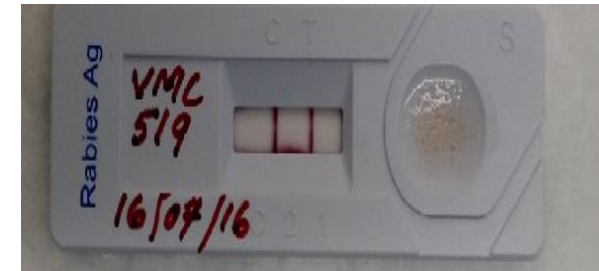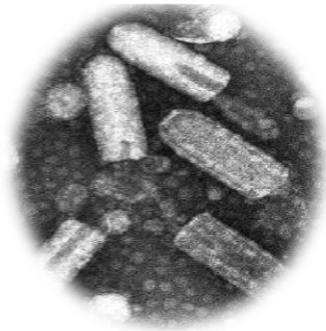# LATERAL FLOW IMMUNOASSAY of Brain samples;
principle, protocol, interpretation and reporting results

Dr.D.Rathnamma

Professor and Head, Biosafety Officer

**KVAFSU-CVA Rabies Diagnosis Laboratory,**
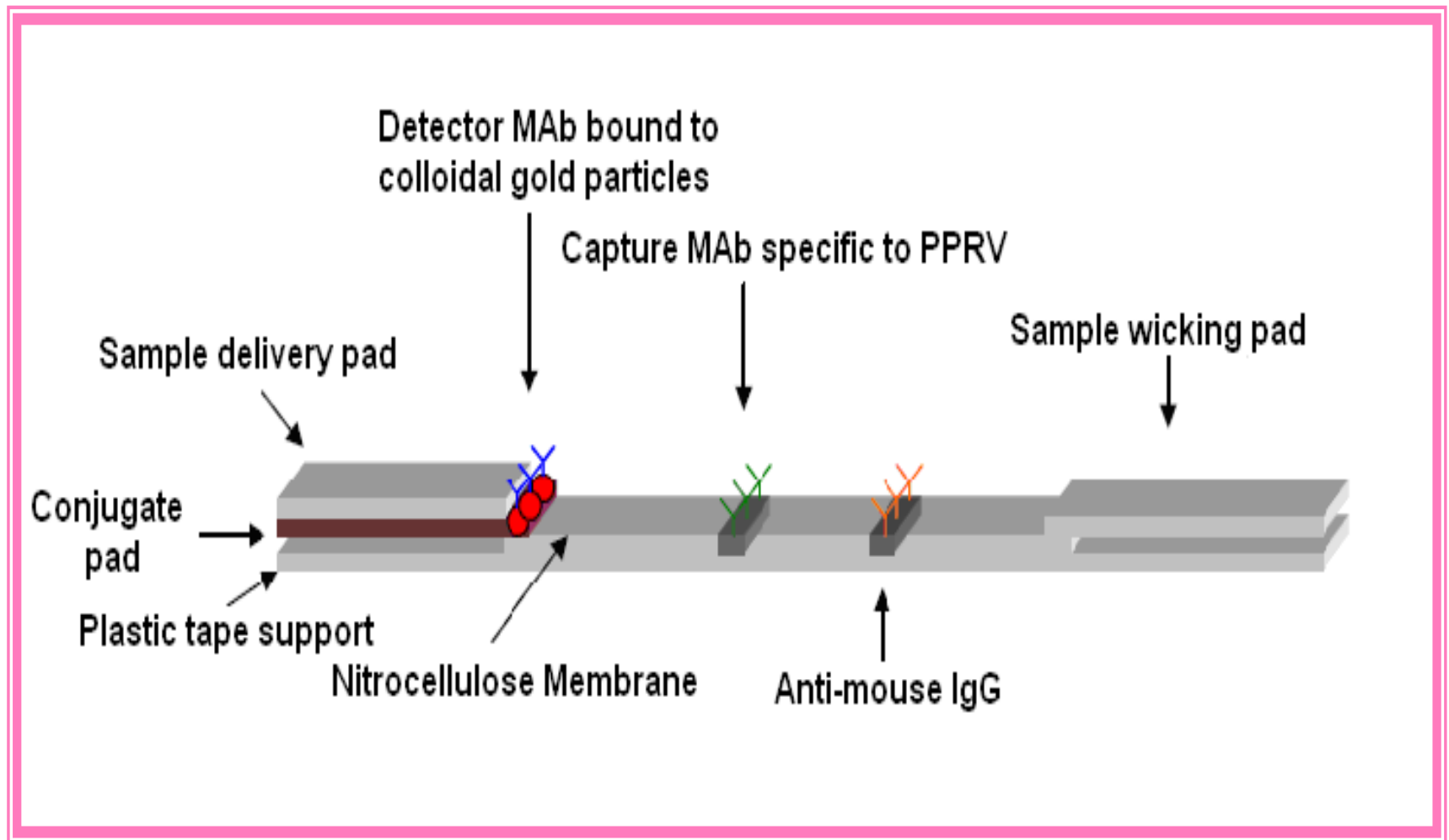
WOAH Reference Laboratory for Rabies

Dept. of Microbiology, Veterinary college, KVAFSU, Hebbal, Bengaluru, India

- Lateral flow immunoassay (LFA) - a rapid test

- Immunochromatographic assay for the qualitative detection of antigen or antibody

- Any type of sample can be tested (brain, saliva, serum, plasma, whole blood,urine etc)

- Test sensitivity can be quite good - HBsAg (1.0 ng HBsAg/ml or less) (Kim *et al.*,2016).

