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Antibody-Antigen Detection Tests for Sleeping Sickness Diagnosis: Are There Opportunities For Advancement?

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Abstract: **Human African trypanosomiasis (HAT; also termed as sleeping sickness) is a parasitic disease caused by African trypanosomes belonging to the genus *Trypanosoma*, species *brucei*. Diagnosis usually involves screening of populations using serological tests, confirmation of infection by parasitological demonstration of trypanosomes in body fluids, and subsequent staging of the disease which ultimately determines the treatment to be administered. This means that diagnosis should be both accurate and timely. Diagnosis is however limited by lack of specific, accurate and field applicable tools. This review focuses on the currently applied serological tests for diagnosis of sleeping sickness, highlighting their merits and limitations, and discuses the emerging advances in the serodiagnosis of this neglected tropical disease. Improved diagnostic tools specific for HAT will certainly contribute towards the successful elimination of the disease from sub-Saharan Africa**

Keywords**: Trypanosomiasis, serodiagnosis, antigen, antibody, sensitivity, specificity.**

1. INTRODUCTION

Human African trypanosomiasis (HAT or sleeping sickness) is a neglected tropical disease caused by two sub species of *Trypanosoma brucei*; *T. b. gambiense* and *T. b. rhodesiense*. The single celled extracellular eukaryotic parasites are transmitted by tsetse flies of the genus *Glossina*. *T. b. gambiense* is responsible for the insidious, chronic form of the disease (Gambian HAT) in central and western Africa, while *T. b. rhodesiense* causes a severe, acute form of the disease (Rhodesian HAT) in east and central Africa [1]. The geographic distribution of the two forms of the disease could however change leading to an overlap of the diseases in Uganda [2]–[4]. In both forms of the disease, progression occurs in two distinct clinical manifestations; the early stage (also termed as haemolymphatic stage) characterized by the proliferation of the parasites in blood, lymph and body tissues, and the second/ late stage (meningo-encephalitic stage) which occurs when trypanosomes cross the blood brain barrier and spread to the central nervous system [5], [6].

Trypanocidal drugs in current use are dependent on the stage of the disease and the trypanosome subspecies involved [7]. Drugs in routine clinical use include pentamidine and suramin for *T. b. gambiense* and *T. b. rhodesiense* early stage disease treatment respectively, while eflornithine/ D,L-alpha-difluoromethylornithine (DFMO) (*T. b. gambiense*) and melarsoprol (both trypanosome species) are administered for late stage disease [8]. The drugs are however old, toxic (none of these drugs would currently be approved by the Food and Drug Administration [9], and, in addition, melarsoprol has been linked with post treatment reactive encephalopathy (PTRE) [6], [10], an acute condition which leads to death in 5% of patients to whom it is administered to. Furthermore, the drugs are becoming ineffective due to the emergence of trypanosome strains that are resistant [11], [12]. The introduction of Nifurtimox-Eflornithine Combination Therapy (NECT) in 2009 for treatment of second stage *T. b. gambiense* HAT has been shown to be less toxic and more effective compared to eflornithine only treatment regimen in a preclinical study conducted in Congo [13]. Other compounds are currently under various stages of development or clinical trials [14]. With no chemoprophylaxis and vaccines available for African trypanosomiasis, there is need for timely and accurate diagnosis and stage determination for proper drug treatment.

Sleeping sickness diagnosis is a multi-procedural complex component in sleeping sickness control, and mainly involves screening populations at risk of infection (passive or active screening), parasitological confirmation of trypanosomes in body fluids, followed by staging of the disease prior to commencement of treatment [15], [16]. Presently, sleeping sickness diagnosis includes examination of clinical presentations, parasitological demonstration by microscopy, molecular amplification of trypanosome subspecies specific nucleic acids and serological assays targeting trypanosome specific antibodies or antigens. Parasitological demonstration of parasites in body fluids offers concrete confirmation of infection, and is considered as the reference/gold standard in the diagnosis of the disease by the World Health Organization (WHO) [15]. However, as trypanosomes are in low quantities especially in *T. b. gambiense* infections, serological tests are relied upon for active population screening before confirmation by microscopy following concentration of the parasites in the body fluids. Molecular detection tests are highly sensitive, but are limited by cost, inapplicability in field environments where constant electricity supply in seldom assured, and the need for qualified personnel. This review describes the most commonly applied serological tests available for sleeping sickness detection, and discuses new and emerging advances in the serological diagnosis of sleeping sickness.

