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## Special Series—Recommendations of a national working group on prevention and control of rabies in the United States

### Article II: Laboratory diagnosis of rabies

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**R**apid, readily available, and accurate diagnosis of rabies is the keystone of prevention. All surveillance activity and description of the complex epizootologic characteristics of rabies in the United States is based on laboratory diagnosis.<sup>1</sup> Veterinarians are the first line of defense against rabies when responding to clinically ill animals. Rabies is an important consideration when an animal has compatible clinical signs because of the diverse potential sources provided by wildlife reservoirs, such as raccoons, skunks, foxes, and bats. Historically, the most imminent threat of rabies exposure to veterinarians and clients originated from domestic dogs. However, rabies in canids has been nearly eliminated throughout most of the United States via vaccination and control of stray dogs. At present, the greatest threat of rabies is among domestic cats, cows, horses, and captive wild animals. Infection with rabies among these animals poses unique risks for exposure of multiple persons, often because rabies is considered late in the clinical course or during postmortem examination.<sup>2-5</sup> The standard diagnostic test consists of direct **fluorescent antibody (FA)** testing of impressions made from fresh brain samples (ie, cerebellum, hippocampus, and brain stem). Fresh brain tissue may not be routinely collected, and this prevents diagnosis with the FA test. If necropsy has been performed, formalin-fixed tissues may be the only available samples. Experimental diagnostic techniques may need to be applied, such as the direct FA test on formalin-fixed material, immunohistochemistry, or polymerase chain reaction assay on paraffin-embedded tissue.<sup>6</sup> Accurate diagnostic capacity and appropriate management of biting animals are essential to the proper handling of potentially exposed animals. In conjunction with local or state health authorities, practicing veterinarians are often directly involved in the 10-day confinement and observation of biting animals or the euthanasia and submission of brain material for testing. They are also routinely consulted in the management of exposed animals; this consists of a 6-month quarantine or the euthanasia of naive animals and the

booster of previously vaccinated animals. A reasonable index of suspicion of rabies among animals with neurologic signs of disease, the preservation of appropriate fresh brain tissue, and demonstrably proficient diagnostic laboratories are essential to the appropriate treatment of potentially exposed humans, as well as identification of at-risk animals for consideration of increased vaccination coverage.

A number of recent cases of rabies in human beings in the United States have been diagnosed either retrospectively or well into the clinical course of the disease, despite compatible clinical observations.<sup>7,8</sup> This lack of early recognition has led to the administration of numerous **postexposure prophylaxes (PEP)** in hospital staff and family members exposed to the patient. Additionally, the recent identification of a rabies virus variant associated with silver-haired (*Lasionycteris noctivagans*) and eastern pipistrelle (*Pipistrellus subflavus*) bats in most of these cases is typically associated with a history in which the patient did not report or recognize a bite.<sup>9-11</sup> Awareness needs to be heightened among physicians of the possibility of rabies in clinically compatible cases (eg, viral encephalitis of unknown cause) where a bite history may be lacking. Physicians and public health officers need to be cognizant of clinical signs and exposure history leading to a suspicion of rabies in human beings; they must also be aware of techniques for appropriate sample collection for antemortem diagnosis and clinical implications of test results.

#### Laboratory Capacity for Prompt Testing of Potentially Rabid Animals

Rapid and accurate laboratory testing allows a physician to initiate timely PEP in a human being who has had contact with a rabid animal. Equally important, the knowledge that an animal is not rabid rules out the necessity for expensive and extended treatment (eg, prophylaxis typically extends > 1 month). Characterization of people receiving PEP would elucidate the critical role of rapid, reliable, available diagnosis and the prevention of unnecessary medical treatment. If laboratory diagnosis is not available within a reasonable period, PEP is often initiated and then discontinued after negative laboratory results are obtained. In addition, rapid testing allows the initiation of appropriate quarantine and booster vaccinations for exposed domestic animals. General recom-

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mentations for the testing of animals suspected of rabies, as provided by the annual Compendium of Animal Rabies Control,<sup>12</sup> should be followed.

**Recommendations**—Timely ad hoc evaluation of specimens, even after business hours and on weekends, is encouraged. Adequate staffing by trained personnel for routine and emergency testing of suspect animals is a basic requirement for those public health laboratories performing diagnosis of rabies. Sufficient staffing enables laboratories to provide diagnostic testing on a routine basis and in emergency situations, such as when a human being is bitten, when the biting animal is suspected of having rabies, and when a physician is awaiting test results to provide PEP.

#### **Precision in Diagnostic Performance of the Direct Fluorescent Antibody Test**

Adherence to established technique among laboratories performing the FA test is essential for accurate and reliable diagnosis of rabies. When performed properly, no other laboratory test for the diagnosis of rabies is as simple, sensitive, specific, and rapid as the direct FA test performed on fresh brain tissue.<sup>13</sup> However, a recent survey of laboratories performing analysis of specimens with public health implications revealed an array of divergent modifications to virtually every step of the FA test.<sup>14</sup>

**Recommendations**—Guidelines for validation and verification of procedural FA test modifications should be compiled. Statistically meaningful validations of the effect of any proposed modifications on the sensitivity and specificity of diagnosis of rabies should be required. National proficiency testing for diagnosis of rabies should be strongly supported, and all state and local laboratories should be encouraged to participate.