- Test use **colloidal gold,** dye, or latex particle conjugates to generate signal.
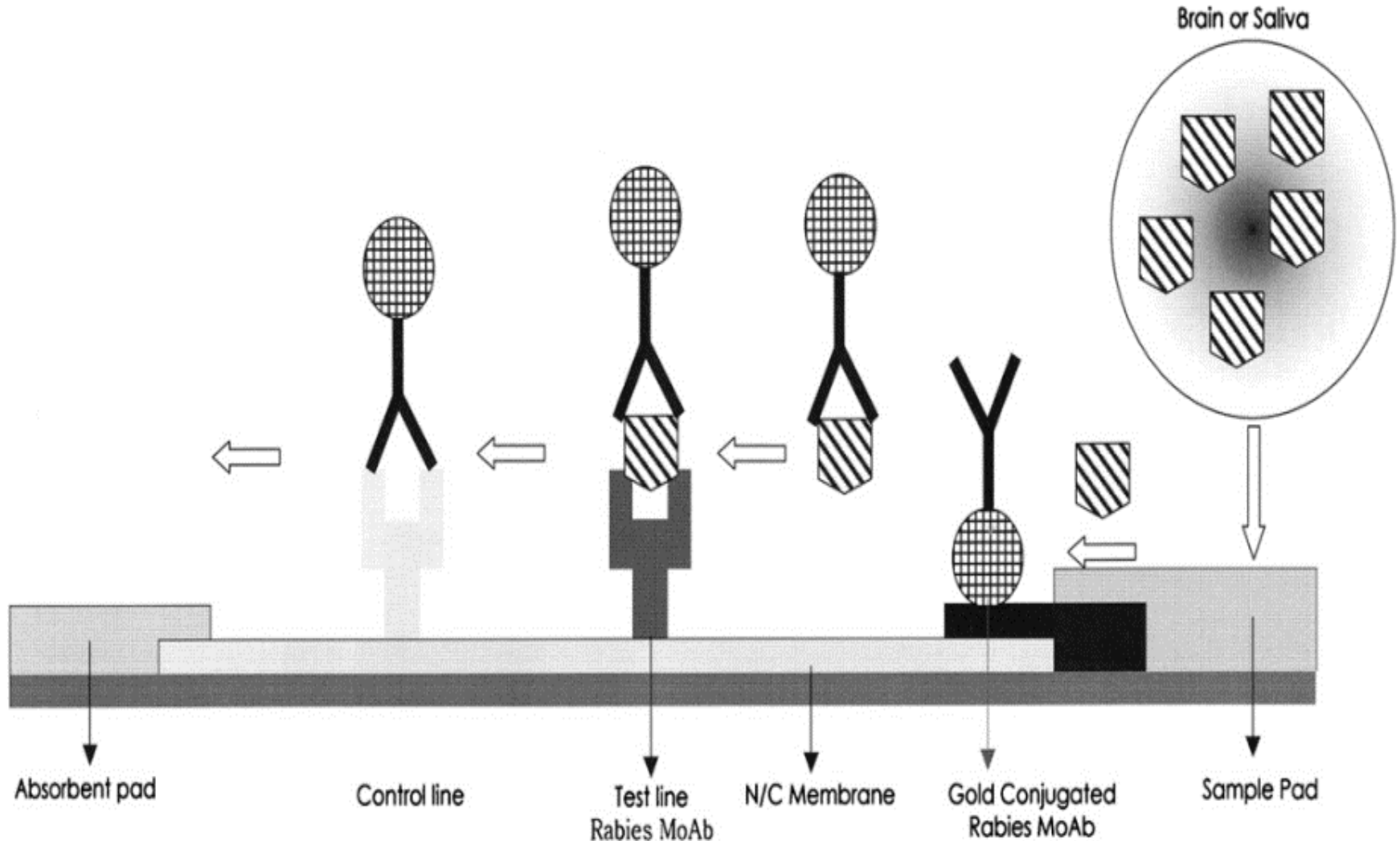
Typical configuration of a lateral flow immunoassay

Sample pad

Conjugate pad

Wick

Backing pad

Test line   Control line
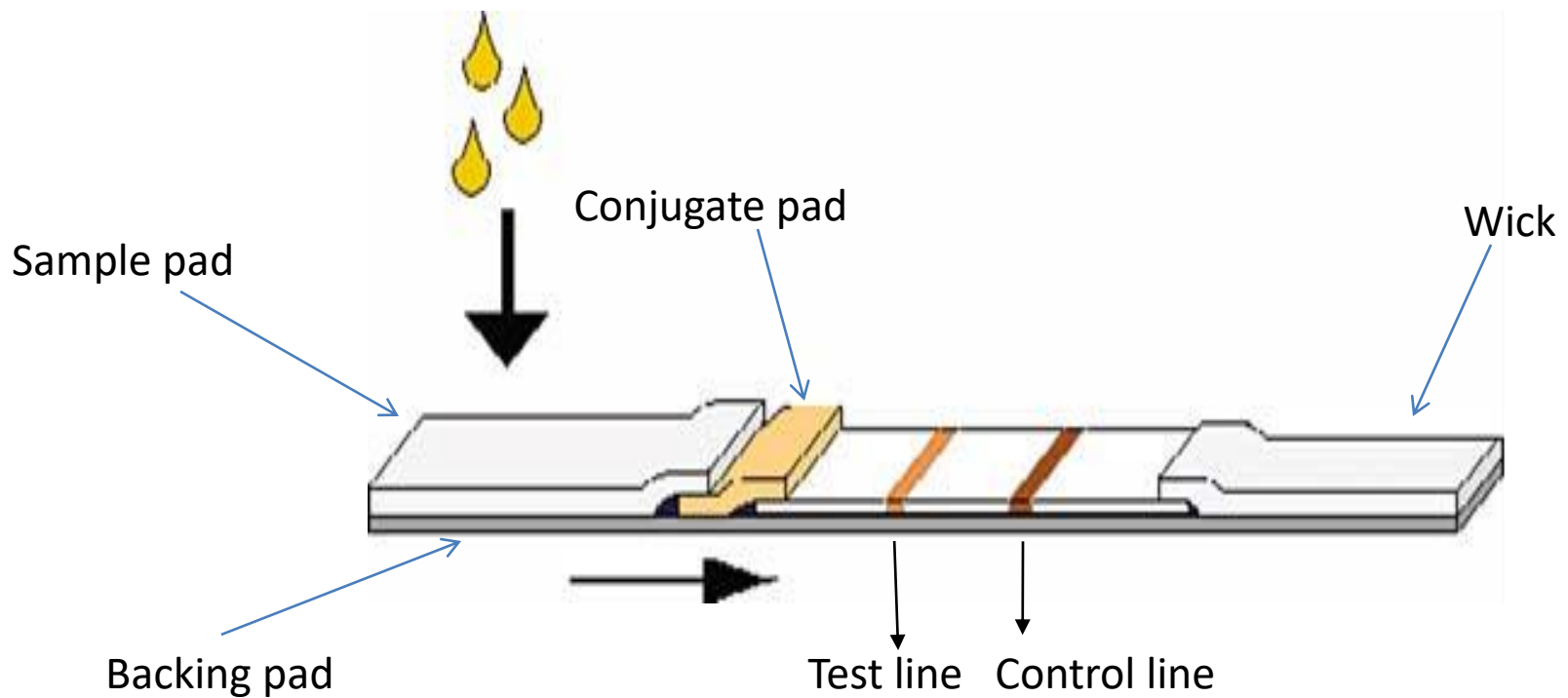
Principle and constituent components of LFA device