2. CURRENT SERODIAGNOSTIC TESTS FOR HAT

African trypanosomes are exclusively extracellular parasites covered by a dense surface coat monolayer, the variable surface glycoprotein (VSG) [17], which shields invariant surface antigens from immune detection [18]–[20]. This surface glycoprotein coat is highly immunogenic, and forms a potential sero-diagnostic candidate for trypanosome detection.

However, trypanosomes undergo antigenic variation, a mechanism which involves change of the VSG to an antigenically different one [21], [22]. The parasite's genome has 1000 - 2000 alternative and highly diverged surface antigen genes and pseudogenes, of which only a single one is expressed in any individual trypanosome cell [23]. Studies have shown that despite the high diversity of expressed antigenic variants due to antigenic variation, some VATs are produced more frequently than others i.e. are predominantly expressed [24]–[26]. Most tests for screening populations at risk in *T. b. gambiense* prone foci are based on antibody detection against the trypanosomes' surface coat, the variable surface glycoprotein. There are three commonly used antibody-antigen detection tests available for HAT diagnosis, namely; the Card Agglutination Test for Trypanosomiasis (*CATT/ T. b. gambiense* or wb-CATT) developed by Magnus and colleagues [27], the LATEX/ *T. b. gambiense* test [28] and ELISA/ *T. b. gambiense* developed in 1998 by Lejon and colleagues [29]. Other serological tests include indirect fluorescent antibody test (IFAT), the card indirect agglutination test for trypanosomes (CIATT) and the immune trypanolytic test (TL).

CATT/T. b. gambiense and its variants:

The card agglutination test for trypanosomiasis (CATT) is a serological test for diagnosis of Gambian HAT developed in 1978 by Magnus and colleges [27]. CATT is the most widely used test applied for both passive and active of populations in *T. b. gambiense* endemic foci due to its simplicity and cost effectiveness. This test is based on a single lyophilized laboratory adapted strain of *T. b. gambiense* expressing predominantly expressed variable antigenic type (VAT) designated LiTat 1.3 on the parasites surface. Applied on whole blood, plasma or serum, the CATT/ *T. b. gambiense* is used in active screening of populations at risk, and seropositive cases are further confirmed by parasitological examination.

Mass production of trypanosomes expressing the variable antigenic type LiTat 1.3 antigen is achieved by culturing the parasites in laboratory rodents and the blood stream stage of the parasite extracted from the blood; a process which puts laboratory staff at risk of infection [30]. The harvested parasites expressing VAT LiTat 1.3 are fixed, stained with Coomassie blue before being lyophilized [15]. The kit consists of the antigen reagent, a control sera and a rotator; and operates by mixing blood and PBS-resuspended antigen reagent in equal volumes (one drop each), the mixture is rotated at 60 rpm for 5 minutes using the rotator and the results analyzed by viewing, and the reported sensitivity and specificity of this test is 87-98% and 95% respectively [15]. CATT on diluted blood is more cost effective than on whole blood (CATT/wb), but its slight complication makes it rather difficult to perform under field conditions [31].

Adaptation of this test to permit the utilization of minute amounts of dried blood samples on filter papers or diluted blood i.e. the Micro-CATT test, is a variant of the CATT/*T. b. gambiense* serological mass screening test and was recommended by WHO for mass screening due to its affordability [32]. This test uses micro-volumes, unlike the classical CATT/*T. b. gambiense* test (a fifth of the standard quantities of antigen and sample). This test is however less sensitive than the classical CATT/*T. b. gambiense* especially when the FP eluate is older than a day or stored at 4°C [33], [34]. Coupled to

the decrease in sensitivity with time, micro-CATT also suffers from difficulties in interpreting agglutination test results due to the micro volumes used in this test [33], [34].

CATT on blood impregnated on filter papers (CATT-FP) was developed at the Department of Parasitology of the Prince Leopold Institute of Tropical Medicine, Antwerp (ITMA), is an improvement of the micro-CATT, and involves using a higher volume of the FP eluate (30 µL). CATT-FP has also to enable the screening of populations in areas outside active screening radius [33]. The maiden test evaluation of this test was carried out in south-Sudan in Kajo-Keji County, and compared well with CATT on plasma, showing a sensitivity of 94% on parasitologically confirmed cases. More so, the CATT-FP test illustrated that samples could be stored at ambient temperatures (25-34 °C) and under refrigeration (2-10 °C) for upto fourteen days to eight weeks without loss of sample integrity [33], unlike micro-CATT test whose sensitivity decreased with time. This can be attributed to the strict dry storage of the filter papers impregnated with blood, ensuring integrity of the blood samples.

