OIE Virtual Training Series on Rabies Diagnosis for SAARC Region

5 to 6 November 2020

9:00 am Kabul; 9:30 am Islamabad and Male'; 10 am Colombo and New Delhi; 10:15 pm Kathmandu; 10:30 am Dhaka and Thimphu; 1:30 pm Tokyo

Via: Zoom

Q & A Session

Note: If further clarifications are required, please directly contact the speakers/ experts ((Dr Shrikrishna Isloor – kisloor@gmail.com) or refer to the relevant publications.

DAY 1 (November 5, 2020)

Introduction to the KVAFSU-CVA RDL's capacity and what support services are available from this OIE Reference Laboratory for Rabies including an overview of the latest advances in rabies diagnostic techniques

1. **Rabies laboratory facilities**

   1.1. What are the basic infrastructure requirements and recommendations in the laboratory to start rabies diagnosis?

   - It can just be a simple laboratory with horizontal laminar flow biosafety cabinet. It is recommended that the laboratory has secured entry and partitions, proper disposal system/ facility, a -20 to -80 deep freezer, an anteroom (change room), sterilization room, a good quality fluorescent microscope in addition to incubator, autoclave, refrigerators, sensitive balance, micropipettes etc.
   - Dr Isloor has shared the image depicting the layout of their laboratory. For further details you can email *rdlkvafsucva@gmail.com*.

Theory session on the Occipital foramen approach for brain sampling, including sample preparation, sample transport and sample preservation, and practical demonstration (video)// Theory session on lateral flow assay and practical demonstration (video)

2. **Cold Chain**

   2.1. What is your recommendation for storage of brain samples in cold chain?

   - For short term storage (a few hours), 4° Celsius is alright. The sample can be kept for up to 1 week if maintained properly in this temperature.
   - If processing might be delayed, then storage in -20° degrees Celsius is preferred.

   2.2. Are gel packs useful for sample shipment?

   - Frozen gel packs, when properly used, can maintain the cold chain for approximately 36 hours.

3. **Sample collection**

   3.1. In the occipital foramen approach for brain sampling, why did they not use a syringe in the straw technique?

   - This was done for convenience during sample collection. The straw was pinched at the end during collection to create a vacuum. This has almost the same principle when using a syringe. We have used the disposable syringe connected to the AI sheath in collection of brain sample through Occipital foramen approach and the same has been demonstrated in the practical session.

3.2. There are biosafety concerns in the field because of exposure risk and improper disposal of rabies suspected carcasses during the sample collection activities. What are your views and experiences on biosafety measures at the field level?

- The use of basic Personal Protective Equipment (PPE) and the occipital foramen approach for sample collection is safer, especially when using the straw method. However, those collecting the brain sample must be immunized against rabies. As for the disposal of the carcass, incineration is the preferred method to use. An acceptable alternative is to bury the carcass at least 4 feet deep and covered with lime.

3.3. How can we conduct an early rabies diagnosis in live animals?

- We can conduct tests using saliva from suspected animals. However, it is to note that virus is shed intermittently in saliva and therefore a single test may not detect the virus. Therefore, multiple sampling is needed. Collect at least 3 samples at 3 different times every 2 hours span. Pool the samples and then test using LFA or PCR. Not DFA.
- For sampling in live animals, utmost consideration should be given for safety of the collector especially with aggressive animals. If an animal is cooperative or if person is well immunized and protected with PPE then saliva can be collected. In dumb form / paralytic form it is easier to collect samples. Safety should be the first priority when dealing with live suspected animals.
- Hair follicles can be used as sample and gives good results, but only using PCR.

3.4. For PCR technique from hair follicles, is it the hair from the neck region we need to collect?

- Yes. Literatures indicate the hair follicles from the neck region. PCR on the hair follicle should be hypothetically working on most species. But availability of PCR in all the labs, expertise, reagents should be ensured.

3.5. If the carcass is completely putrefied, do you still recommend collecting samples?

- Yes, putrefied samples can still be tested using PCR.

4. Disposal

4.1. How do you dispose rabies affected carcass after sample collection?

- Of all the common methods of carcass disposal (incineration, burying, and rendering), incineration is the preferred method to use, however, cost should be considered since it can be expensive. An acceptable alternative is to bury the carcass. For burying, the carcass should be buried at least 4 feet deep and covered with lime to discourage scavengers from uncovering and consuming it.

