

United States Department of Agriculture
National Veterinary Services Laboratories

Standard Operating Procedure

TRACKING PROCEDURES
FOR
BOVINE SPONGIFORM ENCEPHALOPATHY

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Contact Person: Roger E. Brannian, (515) 663-7480

Approvals:

/s/ S. Mark Hall Date: 2/20/04
S. Mark Hall, Head
General Pathology and Pathology Investigations Section

/s/ Arthur J. Davis Date: 2/20/04
Arthur J. Davis, Chief
Pathobiology Laboratory

United States Department of Agriculture
Animal and Plant Health Inspection Service
P. O. Box 844
Ames, IA 50010

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Tracking Procedures for Bovine Spongiform Encephalopathy

- I. LOG-IN (Log-in staff)
 - A. Samples received from Biological Materials Processing Section (BMPS)
 - B. Separate Bovine Spongiform Encephalopathy (BSE) cases from Chronic Wasting Disease (CWD), Scrapie, Tuberculosis (TB) and miscellaneous cases
 - C. Line up cases in order of accession number
 1. Make sure sequential numbers are on each jar if more than one animal per accession
 2. Write sequential numbers if required
 - D. Open each sample bag one at a time and complete information on Veterinary Services Form 10-4, Specimen Submission Form (VS Form 10-4)
 - E. Always check paper work and jar labels for errors and/or discrepancies
 - F. Complete the information on the VS Form 10-4 as follows:
 1. Correct and/or add any missing information you can determine
 2. The type of sample and where it will be placed, sent or stored
 - a. Formalin – shelf
 - b. Fresh – freezer
 - c. Block – sent to Histology Lab
 3. Make sure to account for all submitted tissues
 4. Number of animals per case
 5. Check to see that date received and accession numbers were recorded

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6. Write down test requested, "BSE clinical" or "BSE Prion resistant Protein (PrP) only"
 - a. "BSE clinical" refers to any animal submitted with a history indicating Central Nervous Signs (CNS) and is Rabies Negative, Sick, Food Safety Inspection Service (FSIS) Condemned, Foreign Animal Disease Suspect (FAD), Import, or selected samples from diagnostic laboratories
 - b. "PrP only – BSE" is an animal submitted as a Downer, Dead, Not Listed, or Other
 7. Assign a specific pathologist if BSE clinical case
 8. Record classification for reporting staff on VS Form 10-4:
 - a. Downer
 - b. Dead
 - c. Rabies negative
 - d. Sick
 - e. CNS
 - f. Not Listed (NL)
 - g. Other
 - h. Import
 9. Mark "History in File" if additional paper work was submitted
- G. Assign RA number to each case. THIS NUMBER SERVES AS THE TRACKING NUMBER FOR EACH CASE AS IT PROGRESSES THROUGH THE LAB. All BSE cases have an Reference Assistance (RA) number starting with the letter "B"
- H. RA number stickers should be added to the following:
1. VS Form 10-4

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2. One on outside and one inside of the fixed tissue bag
3. One on the fresh tissue bag (if present)

I. Additional samples

1. If additional samples are received at a later date, the original VS Form 10-4 is retrieved and updated to include the new information
2. The amended VS Form 10-4 is stamped and dated with a "corrected copy" stamp. Write your initials by the stamp

J. Cassette labeling

1. Cassettes are color coded. YELLOW is used for all BSE cases
2. Labeling is performed by the ThermoShandon MicroWriter Plus, an automated computerized cassette printer according to the manufacture's equipment operating manual
3. The appropriate RA number for each animal should be printed on a yellow cassette. If there is more than one animal per accession, the RA number should be followed by the sequential numerals for each animal as recorded on the jars and the VS Form 10-4. If there is only one animal, a numeral does not follow the RA number
4. If the case is classified as BSE clinical, eight additional cassettes are prepared for conventional hematoxylin and eosin (H&E) staining of standard CNS tissues as follows:

A - Obex - anterior face down (in this case the H&E slide will be made from the same block as the IHC slide - see II.B. below)

B - Spinal Cord - serial cuts; Cerebellum

C - Pons and Trapezoid body

D - Mesencephalon - serial cuts

E - Thalamus

F - Cerebrum and Hippocampus

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G – Caudate Nucleus

H - Cerebrum at level of Caudate Nucleus

Note: Histology Lab will know to stain the above slides with H&E because the RA number will be followed by a letter (A, B, C, etc.) rather than a numeral (1, 2, 3, etc.)