Another alternative to the classical CATT/*T. b. gambiense* test, the CATT-EDTA test [35] is an improvement of the classical CATT/*T. b. gambiense* test; the EDTA (10mM) incorporated in the reaction buffer helps reduce complement mediated prozone effect, increasing the sensitivity of CATT. Active complement present in freshly collected blood inhibits the agglutination reaction leading to the formation of a prozone common in classical CATT test [36]. Dilution of blood also helps increase the specificity of the CATT-EDTA test (Jamonneau *et al.*, 2000). The CATT-EDTA test was evaluated in a preliminary study conducted in Cote d'Ivoire [36], and showed a higher specificity (94.6%) compared to that of CATT/*T. b. gambiense* (92.5%).

A limitation of CATT/ *T. b. gambiense* screening tests is the observed absence of VAT LiTat 1.3 in some *T. b. gambiense* foci e.g. Cameroon [37], leading to false negative results. In a study carried out in Fontem, Cameroon, it was observed that the LiTat 1.3 gene was not expressed by *T. b. gambiense* strains, while a LiTat 3 like gene, expressed by some strains, elicited antibodies which could not be detected by the CATT test [37]. This lowers the sensitivity of the CATT/*T. b. gambiense* in such areas where LiTat 1.3 is not expressed.

Card Indirect Agglutination Test for Trypanosomiasis (CIATT):

The card indirect agglutination test for trypanosomiasis (CIATT) or TrypTect CIATT® is a latex agglutination test for both *T. b. gambiense* and *T. b. rhodesiense* infection first described in 1997 by [38]. This test, unlike the other tests previously described, detects trypanosome antigens circulating in the bloodstream using a monoclonal antibody specific to an invariant internal antigen present in both human infective *T. brucei* subspecies [38] - the antibody is coupled to latex particles, forming the reaction reagent. The assay involves mixing on the test card equal volumes (50 µl) of test reagent and serum/ plasma, and after two minutes of incubation the test card is tilted and rotated, macroscopically showing presence of agglutination when the test sample is positive.

Analysis of parasitologically confirmed 244 *T. b. gambiense* samples from Uganda and Côte d'Ivoire, and 132 *T. b. rhodesiense* samples from Uganda by the CIATT illustrated that the test was highly sensitive, recording 95.8 % and 97.7 % sensitivities for *T. b. gambiense* and *T. b rhodesiense* infections respectively [38]. The antibody coupled to latex particles did not agglutinate with 193 negative sera samples assayed, illustrating the high specificity of this test (100 % specificity). This study also showed that the CIATT was more sensitive than some parasitological tests done in parallel (lymph node aspirates, mAECT, mHCT and CSF single and double centrifugation techniques). However, this test could not detect minute antigens in circulation, necessitating the need to test suspect cases after one to two weeks [38].

Antigen detection tests enable detection of active infection, unlike antibody detection tests which can detect persistent antibodies still in circulation months after the infection has been cleared due to treatment or self cure. This means that the CIATT can be used in post treatment follow-up, and the ability to assess post treatment follow up with finger prick blood could eliminate the need for lumber puncture, a painful and intrusive method undergone by patients during post treatment follow ups.

LATEX/T. b. gambiense:

The LATEX/*T. b. gambiense* utilizes the combination of three predominant VATs, namely LiTat 1.3, LiTat 1.5 and LiTat 1.6 (VAT G16/6) of bloodstream *T. b. gambiense* parasites. The incorporation of three VATs predominantly expressed during *T. b. gambiense* infection broadens the geographic user-range of this test.

Partially purified antigens are adsorbed on latex particles/beads, and equal amounts of diluted blood samples are mixed with the latex reagent and spread onto a 1.5 cm reaction zone with a dark background (Jamonneau *et al.*, 2000). Agitating the card at 70 rpm for five minutes enables the antigens to agglutinate with the VAT specific antibodies present in the blood, which are visible as white macroscopic agglutinations [28], [39].

The specificity of LATEX/*T. b. gambiense* test was reported to be 98.1% in a study conducted in Cote d'Ivoire. Despite being highly specific, test requires qualified personnel, is time consuming and the requirement of microplates complicates its application in the field setting [36]. In addition, mixing more than one antigen in the same reaction reagent reduces the reactivity of the individual antigens in the final mixture, reducing the sensitivity of the test.

Enzyme-linked immunosorbent assay (ELISA):

The enzyme-linked immunosorbent assay (ELISA) is an immunological test that indirectly demonstrates the presence of an infecting parasite in body fluids. In contrast to CATT and LATEX tests, ELISA makes use of markers to enable the detection/visualization of antigen–antibody complexes.

