HAT Sero *K*-SeT

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Produced in BELGIUM

***In vitro* rapid detection test of antibodies specific to *Trypanosoma brucei gambiense* in human whole blood, serum or plasma**

FOR IN VITRO USE **EN**

FOR PROFESSIONAL USE ONLY

References: K-15S2, 40 tests per kit, with accessories
K-12S2, 40 tests per kit, without accessories

I. INTRODUCTION

Human African trypanosomiasis (*HAT*) or *sleeping sickness* is a life threatening neglected tropical infection affecting rural populations in sub-Saharan Africa. In west and central Africa, the chronic form of *sleeping sickness* is caused by *Trypanosoma brucei (T.b.) gambiense*[1], a protozoan parasite. It is transmitted by the hematophagus Tsetse fly by biting[2].

HAT causes severe neurological disorders often leading to death if not treated. In addition, affected persons can act as reservoir hosts[3].

With the steadily decreasing prevalence of *HAT*, an individual rapid detection test with high specificity that is stable at ambient temperature and can be performed after minimal training is needed[4].

II. PRINCIPLE OF THE TEST

HAT Sero *K*-SeT test is a ready-to-use lateral-flow test based on a membrane technology. A nitrocellulose membrane is sensitized to catch antibodies of the samples and these are revealed with a colloidal gold conjugate. As antigens, it contains variant surface glycoproteins of *T.b. gambiense* Variable Antigen Types LiTat 1.3 and LiTat 1.5[4].

The sample must be delivered directly in the sample well of the device. When 2 drops of buffer are subsequently added to the same sample well, migration occurs on the strip. Then the solubilised conjugate migrates with the sample by passive diffusion and the conjugate and sample material come into contact with the reagent adsorbed onto the nitrocellulose. If the sample contains anti-*T.b.gambiense* antibodies, the conjugate-antibodies complex will remain bound to the test line and a red line will develop. Solution continues to migrate to encounter a second reagent that binds the migration control conjugate, thereby producing a red control line confirming that the test is working properly. The result is visible in the reading window within 15 minutes.

III. REAGENTS AND MATERIALS

1. **Gambiense Sero *K*-SeT (40)**
Sealed pouches each containing one device and one desiccant. Each device contains one sensitized strip.

2. **Instruction for use (1)**

3. **Procedure card (1)**

4. **BL-A buffer (6 mL)**
Saline dilution buffered to pH 7.5 containing Tris, EDTA, NaN_3 (<0.1%), a detergent and blocking proteins.

5. **Heparinized capillary tubes (50)**

6. **Capillary micropipette (2)**

7. **Materials needed (supplied with item K-15S2)**
   - Blood lancets
   - Alcohol prep pads

Required materials (not provided):
- Gloves
- Sterile cotton pads

IV. SPECIAL PRECAUTIONS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices (GLP).
- The user has to ensure that the technician who is performing the test is trained to handle the provided accessories.
- All reagents are for *in vitro* diagnostic use only.
- Pouch must be opened with care **at the moment** of the test.
- Avoid touching nitrocellulose with your fingers.
- Wear gloves when handling samples.
- **Never use reagents or buffer from another kit.**
- Green lines indicate reagents adsorption sites. Green color disappears during the test.
- Reagents' quality cannot be guaranteed beyond their shelf-life dates or if reagents are not stored under required conditions as indicated on the pouch containing the device.

V. WASTE DISPOSAL

- Dispose of gloves, lancets, capillary tubes, alcohol wipes and used devices in accordance with GLP.
- Each user is responsible for the management of any waste produced, and must ensure that it is disposed of in accordance with the applicable legislation.

VI. STORAGE

- An unopened pouch may be kept at between 4 and 30 °C and used until the shelf-life date indicated on the packaging. Once the pouch is opened, run the test immediately.
- Do not freeze devices and buffer.

VII. SPECIMEN HANDLING AND COLLECTION

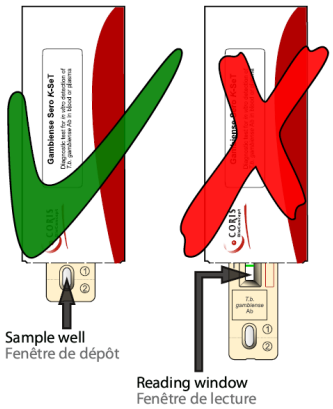
Specimen should be tested as soon as possible after collection. The whole blood specimen must be tested extemporaneously after taking. Serum or plasma may be stored at 2-8°C for 1 week or -20°C for longer periods of time.

