

Implementation Guideline for Transmissible Spongiform Encephalopathy (TSE) Testing

1 Purpose

This guideline aims to ensure the safety of meat, following the identification of Bovine Spongiform Encephalopathy (hereinafter referred to as “BSE”) case in Japan. To relieve the public concerns, tests shall be conducted for Transmissible Spongiform Encephalopathy (hereinafter referred to as “TSE”), based on the Abattoirs Law (Law No. 114 in 1953; hereinafter referred to as “the Law”), to ensure that TSE-infected bovine, sheep and goat shall not be distributed for food, while contributing to the assessment of TSE infection status in Japan.

2 Necessity of TSE tests

To ensure safety of meat at abattoirs, it is necessary to exclude flesh, etc. derived from TSE-infected animals, which may be contaminated by high-density abnormal prion protein, in combination to adequate removal of specified risk materials, in which abnormal prion is accumulated. For this purpose, TSE tests shall be conducted in conformity of Article 14 of the Law.

3 Instructions concerning application for TSE tests, and notes on receiving the tests

(1) Applicants for carcass inspection shall be instructed beforehand on the following issues. Instructions shall be also given to related businesses as necessary.

- a Applicants shall indicate the ages of bovines on a month basis, when submitting application for carcass inspection stipulated in Section 1, Article 15 of the Implementation Rules for the Abattoirs Law (hereinafter referred to as “Rules”). Applicants shall also attach documents identifying month ages of the bovines, such as copy of database for individual identification of cattle, in conformity with the Law Concerning Special Measures for the Management and Transmission of Information on Individual Identification of Cattle (Law No. 72 in 2003); calf registration certificate (issued by the Wagyu Registry Association), and/or pedigree registration certificate (issued by the Japan Holstein Registry Association) as necessary.

As for sheep and goat, it is desirable to attach documents identifying month ages of the sheep or goat, such as sheep/goat pedigree registration certificate (issued by the Japan Livestock Technology Association).

- b Applicants shall also make prior confirmation on the information concerning names, addresses, etc. of senders/raisers of the cattle. This information is necessary for survey by the supervising livestock agencies, in case of TSE tests turn positive.
- (2) Attention shall be paid on the following points, when receiving application for carcass inspection.

- a Confirm the month ages of applied bovines, in both the “age” column of carcass inspection application and documents specified in (1) a.

As for the month ages of sheep/goat, confirm the documents as specified in (1) a, if such documents are attached.

- b If information as specified (1) b is not clear, instruct the applicant to make necessary confirmations.

4 Ante-mortem inspection

- (1) In the inspection based on provisions of Section 1, Article 14 of the Law, conduct the dentition examination for bovines, and make comprehensive judgment of month ages, referring to the documents stipulated in 3 (1) a, attached to the carcass inspection application. If the third incisor tooth is present, the bovine shall be judged 30 months or older, regardless of the information on carcass inspection application.

Conduct the dentition examination for sheep and goat as well. Eruption of the second permanent molars (in both upper and lower jaws) shall be an indicator for the judgment of 12 months old.

- (2) Also check the existence of strange cries, turning around and other abnormal behaviors, ataxia and other nervous symptoms, combined with the results of gait checks.

Refer to the video concerning clinical symptoms of Transmissible Spongiform Encephalopathy, dispatched based on the Clerical Notice dated March 26, 2003, for the implementation of ante-mortem inspection.

5 Measures based on the results of ante-mortem inspection

- (1) If the tested bovine, sheep or goat corresponds to 4 (2) as a result of ante-mortem inspection, and is suspected of TSE-infection (“suspected case” as stipulated in Article 2 of the Domestic Animal Infectious Diseases Control Law), slaughtering or dressing of such bovine/sheep/goat shall be prohibited in accordance with the provisions of Item 1, Article 16 of the Law, because slaughtering or dressing of such livestock may lead to contamination by the infectious agent (i.e. abnormal prion protein).
- (2) If a meat inspection station takes the measure stipulated in (1), that station shall notify the fact to the applicant, the abattoir founder, and other related parties. The station shall also report to food sanitation departments of the relevant prefectures and municipalities operating health centers (hereinafter referred to as “prefectures”), which shall in turn report to livestock departments of the relevant prefectures. The information shall be also provided to food sanitation departments and livestock departments of the prefectures supervising the sender’s location.
- (3) Even if a meat inspection station takes the measure of prohibiting slaughtering or dressing in accordance with the provisions of Item 1, Article 16 of the Law, for bovine/sheep/goat indicating systemic symptoms in which TSE symptoms are not identified, but for sepsis, severe jaundice, or other reasons than the above, that station shall also notify the fact to the applicant, the abattoir founder, and other related parties.