Principle and constituent components of LFA device

Double sandwich antibody model
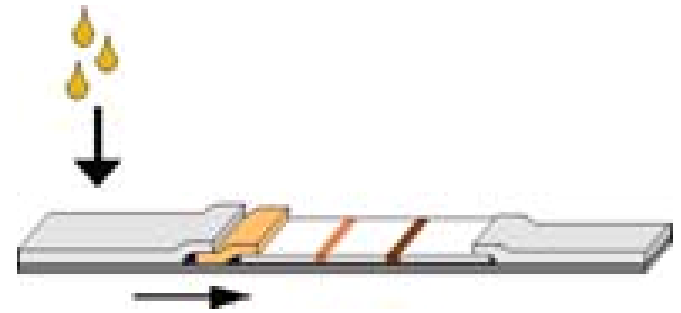

Brain or Saliva

Absorbent pad   Control line   Test line Rabies MoAb   N/C Membrane   Gold Conjugated Rabies MoAb   Sample Pad

Double sandwich antibody model

Pre run strip

Anti species antibody

Positive result

Negative result
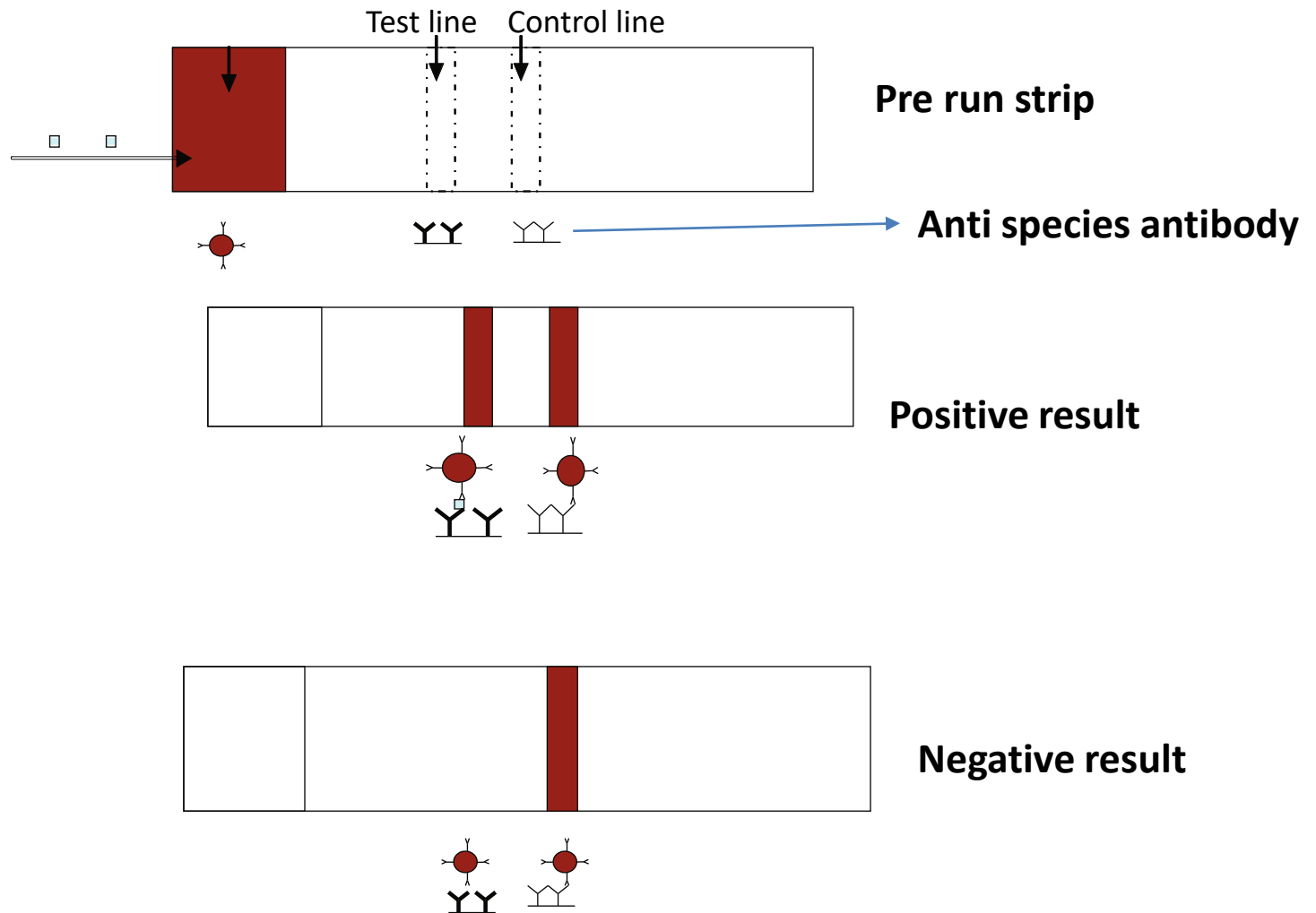
Constituent components of a lateral flow assay

1. **Nitrocellulose membrane(NCM)** solid support for Ag-Ab reaction- appropriate antibodies are coated at test and control zone.
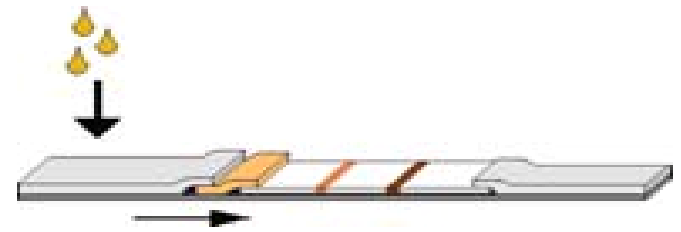
- widely used

nylon and polyvinylidene fluoride (PVDF) membranes- also used with limited success.