ELISA has been used in the serodiagnosis of sleeping sickness to detect anti-trypanosome specific antibodies in serum and CSF [29] or saliva [40]. ELISA has also found application in the staging of the disease, as anti-trypanosome specific antibodies or cerebrospinal IgM immunoglobulin whose levels are elevated in the CSF during infection [41]–[43].

Trypanosome antigens are prepared by infecting laboratory rodents, and the trypanosomes are collected by differential centrifugation, resuspended in saline, lysed via sonication and centrifuged to remove insoluble cell debris [44]. The test antigens (crude or pure) are adsorbed on microplates at different dilution factors, and diluted sera/CSF added. An enzyme conjugate (antigen specific antibody conjugated to an enzyme such as alkaline phosphatase or horse radish peroxidase) subsequently added followed by the enzymes substrate. The optical density is read at a specific wavelength, with the extinction value obtained being a measure of the quantity of enzyme-labeled immunoglobulin bound to the antigen – antibody complex [45].

ELISA/*T. b. gambiense* is highly sensitive [45] and specific (98.5%) [31], cost effective and has acceptable reproducibility. However, this test suffers from variable sensitivities, and can only be applied in reference centers with highly skilled technicians, who are more often lacking in poor remote areas. In addition, cross reactions with antibodies from patients infected with Leishmaniasis is a concern, leading to false positives (Knapen *et al*., 1977). A sensitive micro-ELISA for *T. b. rhodesiense* is also described by [46], but this test too suffers from cross reaction by antibodies specific to, and antigens of *Leishmania donovani* and *T. cruzi*.

An antigen based ELISA for sleeping sickness diagnosis is also described, which is sensitive and not limited by cross reactions with sera collected from patients infected with common tropical diseases. In addition, detection of trypanosome specific antigens circulating in the body fluids provides direct evidence of active infection [47]. Using a monoclonal antibody elicited against invariant surface protein at the procyclic stage of the parasites, [48] describes an indirect ELISA tool for diagnosis of Rhodesian HAT. In this study, the antigen ELISA was 100% specific, and 90% sensitive, and could also detect six samples which were parasitologically negative. However, this test could not detect some parasitologically confirmed cases, a limitation attributable to low amounts of antibody produced or during early stages of the disease [48].

Immune trypanolysis test (TL):

The immune trypanolysis test (TL), is an antibody-compliment mediated serological test that detects antibodies raised against specific VATs, revealing/demonstrating prior contact with the parasite. The variable glycoprotein surface coat of the trypanosomes, being highly immunogenic, elicits an immune response by the host's immune system which involves the production of antibodies specific to the VATs. These antibodies are able to opsonize, agglutinate and lyse circulating trypanosomes [49]. This test can only be applied in reference laboratories, limiting its field applicability.

Immune trypanolysis test involves the intravenous inoculation of 10^7 mouse adapted bloodstream trypanosome clones expressing predominantly expressed VATs and a control VAT in complement rich cavia serum. Human plasma sample (25 µL) is mixed with 25 µL of complement rich serum, and 50 µL of 10^7 trypanosomes/mL suspension prepared from adapted bloodstream form trypanosomes in mice added. The micro-plates are mixed and incubated at room temperature for one and a half hours, and the reaction mixture examined under a phase contrast microscope [50].

In 1990, Isharaza and Van Meirvenne described a trypanolysis test for the detection of *T. b. rhodesiense* antibodies in 85 sera samples collected from Uganda [51]. The study used a panel of twelve *T. b. rhodesiense* specific VATs (ETat 1/1, 1/2, 1/3, 1/10, 1/14, 1/18, 1/19, UTat 3/1, 3/7, 1/1 and 4/1) and one control VAT (AnTat 25/1). All the assayed VATs were able to detect all the seropositive sera samples albeit with varying proportions. In combination, three of the VATs i.e. ETat 1/1, ETat 1/4 and UTat 1/1 showed positive reactions with all sera samples infected with *T. b. rhodesiense*. However, some parasitologically positive sera were missed by the assayed VATs; this can be attributed to low levels of anti VAT specific antibodies in circulation [51].

Another study by Van Meirvenne and colleagues is described, where a panel of twelve predominantly expressed *T. b. gambiense* VATs were used (LiTat 1.1 to LiTat 1.10, and AnTat 11.17 and AnTat 11.20), and a control from *T. b. rhodesiense;* ETatR1 [49]. This study was highly specific (100%) in different studies conducted in *T. b. gambiense* endemic countries such as Equatorial Guinea, Côte d'Ivoire, Gabon, Congo, Uganda (north west part of the country), Zaire, Sudan and Nigeria. However, the sensitivities vary depending on the sera dilution factor [49]. In addition, some of the VATs included in this study were absent in some countries, narrowing the geographical user range of the test.

