

LADDL Rabies Testing FAQ (Frequently Asked Questions)

About the rabies testing service at the LADDL:

The LADDL provides rabies testing, which is independent and supplemental to the state-funded rabies testing services performed by laboratories of the Louisiana Office of Public Health (OPH). The LADDL coordinates rabies surveillance efforts and shares testing data with the Office of State Epidemiologist, particularly when a person may have been exposed to rabies by a positive-tested animal. However, unlike the OPH, which limits testing to certain potential incidental host species and human exposure cases, the LADDL provides service-on-demand for a modest test fee regardless of circumstances. The LADDL is not specifically subsidized by the public for this service, so it collects a fee to offset the costs of testing.

In cases when a person has been exposed to a possible rabid animal, the LADDL strongly encourages the use of the OPH rabies testing services. To access these services, interested parties are to contact the State Public Health Veterinarian of the State Epidemiologist's Office (800/256-2748). Typically, the Public Health Veterinarian investigates the circumstances of the potential exposure, and then determines whether testing is warranted. Parties are informed of specimen handling and shipping details, and whether any fees apply. Additionally, the State Epidemiologist's Office has the ability to expedite access to post-exposure rabies vaccination, if necessary.

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What is the LADDL test fee for rabies testing?

The current LADDL test fee is \$34. Payment is in the form of cash or personal check (made to “LADDL”), which must accompany the specimen to be tested, along with a written request (i.e., the LADDL submittal form) found at the following link:

<http://laddl.lsu.edu/Forms/DCN%20LADDL%20Specimen%20Submission%20Form.pdf>

Alternatively, the LADDL can bill for the testing fee if the submitter has an existing account with the LADDL. Most practicing, licensed veterinarians in Louisiana have an account.

What are the proper specimens for rabies testing?

Rabies testing is performed only on **dead** animals. Following humane euthanasia, the bodies (or heads, or specified areas of brain tissue) of animals to be tested are placed in leak-proof containers (ideally double-bagged) and **chilled immediately**; freezing is discouraged because tissue extraction is complicated by the thawing process, which also contributes to testing delays.

According to the US Center for Disease Control (CDC), the **intact brain cerebellum and brain stem** are required for a complete rabies test; from large animals (i.e., horse, cow), a **bilateral representation** of these tissues is required, plus a sampling of the **cervical spinal cord** (below the brain stem).

From decades of experience in rabies testing, it has been demonstrated that these sites are the most reliable place to look for evidence of rabiesvirus in animals. Furthermore, in approximately 4% of true rabies cases, virus is present only on one side of brain tissue (presumably the side of original exposure); to guarantee detection of the virus in such instances, both sides of the brain are sampled for a complete test.

In instances when the rabies specimen (i.e., brain tissue) is incomplete (i.e., unilateral, or lacking cerebellum and brain stem) or displays decomposition or damage (i.e., blunt trauma or gun-shot to the head), rabies testing proceeds on recognizable, harvestable brain tissue. Test results in such instances are either obviously positive or “indeterminate”, the latter of which meaning that a negative result cannot be arrived at due to the damaged or incomplete specimen. In the state of Louisiana, “indeterminate” test results on cases involving possible human exposure are usually interpreted as “positive”, leading to post-exposure prophylactic (PEP) vaccination as if it were a true positive exposure case. Therefore, the submission of damaged specimens is discouraged because it may result in unnecessary delays if vaccination is warranted or unnecessary vaccination of the bite victim if the animal was truly rabies-negative.

To lower shipping costs, it is acceptable to remove heads from the bodies of rabies suspects. However, the LADDL strongly recommends that the removal of heads and/or collection of brain tissues are performed by trained animal health professionals who

have received pre-exposure prophylactic vaccination for rabies. Until a negative rabies test result has been received, the remains should be handled as if it were bio-hazardous material, and ultimately should be disposed as such according to state law and local ordinances.

How are rabies specimens handled and sent?

Rabies specimens are placed in leak-proof containers (ideally double-bagged) and **chilled immediately**; freezing is discouraged because tissue extraction is complicated by the thawing process, which also contributes to testing delays. Rabies specimens are placed in a properly marked shipping container with a suitable amount of absorptive material and sufficient refrigerant to maintain the chilled state for the duration of transit; one large, or two small frozen gel-packs is recommended **per day** of transit. For specimen volumes greater than 50 cubic centimeters (i.e., animal heads for rabies testing), the item must be triple-packaged to meet shipping regulations for medical specimens.