The station shall also report to food sanitation departments of the relevant prefectures, which shall in turn report to livestock departments of the relevant prefectures. The information shall be also provided to food sanitation departments and livestock departments of the prefectures supervising the sender's location.

6 Post-mortem inspection

(1) Inspection for bovines, etc.

- a As a rule, screening tests shall be conducted on the day of slaughter for bovines aged 21 months or older.

Screening tests may be conducted for bovines aged 20 months or younger, if it is considered necessary by livestock inspectors from disease identification point of view.

- b Results of the screening and confirmatory testing shall be compiled in the following categories:
 - a) Bovine aged 24 months or older, indicating suspected symptoms including movement disorders, perception disorders, reflection/consciousness disorders and other nervous symptoms, and/or systemic symptoms in ante-mortem inspection;
 - b) Bovine aged 30 months or older; and
 - c) Other bovines.

(2) Tests for sheep and goat, etc.

- a As a rule, screening tests shall be conducted on the day of slaughter for sheep/goat aged 12 months or older.

Screening tests may be conducted for sheep/goat aged 11 months or younger, if it is considered necessary by livestock inspectors from the standpoint of disease differentiation.

- b Results of the screening and confirmatory testing shall be compiled.

(3) Sampling, etc.

For samples of bovine/sheep/goat, take medulla oblongata through the foramen magnum opening by using spatula technique and so on. Use part of one side of the medulla oblongata as sample for screening test, conducted in conformity with "Guideline for Screening Tests of Transmissible Spongiform Encephalopathy (TSE)" (Attachment 1). The remainder of medulla oblongata shall be frozen as sample to be sent.

The other side of medulla oblongata shall be fixed with 15 - 20% solution of buffer formalin, as sample to be sent. This fixation shall be performed promptly after sampling. If it is difficult, the fixation must be performed promptly after the results of ELISA test are given.

If medulla oblongata is damaged during the pithing operation, and the position for sampling is not clear, samples shall be taken from multiple locations.

(4) Measures to be taken in case the screening test turns positive

Following the conclusion of the screening test (i.e. end of the second ELISA test), and the test results turn out to be positive, then the meat inspection station shall immediately notify the fact to the applicant, the abattoir founder, and other related parties. The station shall also report to food sanitation departments of the relevant prefectures, which shall in turn report to livestock departments of the relevant prefectures. The information shall be also provided to food sanitation departments and livestock departments of the prefectures supervising the location of the sender of the relevant bovine/sheep/goat.

To conduct the confirmatory testing for the relevant bovine/sheep/goat, send the samples put aside in (3) to institutions designated in a separate notice. If the testing should be conducted by prefectures, the testing shall conform to Separate Attachment 2, "Procedure for Confirmatory Testing of Transmissible Spongiform Encephalopathy (TSE) by Prefectural and City Governments."

(5) Notification on results of the confirmatory testing

Results of the confirmatory testing shall be notified to food sanitation departments of the relevant prefectures, by way of the Inspection and Safety Division, Department of Food Safety, Pharmaceutical and Food Safety Bureau, MHLW (hereinafter referred to as "Inspection and Safety Division"), from the institution stipulated in (4), which has conducted the confirmatory testing. If the testing has been conducted by prefectures, the results shall be notified to the Inspection and Safety Division by way of food sanitation departments of the relevant prefectures.

Food sanitation departments of the relevant prefectures shall notify the results of confirmatory testing immediately to the applicant, the abattoir founder, and other related parties. Food sanitation departments shall also provide information to livestock departments of the relevant prefectures, as well as food sanitation departments and livestock departments of the prefectures supervising the sender's location.

(6) Definitive diagnosis

If the results of screening and confirmatory testing turn out TSE-positive, the Expert Committee for the Testing of Bovine Spongiform Encephalopathy of the Ministry of Health, Labour and Welfare shall make the definitive diagnosis.

(7) Measures concerning carcass, etc. during screening and confirmatory testing

While screening and confirmatory testing are underway, dressed carcass, viscera, blood (to be reused only), bone, hide, heads, legs, tails, etc. of the corresponding bovine/sheep/goat, including the separated parts to be disposed, shall be stored so that identification should be available, and that the edible parts should not be contaminated by microbes, etc. If storage by individual carcass is difficult, several carcasses or a daily handling lot may be stored together. In case of TSE-positive, such lots may be treated (i.e. incinerated) together.

