

## The IHC Test Variables:

- IHC has been the primary confirmatory test for USDA's BSE surveillance program and is recognized by the World Organization for Animal Health, or OIE.
- IHC allows scientists to determine if a sample is positive for BSE in two distinct ways:
  - 1.) A staining technique (presence of abnormal prion protein) that uses antibodies to detect abnormal prion protein in the brain.
  - 2.) A visual examination to determine whether there are lesions (holes or "spongy" appearances) present in the brain.
- **Several variables could yield conflicting results:**
  - o IHC is not a standardized, commercially available test. It involves variables, including several options in types of antibodies and other reactive agents. The sensitivity of any given test is influenced by those variables.
  - o If the level of infectivity in the animal is extremely low, the abnormal prion in the brain will be minimal and therefore more difficult to detect.
  - o Variations in the conditions under which the staining process is performed, such as chemicals and reactive agents used, temperature and length of antibody exposure, can also cause the test to yield different results.

## Testing History on This Animal:

- In November 2004, a sample from this animal returned inconclusive for BSE on a Biorad screening test.
  - The sample was subjected to an IHC confirmatory test, which returned negative.
  - USDA scientists also ran an additional, experimental IHC "rapid" tissue fixation test for academic purposes, which can be conducted more quickly than the IHC confirmatory test and is therefore of interest to the scientific community, but it has not been approved internationally.
- While some abnormalities were noted in the experimental IHC test results, because the test was not a validated procedure, and because the two approved IHC tests came back negative, the results were not considered to be of regulatory significance and therefore were not reported beyond the laboratory.
  - A Western blot test conducted the week of June 5, 2005, returned positive for BSE.
  - An additional IHC confirmatory test conducted the week of June 13, 2005, by USDA scientists utilizing different antibodies from the November 2004 test, confirmed this case as weakly positive for BSE.
  - The Veterinary Laboratories Agency in Weybridge, England, conducted a series of diagnostic tests including an IHC, using different antibodies from those used by USDA in November 2004, which returned positive results for BSE.
  - Experts from the Weybridge lab confirmed the accuracy of the results of USDA's November confirmatory IHC test, concurring that the case could not have been confirmed on the basis of this sample.
  - Weybridge experts also examined the November experimental IHC test and interpreted the results to be positive.

## Potential Causes of Conflicting Results:

- USDA scientists are consulting with Weybridge scientists to determine the cause of the conflicting IHC test results.
- Several factors could cause or contribute to the discrepancy as follows:
  - o This animal had a very low level of infectivity and therefore the sensitivity of USDA's routine IHC test might not have been sufficient to detect the disease.
  - o Weybridge experts indicate that deposits of abnormal prion in the brain tissue were not uniformly distributed and were present at low concentration, which means that even adjacent samples of brain tissue might not give identical results.

## USDA Protocol Review:

- USDA will develop a protocol to conduct dual confirmatory tests, the IHC and the Western blot, when the screening test, the Biorad, returns an inconclusive result.
- USDA and Weybridge scientists are in agreement that the Biorad test is a very effective and appropriate screening test.
- USDA scientists will consult with Weybridge scientists to assess the array of antibodies available for use in IHC confirmatory tests to determine the most appropriate for use in United States confirmatory tests. Those consultations will be repeated periodically.

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