There is no cross-reactivity with EDTA, heparin or citrate.

VIII. PROCEDURE

Preparations of the test:

1. Allow kit components, in unopened packaging, and specimens to reach room temperature before performing a test.
2. Open the pouch. Once opened, run the test immediately.
3. Indicate the patient's name or specimen number on the device (one device per sample).
4. Check that the two green lines are present in the reading window. If not, take another device.
5. For the next phases of the test, slide the device partially into the pouch so that the sample well is visible but the reading window is hidden.



Preparations of fingertip blood:

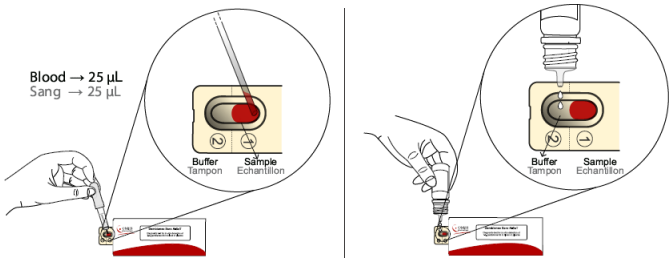
1. Prick the previously disinfected patient's fingertip with a micro-lancet.
2. Wipe away the first drop of blood with a sterile cotton pad.
3. Fill to the end the heparinized capillary tube provided with the kit. Fill volume is approximately 25 µL. Avoid introducing any air bubbles into the capillary tube.

Performing the test:

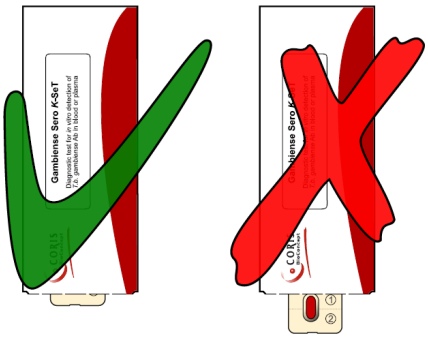
1. A. Whole blood:
   Take 25 µL of blood with a capillary tube (fingertip or venous blood – capillary micropipette provided) or a pipettor (venous blood only - not provided) and dispense into the **inner side** of the device sample well as illustrated below (annotated "1" area).

   B. Serum or plasma:
   Take 15 µL of serum or plasma and dispense into the inner side of the sample well of the *Gambiense* Sero *K*-SeT (sampling device not provided) as illustrated below (annotated "1" area).

2. Add immediately 2 drops (using the provided vial) or 85 µL of BL-A buffer into the **outer side** of the sample well as illustrated below (annotated "2" area). In order to obtain uniform drops, **don't touch the membrane** of the sample well with the vial dispenser and hold it **vertically**.



3. Slide the whole device into the pouch to avoid evaporation of the buffer and to maximize the test efficiency.



4. Leave to react for 15 minutes **into the pouch**.
5. After 15 to 20 minutes, remove the device from the pouch. The results are observed in the reading window.

WARNINGS

If the sample is taken using a capillary tube, the time between the sampling and the deposit cannot exceed 30 minutes.

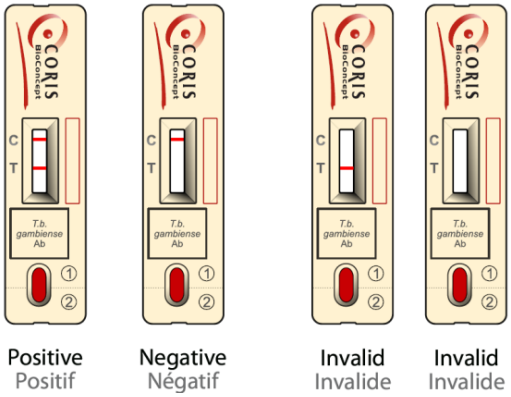
Test must be read immediately after its removal from the pouch.

Do not take the appearance of new lines into account after the reaction time is passed.

Do not use contaminated capillary pipette.