In 2014, Camara *et al.* evaluated the use of blood spotted filter papers as a substitute for serum or plasma in immune trypanolysis test. Blood spotted filter papers can be stored and transported under field conditions, while serum or plasma require a cold chain. Notably, their results showed that use of filter papers resulted in decline in sensitivity, but specificity was not affected [52].

Immunofluorescence Antibody Test (IFAT):

The immunofluorescence antibody test (IFAT) is a serological test for both serum and dried whole blood on filter papers which utilizes IgG antibodies conjugated to fluorescein isothiocyanate. Before the introduction of CATT, the IFAT was the widely applied serological test in the mass screening of *T. b. gambiense* infection in the 1970s [53].

The test procedure includes antigen preparation by inoculating *T. b. gambiense* in guinea pig or albino rats, blood collected after the parasitaemia reaches 2-20 parasites per field; and mixed with heparine-glucose mixture and used to prepare thin blood films consisting of 5-50 parasites/field. This is followed by placing of patient serum on a glass plate with the prepared antigen, the mixture is washed with phosphate buffered saline (PBS) and fluorescein labeled antihuman at a predetermined dilution added. After washing as before, the slides are covered with glycerine-PBS mixture and the slides viewed under UV microscopes [54].

IFAT is easily performed by non specialized laboratory technicians with a read out time of 10 to 30 seconds. However, this test is costly and only amenable to laboratory settings.

3. EMERGING TRENDS IN SERODIAGNOSIS OF HAT

A shift from native antigens to synthetically synthesized or recombinant antigens has recently been the focus in the serodiagnosis of sleeping sickness. Inadequate specificity due to cross reactivity of target antibodies induced by the body against a wide spectrum of native antigens and the necessity to culture infective *T. b. gambiense* in rodents for mass production of native antigens has shifted focus to the synthetic production of peptides that imitate or mimic predominant N terminal domain of LiTat 1.3 and LiTat 1.5 VAT epitopes to replace the native *T. b. gambiense* VSG antigens for screening of populations at risk [30]. The diagnostic performance of the synthetic biotinylated peptides were evaluated in indirect ELISA, and showed high specificities and sensitivities indicating their potential application in diagnosis of *T. b. gambiense* HAT.

Recombinant VSG antigens have also been studied for their diagnostic potentials. Some diagnostic antigens have been expressed in different biological systems and assayed for their diagnostic potential. Recombinant LiTat 1.3 and LiTat 1.5 VATs were expressed as small ubiquitin-like modifier fusion proteins in the yeast *Pichia pastoris* in 2014 by Rogé and colleagues [55], affinity purified and their diagnostic potentials evaluated in ELISA on patient sera. A comparison between the recombinant antigens and the native antigens showed that sensitivities and specificities were significantly lower for recombinant antigens compared to their native counterparts. When combined, however, recombinant antigens recorded sensitivities and specificities of 88.6% and 98.7 % respectively, slightly lower than those of the native antigens (93.2 and 100 respectively) but higher than individual antigens. Thus, the high sensitivity and specificity of the recombinant antigens, especially in combination, form a basis for the replacement of native antigens whose production is costly and unsafe for the laboratory staffs who work on the infective *T. b. gambiense* strain [55].

Rapid diagnostic tests (RDTs) are fast, cheap, and easy to use on whole blood or plasma, do not require electricity or a cold chain, making them applicable in screening populations at risk under field conditions with limited resources. Furthermore, RDTs provide an alternative to the card agglutination test for trypanosomiasis in *T. b. gambiense* endemic foci. Two RDTs for *T. b. gambiense* diagnosis have been developed [56], the HAT Sero-Stip and HAT Sero-K-SeT tests specific for *T. b. gambiense* LiTat 1.3 and LiTat 1.5 specific antibodies. The HAT Sero-Stip is a dipstick while the HAT Sero-K-SeT is a lateral flow device for assaying both blood and plasma, and cost $2.50 each. These tests were evaluated on 296 plasma samples collected from DRC and other samples were obtained from the WHO HAT specimen bank, and showed high sensitivities and specificities.

The HAT Sero K-SET rapid diagnostic test was subsequently developed by Coris Bioconcept and evaluated in DRC on 493 whole blood samples (134 cases and 359 controls), and illustrated high sensitivity (98.5%) and specificity (98.6%) [57]. This kit is stable for long at 4 - 30°C, and the results are ready in 15 min. Another RDT, the SD BIOLINE HAT test, co-developed by Foundation of Innovative New Diagnostics (FIND) and launched in 2012 in DRC, is an immunochromatographic test that uses native antigens to detect antibodies raised against *T. b. gambiense* in blood, serum or plasma This test kit is stable at ambient temperatures, simple and easy to read and is therefore applicable in the field in remote rural areas lacking such amenities as electricity.