Advantages of NCM;

- low cost, true capillary flow characteristics, high protein-binding capacity, ease of handling
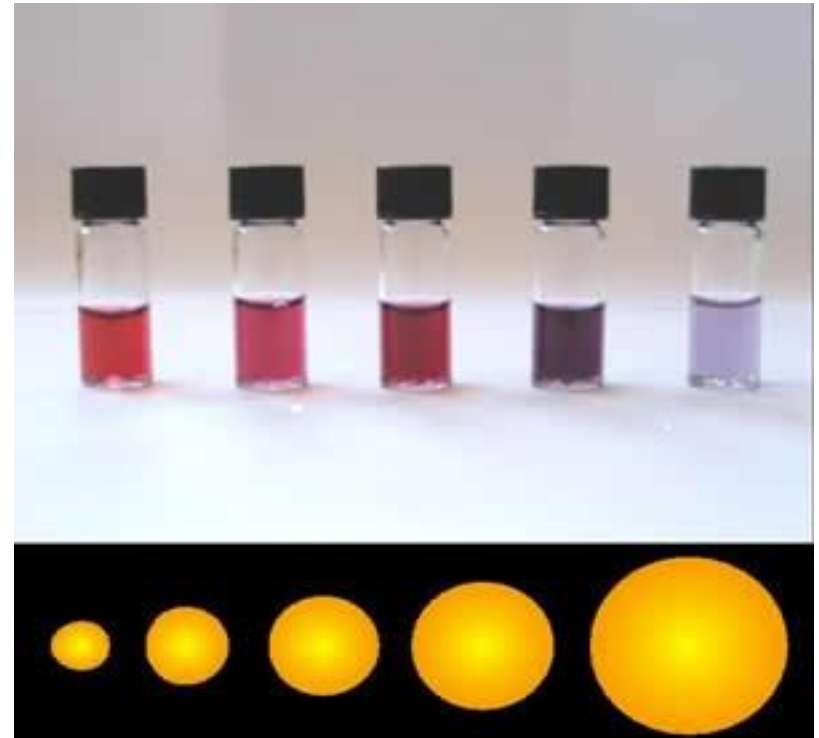
2. Conjugate pad -

particulate conjugate –

colloidal gold or colloidal carbon or a colored, fluorescent, or paramagnetic monodisperse latex particles.

- Conjugated to either antigen or antibody
- Colloidal gold - most widely used label
- easy and inexpensive to prepare.
- the color is intense, and no development process is needed.
- label is very stable in liquid or dried form.
- non-bleaching after staining on membranes.

Preparation of Colloidal Gold particles- various methods

- Gold nano particles are produced in a liquid by reduction of **chloroauric acid , H(AuCl$_4$)**.

- **Sodium citrate reduction method** for producing colloidal gold solution of uniform and controllable size.

- gold particles are typically in the range of 20–40 nm in size – due to smaller size higher packing density can be achieved.

- Solution turns initially from gray to dark gray color, then to a purple, and finally a **red color.**

- Any protein molecule can be attached to gold nano particle.

Suspensions of gold nanoparticles of various sizes. Size difference causes the difference in colors.

First use of an antibody conjugated colloidal gold for a diagnostic immunoassay for detection of hCG in urine (Leuvering *et al.,* 1981).

3. The sample pad
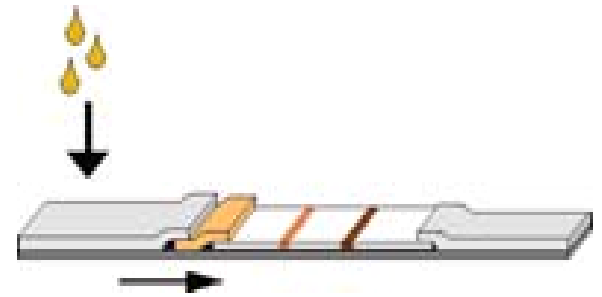
- To accept any kind of sample- whole blood, feces, tissue, serum, sputum, brain etc

- The materials used - cellulose, glass fiber, rayon etc.

- Material must be treated with assay buffer, surfactants, blocking reagents (if required), additives to increase sensitivity of the assay and dried prior to use.

4. The wick
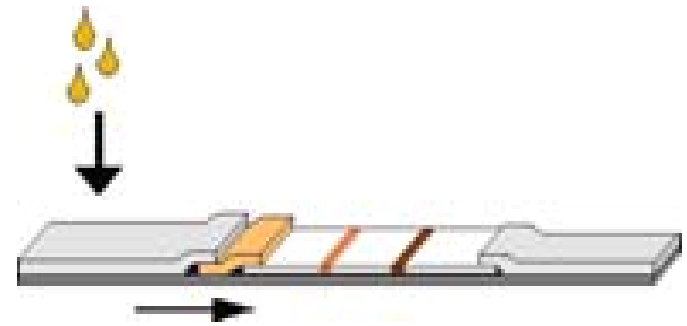
- Engine of the strip, designed to pull all of the fluid added to the strip and to hold it.

- Should not release the fluid back into the assay.

- The material is typically a high density cellulose.

