



STANDARD OPERATING PROCEDURE

SERODIAGNOSIS OF ANIMAL TRYPANOSOMIASIS DUE TO *T.EVANSI* (SURRA) CATT/*T.EVANSI* (*)

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This SOP is based on the notice of the CATT/*T.evansi* (https://www.itg.be/files/docs/TTP/brochures/PDT_BR_0003_4.1.pdf) supplied by the ITM ([https://www.itg.be / production@itg.be](https://www.itg.be/production@itg.be)) and recommended by the OIE for the serodiagnosis of animal trypanosomiasis due to *Trypanosoma evansi* (Surra).

Infection with *Trypanosoma evansi* results in production of circulating antibodies against several surface antigens of the parasite. Such antibodies can be demonstrated in the plasma or serum of the infected host by direct agglutination. The CATT-antigen is a freeze dried suspension of purified, fixed and stained bloodstream form trypanosomes expressing a predominant variable antigen type of *Trypanosoma evansi* (RoTat 1.2).

The test is performed on a plastified card. Twenty-five microliter of diluted serum or plasma are mixed with one drop of reconstituted antigen. When antibodies are present in the test sample, trypanosomes agglutinate within 5 minutes rotation at 70 rpm.

(*) CATT = Card Agglutination Test for Trypanosomiasis

1. COLLECTION OF SAMPLES

1. TISSUE MATERIAL TO BE EXAMINED

The serological diagnosis of surra (*Trypanosoma evansi*) by CATT/*T.evansi* is performed on equine sera. On receipt, the sample tubes must not be opened or damaged. A volume of serum greater than 100 µl must be provided. The serum must not be hemolyzed or coagulated. Before testing, a serum sample should be kept refrigerated (5±3°C).

2. TRANSPORT OF SAMPLES

Sera prepared from the whole blood samples received at the lab must be stored at 5°C ± 3°C. It is advisable for all blood samples to be centrifuged and stored whenever possible without the clot. For long-lasting storage, it is advisable to freeze sera without clot at ≤ -16°C.

2. REAGENTS, PRODUCTS AND APPARATUS

The CATT/*T.evansi* kit is supplied by the Institute of Tropical Medicine Antwerp ([https://www.itg.be / production@itg.be](https://www.itg.be/production@itg.be)).

1. KIT REAGENTS (PACKING SIZE: 250 TESTS)

Contents: - 6 vials CATT Antigen
- 1 vial Positive Control
- 1 vial Negative Control
- 1 vial CATT buffer
- 1 direction for use

2. KIT ACCESSORIES (PACKING SIZE: 250 TESTS)

Contents: - 26 plastified test cards
- 3 stirring rods
- 1 syringe (2.5 ml)
- 8 droppers

3. CARD TEST ROTATOR

A 12VDC-CARD TEST ROTATOR for performing the CATT in the laboratory (connected to a 220VAC power source by means of an AC/DC adaptor) or in the field (connected to a 12V car battery) is also available. (Not included in the kit, should be ordered separately).

IMPORTANT: Never mix up reagents (antigen, controls, buffer) of different deliveries!

1. CATT ANTIGEN (2.5 ml / vial)

- Freeze dried suspension of purified, fixed and stained trypanosomes of VAT RoTat 1.2
- Preservative: sodium azide (0.1 %).
- **Storage:** refrigerator (+2°C / +8°C) or freezer (-20°C).

2. CATT BUFFER (30 ml / vial)

- Phosphate buffered saline (pH 7.2).
- Use for reconstitution of CATT-antigen, positive and negative controls + preparation of sample dilutions.
- Preservative: sodium azide (0.1%).
- Storage: refrigerator (+2°C / +8°C). **DO NOT FREEZE!**

3. POSITIVE CONTROL (0.5 ml / vial)

- Freeze dried goat antiserum.
- Preservative: sodium azide (0.1 %).
- Storage: refrigerator (+2°C / +8°C) or freezer (-20°C).

4. NEGATIVE CONTROL (0.5 ml / vial)

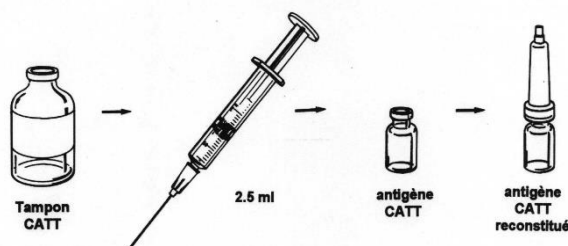
- Freeze dried solution of bovine albumin.
- Preservative: sodium azide (0.1 %).
- Storage: refrigerator (+2°C / +8°C) or freezer (-20°C).

3. EXECUTION DU TEST

1. RECONSTITUTION OF THE CATT ANTIGEN

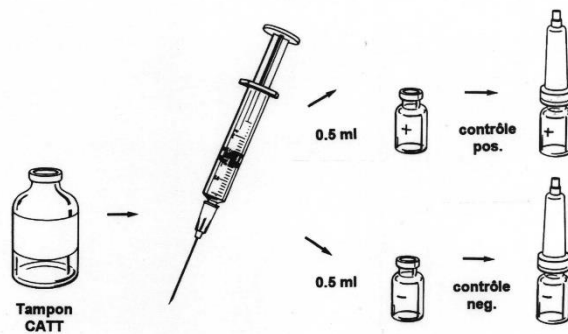
- Using the syringe, add 2.5 ml of CATT buffer to a vial of freeze dried CATT antigen.
- Immediately shake the vial for a few seconds so as to obtain homogeneous suspension.
- Put a dropper on the vial.
- The antigen suspension is ready for use.

- Notes:**
1. Before each use, shake the vial for a few seconds.
 2. Keep the CATT antigen out of the sun and dust.



2. RECONSTITUTION OF THE CONTROLS

Using the syringe, add 0.5 ml of CATT buffer to a vial of the positive and the negative control. Put a dropper on each vial.



Note: After reconstitution of each vial of CATT antigen, test one drop of the positive control and one drop of the negative control to check the quality of the antigen.

3. PREPARATION OF TEST SAMPLES

3.1. SCREENING TEST ON EQUINE DILUTED SERUM OR PLASMA

Prepare a 1:4 dilution in CATT BUFFER.

Using a micropipette, put 25 µl of diluted serum or plasma in a test area on the card.

3.2. QUANTITATIVE METHOD (TITRATION)

Prepare serial twofold dilutions 1:4, 1:8, 1:16, 1:32 and 1:64 of the test sample in CATT BUFFER.

Using a micropipette, put 25 µl of each dilution in a test area on the card.

4. AGGLUTINATION REACTION

1) On a test area of the card put:

- 25 µl of diluted serum or plasma (III.3.1)

or

- 25 µl of the serial twofold dilutions (III.3.2)

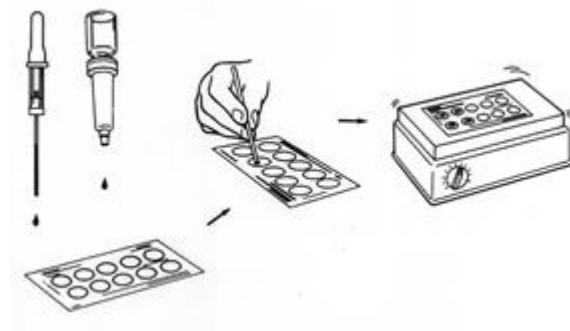
2) Then add:

1 drop (about 45 µl) of the well homogenised CATT antigen in each test area.

Note: In order to obtain drops of constant volume, hold the dropper vertically and allow the drops to fall freely without touching the card.

3) Using a stirring rod, mix and spread out the reaction mixture to about 1mm from the edge of test area. Wipe off the stirring rod after each use.

4) Rotate the test card on a flat bed orbital rotator for 5 minutes at 70 rpm.



Notes:

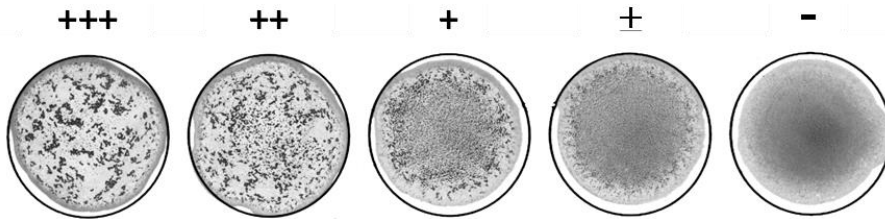
1) In order to prevent the reaction mixtures from drying out, always close the lid of the rotator and put a wet cotton plug beneath.

2) If no electrical rotator is available, rock the card so as to impart to the reaction mixture a constant circular movement by slowly tilting it, always in the same direction, from left-back to right-forth.

4. READING AND INTERPRETATION

4.1. VALIDATION OF THE ANALYTICAL PROCESS

- After 5 minutes rotation, read the results before removing the card from the rotator.
- When working manually, read the results while tilting the card gently.
- Read the results as follows:



- +++ = STRONGLY POSITIVE (very strong agglutination)
- ++ = POSITIVE (strong agglutination)
- + = POSITIVE (moderate agglutination)
- ± = WEAKLY POSITIVE (weak agglutination)
- = NEGATIVE (absence of agglutination)

Note: The CATT/*T.evansi* is not strictly species-specific. This may complicate the interpretation of positive results in areas where other species of salivarian trypanosomes occur.

5. STABILITY, STORAGE AND EXPIRY DATE

1. STABILITY

- The freeze dried reagents (antigen, positive and negative controls) and the CATT buffer remain stable for 1 year when stored in a refrigerator between +2°C and +8°C. At higher temperatures, i.e. + 45°C, the freeze dried reagents retain their activity for at least 1 week.
- After reconstitution, the reagents can be used during 1 week when stored between +2°C and +8°C, or up to 8 hours at 37°C.

Notes:

- These values are only an indication on the stability of the reagents but are not recommendations for prolonged storage!
- Do NOT freeze the reconstituted antigen suspension!

2. RECOMMENDATIONS FOR STORAGE AND SHIPMENT

- Freeze dried reagents (antigen, controls): in the refrigerator (+2°C to +8°C) or in the freezer (-20°C).
- CATT buffer: in the refrigerator (+2°C à +8°C) –Do not freeze!
- During transport, storage and handling: avoid exposure to heat and direct sunlight.
- It is recommended to dispatch the reagents from a central storage centre to the field under refrigerated conditions (cold chain).

3. SHELF LIFE / EXPIRY DATE

- When stored under the prescribed storage conditions, all the reagents will retain their activity until the expiry date mentioned on the “Reagent” boxes and on the packing list.