7 Measures based on the results of post-mortem inspection

The following measures shall be taken under the observance of livestock inspectors, etc., in conformity with Item 3 or 4, Article 16 of the Law:

- (1) In case the screening or confirmatory testing turns positive
 - a Ensure the implementation of disinfection and other measures stipulated in 8, of facilities, equipment, machines, utensils, etc. that have, or might have, come into contact with bovine head (excluding tongue and cheek), spinal cord and distal ileum (two meters from connection to appendix); tonsil, spleen, small and large intestines (including lymph nodes attached to them) of sheep and goat aged 12 months or older; and head (excluding tongue, cheek and tonsil), spinal cord and placenta, as listed in Attached Table 1 of the Rules (hereinafter referred to as “parts listed in Attached Table 1”). As for facilities, equipment, machines, utensils, etc. that do not come into contact with the parts listed in Attached Table 1, thorough washing should be done.
 - b Incinerate as necessary part of dressed carcass, viscera, blood (to be reused only), bone, hide, heads, legs, tails, etc. of the corresponding bovine/sheep/goat, including the separated parts to be disposed, which have been stored as stipulated in 6 (7).
- (2) In case the definitive diagnosis proves TSE

Prefectures receiving notification that the definitive diagnosis has proven TSE from the Inspection and Safety Division shall take the following measures:

- a Ensure the implementation of disinfection and other measures stipulated in 8, of facilities, equipment, machines, utensils, etc. that have, or might have, come into contact with the parts listed in Attached Table 1, but have not been treated in (1) a. As for facilities, equipment, machines, utensils, etc. that do not come into contact with the parts listed in Attached Table 1 as well, thorough washing should be done.
- b Incinerate dressed carcass, viscera, blood (to be reused only), bone, hide, heads, legs, tails, etc. of the corresponding bovine/sheep/goat, including the separated parts to be disposed, which have been stored as stipulated in 6 (7), but have not been incinerated in (1) b.

8 Disinfection methods for parts to be disposed, etc.

Inactivation methods for pathogens, in case TSE-infected bovine/sheep/goat has been identified in tests, stand as follows at present. Ensure the implementation of disinfection and other measures in conformity with the following:

- (1) Complete incineration at Min. 800C° (of carcasses, rubber gloves, protective garments, etc.)
- (2) Pressurized steam sterilization at 132 to 134C° for an hour (of utensils, etc.)

- (3) Treatment in Min. 1-mol solution of sodium hydroxide, at 20C° for an hour (of facilities, dirt, etc.)
- (4) Treatment in sodium hypochlorite solution of Min. 2% effective chlorine concentration, for an hour (of facilities, dirt, etc.)

9 Instructions to founders, managers, slaughterers and their employees

- (1) The parts listed in Attached Table 1 shall be removed in the treatment process, and incinerated following the confirmation of livestock inspectors.
- (2) If the storage cooling equipment is too small for the storage of carcasses during TSE tests, solve the problem by using the existing facilities effectively, such as separation of storage refrigerators, so as the carcasses shall not contaminate others.
- (3) This Guideline stipulates necessary measures with regard to TSE tests. If necessity arises to restrict the use of abattoirs, as a result of implementing this guideline, that restriction shall be regarded as for “due reasons,” stipulated in Article 11 of the Law.

The number of animals processed shall be reviewed as necessary.

If TSE-positive bovine, sheep or goat is identified, the relevant carcass, etc. must be incinerated. Therefore, available incinerating facilities must be designated beforehand.

10 Reporting of test results to the Inspection and Safety Division, and their publication

- (1) If food sanitation departments of prefectures take the measure of prohibiting slaughtering or dressing as stipulated in 5 (1), the relevant sanitation departments shall report the fact to the Inspection and Safety Division immediately, using Attached Form 1.
- (2) As for screening, results from the previous Sunday through to Saturday shall be reported by 6:00 p.m. of Monday next week (If holiday falls on Monday, the day following the holiday(s)). Use the Meat Inspection Support System for bovine, and report by Attached Form 2 for sheep/goat.

If the Meat Inspection Support System is out of order, or if tests are not conducted for bovines aged 20 months or younger, report those facts to the Inspection and Safety Division in Attached Form 3.

- (3) The Inspection and Safety Division discloses TSE-infected cases as soon as the determinant diagnoses are given, and publishes every week the total number of animals proving negative in screening and confirmatory testing. There shall be no omissions in the result reports.

11 Others

In the case of screening or confirmatory testing for bovines without disorders, aged 20 months or younger, etc., which are not required by the Abattoirs Law, this Implementation Guideline shall be also applied to the handling of dressed carcass, viscera, etc. derived from tested bovines.

(Revision history)

October 18, 2001; partial revision: Addition to implementation guideline

November 5, 2001; partial revision: Change to handling of sample mailing

January 23, 2002; partial revision: Revision from daily to weekly reporting of screening

November 11, 2002; partial revision: Change to handling of sample mailing

February 28, 2003; partial revision: Addition to implementation guideline for confirmatory testing

July 1, 2003; partial revision: Addition of BSE diagnostic kit, etc.