5. Backing pad /Backing card
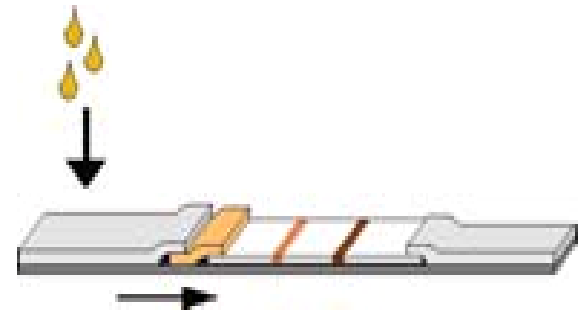
- A supporting backbone or platform

- All components of the LFA are laminated to the backing material to provide rigidity and easy handling of the strip.

- The backing materials are typically polystyrene or vinyl (PVC) or polyester coated with an adhesive.

6. Labels for detection (conjugates, antibodies)
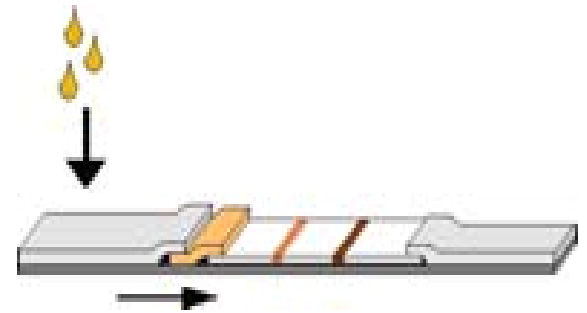
- Gold nano conjugated with specific Mab

- Specific Mab at Test zone

- Anti mouse antibody at Control zone

Lateral flow immunoassay 'Rapid Rabies Antigen Test kit' (Bionote, Hwaseong-si, Korea)

Materials provided in the kit (10 Tests/Kit):

- Ten Rapid Rabies Ag Test devices

- Ten assay diluent tubes

- Ten disposable swabs
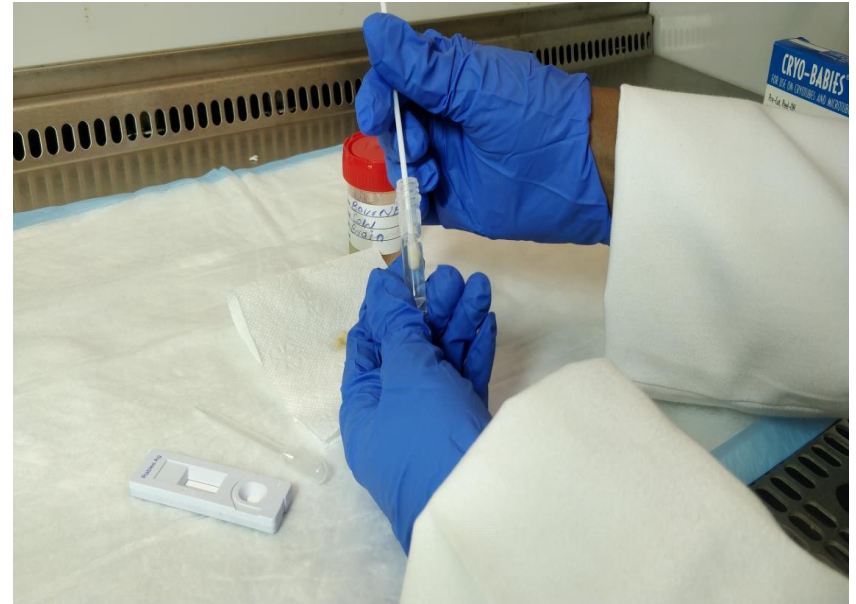
- Ten disposable droppers

- One instruction manual

LFA Protocol:

1. All reagents and samples shall be at room temperature before use.

2. Using the disposable swab provided, collect the sample from the brain tissue or add a small piece of the brain tissue into the assay diluent tube.
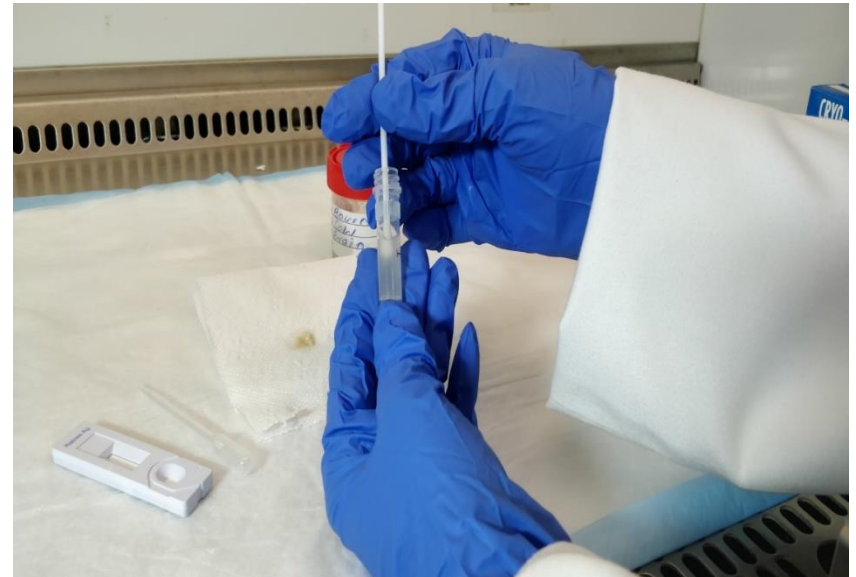
3. Swab shall be inserted into the assay diluent tube.

4. Homogenise the tissue using the swab until the sample has been dissolved into assay diluent.

5. LFA device shall be removed from the foil pouch and placed on a flat and dry surface.

6. Using a disposable dropper, take the sample suspension from the assay diluent tube.

7. Three to four drops of sample suspension shall be added into the sample pad drop by drop vertically and slowly.

8. Test results shall be interpreted after 5-10 minutes.

Reading shall not be taken after 20 minutes.

Interpretation of the LFA results:

- **Positive result**: Presence of red coloured Test (T) line and control (C) lines indicates the presence of rabies viral antigen in the brain tissue sample.

- **Negative result:** Presence of only red coloured control (C) line indicates the absence of rabies viral antigen in the brain tissue sample.