Recently, rapid lateral flow diagnostic tests for diagnosis of Gambian HAT have been developed, which use a combination of native LiTat 1.3 and 1.5 VSG antigens [56]. Two prototype RTDs, one developed by Standard Diagnostics Inc (SD) based on two recombinant VSGs (LiTat 1.3 and LiTat 1.5), and the second one developed by BBI Solutions (BBI) based on recombinant ISG65 and native VSG miTat 1.4 (sVSG117) [58] have been evaluated by Sternberg and colleagues [59] and compared to the SD BIOLINE HAT test. This study showed that a predicted performance of two recombinant antigens (LiTat 1.5 and ISG65) combination would have the best sensitivities and specificities comparable to SD BIOLINE HAT and CATT tests, and could be used in the development of a rapid diagnostic test applicable in the field setting [60].

4. CONCLUSION

Antibody – antigen detection tools are of great importance in the screening of populations at risk of trypanosomiasis infection, and also in the epidemiological surveillance of the disease, and will play crucial role in elimination of the disease due to their ease of application in the field setting. In addition, serological tests are more convinient in detecting circulating infections in animals, especially in Rhodesiense HAT prone foci, which are the carriers of the zoonotic *T. b. rhodesiense*.

The advancement of molecular biology, immunology and technology has improved serological diagnosis of African trypanosomiasis (AT), with the introduction of recombinant antigens, synthetic epitopes to replace native antigens and rapid diagnostic tests showing great improvements in serodiagnosis of HAT. A commercial serodiagnostic tool for Rhodesian HAT detection is however still nonexistent, and more focus need to be channeled towards the acute form of the disease, to enable early disease detection and ultimately evade the use of the toxic drug, melarsoprol, the only drug available for late stage disease treatment.

REFERENCES

[1] S. C. Welburn, E. M. Fèvre, P. G. Coleman, M. Odiit, and I. Maudlin, “Sleeping sickness: A tale of two diseases,” Parasitol. Today, vol. 17, no. 1, pp. 19–24, 2001.

[2] E. M. Fèvre, K. Picozzi, J. Fyfe, C. Waiswa, M. Odiit, P. G. Coleman, and S. C. Welburn, “A burgeoning epidemic of sleeping sickness in Uganda,” Lancet, vol. 366, no. 9487, pp. 745–747, 2005.

[3] K. Picozzi, E. M. Fèvre, M. Odiit, M. Carrington, M. C. Eisler, I. Maudlin, and S. C. Welburn, “Sleeping sickness in Uganda: a thin line between two fatal diseases.,” BMJ, vol. 331, no. 7527, pp. 1238–1241, 2005.

[4] N. a. Wardrop, P. M. Atkinson, P. W. Gething, E. M. Fèvre, K. Picozzi, A. S. L. Kakembo, and S. C. Welburn, “Bayesian geostatistical analysis and prediction of rhodesian human African trypanosomiasis,” PLoS Negl. Trop. Dis., vol. 4, no. 12, pp. 1–10, 2010.

[5] M. Odiit, F. Kansiime, and J. C. K. Enyaru, "Duration of symptoms and case fatality of sleeping sickness caused by Trypanosoma brucei rhodesiense in Tororo, Uganda," East Afr. Med. J., vol. 74, no. 12, pp. 792–795, 1997.

[6] P. G. E. Kennedy, "Human African trypanosomiasis of the CNS : current issues and challenges," vol. 113, no. 4, 2004.

[7] M. Radwanska, "Emerging trends in the diagnosis of Human African Trypanosomiasis Emerging trends in the diagnosis of Human African Trypanosomiasis," Parasitology, vol. 137, no. April 2010, p. pp 19771986, 2010.

[8] C. J. Bacchi, "Chemotherapy of human african trypanosomiasis.," Interdiscip. Perspect. Infect. Dis., vol. 2009, p. 195040, 2009.

[9] M. C. Field, "Drug screening by crossing membranes: a novel approach to identification of trypanocides.," Biochem. J., vol. 419, no. 2, pp. e1–e3, 2009.

[10] J. Pépin, F. Milord, A. N. Khonde, T. Niyonsenga, L. Loko, B. Mpia, and P. D. Wals, "Risk factors for encephalopathy and mortality during melarsoprol treatment of Trypanosoma brucei gambiense sleeping sickness," Trans. R. Soc. Trop. Med. Hyg., vol. 89, no. 1, pp. 92–97, Jan. 1995.

