

# CATT/*T.b.gambiense* (\*)

## Serodiagnosis of human African Trypanosomiasis (sleeping sickness) due to *T.b.gambiense*

### I. PRINCIPLE

Infection with *Trypanosoma brucei gambiense* results in production of circulating antibodies against several surface antigens of the parasite. Such antibodies can be demonstrated in the blood, 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 brucei gambiense*.

The test is performed on a plastified card. One drop of undiluted blood (screening test) or 25 µl of diluted plasma/serum (confirmation test) are mixed with one drop of reconstituted antigen. When antibodies are present in the test sample, trypanosomes agglutinate within 5 minutes rotation at 60 rpm.

(\*) CATT = **C**ard **A**gglutination **T**est for **T**rypanosomiasis

### II REAGENTS

#### 1. CATT-ANTIGEN (2.5 ml / vial)

- Freeze dried suspension of purified, fixed and stained trypanosomes
- 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)

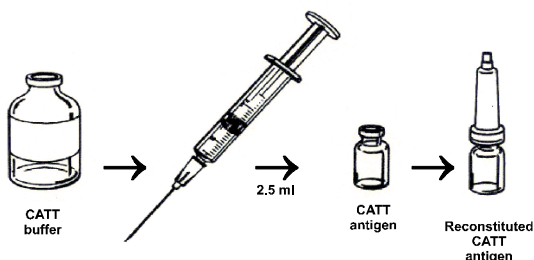
- Freeze solution of bovine albumin
- Preservative: sodium azide (0.1 %).
- **Storage:** refrigerator (+2°C / +8°C) or freezer (-20°C).

### III. EXECUTION OF THE TEST

#### 1. Reconstitution of the CATT-ANTIGEN

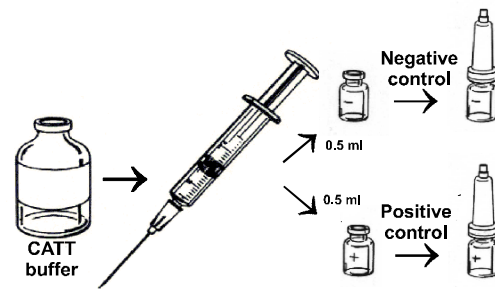
- Using the syringe, add 2.5 ml of CATT buffer to a vial of freeze dried CATT antigen.
- Immediately shake the vial for a few seconds so as to obtain a homogeneous suspension.
- Put a dropper on the vial.
- The antigen suspension is ready for use.

**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.

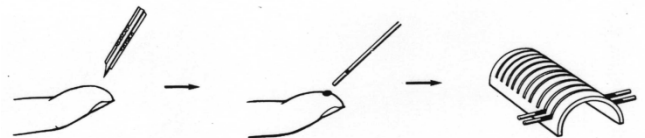


**Note:** After reconstitution of each vial of CATT antigen, test one drop of the positive control and one drop of the negative control to check the quality of the antigen.

#### 3. Preparation of test samples

##### 3.1 Screening test on undiluted blood

- Disinfect fingertip and prick with a microlancet.
- Wipe off the first drop of blood.
- Fill a heparinized capillary tube for about ¾ of its length avoiding air bubbles.
- Incline the capillary tube several times in order to mix the blood with the heparin.
- Place the capillary tubes horizontally on a capillary tube holder.



Using the suction bulb, put 1 drop of undiluted blood in a test area on the card

During field surveys:

- Collect blood samples of 5 to 10 persons.
- Proceed immediately afterwards to execution of the test.
- Should sedimentation of red blood cells occur in the capillary tube, mix the blood again before testing.
- Should coagulation of the blood occur, take a new sample.
- In order to avoid drying out of the blood at the extremities of the capillary tube, put the holder in a box containing a wet cotton plug

##### 3.2 Confirmation test on diluted blood, serum or plasma

- Prepare a serum or plasma sample by any suitable method.
- Dilute blood (1/2) or serum/plasma (1/4) in CATT buffer.

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

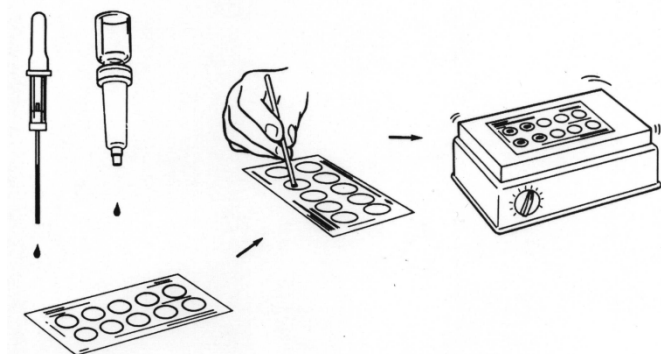
##### 3.3 Quantitative method (titration) on serum/plasma

- Prepare twofold dilutions 1/4, 1/8, 1/16, 1/32 and 1/64 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:
    - 1 drop of undiluted blood (III.3.1)
    - or
    - 25 µl of diluted blood, serum or plasma (III.3.2)
    - or
    - 25 µl of the twofold dilutions (III.3.3)
  - 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 the 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 60 rpm**.



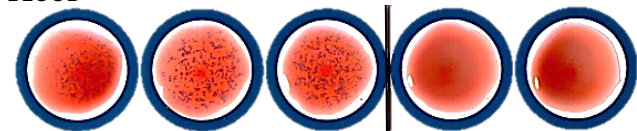
#### Notes:

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.

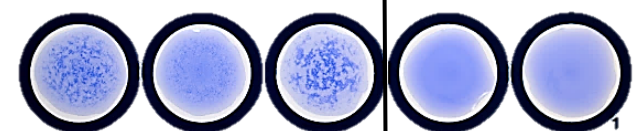
#### IV. READING and INTERPRETATION

- 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:

#### BLOOD



#### SERUM/PLASMA



POSITIVE

NEGATIVE

#### V. 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.

#### VII. PRESENTATION

##### 1. KIT REAGENTS (Packing size: 250 tests)

- Contents :
- 5 vials CATT-Antigen
  - 1 vial Positive Control
  - 1 vial Negative Control
  - 1 vial CATT-Buffer
  - 1 directions for use

##### 2. KIT REAGENTS (Packing size: 500 tests)

- Contents :
- 11 vials CATT-Antigen
  - 1 vial Positive Control
  - 1 vial Negative Control
  - 1 vial CATT-Buffer
  - 1 directions for use

##### 3. KIT ACCESSORIES (Packing size: 250 tests)

- Contents :
- 300 heparinized capillary tubes
  - 26 plastified test cards
  - 3 stirring rods
  - 1 suction bulb
  - 1 syringe (2.5 ml)
  - 8 droppers

##### 4. 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)

**Notes:** Microlancets are not included in the Accessory Kits and should be ordered separately.

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