**United States Department of Agriculture**
**National Veterinary Services Laboratories**

**Standard Operating Procedure**

# Submission of Rabies Negative Brain Samples from Rabies Testing Laboratories to NVSL for Bovine Spongiform Encephalopathy Immunohistochemistry Testing

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Animal and Plant Health Inspection Service
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## Purpose

The purpose of this Standard Operating Procedure (SOP) is to outline the proper procedure for the submission of brain samples from rabies negative cattle tested by rabies testing laboratories to be sent to the National Veterinary Services Laboratories (NVSL). The brain samples from rabies negative cattle are tested for bovine spongiform encephalopathy (BSE) by immuno-histochemistry (IHC) testing at the Pathobiology Laboratory, NVSL.

## Background

BSE is a transmissible spongiform encephalopathy found in cattle. The BSE agent is considered zoonotic and is generally believed to be responsible for a new variant of Creutzfeldt-Jakob Disease (vCJD). CJD is a slowly progressive, fatal, neurological disease of humans (see Appendix 2 for additional background on BSE). Everyone working with potentially infected BSE samples should understand and follow BSL-3 guidelines to the extent the laboratory facilities permit. The Centers for Disease Control and Prevention (CDC) publication, Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, should be consulted for current recommendations concerning the handling of prion infected or potentially infected materials.

There are no clinical signs that are pathognomonic for BSE in cattle. However, many of the clinical signs associated with rabies in cattle are indistinguishable from clinical signs that may be seen with BSE. For this reason, rabies testing laboratories are encouraged to submit rabies negative samples to the NVSL for BSE IHC testing.

## Sample Collection

The following collection procedure will provide acceptable brain specimens for testing both rabies and BSE and is consistent with the CDC, Protocol for Postmortem Diagnosis of Rabies in Animals by Direct Fluorescent Antibody Testing.

1. The entire brain should be removed intact with a portion of the cranial cervical spinal cord attached.

2. The brain should be submitted intact for bilateral sampling to the local rabies laboratory for testing following the submission requirements of the local laboratory. If appropriate and agreed upon in advance with the rabies laboratory personnel, the medulla can be removed at the level of the obex as described below. The obex and cranial cervical spinal cord can be submitted to NVSL for BSE sampling and the remainder of the brain can be used for rabies testing.

3. If the sample is rabies negative:

    a. The brain stem is transected at the level of the medulla (caudal to the cerebellar peduncles at cranial nerve X) and at the junction of the medulla and the spinal

cord. The section of the brain stem containing the obex is placed in a leak-proof, screw-top jar containing at least 200 ml of 10% neutral buffered formalin. The portion of the cranial cervical spinal cord (approximately ½ inch section) is placed in a plastic bag and frozen. This is the MINIMUM sample required for BSE testing.

b. The remainder of the brain can be divided in half by cutting along the midline in the space between the cerebral hemispheres, through the cerebellum, and through the remainder of the caudal brain stem (pons and medulla). One of the cerebral hemispheres with the attached midbrain should be placed in a Whirl-Pak® or in a plastic bag and frozen. The other half of the cerebrum with the attached midbrain should be placed in a liter bottle of 10% neutral buffered formalin. The fixed samples are sent to NVSL while the frozen samples should be held at the submitting laboratory until initial testing is completed.

## Sample Packaging and Shipping

Samples should be shipped in 10% neutral buffered formalin in a leak-proof container and double bagged with an amount of absorbent material between the outer and inner bags sufficient to absorb all liquid contents of the primary container. Specimen jars containing 10% formalin and certified shipping containers can be obtained at no charge from the Biological Materials Processing Section of NVSL:

Phone: (515) 663-7530
FAX: (515) 663-7378

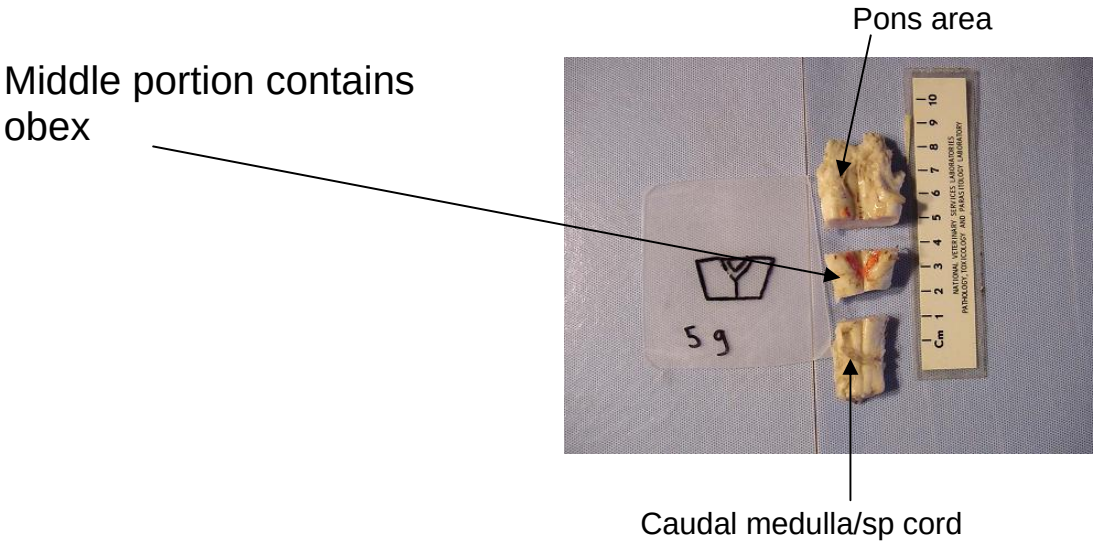
Samples may be submitted to NVSL by any standard carrier willing to accept the sample. However, Veterinary Services will pay Federal Express shipment expenses. Contact the Area Veterinarian-in-Charge (AVIC) for pre-paid Federal Express shipping labels. Include the submitter's phone number when shipping samples. Samples should be shipped to:

National Veterinary Services Laboratories
1800 Dayton Avenue
Ames, Iowa 50010

**Submission of Rabies Negative Brain Samples from Rabies Testing Laboratories to NVSL for Bovine Spongiform Encephalopathy Immunohistochemistry Test**

Appendix 1

# Brainstem Including Obex



# Appendix 2

## Background on BSE

Bovine spongiform encephalopathy (BSE), widely known as "mad cow disease," is a chronic, degenerative disease affecting the central nervous system of cattle. Worldwide there have been more than 180,000 cases since the disease was first diagnosed in 1986 in Great Britain. BSE has had a substantial impact on the livestock industry in the United Kingdom. The disease has also been confirmed in native-born cattle in Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Japan, Luxembourg, Liechtenstein, the Netherlands, Northern Ireland, Poland, Portugal, Slovakia, Slovenia, Spain, and Switzerland. However, over 95% of all BSE cases have occurred in the United Kingdom. There have been two confirmed cases of BSE in North America, both originated from herds in Canada. One was confirmed in May, 2003, in Alberta, Canada while the other was confirmed in an animal in Washington State in the United States in December 2003.

BSE belongs to the family of diseases known as the transmissible spongiform encephalopathies (TSE). These diseases are caused by a transmissible agent which is yet to be fully characterized. TSEs share the following common characteristics:

a. a prolonged incubation period of months or years;
b. a progressive, debilitating, neurological illness which is always fatal;
c. when examined by electron microscopy, detergent treated extracts of brain tissue from animals or humans affected by these diseases reveal the presence of scrapies associated fibrils (SAF);
d. pathological changes appear to be confined to the central nervous system (CNS) and include vacuolation and astrocytosis;
e. the transmissible agent elicits no detectable specific immune response in the host which has inhibited the development of a preclinical live animal diagnostic test to date.

### Clinical Signs of BSE in Cattle

Affected animals may display changes in temperament, such as nervousness or aggression; abnormal posture; incoordination and difficulty in rising; decreased milk production; or loss of body condition despite continued appetite. There is no treatment, and affected cattle die.

The incubation period ranges from 2 to 8 years. Following the onset of clinical signs, the animal's condition deteriorates until it dies or is destroyed. This usually takes from 2 weeks to 6 months. Most cases in Great Britain have occurred in dairy cows between 3 and 6 years of age.

### How BSE Is Currently Diagnosed

There is currently no test to detect the disease in a live animal. Currently, there are two laboratory methods to confirm a diagnosis of BSE: 1) microscopic examination of the brain tissue to identify characteristic changes; 2) techniques to detect the partially-proteinase resistant

form of the prion ($PrP^{res}$) protein. These techniques are immunohistochemistry, immunoblotting, and ELISA.

## Similar Diseases of Humans and Other Animals

TSEs are caused by similar uncharacterized agents which usually produce spongiform changes in the brain. According to the leading hypothesis, TSEs are caused by an unconventional transmissible agent, an abnormal protein (i.e., prion) that is able to induce abnormal folding of normal cellular proteins, leading to neuronal death. TSEs include scrapie (which affects sheep and goats), transmissible mink encephalopathy, feline spongiform encephalopathy, and chronic wasting disease of deer and elk. TSEs in humans include kuru, classical Creutzfeld-Jakob disease (CJD), Gerstmann- Straussler syndrome, fatal familial insomnia, and new variant CJD (vCJD).

## BSE and vCJD—Human Health Concerns

Variant CJD (vCJD) was first described in 1996 in the United Kingdom and has different clinical characteristics than classic CJD. The median age at death for vCJD patients is 28 years, compared with 68 years for patients with classic CJD. In addition, all vCJD cases have neuropathologic findings distinctly different from those of classic CJD. Except for a patient with preclinical vCJD related to bloodborne transmission, all vCJD patients had a particular genetic profile (i.e., homozygosity for methionine) at codon 129 of the prion protein gene. Thus, cases of vCJD can be distinguished from classic CJD on the basis of clinical and pathologic data. Epidemiologic and laboratory evidence suggests that the agent causing BSE in cattle can be transmitted to humans via consumption of BSE-contaminated cattle products, causing vCJD. However, the risk for acquiring vCJD from consumption of BSE-contaminated product is low presumably because of a "species barrier" that provides substantial but incomplete protection against development of vCJD

## Where has vCJD been detected?

In the UK, the number of reported probable and confirmed cases of vCJD is 150 as of September 6, 2004, including those of three persons residing in Ireland, Canada, and the United States who are believed to have been exposed to BSE in the UK. Eight additional cases, not directly linked to the BSE outbreak in the UK, also have been reported (seven in France and one in Italy).

## Transmission of BSE

There is no evidence that BSE spreads horizontally, i.e., by contact between unrelated adult cattle or from cattle to other species. Some evidence suggests that maternal transmission may occur at an extremely low level. Results of British research show that there is approximately a 9-percent increase in the occurrence of BSE in offspring of BSE-affected dams as compared to calves born to dams where BSE was not detected. The study did not ascertain if this was the result of genetic factors or true transmission. The research did, however, point out that at this

level if maternal transmission does occur it alone will not sustain the epidemic (Wilesmith, et al. 1997).

A recently published study found no evidence of disease transmission via embryos collected from cows with BSE. The embryos were collected and handled in accordance with international health standards (Wrethall, et. al., 2001).