Federal statutes and the Certified Federal Register (CFR) define medical specimens as shippable “hazardous materials”, which ordinarily cannot be shipped by public conveyance in a conventional manner. This designation applies to both human-source and animal-source specimens. Senders must package and label medical specimens according to federal regulation, or suffer possible fines or incarceration for non-compliance. Individuals unfamiliar with such tasks are strongly urged to review available public information governing the shipping of medical specimens. The US Dept of Transportation and the US Postal Services have web-based information on this topic. An example of such can be found at:

<http://www.usps.com/cpim/ftp/pubs/pub52.pdf>

Before medical specimens are consigned through commercial overnight parcel services, LADDL clients are strongly recommended to contact their company-of-choice for information regarding their preferred methods of handling medical specimens for rabies testing; commercial parcel delivery companies often have more stringent suitability-to-ship criteria than public authorities.

What documentation is needed for rabies testing?

All specimens and testing requests sent to the LADDL require accompanying documentation. The documentation represents the formal service request to LADDL to perform the specified test(s), and agreement to pay the currently published fee for the test(s). The documentation must include:

- The name and contact information of the party to be responsible for the testing fees (and ultimately to receive the test report)
- The name of the animal owner
- The species, age, sex, and identification (i.e., pet name) of the patient

- A brief description of the circumstances justifying the need for the test
- Indication of the specific test(s) to be run, i.e., checked “rabies test” on the LADDL submittal form, found at:

<http://laddl.lsu.edu/Forms/DCN%20LADDL%20Specimen%20Submission%20Form.pdf>

For rabies test requests, the following additional information is included in the test request documentation:

- Whether there was a human exposure
- If there was an exposure, the name and contact information of the person exposed
- The contact information for any other third parties (i.e., family physician) who require the test report

Where are rabies specimens sent?

Specimens with documentation are hand-delivered or sent by **next day delivery** to:
LADDL, Room 1519
LSU School of Veterinary Medicine
1909 Skip Bertman Drive
Baton Rouge, Louisiana 70803
225/578-9777

To assure that specimens arrive in optimum condition for testing, specimens are sent for arrival during business hours; after-hour, weekend, or holiday deliveries should be avoided.

When is rabies testing performed?

Rabies testing is performed each business day. Typically, complete requests (intact specimens with documentation) arriving before noon are performed and reported by the end of the business day. Requests arriving in the afternoon or outside of business hours typically are performed and reported by the next business day. Occasionally, deviations to this schedule arise when facilities and resources to remove the brain from the animal head are not immediately available, when material arrives frozen, or when additional testing on a given specimen is required beyond the original test run.

How are rabies test results reported?

All rabies testing results are reported by fax copy to designated telephone number(s) indicated on the documentation; reporting often occurs by the end of the business day following testing, if not same day. As a courtesy, a telephone report can be made on request.

In positive rabies exposure cases, testing information and results are forwarded to the Louisiana State Epidemiologists office, which may disseminate test results to the indicated contacts and other interested parties, depending on its follow-up investigation on the circumstances of the case. It may also advise exposed individuals whether post-exposure prophylactic (PEP) vaccination is warranted, and if so, facilitate the availability and administration of vaccine in collaboration with the contact's designated personal physician or with an LSU Health Services physician. In positive exposure cases, the primary test report may come from a representative of the State Epidemiologist's Office; later, the LADDL issues a formal written report, typically accompanying the test fee invoice. Additional reports can be generated upon request by the original responsible party for a nominal fee.

What is the LADDL confidentiality policy on rabies testing data?

Information from rabies testing requests and results are among the few exceptions to the LADDL confidentiality policy, and are considered and handled as public information as necessary, as determined by the LADDL director or associate director. **By default, submitters of rabies test requests to the LADDL are understood to have waived any right of privacy regarding information directly or indirectly associated with rabies testing.**

Given the special public health aspect of rabies testing, the LADDL must be ready to respond quickly with information from positive cases, should they arise. This means conveying information to any state public health authority, personal physicians, administration, clinicians, staff, and students of the LSU School of Veterinary Medicine, animal control personnel, and to other third parties that, from follow-up investigation, may have had a role in handling the rabid material. An unfortunate delay in response to a potential rabies exposure could arise if formal permission must be sought from all listed parties before information is released on an individual case.

