



## Rapid detection of Evans syndrome (IMHA and IMTP) in dogs by Flow cytometry



(A Biotechnological service offered for the first time in the country)

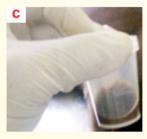
#### Backdrop

- Immune-mediated haemolytic anaemia (IMHA) and Immune meidated Thrombocytopenia (IMTP) are collectively called as Evans syndrome.
- Evans syndrome is a common cause of anemia in dogs and cats.
- Evans syndrome leads to damage of RBCs/platelets in the blood vessel and/or tissues leading to intravascular or extravascular haemolysis.
- The Direct Antiglobulin Test (DAT) is the conventional test for Evans syndrome. It has several disadvantages including false negative results, requirement of high quantity of antiserum, RBCs, time and high cost.
- Flow cytometry proved to be highly sensitive in detecting the RBC and platelet - bound immunoglobulins in Evans syndrome.
- Flow cytometry provides a more rapid, sensitive, specific and early detection method for IMHA and IMTP using lower volume of cells.

#### Clinical signs







- **Evans syndrome in dogs may manifest as:**
- a) Blanched mucous membrane;
- b) Ecchymosis (subcutaneous purpura) and
- c) Blood in urine

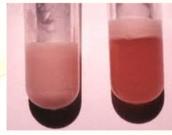
#### Test Methodology

1. Blood collection from suspected dogs in EDTA vials (1ml).



2. Blood should reach lab within 24 hours at +4°C for RBC and platelets separation





3. Staining of RBC and platelets with Flurochrome (IgG and IgM)







4. Flow cytometry reading for suspected dogs stained RBC and platelet

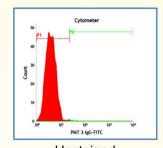


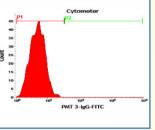
#### Cost of Service

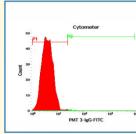
- i. Cost of testing Rs. 500 per sample
- ii. Results will be provided after 24 hours

#### Results

Normal Dog - Flow cytometry results MFI less than 6





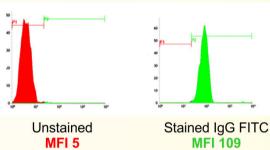


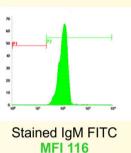
Unstained MFI 5

Ig G FITC stained
MFI 5.43

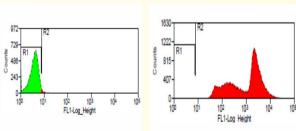
Ig M FITC stained MFI 5.36

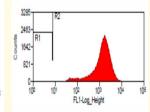
### IMHA positive dog - Flow cytometry results MFI should be more than 6





# IMTP positive dog - Flow cytometry results MFI should be more than 6





Unstained MFI 4.0

Stained IgG MFI 2523.4

Stained IgM MFI 2874.9

### Sensitivity and specificity

- Flowcytometry showed 93.1% sensitivity and 100 % specificity
- Conventional DAT showed 17.2% sensitivity and 100 % specificity