- **Invalid result:** If the red coloured control (C) line does not appear in the result.
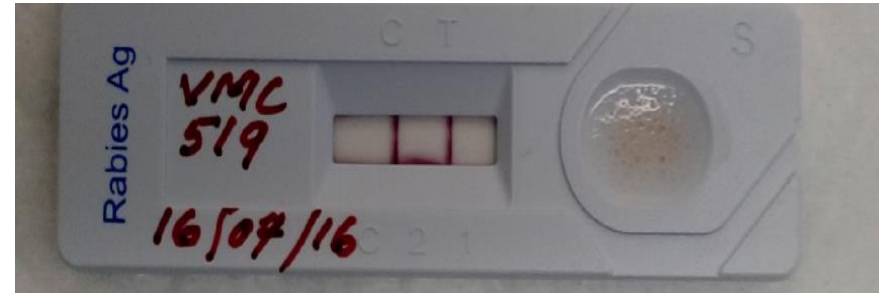
Detection of rabies virus in
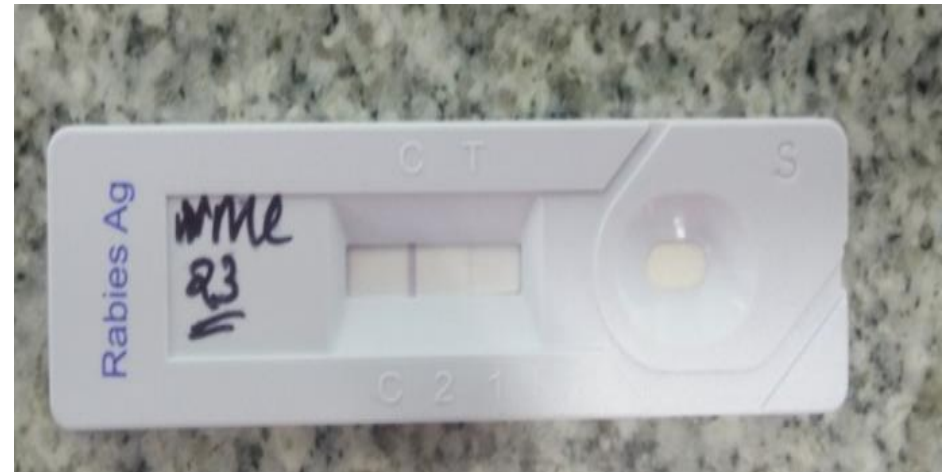## cell culture supernatants
(Sharada *et al.,* 2015)
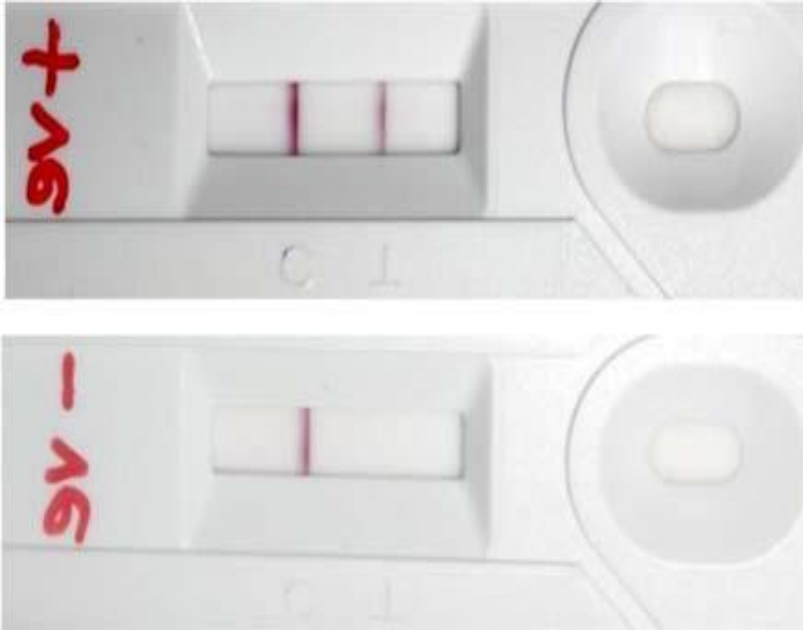
Brain tissues of Dogs

Gradations based on intensity of band at "T" line in cell cultures (Sharada *et al.,* 2015).

