

## BSE Confirmatory Tests

### Immunohistochemistry (IHC) Western Blot

- Primary confirmatory test for USDA's BSE surveillance program.
- Recognized by the World Organization for Animal Health, or OIE.
- Allows scientists to determine if a sample is positive for BSE in two distinct ways: visually (spongiform changes), and through a staining technique (presence of abnormal prion protein).
- Involves looking at an intact portion of the brain, the obex, to see if there are lesions (holes or a "spongy" appearance) present that are characteristic for BSE, and using a staining process using antibodies that detect the abnormal protein prion.
- Takes four to seven days to run.
- Freezing samples does not interfere with performing the IHC test as long as the sample is confirmed as obex.
- Several types, with the SAF Immunoblot being the one recognized by OIE.
- Used under USDA protocol when a sample is "not suitable for IHC", i.e., if it is autolyzed (or degraded) or brain stem architecture is not evident microscopically.
- Uses a large portion of obex brain tissue; the abnormal prion protein in brain material is enriched by ultracentrifugation, and the sample is exposed to protease, an enzyme, to destroy any normal prion proteins that may be present, leaving only abnormal prion proteins. Remaining sample is then run through a gel to separate the abnormal prion protein components by molecular weight. After the transfer of the proteins to a membrane, proteins are stained using antibodies that can identify a specific banding pattern associated with prion diseases including BSE. A diagnosis is made by recognizing three distinctive bands that are identified as a result of a reaction with the anti-prion protein antibody.
- Freezing samples does not interfere with the performance of western blot tests.

### Similarities/Differences:

- Both IHC and the SAF Immunoblot (Western blot) are internationally recognized as confirmatory tests for BSE.
- The tests use different methods to determine if the abnormal prion protein is present in brain tissue of an animal.
- The IHC test additionally allows for the viewing of brain tissue to determine if lesions characteristic to BSE are present.
- Both tests are equally effective at detecting the classical form of BSE.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, sex, religion, age, disability, political beliefs, sexual orientation, or marital or family status. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD).

To file a complaint of discrimination, write USDA, Director, Office of Civil Rights, Room 326-W, Whitten Building, 1400 Independence Avenue, SW, Washington, DC 20250-9410 or call (202) 720-5964 (voice and TDD). USDA is an equal opportunity provider and employer.