[11] J. Nok, "Arsenicals (melarsoprol), pentamidine and suramin in the treatment of human African trypanosomiasis.," Parasitol. Res., vol. 90, no. 1, pp. 71–79, 2003.

[12] M. P. Barrett, D. W. Boykin, R. Brun, and R. R. Tidwell, "Human African trypanosomiasis: pharmacological re-engagement with a neglected disease.," Br. J. Pharmacol., vol. 152, no. 8, pp. 1155–71, Dec. 2007.

[13] G. Priotto, S. Kasparian, D. Ngouama, S. Ghorashian, U. Arnold, S. Ghabri, and U. Karunakara, "Nifurtimox-eflornithine combination therapy for second-stage Trypanosoma brucei gambiense sleeping sickness: a randomized clinical trial in Congo.," Clin. Infect. Dis., vol. 45, no. 11, pp. 1435–1442, 2007.

[14] M. P. Barrett and S. L. Croft, "Management of trypanosomiasis and leishmaniasis," Br. Med. Bull., vol. 104, no. 1, pp. 175–196, 2012.

[15] Chappuis et al., "Options for Field Diagnosis of Human African Trypanosomiasis Options for Field Diagnosis of Human African Trypanosomiasis Franc," Clin. Microbiol. Rev., vol. 18, no. 1, pp. 133–146, 2005.

[16] WHO, "Report of a WHO meeting on elimination of African trypanosomiasis (Trypanosoma brucei gambiense)," World Heal. Organ. WHO Press, no. December, pp. 1754–1824, 2012.

[17] L. Marcello and J. D. Barry, "Analysis of the VSG gene silent archive in Trypanosoma brucei reveals that mosaic gene expression is prominent in antigenic variation and is favored by archive substructure," Genome Res., vol. 17, no. 9, pp. 1344–1352, Sep. 2007.

[18] G. a Cross, "Glycolipid anchoring of plasma membrane proteins.," Annu. Rev. Cell Biol., vol. 6, pp. 1–39, 1990.

[19] G. a Cross, "Cellular and genetic aspects of antigenic variation in trypanosomes.," Annu. Rev. Immunol., vol. 8, pp. 83–110, 1990.

[20] Ferrante and A. C. Allison, "Alternative pathway activation of complement by African trypanosomes lacking a glycoprotein coat.," Parasite Immunol., vol. 5, no. 5, pp. 491–498, 1983.

[21] P. Borst and S. Ulbert, "Control of VSG gene expression sites," Molecular and Biochemical Parasitology, vol. 114, no. 1. pp. 17–27, 2001.

[22] P. Borst, "Antigenic variation and allelic exclusion.," Cell, vol. 109, no. 1, pp. 5–8, 2002.

[23] K. a Lythgoe, L. J. Morrison, A. F. Read, and J. D. Barry, "Parasite-intrinsic factors can explain ordered progression of trypanosome antigenic variation.," Proc. Natl. Acad. Sci. U. S. A., vol. 104, no. 19, pp. 8095–8100, 2007.

[24] V. M. N., J. P. G, and M. E, "Antigenic variation in syringe passaged populations of Trypanosoma (Trypanozoon) brucei," Ann. Soc. belge Med trop, vol. 55, no. 1, pp. 1–23, 1975.

[25] J. D. Barry and C. M. Turner, "The dynamics of antigenic variation and growth of African trypanosomes.," Parasitol. Today, vol. 7, no. 8, pp. 207–211, 1991.

[26] S. a Frank, "A model for the sequential dominance of antigenic variants in African trypanosome infections.," Proc. R. Soc. B Biol. Sci., vol. 266, no. 1426, pp. 1397–1401, 1999.

[27] E. Magnus, T. Vervoort, and N. Van Meirvenne, "A card-agglutination test with stained trypanosomes (C.A.T.T.) for the serological diagnosis of T. B. gambiense trypanosomiasis.," Ann. Soc. Belg. Med. Trop. (1920)., vol. 58, no. 3, pp. 169–176, 1978.

[28] P. Büscher, E. Draelants, E. Magnus, T. Vervoort, and M. N. Van, "An experimental LATEX agglutination test for antibody detection in human African trpanosomiasis," Ann. Soc. beige Med. trop, vol. 71, pp. 267–273, 1991.

[29] V. Lejon, P. Büscher, E. Magnus, a. Moons, I. Wouters, and N. Van Meirvenne, "A semi-quantitative ELISA for detection of Trypanosoma brucei gambiense specific antibodies in serum and cerebrospinal fluid of sleeping sickness patients," Acta Trop., vol. 69, no. 2, pp. 151–164, 1998.