LFA also detected positive in saliva samples of dogs as an ante mortem diagnosis of rabies (Sujith, 2017).
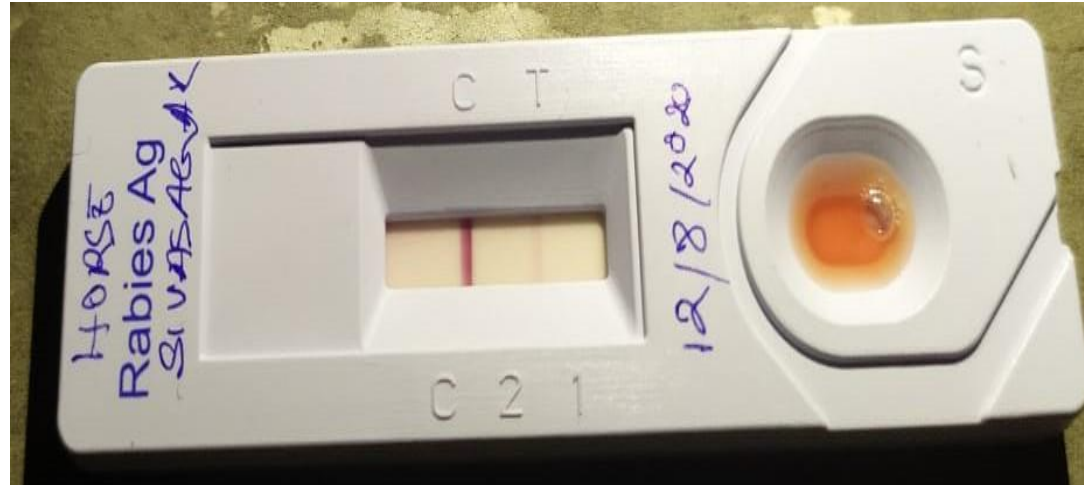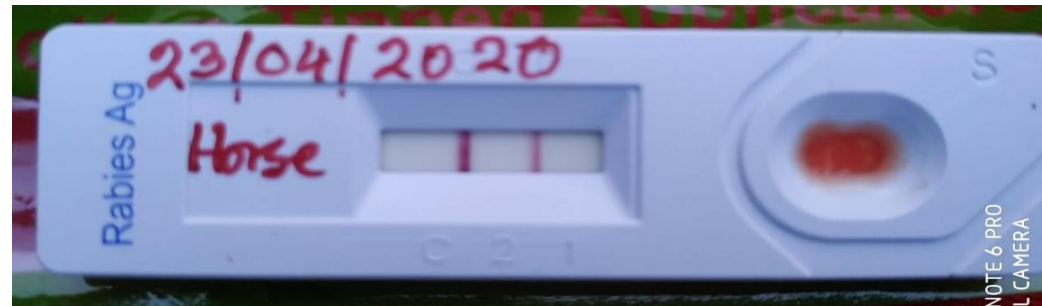
Ante mortem detection of rabies virus in saliva of dog with paralytic form, Pookot, Kerala, confirmed positive by DFA
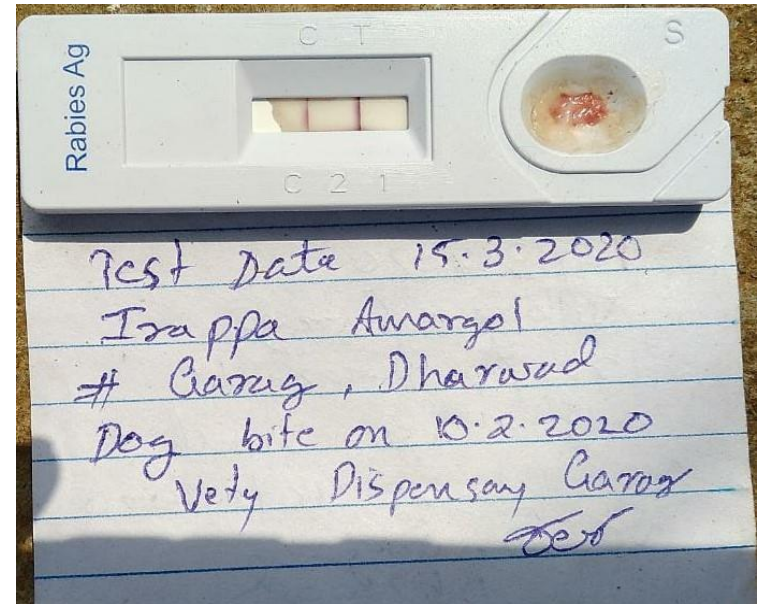
Horse positive for rabies by LFA on 12th August, 2020 in Assam

Horse from Assam Police Battalion positive by LFA

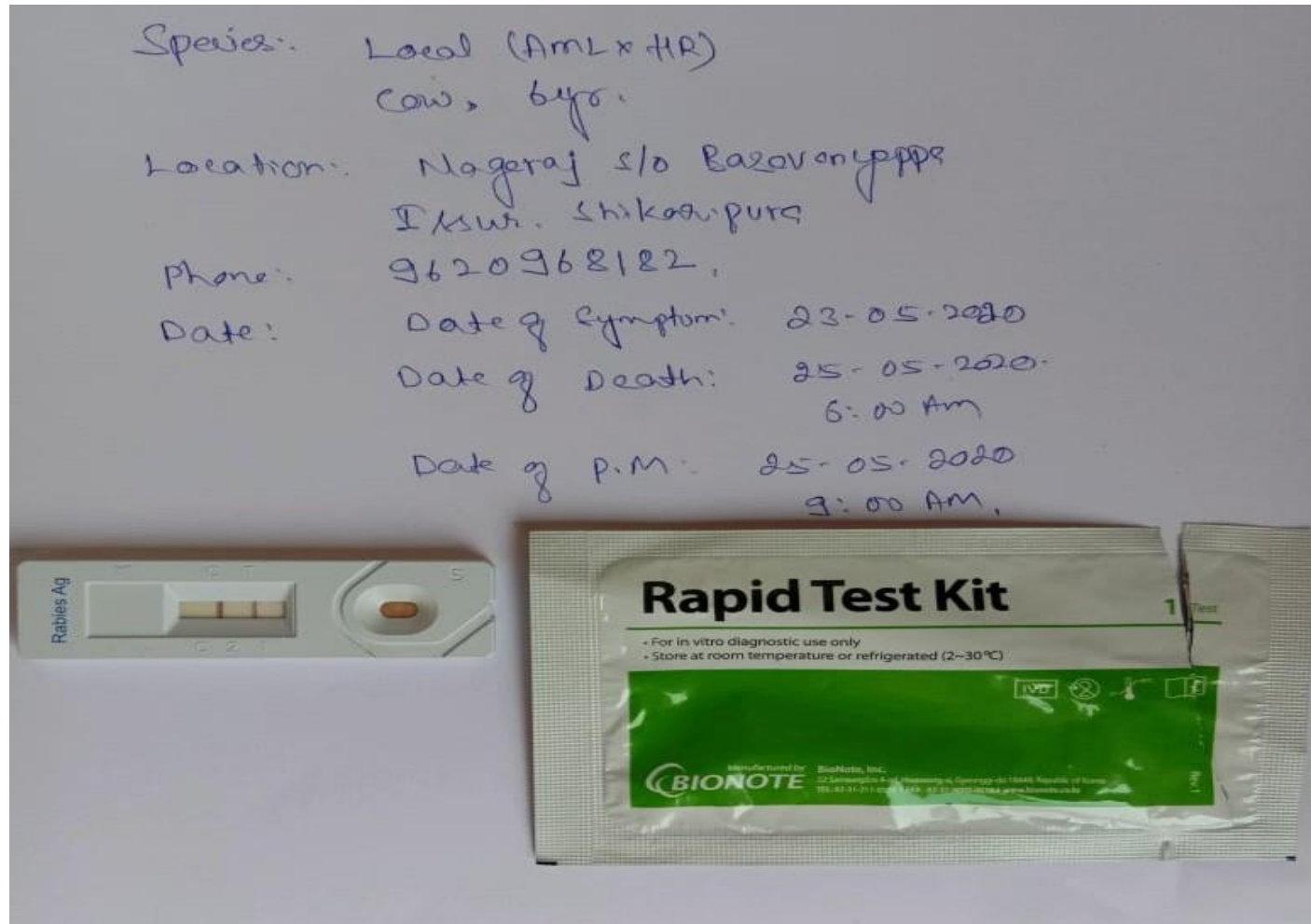# Calf positive by LFA done at carcass side by a field Veterinarian

