RABIES WATCH
THE COMPREHENSIVE RABIES RESOURCE
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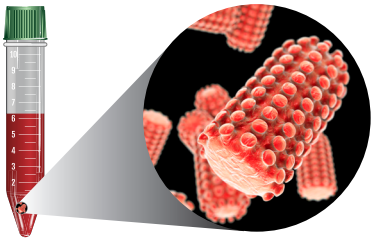
New CDC LN34 Test for Rabies Has the Potential to Improve Disease Management



In May 2018, the United States (US) Centers for Disease Control and Prevention (CDC) announced that it had developed a new rabies test that could "revolutionize testing and treatment."[1] The goal for this new test, known as the LN34 test, was to improve the correct identification of rabies-positive animals with fast turnaround times in laboratory settings outside those that perform the current gold-standard direct fluorescent antibody (DFA) test.[2] Achieving this goal would enable healthcare providers to differentiate between people who have been exposed to the rabies virus and therefore do require postexposure prophylaxis (PEP) from people whose animal exposures did not involve the rabies virus and so do not need to undergo PEP.[1] The CDC and the Association of Public Health Laboratories are working to develop guidance on implementation of rabies testing with the LN34 test on its own or in conjunction with other rabies tests.[1]

The CDC announcement touts the new LN34 test as "simpler and easier to use than current tests" and as producing "no false negatives, fewer false positive[s], and fewer inconclusive results." The LN34 test has a number of methodologic and logistic advantages that may make it an attractive alternative to the DFA test. For example, the LN34 test can be used to evaluate samples from animal tissue in a range of conditions, including fresh, frozen, or even states of decomposition. Additionally, this test does not require any additional expertise or specialized lab equipment beyond the polymerase chain reaction (PCR) technology that is already widely used to test for flu viruses, HIV, and tuberculosis. In contrast, the DFA test can only be performed on fresh tissue samples that have been kept cold, and this test does require specialized training and equipment (fluorescence microscope), which limits its utility.[1]

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The capabilities of the LN34 test were compared with those of the DFA test in a recent publication by Gigante et al. The authors noted that theirs was the largest study ever conducted to validate the use of real-time reverse-transcription PCR (RT-PCR) to diagnose rabies virus in animal samples. Test results obtained from 2120 samples from US sources showed a high degree of concordance between the 2 tests. All DFA-positive samples were correctly identified as positive by the LN34 test. The single false-positive reading (LN34 positive/DFA negative) with the LN34 test was attributable to contamination of the sample with genetic material that was used as a positive control within the laboratory performing the tests, but that did not occur naturally in the area from which the sample was collected. Based on these data, the LN34 test was found to have a diagnostic sensitivity of 100% (95% confidence interval: 99.36%-100%) and a diagnostic specificity of 99.93% (95% confidence interval: 99.62%-100%).[2]

Evaluation of Concordance Between LN34 RT-PCR and DFA Tests on US Samples Analyzed for Rabies Virus (N=2120)[2]

	LN34 positive	LN34 negative	LN34 inconclusive
DFA positive	577	0	0
DFA negative	1	1474	0
DFA indeterminate	23	45	0

Another analysis of test results obtained with 2978 samples from US and ex-US sources supported the high degree of concordance between the 2 tests. All DFA-positive samples except 1 were correctly identified as positive on the LN34 test; the single LN34 inconclusive result occurred with an international sample for which no additional sample was available for validation by the CDC. All but 6 of the 1848 samples identified as DFA negative were found to be LN34 negative. One of the 3 false-positive readings (LN34 positive/DFA negative) was attributable to contamination of the sample, as described above. The 2 other false-positives and the 3 LN34 inconclusive results were all obtained from international sources for which no additional samples were available for verification. Overall, in this analysis, the LN34 test was found to have a diagnostic sensitivity of 99.90% (95% confidence interval: 99.47%-100%) and a diagnostic specificity of 99.68% (95% confidence interval: 99.29%-99.88%).[2]

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Evaluation of Concordance Between LN34 RT-PCR and DFA Tests on All Global Samples Analyzed for Rabies Virus (N=2978)[2]

	LN34 positive	LN34 negative	LN34 inconclusive
DFA positive	1048	0	1
DFA negative	3	1842	3
DFA indeterminate	29	51	1

The CDC notes that the World Health Organization (WHO) and the World Organization for Animal Health are evaluating the results with the LN34 test and considering designation of PCR-based tests for use as primary, stand-alone tests to establish a rabies diagnosis, rather than solely as confirmatory tests used in conjunction with the current gold-standard of DFA tests.[1]

Two experts in the field of rabies disease and prevention, Charles E. Rupprecht, VMD, MS, PhD, and Stephen J. Scholand, MD, provided their perspectives to put the new test into the context of current practice. They praised the CDC for pursuing the goal of improving rabies diagnosis in animals by means of advanced molecular detection, noting that having another option against a disease that is virtually 100% fatal may help improve clinical management in various settings. Dr Scholand commented that the high degree of sensitivity of the new test and its ability to evaluate animal samples regardless of the state of decomposition may prove to be important features. Dr Rupprecht noted that the early detection of rabies-positive animals with a RT-PCR test may be especially relevant in geographic areas in which the technology and expertise to perform DFA testing is limited now.

“The RT-PCR test patented by the CDC may be particularly useful in parts of the world that lack the resources required for DFA testing but do have the capability for molecular testing at a centralized lab, which came about in response to HIV.”—Charles E. Rupprecht, VMD, MS, PhD

"When the animal involved in a potential rabies virus exposure incident is available, testing for rabies is always helpful because it proves without a doubt what the diagnosis really is."
—Stephen J. Scholand, MD

These experts said that certain counterpoints will need to be assessed before the new test is implemented in the US. Dr Rupprecht commented that the RT-PCR test may not provide results to clinicians and patients much faster than the DFA test. While the difference in time required to perform the RT-PCR and DFA tests is a few hours, the rate-limiting step for both tests is the time for delivery of the report to the healthcare provider who can then act upon the new information. He noted that RT-PCR would seem to be of greatest use outside the US in areas where access to specialized labs may be limited or to situations in which an animal's decomposed remains need to be tested for rabies. Dr Scholand observed that in the US, where wildlife account for more than 90% of reported rabies cases,[3] patients do not typically capture the suspect animal and bring it to the clinic for evaluation. Instead, the wild animals usually escape so no sample is available for rabies testing. Exposures to domestic animals that are captive and available for evaluation can be followed up through observation of the suspect animal for 10 days for evidence of rabies disease.[4]

"We rarely have animal tissue to test for rabies following potential exposure to a wild animal in the United States. Most bats fly away and most other wild animals escape."—Stephen J. Scholand, MD

"The DFA test takes a few hours to perform, while the RT-PCR test takes less time. The real delay, regardless of test technique, is the time to report the results to the end user, the healthcare professional in the clinic."—Charles E. Rupprecht, VMD, MS, PhD

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Potential Benefits and Considerations With the LN34 Rabies Test

Potential benefit	Consideration
• Timely detection of rabies through diagnostic testing with a fast turnaround time following an animal exposure may improve clinical management[1] • Excellent agreement with the gold-standard DFA test; similar sensitivity and specificity for detection of rabies virus in animal tissue samples[2] • Useful for evaluation of decomposed samples, in contrast to DFA test[2] • RT-PCR testing platform widely available for other forms of testing (eg, flu, HIV)[1] • Simpler logistics (sample storage at room temperature, broader availability of PCR technology and technician expertise) may expand the geographic reach of rabies testing in resource-constrained areas[1,2]	• Difference in turnaround time with LN34 test and DFA test is small (hours) • Clinical impact of slightly quicker test results remains to be proven; the rate-limiting step in clinical practice is typically result reporting, not test turnaround time • Samples from wild animals implicated in exposure incidents (>90% of US cases[3]) are typically not available for rabies testing • Logistical advantages of LN34 vs DFA test[2] are largely not relevant in the US

For now, the impact of the RT-PCR on clinical management of rabies is unclear. Expert opinion suggests a range of possibilities. At one extreme, this new test may not have a major impact on rabies control and prevention in the US given the predominance of wildlife as viral vectors and the typical absence of the source animal for evaluation. On the other hand, this new test could play an important role if its actual time from sample collection to delivery of results to the clinician favorably impacts care and if total costs are acceptable. The value of flexibility to evaluate decomposed samples for rabies virus remains to be shown.

The quotations and opinions of Charles E. Rupprecht, VMD, MS, PhD, and Stephen J. Scholand, MD, in this article were provided through email correspondence in May 2018.

REFERENCES

1. Centers for Disease Control and Prevention. New rapid rabies test could revolutionize testing and treatment. https://www.cdc.gov/media/releases/2018/p0516-rapid-rabies-test.html. Updated May 16, 2018. Accessed June 15, 2018. **2.** Gigante CM, Dettinger L, Powell JW, et al. Multi-site evaluation of the LN34 pan-lyssavirus real-time RT-PCR assay for post-mortem rabies diagnostics. *PLoS ONE*. 2018;13(5):e0197074. https://doi.org/10.1371/journal.pone.0197074. **3.** Ma X, Monroe BP, Cleaton JM, et al. Rabies surveillance in the United States during 2016. *J Am Vet Med Assoc.* 2018;252(8):945-957. **4.** Manning SE, Rupprecht CE, Fishbein D, et al; Advisory Committee on Immunization Practices Centers for Disease Control and Prevention (CDC). Human rabies prevention—United States, 2008: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep.* 2008;57(RR-3):1-28.

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