidney
		Perirenal fat
		Aorta
		Lung left caudal
		Pericadium
		Heart left ventricle
		Heart valve
		Trachea
	Muscular-skeletal	Psoas
		Diaphragm
		Intercostal muscle
		Sternocephalicus
		Triceps
		Achilles tendon
		Semitendinosus
		Longissimus dorsi
		Bone marrow (sternum)
		Bone (femur)
	Other	Pancreas
		Adrenals
		Faeces
		Skin (from back)
	Female organs	Teat
		Udder
		Ovary
		Fallopian tube
		Endometrium
		Caruncle
	Urine	