Dear Colleagues:

In May 2018, CDC announced the availability of a PCR assay to detect Rabies virus (RABV) and related viruses of the Lyssavirus genus, family Rhabdoviridae. The test, known as the LN34 assay, is able to rapidly detect a wide range of Lyssaviruses. Currently, the most widely used test for rabies diagnosis is direct fluorescent antibody (DFA) testing to confirm the presence of RABV antigen.

Rapid diagnosis of rabies is critical in initiating prompt infection control and public health actions to detect and treat potential rabies exposures and infections. The APHL Rabies Diagnostics Workgroup is currently working to develop recommendations for the laboratory diagnosis of rabies infection. The work group is comprised of subject matter experts from public health and veterinary laboratories. The goal is to make recommendations on the possible utility of the LN34 assay in rabies diagnostics to inform national guidelines. The workgroup's current task is a systematic literature review, which will guide the recommendations and algorithm development. In the meantime, the Rabies Diagnostic Workgroup has developed the following considerations for laboratories interested in implementing the LN34 assay.

At this time, the workgroup does not recommend using only the LN34 assay for laboratory diagnosis of rabies. CDC and public health laboratories are continuing to evaluate the assay and strategies for implementation. The LN34 assay should be used in conjunction with DFA while being evaluated.

Currently, the LN34 assay may be used to confirm inconclusive, unsatisfactory and discordant DFA results. In most cases, discordant and inconclusive DFA results are currently sent to CDC for resolution, resolving discordant DFA results in-house would decrease turnaround time.

Laboratories implementing the LN34 assay must validate it prior to using it for diagnostic purposes as a laboratory developed test (LDT). Baseline validation of the assay includes testing a panel of known positive and negative samples that include representatives of all circulating lyssavirus variants in the area. A performance evaluation panel will be generated and distributed by CDC. It will contain inactivated, homogenized brain tissue from 10 blinded samples, and will include rabies positive and negative samples. At least one rabies positive sample will be diluted in rabies negative brain tissue samples to approach the assay's limit of detection. Along with the CDC provided panel, laboratories implementing the LN34 assay should follow their internal standards for implementing a new test. A reasonable evaluation of the assay should include parallel testing for a certain amount of time and number of samples that cover a wide variety of Lyssaviruses. Before implementing the assay alone. It is also important to ensure that the positive control RNA produces a Ct value within the acceptable range (provided by CDC).

Additional considerations include:

- Turnaround Time: The LN34 assay does not necessarily result in a shorter turnaround time than DFA. However, combining LN34 with sequencing to determine strain types may result in faster characterization of circulating rabies strains.
- Biosafety: Laboratories should follow safety and quality procedures determined by their institution when implementing a new diagnostic test and complete a risk assessment. Samples should be considered infectious until inactivation processes are complete. Pay special attention to any movement of specimens from the rabies testing space to common facilities. For example, if using a PCR instrument located in a core molecular testing laboratory, it is important to ensure that the samples are inactivated before transporting them outside of a dedicated rabies testing area. Pre-exposure rabies vaccination, regular periodic antibody titer checks, and booster immunizations as necessary are also required for all persons prior to working with lyssaviruses, or with known or potentially infected specimens, or engaging in diagnostic, production or research activities with these viruses.
- Costs: Costs associated with implementing new testing. During the validation and evaluation period, both assays will be run in parallel, which increases costs, is a strain on reagents, time and requires maintaining competency for laboratory scientists. In general, PCR testing is more costly than DFA. However, if the PCR product is further used in variant typing for strain characterization then it might be more economical than DFA.
- Results Reporting: The laboratory will need to develop new reporting language including disclaimers describing any assay limitations and build the new test into the laboratory information management system. The laboratory will need to inform providers of the new test's implementation and educate them on how to interpret the results.

Currently the APHL Rabies Diagnostics Workgroup is completing a systematic literature review of laboratory diagnosis of rabies. The results of the literature review will be used to answer key questions on rabies testing practices with the goal of providing evidence-based recommendations and identifying gaps of available data in the literature. The workgroup plans to release the recommendations in summer 2021.

Sincerely,

Cecilia Kretz, PhD
On behalf of the "APHL Rabies Diagnostics Workgroup"

Resources

https://www.cdc.gov/rabies/specific_groups/laboratories/index.html
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3848253/
WHO Guide – laboratory Techniques in Rabies