What is considered a possible rabies exposure?

According to the Louisiana Office of Public Health, exposure in most instances is defined as either a bite or a scratch possibly exposed to saliva. Additionally, exposure is understood to have occurred in following instances:

- Any direct contact with a bat, a known reservoir of rabiesvirus in Louisiana
- Persons waking from sleep in a room where a bat is present
- Mentally handicapped persons, persons under the influence of alcohol or drugs, infants, or children alone in a room with a bat
- Instances of exposure to salivary secretions or brain tissue of rabid or potentially rabid animals
- If during the handling of a rabid animal, its saliva, secretion or excretion, or any bodily fluids accidentally have been ingested or come into contact with eyes, mucus membranes, scratch, or other skin lesion

- Any set of circumstances judged to be a rabies exposure by the State Public Health Veterinarian

Any questions regarding exposure should be addressed to the State Public Health Veterinarian of the Office of State Epidemiologist, or to one's personal physician.

When is it necessary to have a rabies test performed?

In many exposure cases, testing is performed to determine the need for rabies vaccination (post-exposure prophylaxis, or PEP). It is popularly understood that when an animal (specifically, an individual of a mammalian species) bites a person or another animal, then rabies testing is warranted. While this may be prudent, even mandated by state or local laws in certain circumstances, the biting of a person by an animal doesn't need to result in rabies testing in all instances. Several factors go into this decision, which when not dictated by law, is made by the bitten individuals (or for minors, the parents or guardians) in collaboration with public health authorities and personal physicians. The ultimate public authority in determining the necessity of rabies testing lies with the State Public Health Veterinarian of the Office of State Epidemiologist, Louisiana Department of Public Health. **The LADDL does not participate in this determination.** The risk assessment of a possible rabies exposure, and the decision whether or not to test and vaccinate for rabiesvirus often involves many factors, including the following:

- **State laws and local ordinances**—Because proper rabies testing necessitates the destruction of the animal, the decision to test sometimes involves some contention between the various parties. Animal control or local law enforcement officers often are involved in potential instances of rabies exposure to ensure the rights of both animal owners and exposure victims, and the community's public health concerns. To a certain extent, the actions of citizens and public officials are directed by state laws and local parish or municipal ordinances, where they exist.

According to Louisiana's Sanitary Code, when a dog, cat, or ferret bites a human, the animal must be killed and tested, or confined for 10 days (**not** tethered, but caged or penned to prevent human contact) and observed for the development of neurological signs compatible with rabies. Such determinations are made only by a licensed veterinarian or the State Public Health Veterinarian. If signs become evident, the animal must be destroyed and tested for rabies.

In instances when an unvaccinated dog, cat, or ferret has been bitten by a potentially rabid animal, either the bitten animal must be destroyed and tested, or at the animal owner's discretion, the animal must be confined and observed for 6 months, and vaccinated at the 5th month of confinement. Exposed dogs, cats, ferrets, or livestock with current vaccination are revaccinated, confined, and observed for 45 days. Generally, other exposed, unvaccinated animals are humanely euthanized for testing at the discretion of the State Public Health

Veterinarian. Details of the Public Health—Sanitary Code of the State of Louisiana governing potential rabies exposures are found at:

<http://www.dhh.louisiana.gov/offices/miscdocs/docs-206/SanitaryCode.pdf>

Many local jurisdictions have rabies ordinances at least reflective of the State's Sanitary Code, but some may be more restrictive. The LADDL urges parties to follow instructions by local authorities or ordinances governing potential rabies exposure wherever they exist. An example of such, applicable to situations in East Baton Rouge Parish, can be found at:

<http://library.municode.com/index.aspx?clientId=10107&stateId=18&stateName=Louisiana>

- **Unprovoked vs. provoked bite**—One of the questions following a potential rabies exposure should be whether the bite was unprovoked. An unprovoked bite by virtually any domesticated species and most wild mammalian species in North America usually represents a significant change in mental status and behavior by the animal. Aggression is one of several clinical signs resulting from rabiesvirus infection, especially in carnivores.

A typical instance of an unprovoked bite might involve a skunk or raccoon racing out from a shrub-line, and chasing down a horse or cow in pasture, or perhaps a family pet or child playing behind a residence near the woods. Such behavior suggests altered mental status in the animal; both species in the above example are somewhat nocturnal, and by nature would avoid rather than approach persons or other animals.

The LADDL has performed hundreds of negative rabies tests on animals that had bit a person while that person was attempting to administer care or handle the animal for some reason. This would not be considered an unprovoked bite; the animal may have been ill or in pain, thus reacting instinctively and adversely to being handled. In instances of bites by pet dogs, cats, or ferrets, factors that argue against the need for testing would be recent rabies vaccination and a lack of neurological signs specific for rabies. Of course, nothing would be known of the general health condition or rabies status of wild or feral animals, so capture, euthanasia, and testing is warranted.

- **Vaccinated vs. unvaccinated**—Vaccination status is another factor contributing to whether testing is necessary. A new understanding of the potency of rabies vaccination has extended the length of immunity for the vaccinated animal to up to 3 years for certain vaccine regimens. Bites by animals documented to have been vaccinated within the last 3 years need not result in automatic rabies testing. In such instances, a time-course of quarantine and professional observation mitigate the risk of rabies from exposure by a vaccinated animal.

Conversely, bites by wild or feral animals, or animals with uncertain health records increase the risk of rabies and need for testing.

- **Domestic vs. wild or feral animal**—The inherent risk in a potential exposure by a domestic animal is less than feral or wild animals. The course of rabies infection in most domestic animals in many instances is relatively quick, usually within 90 days. Rabiesvirus infection of a domestic animal often is clinically apparent at the moment when that animal bites or exposes another. Animal control agencies function to reduce the possibility of domestic animals coming into contact with potentially rabid wildlife. Responsible pet owners often can demonstrate current vaccination documentation. Even more specifically, the habitat of a house pet would preclude exposure and the risk of rabies. Pastured domestic animals (i.e., horse, cow) receive occasional observation and attention, and become noticeable when befallen by illness, especially by some non-specific neurological disorder suggestive of rabies. However, for certain wild, feral, or reservoir host species, the signs of rabies infection may be subtle or non-apparent, even to animal health professionals.
- **Reservoir vs. incidental host species**—Potential exposure resulting from interaction with a reservoir host species obviously represents a much greater risk of rabies than would an exposure involving other mammalian species. In Louisiana, **wild skunks and bats** are recognized reservoir species for rabiesvirus. Infection of other animals often begins with exposure from one of these species. Rabiesvirus is transmitted within family groups of reservoir host species by natural grooming habits. Once infected, reservoirs can harbor the virus for a significant portion of the animals' lifespan without exhibiting obvious clinical signs of infection. The virus has evolved and adapted to these host species for protracted co-existence and shedding.

Coyotes, foxes, and raccoons are species most often infected with rabies, even serving as reservoir hosts in other regions of North America. Rabiesvirus subtypes from these reservoir species have yet to appear in animals in Louisiana. Rather than reservoir hosts, these species are more likely to be incidentally infected, probably by a rabid skunk bite. The LADDL has considerable testing data on neurologically affected raccoons, which are rabies-negative but often found to be infected by Canine Distemper Virus. However, all bites by such wildlife must be considered potential exposures to rabiesvirus.

In Louisiana, known incidental hosts of rabies include dogs, cats, horses, and cattle. Certain wild animal species, such as small rodents (e.g., squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, and mice) and lagomorphs (including rabbits and hares) have almost never been found infected with rabiesvirus, and have not been known to transmit rabies to humans. However, any mammalian species theoretically can become incidental hosts for rabies.

- **Presentation of certain neurological signs**—A veterinarian is trained to recognize the clinical signs of neurologically affected animals, and link them to possible nervous system lesions. The characteristics and subtleties of these signs may not be readily distinguished by untrained persons, so according to the Louisiana Sanitary Code, such determinations are left to licensed veterinarians or the State Public Health Veterinarian.

Rabiesvirus infection often results in certain patterns of neurological signs, typified by descriptions of “furious” rabies (i.e., hyper-aggressiveness), as is often seen in rabid carnivores (i.e., dog, cat), or “dumb” rabies (i.e., a virtual lack of responsiveness) as is usually observed in large farm animals infected by rabies. The signs of rabies may not be limited to “furious” or “dumb” descriptions, and may include other neurological presentations. Although rabies is possible, a dog exhibiting a combination of twitches, seizures, or “chewing-gum” fits is more likely to be a victim of distemper. Ultimately, the risk of rabies in an animal lacking neurological signs is significantly less than in neurologically affected animals.

How is rabies testing performed?

The standardized test for public health and clinical diagnosis of rabies is the Direct Fluorescent Antibody (dFA) test, which is performed only on post-mortem brain tissue. On requests specifying the rabies test, the LADDL currently performs only the dFA test.

From over 50 years of experience, the dFA technique is recognized by the public health community and the consensus of laboratory scientists involved with rabies testing as the most rapid and reliable of all routine tests for rabies. Rabies dFA procedure has been optimized for sensitivity and specificity approaching 100%, and has been standardized for consistency between rabies testing laboratories. As an immune-based test, the critical ingredient of dFA is the FA conjugate, several of which are commercially available from FDA-recognized sources to perform rabies testing. Ideally, the test is run simultaneously using two separate FA conjugates for double-redundancy, which also adds to the sensitivity and specificity of the procedure. Additional steps adopted by the LADDL to maintain a high quality of testing include internal positive and negative controls, intramural and extramural training, equipment certification, and subscription to an independent proficiency testing service. Ultimately, the LADDL procedure meets or exceeds the CDC recommended procedure, which can be found in *Protocol for Postmortem Diagnosis of rabies in Animals by Direct Fluorescent Antibody Testing*, A Minimum Standard for rabies Diagnosis in the United States. For further details on the dFA test, please see:

http://www.cdc.gov/rabies/docs/standard_dfa_protocol_rabies.pdf

Other technologies proven useful in determining rabies infection status include the following:

- Histopathologic examination of brain tissue is an older, more traditional method used in the laboratory diagnosis rabies infection. As dFA, rabies histopathology is performed only on brain tissue extracted from dead animals. Trained pathologists microscopically examine the brain tissue for characteristic changes indicative of rabies encephalomyelitis (inflammation). However, some of these changes may also appear in brain tissue in the absence of rabies infection, such as in some types of non-rabies viral encephalitis. Also, rabiesvirus infection of certain species (i.e., reservoir hosts) fails to induce much microscopic change in brain tissue. From this lack of specificity, the sensitivity of histological techniques in diagnosing rabies is only 60-80%, much less than that of immunological methods, particularly when the tissue has decomposed.
- Immunohistochemistry (IHC) is a method that combines the ability to distinguish cells and tissues during histopathological examination with the immunological identification of rabiesvirus in brain tissue. Due to the length of time required for processing, and possible alteration of rabiesvirus components by tissue fixation, this technique is not used as a routine screening test. The LADDL currently does not offer this method for rabies diagnosis.
- Viral isolation (VI) techniques are traditionally regarded as the principle, standard method to verify dFA positive rabies tests. Such attempts essentially try to recover infectious rabiesvirus from brain tissue specimens using culture methods. Viruses are so primitive that they require living cells to grow. Rabiesvirus culture techniques require that the processed brain tissue specimen be inoculated into something alive, often either laboratory mice or certain types of cell or tissue culture.

Mouse inoculation (MI) is understood to be the most sensitive method of rabies detection. Typically, suckling mice (5-10 per case) are inoculated intracranially using humane analgesia with a sterilized 20% tissue suspension. The inoculated mice are observed for neurological signs over a 28-day course. To verify the Rabiesvirus culture, dFA is performed on brains of mice that die exhibiting neurological signs.

The Rapid Tissue Culture Infection Test (RTCIT) involves the use of specialized types of cell cultures known to be sensitive to permit growth of most rabies strains. These include the mouse neuroblastoma cell line (American Type Culture Collection CCL 131), or the BHK-21 cell line (ATCC CCL 10). Brain tissue suspension is inoculated onto the cultured cells, which are then incubated at 35-37°C for 4 days. In positive cultures, dFA on inoculated, fixed cells reveals rabies antigen in the cytoplasm of infected cells. To validate the RTCIT method for rabies diagnosis, each lab must test the selected cell line for susceptibility to the locally dominant variants of rabiesvirus.

Because of lessened sensitivity with poor specimens and the time required to run the test, VI techniques are relegated to test verification. With the high-quality of commercial rabiesvirus dFA testing reagents, the LADDL no longer practices

culture techniques in order to verify rabiesvirus test reactions; verification of questionable dFA tests is performed with the help of US South-central regional State Public Health Laboratories.

For a discussion of rabievirus PCR testing, see below under “**Why can’t rabies testing be performed on live animals?** “

Why can’t rabies testing be performed on live animals?

With the development of Polymerase Chain Reaction (PCR) testing for rabiesvirus, the possibility now exists for a highly sensitive and specific test that could be applied to a great variety of specimens, such as saliva, skin biopsies, and even decomposed samples. The PCR technique can be run on specimens from live animals, and eventually could obviate the need for humane euthanasia of the animal for rabies testing.

PCR tests target a subset of a gene within the known genetic code of the biological entity of interest, in this case, rabiesvirus. The targeted genetic sequence must be known to appear in all rabiesvirus strains. During the test run, the subset sequence is artificially amplified many times over, and then chemically verified to be identical to the original sequence appearing in the rabiesvirus gene.

PCR testing has not yet evolved to become useful in routine screening for rabies due to several factors. A practical limitation is that the technology is expensive; current costs of test ingredients, processing, and controls would lead to a test fee of \$75-100, or perhaps higher. Other factors include legal liability and risk mitigation (i.e., insurance), which would drive costs even higher for labs willing to practice the technology. A technical problem is that PCR sometimes renders false positive reactions; usually such reactions are “weak”, but when they occur, they must be adjudicated using other methods, ultimately requiring the destruction of the animal. Biological issues include possible false negative reactions from saliva samples; theoretically, animals can be infected with rabies, but not yet shedding the virus in saliva at the moment of sampling. Another issue has to do with the rabiesvirus gene itself, which like certain other viruses, departs from the genetic code-model of double-stranded DNA. The genetic coding of rabiesvirus is provided by single-stranded RNA, which is much more fragile than DNA, thus would require special attention during specimen handling. Compared to DNA, the fidelity of viral RNA replication is poor, allowing RNA viruses to mutate readily. If a single mutation should occur in the region targeted by the rabies PCR test, a false negative reaction could lead to tragic circumstances in human exposure cases. Because of this possibility, it is uncertain whether PCR testing can detect *all* possible field strains of rabiesvirus.

With time and experience, the rabiesvirus PCR technique will be optimized and standardized, and then generate solid data on its sensitivity and specificity. Ultimately, its limitations will be overcome, or at least become better known and factored into a test-risk assessment. Until then, rabies PCR is relegated to confirmation and viral

biotyping applications. The LADDL currently does not practice rabies PCR testing; when necessary, testing is performed on a referral basis.

Is a serum test available for rabies?

Given the time course of response of the mammalian immune system, a serum test designed to detect antibodies against rabiesvirus is not used in routine diagnosis. Such techniques performed on serum or cerebrospinal fluid have been used on a retrospective basis to verify clinical diagnosis of human rabies infections, which are often fatal once clinical signs appear and serological testing becomes positive.

For similar reasons, serological techniques are not useful in the diagnosis of rabies in animals. However, such methods are significant in rabies surveillance and immunization status determinations. Certain domestic and international jurisdictions have importation restrictions regarding pet animals; many require lengthy periods of quarantine and/or certification of the animals' rabies immune status by serum testing. Two principle methods are the Rapid Fluorescent Focus Inhibition Test (RFFIT) and the Fluorescent Antibody Virus Neutralization (FAVN), the procedures of which are proscribed by international animal health authorities. For more details on these types of tests, please see:

http://www.oie.int/eng/normes/mmanual/A_00044.htm

The LADDL does not perform these techniques, but can have testing done on a referral basis. Interested parties wanting to relocate pets to rabies-controlled territories are invited to work through your veterinarian-of-choice to have such testing performed. A simple serum sample is required, but the type of technology (along with other criteria) varies by jurisdiction. Anyone interested in relocating animals to other state in the US, or to another country or international sovereignty is encouraged to contact the animal health authorities of the destination territory for instructions governing pet importation and rabies control.

Other questions? Please see:

<http://www.cdc.gov/rabies/>

<http://www.dhh.louisiana.gov/offices/miscdocs/docs-249/Manual/rabiesManual.pdf>

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