



## LATERAL FLOW ASSAYS IN VETERINARY DIAGNOSIS

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One of the main challenges for a versatile application of monitoring technologies in the veterinary and food industry is to develop fast, quantitative and low cost devices that can be used with minimal expertise. Most of the diagnostic technologies in use today require laboratory facilities, expensive equipments and trained personnel. During the last decade, a few technologies have been proposed and developed that fulfill most requirements of versatility mentioned above. One of the most promising approaches is the lateral flow immunoassay technique.

Immunoassays are analytical measurement systems that use antibodies as test reagents. These antibodies are attached to some kind of label and then used as reagents to detect the substance of interest. The label can either be an enzyme or coloured particles like colloidal gold, latex beads or carbon/silica nano-particles, and it is used for direct visualization of the reaction. The most commonly used labels are 20-40nm gold particles. These lateral flow assays are especially designed for use at point of care and the results are usually available within 10-20 minutes.

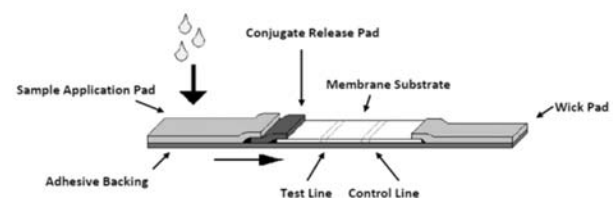
### Lateral flow assay principles.

The principle of the assay can be illustrated with a canine Parvovirus antigen assay as an example. The canine Parvovirus assay is an immunochromatographic assay as shown in the diagram above. The membrane substrate has a test line which is formed by monoclonal anti-canine Parvovirus antibody, and a control line formed by anti-mouse IgG. The virus in the fecal sample dropped into sample well moves along the sample pad and react with polyclonal anti-canine Parvovirus antibody labeled with colloidal nano-gold on the conjugate pad. The antibody-antigen complex then

moves along the membrane substrate and binds to the monoclonal antibodies on the test line, forming a test line which is colored red by the nano-gold. The excess complex moves further along the substrate and binds with the anti-mouse antibody on the control line, which causes red color to develop along the control line as well. If virus is absent in the sample, only gold-labeled antibody flows along the substrate, and it does not bind to the test line, which causes only the control line to appear.

### Lateral flow assays in companion animal diagnosis

These patient side rapid tests are commonly used for diagnosis of canine infectious diseases like Canine distemper, Canine Parvovirus, Canine Adenovirus, Corona virus, Canine brucellosis, *Ehrlichia canis*, Giardia and Heartworm. In case of Canine Distemper, nasal or ocular discharge can be used for virus detection. Fecal swabs are the specimen of choice for canine Parvovirus detection. In antibody tests like Ehrlichia and *Brucella canis*, whole blood or serum can be used depending on the test format. Similar antibody tests are also available for distemper and parvovirus which is helpful for assessing the vaccination status of animals. These tests usually measure IgG response in the serum. Another important application of these rapid tests is detection of rabies virus in suspected cases. The virus can be detected in saliva or brain samples without much exposure to rabid specimens.



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### Lateral flow assays in Farm animal practice

The most important application of these rapid tests in farm animal practice is for screening and surveillance. Tests for Brucella and TB antibody can be used for herd screening and elimination of infected animals. Non-structural protein(NSP) antibodies in bovine serum can be detected by these rapid tests, and these tests help in assessing the Foot and Mouth disease status of the herd. This NSP test can also be used for effective and rapid screening of animals at border check posts and to certify animals imported from other areas as disease-free. Similar tests are also available for Rota virus, anthrax and many parasitic diseases.

### Avian Tests

All avian screening tests can be produced in this format. However the most widely used tests for poultry farms are avian influenza tests. Throughout the world this test is accepted as a screening method.

### Food testing and feed testing

Aflatoxin B1 is a severe problem in cattle and poultry feeds affecting the health and productivity of these animals. A rapid screening test which can detect Aflatoxin B1 in feed is very of critical importance in farms and feed manufacturing plants. Lateral flow assays can be designed for quantification of Aflatoxin B1 using a competitive immunoassay format. These tests are supplemented with an easy and rapid extraction procedure using ethyl acetate. By this method Aflatoxin B1 levels as a low as 2ppb can be detected in feed. This test is easier and cheaper compared to HPLC or TLC methods and there is no need to handle toxic Aflatoxin standards and solvents.

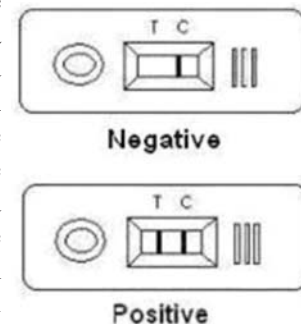
### Rapid tests advantages.

The advantages of lateral flow based rapid tests are-

1. Quick results, available in just a few minutes.
2. Requires no instrumentation.
3. Easy to use.
4. Allows decentralized implementation (serum transport not required)
5. No, or low, capital expenditure.
6. Able to execute the test and read result in the field itself.
7. Does not require refrigerated storage.

### Assay methodology

These rapid tests are designed so that even a technician with limited expertise can perform the test easily. The veterinarian or the technician draws blood (or other suitable specimen depending on the test) from the animal using a syringe or a



vacutainer, optionally coated with a clot-activator for faster serum separation. The syringe is kept till the serum separates, and then, 3 drops of serum/sample are added to the sample hole on the test card using a dropper. The results can be read as shown in the diagram above, in just 5-10 minutes. After the test is complete and result recorded, the used kits should be disposed of according to standard disposal procedure followed for clinical specimens in veterinary hospitals and labs.

### Lateral flow assay kits in India.

In India these kits are manufactured by ubio Biotechnology Systems Pvt Ltd, a biotechnology company located in Cochin, Kerala under the brand quickVET. These tests are available in single tests and 10 test format to veterinarians. Further details can be obtained from [www.ubio.in](http://www.ubio.in)

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