IX. INTERPRETING RESULTS

The results are to be interpreted as follows:



Positive test result: in addition to a reddish-purple band at the Control line (C), a visible reddish-purple band appears at the Test line position (T). Intensity of the Test line may vary. Any reddish-purple line (T), even weak, should be considered as a positive result.

Negative test result: a reddish-purple line appears across the central reading window at the Control line (C) position. No other band is present.

Invalid test result: The absence of a Control line indicates a failure in the test procedure, even if a Test line is present. Repeat invalid tests with a new test device.

Note: during the drying process, which begins after 20 minutes of running, a faint shadow may appear at the Test line position. It should not be regarded as a positive result.

X. PERFORMANCE

A. Sensitivity - Specificity

A phase II trial in endemic area was conducted on 493 persons in endemic area in the Democratic Republic of the Congo (DRC). The results were compared with parasitology.

Parasitology / *HAT* Sero *K*-SeT	Positive	Negative	Total
Positive	132	5	137
Negative	2	354	356
Total	134	359	493

		95 % Confidence Interval[i]
Sensitivity:	98.5%	(94.2 to 99.7%)
Specificity:	98.6%	(96.6 to 99.5%)
Positive Predictive value:	96.4%	(91.3 to 98.6%)
Negative predictive value:	99.4%	(97.8 to 99.9%)
Agreement:	98.6% (486/493)	

B. Repeatability

To check intra-batch accuracy (repeatability), the same positive samples and a buffer solution were processed 15 times on kits of the same production batch in the same experimental conditions. All observed results were confirmed as expected.

[i] Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," *Statistics in Medicine,* **17**, 857-872 (1998).

XI. LIMITS OF THE KIT

The test is qualitative and cannot predict the quantity of antibodies present in the sample. Additional parasitological tests must be performed to establish diagnosis. Clinical presentation and other test results must be taken into consideration to establish diagnosis.

This test detects antibodies present in the serum, plasma or whole blood of the patient but not the antigens. So, false positive can appear if patient has been in contact with the *T.brucei gambiense* even if he is not infected anymore. Also, low level of these antibodies could lead to false negative result.

Patients infected with neither LiTat 1.5 nor LiTat 1.3 *T.brucei* strains cannot be detected with this test.

A positive test does not rule out the possibility that other pathogens may be present.

XII. TECHNICAL PROBLEMS / COMPLAINTS

If you encounter a technical problem or if performances do not correspond with those indicated in this package insert:

1. Record the kit batch number
2. If possible, keep the clinical sample in the freezer during the complaint management
3. Contact Coris BioConcept (client.care@corisbio.com) or your local distributor

XIII. BIBLIOGRAPHIC REFERENCES

1. **Simarro PP, Jannin J and Cattand P**: *Eliminating human African trypanosomiasis: Where do we stand and what comes next?*, PLoS Medicine 2008; 5: 174-180.
2. **Malvy D and Chappuis F**: *Sleeping sickness*, Clinical Microbiology and Infection 2011; 17: 986-995.
3. **Chappuis F, Loutan L, Simarro P, Lejon V and Buscher P**: *Options for Field Diagnosis of Human African Trypanosomiasis*, Clinical Microbiology Reviews 2005; 18.1: 133–146.
4. **Büscher P, Gilleman Q and Lejon V**: *Rapid Diagnostic Test for Sleeping Sickness*, The New England Journal of Medicine 2013; 368-11: 1069-1070.
5. **Büscher P, Mertens P, Leclipteux T, Gilleman Q, Jacquet D, Mumba-Ngoyi D, Pati Pyana P, Boelaert M and Lejon V**: *Sensitivity and specificity of HAT Sero-K-SeT, a rapid diagnostic test for serodiagnosis of sleeping sickness caused by Trypanosoma brucei gambiense: a case-control study*, Lancet Glob Health 2014; Vol2: e359-e363.

Last update: SEPTEMBER 2014

REF	Catalogue number		Manufactured by
IVD	In vitro diagnostic medical device		Temperature limitation
	Contains sufficient for <n> tests	DIL SPE	Diluent specimen
	Consult instructions for use		Do not reuse
	Keep dry		Use by
DIL AS	Diluent assay	CONT NaN_3	Contains Sodium azide