April 13, 2004; partial revision: Addition of video concerning ante-mortem inspection, etc.

August 4, 2004; partial revision: Addition of screening kit, etc.

July 1, 2005; partial revision: Revision of month ages covered by the tests, etc.

(Attached Form 1)

Attn: Inspection and Safety Division,
Department of Food Safety,
Pharmaceutical and Food Safety Bureau,
Ministry of Health, Labour and Welfare

FAX: 03-3503-7964

Report on Identification of TSE-suspected Bovine, Sheep and Goat

1	Municipality		Abattoir name				
2	Date & time of slaughter prohibition	Date: Time:					
	Reason for the slaughter prohibition						
3	Relevant animal	Type Species		Sex	M / F	Age	month(s) old
4	Findings from ante-mortem inspection						
5	Remarks						
	No. of ear tags, etc.						

Weekly Results of Screening Test for Transmissible Spongiform Encephalopathy (TSE) of Sheep and Goat
Brought in : Month Date - Month Date

Municipality _____

	Sheep and goat indicating symptoms* ¹			Other sheep and goat			Total			No. of sheep and goat below 12 months old, for which tests were not conducted
	Negative	Positive	Total	Negative	Positive	Total	Negative	Positive	Total	
Month - Date										
Month - Date										
Month - Date										
Month - Date										
Month - Date										
Month - Date										
Month - Date										
Month - Date										
Month - Date										
Month - Date										
Month - Date										
Month - Date										
Total										

*1 Sheep and goat indicating suspected symptoms including movement disorders, perception disorders, reflection/consciousness disorders and other nervous symptoms, and/or systemic symptoms in ante-mortem inspection

No. of incidents in which slaughter was prohibited due to suspected TSE between Month Date and Month Date : (incidents)

Weekly Results of Screening Test for Bovine Spongiform Encephalopathy (BSE)
Brought in : Month Date - Month Date

Municipality _____

	Bovine indicating symptoms *1			Bovine aged 30 months or older			Other bovine			Total			No. of bovine below 21 months old, for which tests were not conducted
	Negative	Positive	Total	Negative	Positive	Total	Negative	Positive	Total	Negative	Positive	Total	
Month - Date													
Month - Date													
Month - Date													
Month - Date													
Month - Date													
Month - Date													
Month - Date													
Month - Date													
Month - Date													
Month - Date													
Total													

*1 Bovine aged 24 months or older, indicating suspected symptoms including movement disorders, perception disorders, reflection/consciousness disorders and other nervous symptoms, and/or systemic symptoms in ante-mortem inspection

No. of incidents in which slaughter was prohibited due to suspected BSE between Month Date and Month Date : (incidents)

Procedure for Screening of Transmissible Spongiform Encephalopathy (TSE)

Part 1. Handling of Prion Materials within the Laboratory

- Operations shall be performed in a sectioned exclusive laboratory and, as a general rule, within a safety cabinet.
- The operator shall wear latex or vinyl gloves, a mask, protective clothing and cap, as well as protective eyeglasses, etc. where necessary, in order to avoid contamination through wounds or droplet contamination through eyes or mouth.
- Disposable products shall be used where possible, including work clothing and various instruments and equipment.
- Handle test materials on a bench protection sheet, taking care to avoid generating droplets and aerosols.
- Upon spilling of any test materials or at the end of operation, wipe the work surface clean with sodium hypochlorite solution.
- Place used bench protection sheets and disposable instruments in an autoclave bag and sterilize by steam under pressure at 132-134°C for 1 hour.
- Apparatus such as scissors and tweezers, etc. which are to be reused must be wiped clean with paper such as tissue paper or alcohol-soaked cotton and soaked in 3-5% SDS and boiled for 5-10 minutes (metal corrosion may be controlled by adding sodium carbonate in the amount of 1%), or sterilized by steam under pressure at 132-134°C for 1 hour.
- Soak plastic instruments which can not be heated in sodium hypochlorite of 5% or higher, or in NaOH of 2-molar or higher, for 2 hours or longer.
- Place combustible materials in an autoclave bag and sterilize by steam under pressure at 132-134°C for 1 hour.
- If a medical waste incinerator is available for use, place plastic instruments and combustibles in biohazard bags and incinerate.
- If the exterior of a centrifuge tube which is required in subsequent procedures becomes contaminated, transfer the contents into a fresh tube and continue operations.
- In cases where only a regular autoclave is available, an effective means of sterilization is to place contaminated items in an alkali-proof container and soak in 1- to 2-molar NaOH and treat at 121°C for 30 minutes.
- As a general rule when decontaminating equipment and instruments which can not be incinerated, wipe highly contaminated items or masses of tissue having a high residual prion level first with combustibles such as paper or alcohol-soaked cotton to remove contaminants as much as possible before sterilizing.

Part 2. Sample Region

The head is removed along the dotted line in the figure. A spoon or spatula is inserted from the foramen magnum to collect a sample including the obex shown in Figure 1 (the region colored in black) and Figure 2. The other black regions are the skull and cervical vertebra. As lesions and prion accumulation occur roughly symmetrically, sample collection is divided along the midline. The sample from one side is fixed in 15-20% concentration buffered formalin as material for the histopathological and immunohistochemical examinations, while the remaining side is used as material for the immunobiochemical examination (ELISA, Western blot, etc.).

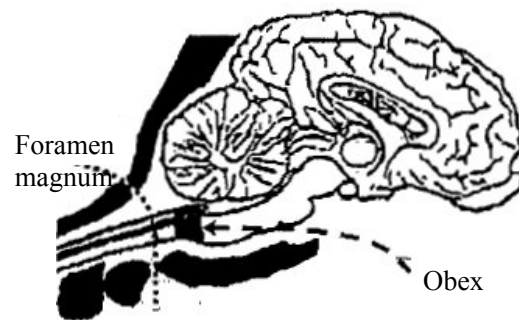


Figure 1. Bovine brain cross-section

Using the material for the immunobiochemical examination, the tissue is sectioned in cross sections containing the obex to serve as the samples for screening. As prion is not accumulated consistently throughout the obex, the sample needs to be balanced out. Therefore, in sample preparation, the tissue slices are roughly cut up into small pieces using scissors and averaged out before collecting the required amount. However, this method does not apply in cases where sampling is conducted using methods other than sectioning.

Part 3. Bovine Spongiform Encephalopathy (BSE) Test Kit Procedures

The screening test is to be performed using one of the following test kits: Platelia BSE, Dynabott Enfer BSE Test, or Frelisa BSE. The procedures for these kits are described in Separate Attachments 1-1, 1-2 and 1-3 respectively.

Part 4. Storage of Test Results

The raw data read by the microplate reader is to be entered in Separate Form 1-1. Once the remaining items in Separate Form 1-1 are filled in, the signed sheet and electronic data shall be stored. The sheet shall be stored pasted with the raw data read by the microplate reader (data on photosensitive paper shall be copied onto plain paper). If the sample is to be dispatched for confirmatory testing, a copy of the stored data pasted with raw data is to be attached.

Part 5. Dispatching Sample for Confirmatory Testing

Samples which tested positive¹ using a BSE test kit shall be dispatched for confirmatory testing according to the procedures indicated below, with the exception where confirmatory testing is to be conducted by the prefectural or city government.

¹ Refers to cases tested positive in the third repeat test.

1. Delivery address

The delivery address is indicated in the Notification No. 0407001 of the Inspection and Safety Division, Department of Food Safety of the Pharmaceutical and Food Safety Bureau (PFBSB), Ministry of Health, Labour and Welfare, dated April 7, 2004, attached separately.

2. Method of notification of confirmatory test results

The Inspection and Safety Division shall inform the local authority who requested the confirmatory test.

Inquiries regarding the confirmatory test should be addressed to the Dairy and Meat Safety Section of the Inspection and Safety Division, at phone number 03-3595-2337.

3. Material for dispatching (As shown in Figure 2)

The obex and surrounding tissue is divided into two along the midline.

(1) One side constitutes material for histopathological and immunohistochemical examinations, fixed in buffered formalin of 15-20% concentration and shipped at room temperature. The sample shall be placed in a 50 mL container filled with buffered formalin.

(2) The remainder constitutes material for the immunobiochemical examination (ELISA, Western blot, etc.), to be shipped frozen. Any materials remaining after sample collection or after ELISA use (homogenized emulsion samples, etc.) are to be shipped frozen as well.

4. Method of dispatch notification

When dispatching samples which tested positive in the screening, a Table of Test Results (Separate Form 1-1) and a Notice of Sample Dispatch (Separate Form 1-2) shall be attached, and the date and time (9-12 am or 1-4 pm) of delivery to the testing laboratory shall be specified.

The Table of Test Results (Separate Form 1-1) and the Notice of Sample Dispatch (Separate Form 1-2) shall be faxed in advance to the Dairy and Meat Safety Section of the Inspection and Safety Division (Fax: 03-3503-7964), together with notification to the same by telephone (Phone: 03-3595-2337).

An emergency number for contact during non-working days, etc., will be provided at a later date.

5. Notes on sample dispatching

The samples shall be dispatched packaged in containers complying with UN specifications, based on Nos. 2 and 3, Article 8 of the Postal Regulations (Notification No. 34 of the Ministry of Communications, 1947).

Additional information regarding the sample shipping container as well as notes on packaging are to be announced separately.

Prior to depositing the package, an inquiry shall be made at the post office in charge of collection and delivery in your district (referred to hereinafter as the “district post office”) regarding the mode of transportation of the postal package. Depending on the mode of transportation, measures shall be made, as indicated below, before depositing the package at the post office.

- (1) Sample-containing postal packages whose shipping process does not include air transport

A label in the following format with all necessary items filled shall be pasted on the postal package surface where clearly visible.

Item: Bovine tissue, etc. “Hazardous Substance” (Note 1) Sender: Name of Local Authority: Name of Laboratory: Address: Phone No.: Qualification: Livestock Inspector (Veterinarian) Name:
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Note 1: Marked in red

- (2) Sample-containing postal packages whose shipping process includes air transport (Note 3)

- 1) A label in the following format with all necessary items filled shall be pasted on the postal package surface where clearly visible.

Item: Bovine tissue, etc. “Hazardous Substance” (Note 1) UN No.: Sender: Name of Local Authority: Name of Laboratory: Address: Phone No.: Qualification: Livestock Inspector (Veterinarian) Name: Contains xx kg dry ice (Note 2)
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Note 1: Marked in red

Note 2: To be marked in red if packaged in dry ice

- 2) Samples shall be stored in containers complying with UN specifications.
- 3) The contents of one container shall be limited to less than 1000 mL for liquids and 50 g for solids.
- 4) A Transportation Permit Label (Classification No.: 6.2) shall be pasted on the

postal package surface where clearly visible (Note 4).

- 5) If the container complying with UN specifications is surrounded by dry ice and packaged in a carton box, a Transportation Permit Label (Classification No.: 9) shall be pasted on the postal package surface where clearly visible (Note 4).
- 6) In the case of 5) above, the post office personnel may request opening of the external carton box when accepting the postal package in order to confirm that the sample is stored in a container complying with UN specifications, in which case the request should be catered to.
- 7) Two copies of the Hazardous Substance Declaration Form shall be prepared and presented with the package (Note 5).

An open envelope stating “Contains Hazardous Substance Declaration Form” shall be pasted on the package. At the post office, the Hazardous Substance Declaration Form, after being reviewed and returned, it is to be enclosed in the pasted envelope in the presence of the post office personnel.

- Note 3: Transportation by air is regulated by Article 86 of the Aviation Law, Article 194 of the Enforcement Regulations for the Aviation Law, and other relevant notifications.
- Note 4: The format for the label is shown in Separate Form 1-3. (Request the required number of labels from the local post office.)
- Note 5: The Hazardous Substance Declaration Form is shown in Separate Form 1-4. (This Form has been established by the Postal Service and various air carriers exclusively for the purpose of transporting the samples of this operation, and is not for use otherwise.)

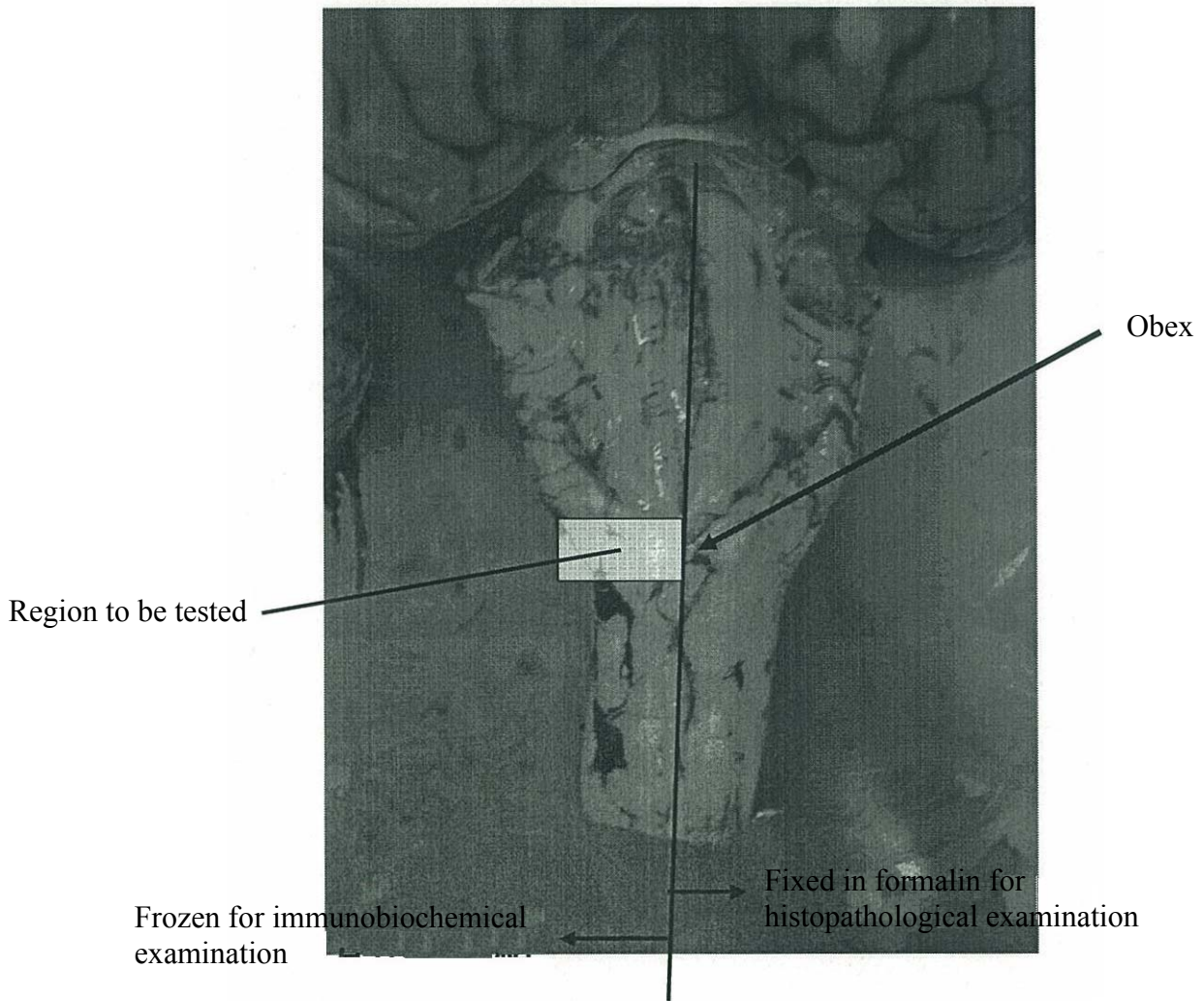
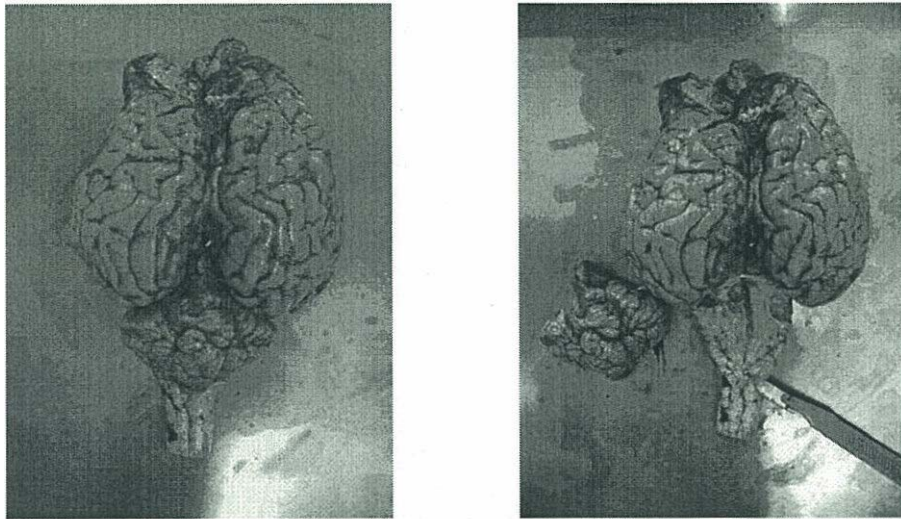


Figure 2. Sampling region for BSE screening

Treatment to Completely Inactivate Abnormal Prion Protein

Table 1. Treatment to completely inactivate abnormal prion protein²

Chemical Agent	Concentration	Treatment Time	Temperature
Formic acid	≥60%	2 hours	Room temp.
Guanidine thiocyanate	≥4 M	2 hours	Room temp.
Guanidine hydrochloride	≥7 M	2 hours	Room temp.
Trichloroacetic acid	≥3 M	2 hours	Room temp.
SDS	≥3%	5 min.	100°C
Phenol	≥50%	2 hours	Room temp.

Table 2. Methods for sterilizing contaminated materials³

Chemical Agent/ Method, etc.	Temperature (°C)	Time (min.)	Applicable items
Incineration	≥800	-	Organs, combustibles, etc.
Autoclave	134	60	Instruments, equipment, organs, etc.
Soaking in 3% SDS**	100	5	Instruments, equipment, etc.
Soaking in 2-molar NaOH	Room temp.	60	Instruments, equipment, etc.
Soaking in 1-molar NaOH	Room temp.	120	Instruments, equipment, etc.
Soaking in 1-5% sodium hypochlorite	Room temp.	120	Instruments, equipment, etc.

Each case is autopsied on a plastic sheet spread in an autopsy room, etc., and only minimal dissection shall be performed. When removing the head, blood is received in a container to minimize contamination. The removed head is placed in a plastic bag, and measures such as covering the neck with a plastic bag are taken to control the spread of contamination.

² Onodera, T., T. Kitamoto, T. Kurata, T. Sato, J. Tateishi. *Creutzfeldt-Jakob Disease Treatment Manual* (Under editorship of Disease control Division, Health Service Bureau, Ministry of Health and Welfare). Tokyo: Shinkikaku Publishing Co., Ltd., 1997, pp18-23.

³ See 2 above

(Separate Form 1-1)

(Test / Repeat test)

Test date

D/M/Y: _____

Controlling local authority

Laboratory name

Analyst name (Signature)

Cutoff value

-10% from cutoff value

Number of samples

(No. of positive samples, no. of negative samples)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

← Record name of sample (Identification code, etc.)
 ← Record measured value

- *1 The table above represents a 96-well plate. Record data corresponding to the actual wells used in the test.
- *2 Mark a diagonal slash in unused well positions.

(Separate Format 1-1) (Example)

(Test) / Retest)

Test date	<u>D/M/Y: 18/10/2001</u>
Controlling local authority	<u>×× City</u>
Laboratory name	<u>×× Meat Inspection Laboratory</u>
Analyst name (Signature)	<u>John Doe</u>
Cutoff value	<u>0.251</u>
-10% from cutoff value	<u>0.2259</u>
Number of samples	<u>18</u>
(No. of positive samples, no. of negative samples)	<u>(18, 0)</u>

	1	2	3	4	5	6	7	8	9	10	11	12
A	Negative control	bovine-3	bovine-11	/	/	/	/	/	/	/	/	/
	0.041	0.172	0.104	/	/	/	/	/	/	/	/	/
B	Negative control	bovine-4	bovine-12	/	/	/	/	/	/	/	/	/
	0.040	0.132	0.082	/	/	/	/	/	/	/	/	/
C	Negative control	bovine-5	bovine-13	/	/	/	/	/	/	/	/	/
	0.042	0.156	0.101	/	/	/	/	/	/	/	/	/
D	Negative control	bovine-6	bovine-14	/	/	/	/	/	/	/	/	/
	0.041	0.122	0.166	/	/	/	/	/	/	/	/	/
E	Positive control	bovine-7	bovine-15	/	/	/	/	/	/	/	/	/
	2.53	0.098	0.145	/	/	/	/	/	/	/	/	/
F	Positive control	bovine-8	bovine-16	/	/	/	/	/	/	/	/	/
	2.45	0.087	0.191	/	/	/	/	/	/	/	/	/
G	bovine-1	bovine-9	bovine-17	/	/	/	/	/	/	/	/	/
	0.083	0.115	0.072	/	/	/	/	/	/	/	/	/
H	bovine-2	bovine-10	bovine-18	/	/	/	/	/	/	/	/	/
	0.106	0.095	0.126	/	/	/	/	/	/	/	/	/

← Record name of sample (Identification code, etc.)
 ← Record measured value

- *1 The table above represents a 96-well plate. Record data corresponding to the actual wells used in the test.
- *2 Mark a diagonal slash in unused well positions.

(Separate Form 1-2)

Confirmatory Testing Laboratory: _____
 Recipient: _____

Name of Local Authority: _____
 Person in Charge: _____
 Phone No.: _____

Notice of Dispatch: Sample for Transmissible Spongiform Encephalopathy Confirmatory Test

1	Dispatch Date	Dispatching Institution (Laboratory name)	Sample No.	Sample Weight (g)	Date of Sampling (D/M/Y)	Data Regarding Animal of Sample Origin					Remarks
						Breed/ Variety	Sex	Age in Months	Clinical Symptoms	Slaughter Date (D/M/Y)	
				Emulsion for ELISA							
				For WB							
				For pathological examination							
Shipper					Breeder						
Name		Address			Phone No.	Name		Address		Phone No.	
2	Dispatch Date	Dispatching Institution (Laboratory name)	Sample No.	Sample Weight (g)	Date of Sampling (D/M/Y)	Data Regarding Animal of Sample Origin					Remarks
						Breed/ Variety	Sex	Age in Months	Clinical Symptoms	Slaughter Date (D/M/Y)	
				Emulsion for ELISA							
				For WB							
				For pathological examination							
Shipper					Breeder						
Name		Address			Phone No.	Name		Address		Phone No.	

Expected date and time of arrival of sample at confirmatory testing laboratory
 Date: _____, Between _____ and _____ AM
 Between _____ and _____ PM

Screening Kit Type/ Lot No.: