RABLAB statement on the use of commercial rapid immunochromatographic tests for rabies surveillance purposes.

There are a number of commercial rapid immunochromatographic tests, also referred to as lateral flow devices (LFDs), for rabies virus (RABV) antigen detection available on the market. The test principle is based on immunoaffinity partition of the analyte between sample migrating laterally through a porous membrane and a finite “affinity zone” prepared by immobilizing an antibody on the membrane.

Regarding test characteristics and specificities of RABV LFDs, WOAH's network of Reference Laboratories for Rabies (RABLAB) generally refers to the recent amendments in Chapter 3.1.18. – Rabies (infection with rabies virus and other lyssaviruses) of the WOAH *Terrestrial Manual*.

LFDs for routine use

From RABLAB's perspective, the routine use of these tests in the context of statutory rabies surveillance is currently not recommended. This opinion is based on the general lack of (1) quality control, (2) clarity on assay methods, and (3) compliance with international standards for validation as a test kit. Given the severity of rabies and potential human and animal health consequences of incorrect laboratory diagnosis, RABLAB can only recommend the routine use of diagnostic methods that have been endorsed by WOAH through the Terrestrial Manual or the WOAH Register of Diagnostic Kits.

1. Numerous studies have demonstrated a wide and concerning variability in the sensitivity and specificity of commercially available LFDs (Eggerbauer et al., 2017, Klein et al., 2020). To analyze their utility, the test performance of LFDs was assessed through several international initiatives (e.g. national and/or WOAH/WHO/FAO reference laboratories). These studies showed that if instructions of manufacturers are strictly adhered to, LFDs showed extreme variation in test performance in terms of diagnostic sensitivity (range between 0 and >95%), with inconsistencies between batches and based on the nature of the diagnostic samples.

2a. WOAH recommends transparency in assay methods, to ensure that they comply with international standards and can be properly evaluated by laboratory experts. The performance of an LFD kit is largely dependent on the monoclonal (or polyclonal) antibodies and their affinity to bind to rabies virus antigenic variants. It is well-established that antibody affinity is variant-specific, and as such the selection of antibodies for a diagnostic assay can influence which rabies virus variants it will detect. Prior to official use of a commercially available LFD, veterinary authorities should review the product details, including the antibodies used by the kit and evidence of their affinity for locally circulating rabies viruses; not all kits are likely to have similar sensitivity and specificity for all rabies virus variants found globally.

2.b Another problem is that positive and negative rabies controls cannot be performed for these tests in parallel as required for any other diagnostic assay, especially when used in the

field, and therefore LFDs would not meet the diagnostic quality assurance requirements of accreditation. The internal test control strip is only an indication whether there was a flow of the respective detection antibody or not, but is no proof that the target antigen can be correctly identified or not. For reasons mentioned above and because LFDs are not yet considered suitable primary diagnostic tests for rabies, it also remains open whether the test results would be accepted and reported by national authorities.

3. As the rapid immunochromatographic method does not comply with standards for a suitable laboratory test method, recognition of LFDs must be made through the WOAH Register of Diagnostic Kits, as a fit for purpose assay. While recent data suggest that several commercially available kits may have superior sensitivity and specificity, many of these studies have deviated from manufacturer instructions resulting in the data being unsuitable for WOAH validation purposes. It would be the responsibility of the manufacturer to validate the kit based on the package leaflets according to international standards (e.g. WOAH) to ensure reliable sensitivity and specificity for the consumer and thus high quality of their product. However, little to no information is provided from manufacturers on the validation of their tests, and unfortunately, no test has yet been submitted or registered for inclusion as WOAH diagnostic kits.

LFDs for research purposes

WOAH cannot recommend LFDs as an official test. However, considering the ease of performance, low cost, and the need for improved rabies surveillance, LFDs may be useful for research purposes, e.g. as a screening tool. If considered for field research, only tests with a high test performance should be used, and test results should never be used to dissuade an exposed individual from pursuing Post Exposure Prophylaxis. All test results should be confirmed by standard techniques as recommended in Chapter 3.1.18. – Rabies (infection with rabies virus and other lyssaviruses) of the WOAH *Terrestrial Manual*, particularly if the data are intended to be used to support a WOAH diagnostic kit endorsement. The cost-benefit of these tests for improving rabies surveillance is currently debatable, and likely is dependent upon the programmatic goals and rabies epidemiology. Official reporting of rabies cases should comply with WHO and WOAH standards, which include clinically confirmed animals and those confirmed with an officially recognized test.

RABLAB recommends that LFD research focus on the following qualities:

- Clearly defined purpose of the test
    - Tests evaluated for primary diagnostics must show sensitivity and specificity comparable to the officially recognized tests in the WOAH Terrestrial Manual
    - Tests evaluated for a screening assay should demonstrate a very high (nearing 100%) sensitivity and a suitable specificity
- Cost-benefit of the utility of the test under the proposed use-scenario
- Clear definition of the protocol used, ideally complying with manufacturer instructions if the data will be used for official WOAH validation
- Adequate performance across rabies virus variants and animal species that are likely to undergo testing and for which validation will be sought
- Inclusion of a positive and negative control within the Kit

- Transparency as to the Kit methods, including the antibodies used in the Kit (understanding that there may be conflicts with proprietary information)
- Any additional data deemed useful or required for official recognition under the WOAH Register of Diagnostic Kits

Eggerbauer E., Pfaff F., Finke S., Hoper D., Beer M., Mettenleiter T.C., Nolden T., Teifke J.P., Muller T. & Freuling C.M. (2017). - Comparative analysis of European bat lyssavirus 1 pathogenicity in the mouse model. PLoS Neglect. Trop. Dis., 11.

Klein A., Fahrion A., Finke S., Eyngor M., Novak S., Yakobson B., Ngoepe E., Phahladira B., Sabeta C., Benedictis P. de, Gourlaouen M., Orciari L.A., Yager P.A., Gigante C.M., Knowles M.K., Fehlner-Gardiner C., Servat A., Cliquet F., Marston D., McElhinney L.M., Johnson T., Fooks A.R., Müller T. & Freuling C.M. (2020). - Further Evidence of Inadequate Quality in Lateral Flow Devices Commercially Offered for the Diagnosis of Rabies. Trop. Med. Infect. Dis., 5, https://doi.org/10.3390/tropicalmed5010013