Training courses should be conducted by the **Centers for Disease Control and Prevention (CDC)** in association with the National Laboratory Training Network and other experts, consisting of lectures and routine laboratory sessions (eg, annually or biannually). The CDC should coordinate efforts to identify and promote aspects of the FA test that would provide a minimum national standard of diagnosis of rabies. This assessment should focus on equipment, procedures, training of personnel, and the number of tests performed annually by state and local laboratories.

Professional associations, such as the Association of State and Territorial Public Health Laboratory Directors (currently the **Association of Public Health Laboratories [APHL]**) and the American Public Health Association, should promote the importance of consistent methods for diagnosis of rabies among their members. Confirmatory methods (eg, virus isolation) for evaluating the sensitivity and specificity of a direct FA test should be available to and used by every laboratory performing rabies testing, with partnering as necessary, when a laboratory does not have the capacity for an in-house confirmatory test. Alternative tests for diagnosis of rabies should be evaluated by use of the FA test and virus isolation as standards, when such tests become available. This evaluation should include tests of fixed tissue from animals for which fresh tissue is unavailable.

#### **Status of Rabies Virus Diagnostic Reagents**

The FA test is the method of choice used by public health laboratories<sup>14</sup> for routine diagnosis of rabies. At one time, the CDC and many state facilities produced diagnostic conjugates. Gradually, commercially available products met the widespread need for reliable reagents. In the past decade, however, great concern has evolved regarding ongoing problems with availability and variations in the quality of commercial diagnostic reagents and a decreasing number of commercial producers. Given the reemergence of rabies in the United States, high quality and reliably available reagents are a fundamental requisite for diagnosis and prevention of rabies.

**Recommendations**—A reference reagent for quality control comparisons to commercial lots of diagnostic conjugates should be produced and maintained in adequate quantities at the CDC or at designated reference laboratories for the FA test. The CDC or designated reference laboratories should ensure that these reagents are readily available for diagnosis of rabies.

#### **Discussion of Current Laboratory Issues and Dissemination of Information**

Given the complex epizootic characteristics of rabies in the United States,<sup>15</sup> communication among individuals at diagnostic laboratories is critical. For example, early detection of unusual epizootiologic patterns may reveal problems with animal translocation or an emerging rabies virus variant, as revealed by the emergence of rabies in raccoons and coyotes. In addition, effective communication is essential for timely warnings of issues that require problem solving (eg, identification of a poor quality diagnostic reagent) and for discussion and updates of current methods.

**Recommendations**—An annual or biannual workshop on rabies should be held in conjunction with the American Society for Microbiology. The workshop format should include lectures and discussion of laboratory issues in the diagnosis of rabies. In addition,  $\geq 1$  annual or biannual bench training workshop should be held at different sites in the United States. This training should be open to individuals from all state and local laboratories and should cover all aspects of diagnosis of rabies. Emphasis at this workshop should be on the exchange of ideas between participants. Within each region, a reference laboratory could be identified by agreement among states in the region with the cooperation of the CDC. Each state laboratory should be encouraged to provide training with the support of a reference laboratory and the CDC. A forum (eg, a moderated computer bulletin board or listserver) should be established for informal exchange between individuals at diagnostic laboratories. Membership in this forum would be by subscription.

#### **National and Regional Capability for Typing Rabies Strains**

Modern virologic techniques have been essential in clarifying the role of divergent rabies reservoirs in wildlife in the United States.<sup>14-16</sup> Although sophisticat-

ed, these tools are increasingly becoming standard diagnostic procedure because of their capacity to elucidate characteristics of rabies. A clearer understanding of epizootiologic patterns will facilitate the formulation of appropriate public health information and policy for prevention and control of rabies. Support for the continued transfer of reagents and expertise from the CDC to state diagnostic laboratories is critically needed with regard to advanced diagnosis of rabies beyond the routine FA test, such as monoclonal antibody analysis and polymerase chain reaction-based typing methods.

**Recommendations**—A strong commitment should be made to expand resources and to continue a leadership role for the national rabies laboratory at the CDC. The CDC should be encouraged to maintain an experienced laboratory staff familiar with routine diagnostic procedures, to develop new technologies, and to facilitate practice of these technologies at appropriate federal, regional, and state agencies. Proposed reference laboratories should be identified and supported by the CDC and the APHL to provide confirmation of diagnostic tests, serologic testing, and virus typing. A format (eg, a limited access Web site) should be established for the routine sharing of information among any reference laboratories and the national laboratory at the CDC.

At the present time, instructions to professionals with regard to samples for rabies testing in human beings have been made more widely available through postings in a professional information section on the CDC rabies Web page (<http://www.cdc.gov/ncidod/dvrd/rabies>). A videotape on the proper removal of animal brains for diagnosis of rabies has been produced and distributed to state health laboratories. A comprehensive national rabies laboratory training session was held in January 1998 in San Antonio, Tex; another event is in the planning stages for 2000. Unfortunately, limitations in the travel funding of laboratorians preclude the participation of some individuals. An additional monoclonal antibody preparation has become available, but the only commercial polyclonal rabies diagnostic reagent continues to vary in availability and quality. Although training by region has advanced, the need for additional transfer of viral typing technology to state laboratories remains high.

### Preview of Article III

The third and final article in this series will be published in the Dec 1, 1999 issue of *JAVMA* and will review the incidence of rabies in wildlife; it will conclude with recommendations for control of wildlife rabies.

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