DAY 2 (November 4, 2020):

Continued discussion on lateral flow assay (LFA)

5. LFA: specificity, sensitivity, availability, cost, and how to discard

5.1 What is the specificity and sensitivity of the LFA test under field conditions? Any precautions to consider while performing the test?

- Current studies at KVAFSU-CVA-RDL showed test sensitivity between 98.5 to 99% and specificity nearly 100% (unpublished). If the LFA tests negative, it must be confirmed by DFA (gold standard test).
- Currently, the LFA test provided by the WHO-SEARO is undergoing validation. It is working almost at par with the Direct Fluorescent Assay (DFA). At times when partially putrefied brain samples are tested, the virus can still be detected by the LFA while such samples are unfit to be tested by DFA. However, this test is still used as a preliminary test.
- It should be considered that while the LFA is a user friendly and rapid test, it is important to

select the best quality kit available, that is well validated.

5.2 Where is the LFA kit available and what is preferred brand in the market?

- The LFA kit (@BioNote) used by KVAFSU-CVA Rabies Diagnostic Laboratory is supplied free by WHO SEARO.
- Preference purely depends on how the kits are validated. Some LFA kits available in the market are yet to be validated and standardized/ optimized. India is actually developing an indigenous LFA kit through a PhD student, to make it more localized and cost effective.

5.3 What is the cost of an LFA test?

- It costs around 400 to 500 Indian Rupees for one cassette. One pack comes in a set of 10. DFA is cheaper and recommended, however LFA is more user-friendly and can be used in field conditions, if made more cost effective.

5.4 How can we properly discard the used LFA cassette?

- It can be done through spraying of 70% ethyl alcohol on the cassettes or dipping it in 2% sodium hydroxide to inactivate the virus before discarding.

Theory session on Direct Fluorescent Assay (DFA) and practical demonstration (video)

6. Sample processing and reading

6.1 Is FITC filter with green light excitation usable or should it be a blue filter?

- Excitation filters will filter all colors except blue. The FITC conjugated to anti N protein Monoclonal antibody (which in turn is bound with N antigen of the rabies virus in the tissue impression) on being exposed to blue light, emits green light. The emission filter allows transmission of green light which can be seen through the microscope.

6.2 What is the difference between double dilutions and log dilutions?

- 1:2, 1:4, 1:8 and 1:16 are double dilutions. Likewise, 1:10, 1:20, 1:40 and 1:80 are also double dilutions or two-fold dilutions. Whereas, 1:10, 1:100, 1:1000 are tenfold dilutions or log dilutions. We employ the 10-fold or log dilutions only when we need to titrate the load /titre of virus.

6.3. Can you briefly describe the process of washing of sample with PBS?

- Take the PBS in a petri dish. Immerse the brain tissue, gently shake...discard the wash carefully...then repeat a couple of times.

6.4. Does sparse or weakly staining inclusions mean sample is negative for rabies or do you do a re-test?

- Declaring a positive result is easy but if it is negative, then we need to retest 2 times more (total 3 times). Visualization could be done at 200 - 400X. DFA's sensitivity depends on the quality of reagents, especially conjugates.

6.5. How do you store the positive and negative samples and for how long?

- Storing the samples at -80° deep freezer is the best way to keep it. If it is not there, -20° is also OK. But ensure that there is no power failure to avoid repeated freezing and thawing that could deteriorate the quality of samples.
- Such samples can be used for retrospective molecular epidemiological studies, or for other purposes.

6.6. What is the concentration and dilution of all types of reagents and washing solution?

- Dilutions of reagents are provided in the manual. However, with reagents we must employ

end point titration, especially for conjugates, and then decide the working strength.

6.7. Does the virus lose its infectivity following acetone fixation?

- Yes, acetone fixation fixes the tissue as well as almost inactivates the infectivity of the virus. Hence one must use double gloves while processing and handling DFA slides.

6.8. Which FITC reagent/kit are you using in your laboratory?

- We use Millipore-Light diagnostics or Fujirebio anti N protein Mab based FITC conjugates .

7. General

7.1. Can we use the same diagnostic tests used in domestic animals for diagnosis of rabies in wild animals?

- Yes, you can use the same tests for diagnosis of rabies in wild animals too.