LFA conducted at carcass side
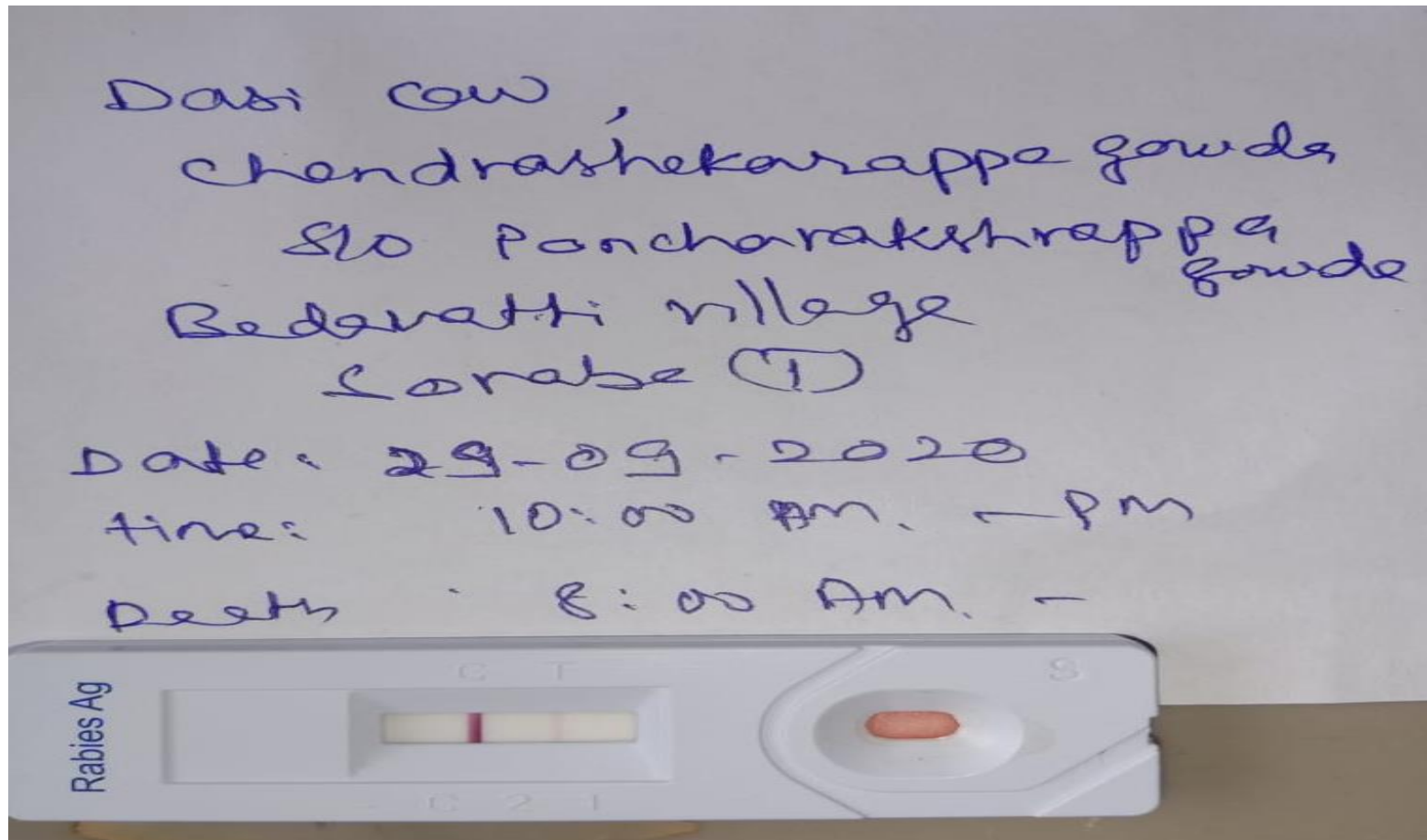
LFA at field in Assam, 30th July 2020
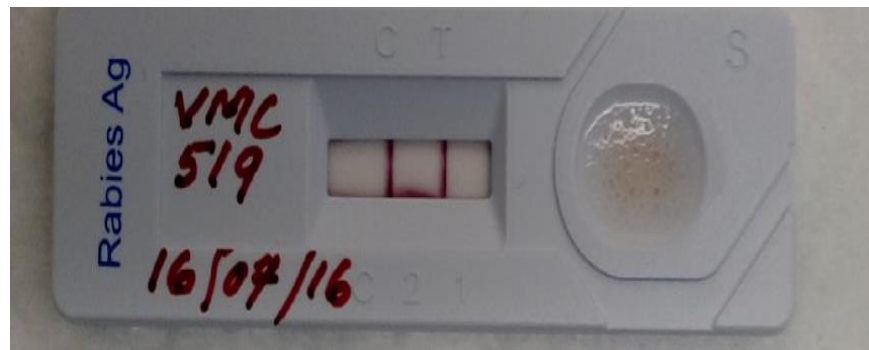
LFA positive in cow in a village by field Vet

LFA conducted in another village at carcass side by field Vet

Lateral flow assay –
a rapid immunodiagnostic test

- Clinical samples: brain stem, saliva, cell cultures

- Dogs, cattle, horse, cats, elephant etc.

- Advantages rapidity, simplicity, diversity of sample (including saliva) as opposed to DFA which can only test brain tissue.

- Field level at carcass side

- Constraints: LFA kits are expensive, not affordable

- Need to develop Indigenous LFA kits

THANK YOU