[30] L. Van Nieuwenhove, P. Büscher, F. Balharbi, M. Humbert, T. Dieltjens, Y. Guisez, and V. Lejon, "Identification of mimotopes with diagnostic potential for Trypanosoma brucei gambiense variant surface glycoproteins using human antibody fractions.," PLoS Negl. Trop. Dis., vol. 6, no. 6, p. e1682, Jan. 2012.

[31] E. Elrayah, M. a. Rhaman, L. T. Karamalla, K. M. Khalil, and P. Büscher, "Evaluation of serodiagnostic tests for T.b. gambiense human African trypanosomiasis in southern Sudan," East. Mediterr. Heal. J., vol. 13, no. 5, pp. 1098–1107, 2007.

[32] T. Miezan, F. Doua, P. Cattand, and P. de Raadt, "Evaluation du Testryp CATT appliqué au sang prélevé sur papier filtre et au sang dilué, dans le foyer de trypanosomiase à Trypanosoma brucei gambiense en Côte d'Ivoire.," Bull. World Health Organ., vol. 69, no. 5, pp. 603–606, 1991.

[33] F. Chappuis, A. Pittet, P. A. Bovier, K. Adams, V. Godineau, S. Y. Hwang, E. Magnus, and P. Büscher, "Field evaluation of the CATT/Trypanosoma brucei gambiense on blood-impregnated filter papers for diagnosis of human African trypanosomiasis in southern Sudan," Trop. Med. Int. Heal., vol. 7, no. 11, pp. 942–948, 2002.

[34] P. Truc, V. Lejon, E. Magnus, V. Jamonneau, A. Nangouma, D. Verloo, L. Penchenier, and P. Büscher, "Evaluation of the micro-CATT, CATT/Trypanosoma brucei gambiense, and LATEX/T.b. gambiense methods for serodiagnosis and surveillance of human African trypanosomiasis in West and Central Africa," Bull. World Health Organ., vol. 80, no. 11, pp. 882–886, 2002.

[35] R. Pansaerts, N. Van Meirvenne, E. Magnus, and L. Verhelst, "Increased sensitivity of the card agglutination test CATT/Trypanosoma brucei gambiense by inhibition of complement," Acta Trop., vol. 70, no. 3, pp. 349–354, Jul. 1998.

[36] V. Jamonneau, P. Truc, a. Garcia, E. Magnus, and P. Büscher, "Preliminary evaluation of LATEX/T. b. gambiense and alternative versions of CATT/T. b. gambiense for the serodiagnosis of Human African Trypanosomiasis of a population at risk in Cote d'Ivoire: Considerations for mass-screening," Acta Trop., vol. 76, no. 2, pp. 175–183, 2000.

[37] P. Dukes, W. C. Gibson, J. K. Gashumba, K. M. Hudson, T. J. Bromidge, A. Kaukus, T. Asonganyi, and E. Magnus, "Absence of the LiTat 1.3 (CATT antigen) gene in Trypanosoma brucei gambiense stocks from Cameroon.," Acta Trop., vol. 51, no. 2, pp. 123–134, 1992.

[38] V. M. Nantulya, "TrypTect CIATT®—a card indirect agglutination trypanosomiasis test for diagnosis of Trypanosoma brucei gambiense and T. b. rhodesiense infections," Trans. R. Soc. Trop. Med. Hyg., vol. 91, no. 5, pp. 551–553, Sep. 1997.

[39] V. Lejon, P. Büscher, N. H. Sema, E. Magnus, and N. Van Meirvenne, "Human African trypanosomiasis: a latex agglutination field test for quantifying IgM in cerebrospinal fluid.," Bull. World Health Organ., vol. 76, no. 6, pp. 553–8, Jan. 1998.

[40] V. Lejon, J. Kwete, and P. Büscher, "Short communication: Towards saliva-based screening for sleeping sickness?," Trop. Med. Int. Heal., vol. 8, no. 7, pp. 585–588, 2003.

[41] B. M. Greenwood and H. C. Whittle, "CEREBROSPINAL-FLUID IgM IN PATIENTS WITH SLEEPING-SICKNESS," Lancet, vol. 302, no. 7828, pp. 525–527, Sep. 1973.

[42] H. C. Whittle, B. M. Greenwood, D. E. Bidwell, A. Bartlett, and A. Voller, "IgM and antibody measurement in the diagnosis and management of Gambian trypanosomiasis.," Am. J. Trop. Med. Hyg., vol. 26, no. 6 Pt 1, pp. 1129–34, Nov. 1977.

[43] V. Lejon, D. Legros, M. Richer, J. a. Ruiz, V. