

Tissue Sample Collection and Handling for Rabies Testing

When an animal develops rabies, usually from the bite of another animal whose saliva is infected with the rabies virus, the virus moves trans-neuronally from the site of entry to the spinal cord and brain. Due to patterns of progression, a thorough histologic examination of the brain stem is critical to rabies diagnosis. Viral antigen is widespread in the brain of most animals positive for rabies, but because of the possibility of unilateral spreading of the virus, especially in larger animals, a negative finding for rabies can be made only if a complete cross-section of the brain stem is examined.

The complete brain of the animal should be submitted for testing. Although brain stem is the tissue most reliably found to contain viral antigen, the characteristic size and shape of the rabies virus that accumulates in the large neurons of foliar regions of the cerebellum are easily detected and recognized by direct fluorescent antibody (DFA) testing. Inclusion of this tissue yields a more confident diagnosis than examination of brain stem alone. Although the hippocampus was once the tissue of choice for histologic tests for Negri bodies, hippocampus is of limited additional value when brain stem and cerebellum are examined. If the cerebellum is missing from tissue submitted for rabies testing, however, a negative finding for rabies may be made from examination of brain stem and hippocampus. While a negative finding for rabies can be made only if brain stem tissue is among the tissues examined, incomplete specimens should be tested, if possible. Specific staining in any tissue reacted with anti-rabies antibody is diagnostic of rabies infection.

Virus is present in the saliva of an infected animal only after virus proliferation in the central nervous system and subsequent progression from the brain to the salivary glands. A negative DFA test for the presence of rabies virus in brain tissue assures that contact with saliva of a biting animal **could not** have transmitted rabies. Because virus may not be spread to all salivary glands and may be present only intermittently in saliva, negative tests of salivary glands or saliva cannot rule out rabies infection.

Shipment of Samples

Because rabies prophylaxis is usually delayed pending a laboratory report, specimen transit time to the laboratory should be as short as possible, preferably within 48 hours. A fresh, unfixed brain sample is critical to a rapid and accurate diagnosis of rabies. Refrigeration will preserve a sample for at least 48 hours. Freezing of the sample for transit will not reduce the sensitivity of the test, but may introduce additional testing delays and impede recognition and dissection of appropriate test samples. Repeated freeze-thaw cycles may reduce test sensitivity and should be avoided. Biocontainment during specimen transport is critical, to prevent both contamination of the outside of the package and cross-contamination between samples within the package.