- K. Labeled cassettes and completed VS Form 10-4 are placed back in bag awaiting cut-in
- II. CUT-IN (Log-in staff, part-time students, pathologists)
- A. BSE cut-in is performed in Room 04 under the biological safety hood
 - B. The Obex is preferred site for sectioning. A cross section should be Made at the tip of the "notch" or "V" on the dorsum of the obex. Make a second parallel section 2-3 mm anteriorly to the first. The anterior face of the section should be placed face down in the cassette. Avoid trimming tissue from the edges of the section if at all possible. If it is necessary to trim the section because it is too large to fit in the cassette, trim only the minimum amount required for accommodation in the cassette and trim lateral and ventral portions only (to retain the dorsal vagal nuclei). Save all unused tissues in formalin for future reference.
 - C. ALWAYS cut only one tissue at a time with only the appropriate correspondingly labeled cassette in the immediate work space
 - D. Make sure that the numbers on the VS Form 10-4, the tissue jar, and the cassette all match for each specimen while proceeding. After sectioning place the loaded cassettes in a new jar of formalin labeled for BSE
 - E. Upon completion, indicate on the VS Form 10-4 if each tissue specimen was acceptable or if there was damage and if so the nature of the damage
 - F. Fill out a cut-in sheet for each sample:
 - 1. Yellow sheet for BSE Prp only cases
 - 2. Tan sheet for BSE clinical cases
 - 3. Cut-in sheets will be sent to reporting staff and be filed with case file

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- G. When finished cutting-in an accession write the date and place your initials on the VS Form 10-4
- H. Computerized tracking reports (Log-in staff)
 - 1. 03RA Track Daily Histo Report
 - a. Lists what was logged in – date and RA number
 - b. When tissues are cut-in, cut-in date is recorded
 - c. When cut-in recorded, copy sent to Histology Laboratory to serve as notice that they should be expecting these cases
 - d. If cases are not received by Histology Laboratory in timely fashion, serves as feedback to investigate
 - 2. 03RA Track IHC Daily Report
 - a. Notifies ImmunoHistoChemistry (IHC) Laboratory of what cases they should be receiving
 - b. IHC Lab verifies they received slides and records date when they received them
 - c. If cases are not received in a timely fashion, serves as feedback to investigate
 - d. Case numbers are listed in order so can track a given case if necessary
- I. Cut-in date and color coding
 - 1. Tissues need to be in formalin at least five days before cut-in
 - 2. Person cutting – in should check on BSE shelf or retained shelf to make sure that all samples that are ready to cut - in (check cut-in date) on a particular day are done. Samples are color coded by colored tape which was placed on the sample bag when it was received:
 - a. Monday – pink

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- b. Tuesday – green
- c. Wednesday – yellow
- d. Thursday – orange
- e. Friday – blue

III. HISTOLOGY LABORATORY (Histology Laboratory staff)

- A. Loaded cassettes and accompanying paper work are received
- B. Automated tissue processing (Ref. Current Version GPPISOP0020)
- C. Paraffin embedding (Ref. Current Version GPPISOP0019)
 - 1. One cassette at a time
 - 2. Make sure tissues do not get flipped over so that opposing faces remain in down position when transferred to mold
- D. Blocks are lined up in box in order of RA number. A check is made to see if there are any discrepancies between the blocks and what is written on the accompanying 03RA Track Daily Histo Report
- E. Blocks are removed, one at a time, in order, from the box and placed on work counter
- F. Glass slides are hand labeled, one at a time, and the RA number is carefully checked to make sure it matches the cassette number on the counter. (Slide labels are color coded YELLOW)
- G. Microtomy procedure (Ref. Current Version GPPISOP0018)
 - 1. Make sure block number matches slide number
 - 2. Paraffin ribbons are placed on slide, one cassette – one slide at a time
- H. Upon completion slides are sent to IHC Lab

IV. IHC LAB (IHC Lab staff)

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- A. 03RA Track IHC Daily Report (from Log-in staff)
 - 1. Printed copy received from log-in
 - 2. Record date received
 - 3. Record number of slides received
 - 4. Recent reports are stored in green notebook in IHC Lab Room 108 on log-in counter
 - 5. Older reports are stored in black notebooks on shelves above counter

- B. Separate BSE slides from other slides and place them in a separate flat

- C. Fill out yellow BSE Brain PrP Worksheet from the slides in the flat. This sheet will accompany the flat

- D. Slides are processed through the following general steps (Ref. Current Version GPPISOP0026):
 - 1. Slides placed in drying oven
 - 2. Deparaffinization procedure
 - 3. Decloaking procedure
 - 4. Slide cooling
 - 5. Slide labeling
 - a. Performed by computerized slide label printer using RA number bar code
 - b. Labels are checked against numbers on BSE Brain Worksheet
 - c. Labels are checked against hand-written label on slide
 - d. Printed labels have adhesive and are placed on slide by hand after printing

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6. Slides are placed in Ventana Automated Processing Machine
 7. Cover slips are placed on the slide by automated equipment
- E. Following preparation of the slides additional information is added to the yellow BSE Brain Worksheet:
1. Ventana run number
 2. Date of run
 3. Person who performed the run
 4. A copy of the sheet stays in IHC Lab
- F. Numbers and information on the worksheet are again checked against the slides in the flat
- G. Original BSE Brain Worksheet and slides are sent to pathologist
- V. **EVALUATION BY PATHOLOGISTS (Veterinary Pathologists)**
- A. Check to see that slide numbers match numbers on BSE Worksheet
 - B. Evaluate control slide to make sure both positive and negative controls are valid
 - C. Examine all tissue on each slide and look for red positive staining especially in the dorsal vagal nuclei, the cuneate nuclei, the lateral cuneate Nuclei, the spinal tract of the trigeminal, and the reticular formation. In the event that the test may be positive, the case should be reviewed by multiple pathologists. The ultimate determination will rest with the chief of the pathology section.
 - D. Report results on the worksheet according to the criteria and terminology outlined in the document entitled "Abbreviations for Diagnostic Terms Used in Computer Automated Laboratory System (CALs)"
 - E. If the anatomical location is not appropriate, this should be verified and initialed by a second pathologist
 - F. Upon completion sign and date the worksheet
 - G. Return slides along with original worksheet to IHC Lab

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VI. RETURN TO IHC LAB (IHC Lab staff)

- A. IHC staff checks to see if signed, dated, reported completely (eg. – location statements verified, etc)
 - 1. If information is complete IHC staff member should sign and date
 - 2. If a slide is positive the block should be checked against the slide to make sure they match
 - 3. Special instructions and requests such as “repeats” and “recuts” should be handled as outlined in “G.” below
 - 4. “Failed runs” should be handled as in “G.3.” below
- B. Copy of completed BSE Worksheet sent to reporting office (Reporting staff)
- C. Original copy of BSE worksheet filed in IHC
- D. All cases are entered into computer by sequential RA number and date for easy retrieval and tracking if required
- E. To access above computer (23AD685) located in IHC Lab Room 108:
 - 1. Folder - “IHC Log Master”
 - 2. Go to “PrP Test Log Current Master”
 - 3. Tab for BSE
- F. Slides are held on shelf for two weeks for drying, then permanently filed
- G. Special instructions and requests:
 - 1. Repeats
 - a. Requested by pathologist if questionable slide
 - b. IHC staff pulls and checks block against original slide
 - c. Repeat slide is requested from Histo Lab

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- d. Repeat slide is labeled with the letter "R" following the original number
 - e. New slide is returned to IHC Lab, logged into green notebook, and processed as the original
 - f. Repeat slide and original slide goes to pathologist (not necessarily the original requesting pathologist)
 - g. Pathology evaluation process is repeated as originally
2. Requests for additional slides (recuts)
- a. Initiated by pathologist for variety of reasons (eg. – artifact, protocol deviations, education, training, etc.)
 - b. Pathologist should fill out "Special Request for Additional Slides" form including RA and animal identification numbers, block verses wet tissue, IHC or IHC deviations, etc. The pathologist may also request an H&E stain or special stain
 - c. Slide is prepared by Histo Lab per usual procedures
 - d. Processed by IHC Lab as usual (or with requested protocol deviations)
 - e. Slides are returned to the REQUESTING PATHOLOGIST
3. "Failed runs"
- a. A failed run includes, but is not limited to, poor staining quality, severe artifact, questionable or invalid control slides, tissue which fell off the slide, neuronal drop out, etc.
 - b. A failed run is determined by the pathologist who should fill out an "IHC Failed Run Report"
 - c. IHC technical staff and pathologist attempt to determine cause of the failed run and take corrective action
 - d. The run is then repeated (Note: This is not considered the same as a "repeat" request, however, because the "failed"

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set of slides are not retained permanently due to their poor quality)

- e. Slides are processed and reported in the usual manner (assuming the problem is resolved)

VII. REPORTING (Reporting staff)

A. Initial entry of VS Form 10-4 into computer system

1. Receive VS Form 10-4 forms each day from log-in area
2. Enter each VS Form 10-4 into CALS system in order to assign the appropriate test to each case:
 - a. Open CALS
 - b. Click on "Login"
 - c. Select "Login Diagnostic Samples"
 - d. Enter species: "Cattle"
 - e. Enter specimen: FIXTIS
 - f. Enter disease: Transmissible Spongiform Encephalopathy (TSE)
 - g. Enter accession number
 - h. Enter sample identification (ID)
 - 1) Enter ID number for single samples
 - 2) If no sample ID is written on VS Form 10-4, enter a "1"
 - 3) For multiple samples enter 1 – n (n=number of samples written on VS Form 10-4)
 - i. Enter animal ID located on VS Form 10-4
 - 1) For single samples enter the number (provided one is listed)

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- 2) For multiple animals, enter "Per your Veterinary Services Form 10-4"
 - j. Enter RA number from VS Form 10-4
 - k. Enter age of animal if single animal; if multiple animals do not enter ages
 - m. Click on the box located below the spot that says "Test"
 - n. Click "Initiate" located on toolbar above
 - o. When "PRP" appears, click on "Add Test"
 - p. Click on "Process" when it becomes highlighted
 - q. Repeat steps d – p for each VS Form 10-4
3. Enter VS Form 10-4 into Access database - record information from VS Form 10-4 in order to track all cases received:
 - a. Open F: drive\RA Data file\FY03RA Log
 - b. Select "Forms" and then choose FY03 Case Log
 - c. Hit the far right button (>*) at the very bottom of the form for a blank record to begin
 - d. Enter accession number
 - e. Enter RA number
 - f. Enter "BSE" in exam field
 - g. Enter date received from VS Form 10-4
 - h. Enter referral number in box # 14 on VS Form 10-4 (Leave blank if not listed)
 - i. Tab through "Fee" – should say "N"
 - j. Enter owner name

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- k. Enter two digit state abbreviation
 - l. In "Purpose" field enter either "SURV", "FAD" or "Import"
 - m. Type in "Cattle" under species
 - n. Type in number of animals from VS Form 10-4
 - o. Tab to the bottom portion of form and fill in each animal ID listed on VS Form 10-4
 - p. Enter breed, age, and sex of animal in appropriate if listed; enter "NL" if not listed
 - q. For clinical cases enter Downer, Dead, Rabies-negative, Sick, CNS, Other, Import as appropriate. Enter "NL" if nothing is listed
 - r. Hit the far right button (>*) at the very bottom of the form for a new blank record
- 4. Place VS Form 10-4 into a folder with the RA number
 - 5. File each case in the pending file
 - 6. Read only Access database (for designated individuals – eg. pathologists to find status of case)
 - a. Open F: drive\RA data file\FY03RA log.mbd
 - b. Forms->case log
 - c. Search to find record by accession number, RA number etc. (Select on the field on the record used to search on the "Binoculars" icon)
- B. Receiving results
- 1. IHC results (PrP results) are placed in the purple bin in the office (703) by IHC staff (check to see that the result sheet is signed by pathologist)

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2. Photocopy result sheet so there is one copy per RA number on the sheet
3. For each separate case, highlight the RA number, animal number and result
4. Match result sheet with the appropriate folder by matching the RA numbers – look in the pending file for the folder. If no results have been received yet, it should be there. If it is not there then look on the table near the desk in Room 03 (this means some of the results have already been received)
5. Paper clip result sheet to the folder and place folders in the stack of BSE “Waiting for Results to be Entered” table near desk in Room 03. Place in numerical order.
6. BSE clinical cases will also have an H&E report which will be found in the second bin below the purple bin or sent by e-mail

C. Entering results into CALS

1. Look through case folders sorted numerically on table near desk in Room 03 (see B.5. above) to determine which cases are ready to report out
2. For each case, look at number of animals listed on the VS Form 10-4 and compare it to the number of results received on the IHC result sheet. If the numbers match and all animals have a result (no repeats), the case is ready to report out (PrP only cases)
3. If the case is a BSE clinical and has been assigned to a pathologist, then once the PrP result and the H&E results are received, the case is ready to report out
4. Enter results into CALS system
 - a. Verify that the RA number on the VS Form 10-4 form and the RA number on the IHC result worksheet match
 - b. Verify that the number of animals sampled on the VS Form 10-4 form matches the IHC worksheet and that there are no repeats (designated with the letter “R”)

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- c. Check to make sure the cut-in card is in the back of the folder and that the RA number and accession number on the cut-in sheet match the respective numbers on the VS Form 10-4

- d. Log into CALS: File->Another Session (open four sessions)-> Window->Tile(to display four screens)
 - 1) Screen 1 - Login->update samples->update samples-> update samples long version
 - 2) Screen 2 - Accessions->edit submission VS Form 10-4
 - 3) Screen 3 - Test->Test Single Sample
 - 4) Screen 4 - Validate->Validate Multiple

- e. Screen 1
 - 1) Enter accession number (six digits in bottom right corner of VS Form 10-4)
 - 2) Hit F2 to search or display
 - 3) Verify sample ID, animal ID, and RA number
 - 4) Note: All BSE cases are entered as one sample. If the case has only one animal the sample ID and the animal ID are all that is written on VS Form 10-4
 - 5) If the case has more than one animal the sample ID will say "1- n" (n=number of animals) and the animal ID will say "Per your VS Form 10-4"
 - 6) If any changes are made to this screen hit "Replace Entry" icon to update

- f. Screen 2
 - 1) Hit "Clear Screen" icon if data is already displayed
 - 2) Enter accession number

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- 3) Hit F2 or search to display
- 4) Verify that information on this screen matches that on VS Form 10-4
- 5) If any changes need to be made to this screen hit "Replace Entry" icon to update

g. Screen 3

- 1) Enter sample key number (found on screen 1)
- 2) Hit F2 or search to display
- 3) Verify that the RA number displayed matches the number on the VS Form 10-4 and IHC result worksheet to ensure you are entering the results on the correct case
- 4) Result field->use drop down to select the result given on the IHC result worksheet. NOTICE THERE IS NO OPTION OF CHOOSING "POSITIVE" AS A RESULT

Note: If this is a Prp only case this is the only result you will need to enter, but if it is a BSE clinical case you will need to add HISTSE test and also enter H&E results

- 5) Billing Units->always 1
- 6) Analyst->name of pathologist who read the slide (first initial and last name)
- 7) Date tested->found on upper left corner of IHC result worksheet above pathologist's name
- 8) Hit "Write Up" icon
 - a) For PrP only cases type in "BSE". If the case has only one animal no editing is required. If the case has more than one

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animal edit the write-up to match the attached samples

- b) For PrP clinical cases type in "NBSE". Above the write up, type in the pathologist's histopath description. Remove your name and the dashed line from the bottom of the report. Paste in the name of the pathologist that read the slides
- c) If there are any results other than ND the report will need to be modified accordingly. Examples: Location statements (LOC), Unacceptable (UNA), etc. If there is a UNA result, include in the narrative the reason (indicated by the pathologist) the sample is unacceptable

9) Hit "Save" icon

10) Hit "Enter Data" icon to enter results you just entered

h. Screen 4

1) Enter accession number

2) Hit F2 or search to display

3) Select sample

4) Hit "Results" icon

5) Make a final review of the report to make sure there are no errors

6) Hit "Print" icon to print the preliminary report.

5. Attach preliminary report to the outside of the folder and place in the assigned pathologist's box

D. Finishing cases

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1. Pathologists will return case folders to one of three bins located on table in front of the desk in Room 703 according to pathologist's review and case disposition:
 - a. Completed cases bin if there are no corrections and pathologist has validated the case
 - b. Red tagged bin if case needs to be faxed out right away
 - c. Corrections bin if there are corrections to be made (case not yet validated by pathologist)
2. Check to verify that the case has been completely validated by the pathologist
3. Fax results if necessary
4. Enter results into Access database:
 - a. Open F:\RA Data\FY03 RA log.mdb
 - b. Forms->case log
 - c. Search to find record by: case # = RA # (Select the field on the record used to search on and click on the "Binoculars" icon)
 - d. Enter the results – find the records searching on the case # /RA # (folder tab)
 - 1) Enter the date reported
 - 2) Enter all result fields in ALL CAPS for the abbreviation entered
 - a) ND (Not Detected)
 - b) LOC (Location)
 - c) R (Repeat)
 - d) SC (See Comments)
 - e) UNA (Unacceptable)

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- f) -- no result (when other animals have the result entered
 - 3) . NEVER REPORT a "P" (Positive) BSE RESULT WITHOUT THE EXPRESS APPROVAL OF THE CHIEF OF THE PATHOLOGY SECTION
5. File completed case in file cabinet
