



NATIONAL CENTRE FOR ANIMAL HEALTH
NATIONAL VETERINARY LABORATORY
STANDARD OPERATING PROCEDURE



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<i>SOP No: NCAH/LSU/LFA 01</i>
<i>Title: Lateral Flow Assay for Antigen detection</i>
<i>Version No: 1, Total Pages: 2</i>
<i>Issue Month/Effective Date: May 2026</i>
<i>Revision: Summary: first version</i>
<i>Supersedes Version No:</i>
<i>Prepared by: Dr. Tshering Choden, Kelzang Lhamo</i>
<i>Reviewed by:</i>
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<i>Application/Distribution: NCAH, RVH,SVL,DVL</i>



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1. Scope

This SOP covers the application of lateral flow assays for rapid detection of antigens in veterinary diagnostic samples. The scope includes handling, processing, and interpretation of test kits for multiple pathogens of veterinary importance.

2. Objective

To strengthen early disease detection and surveillance by enabling quick screening at different laboratory levels, ensure consistent interpretation of lateral flow assay results across the veterinary laboratory network lastly to minimize diagnostic delays by integrating LFA into routine workflows for priority pathogens.

3. Principle

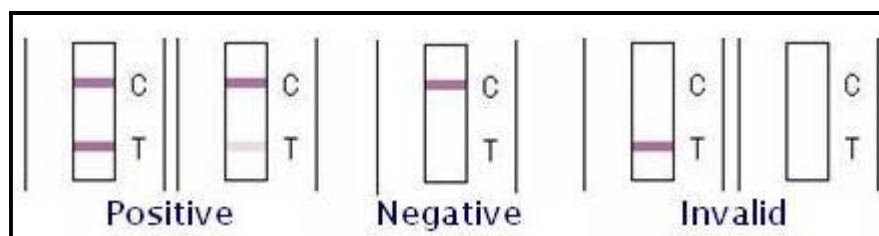
Lateral flow assays are rapid, paper based, point of care diagnostic tools that detect specific antigens, such as proteins from, within 5–30 minutes. Using capillary action, the sample flows through a strip containing pre-coated antibodies, resulting in a visible colored line if the target antigen is present.

4. Procedure

Based on the kit catalogue attached.

5. Result Interpretation

- i. **Negative:** Only the control line region (C) shows a line in the observation well.
- ii. **Positive:** Both the test line region (T) and the control line region (C) show a line in the observation well.
- iii. **Invalid:** No line shows in the observation well of the control line region (C).





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<i>SOP No: NCAH/LSU/02</i>
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<i>Prepared by: Dr. Tshering Choden, Kelzang Lhamo</i>
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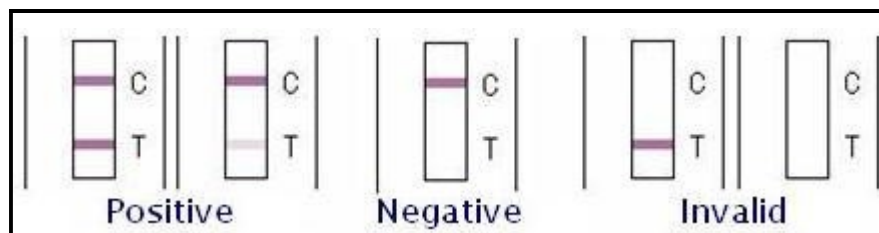
Rapid antibody-based tests (RDTs), or lateral flow immunoassays, provide fast, qualitative results to detect IgM/IgG antibodies against a pathogen from blood sample. These point of care tests offer results in ~15-20 minutes, indicating prior infection or vaccination, but are generally less accurate than laboratory methods and ineffective for diagnosing early infection.

4. Procedure

- Based on the kit catalogue

5. Result Interpretation

- Negative:** Only the control line region (C) shows a line in the observation well.
- Positive:** Both the test line region (T) and the control line region (C) show a line in the observation well.
- Invalid:** No line shows in the observation well of the control line region (C).





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Important Notes for Lateral Flow Assays Use

- **Sample integrity:** Proper collection and handling of specimens (blood, serum, swabs, etc.) is critical
- **Kit storage**
 - Maintain manufacturer-recommended conditions (temperature, humidity) to avoid false results.
 - Most kits should be stored between 2°C–30°C (36°F–86°F), making them suitable for room-temperature storage in cool, dry areas.
 - Store away from direct sunlight, moisture, and extreme heat.
 - Shelf Life: Usually, 18–24 months is standard (check catalogue)
- **Timing:** Read results within the specified time window; delayed reading may cause misinterpretation.
- **Controls:** Always run positive and negative controls to validate test performance.
- **Documentation:** Record results systematically for traceability and reporting.

Specificity and Sensitivity

- **Specificity:** LFAs are generally highly specific when designed with pathogen-specific antibodies, reducing false positives.
- **Sensitivity:** Sensitivity can vary; LFAs may miss low level infections compared to molecular methods (e.g., PCR).
- **Influencing factors:** Sample quality, pathogen load, and kit design directly affect sensitivity and specificity.

Recommended Performance Benchmarks for LFAs

- Sensitivity $\geq 80\%$ (WHO minimum for rapid antigen tests)
- Specificity $\geq 97-99\%$ Ensures very low false-positive rates; critical for surveillance and outbreak control.
- However, Accuracy is dependent on pathogen load and sample quality

Drawbacks of Lateral Flow Assays

- Lower sensitivity compared to gold-standard methods (PCR, ELISA).
- Limited quantitative capability (results are usually qualitative or semi-quantitative).
- Risk of false negatives in early infection stages or low pathogen load.
- Dependence on kit quality and storage conditions.
- Interpretation errors if results are read outside the recommended time frame.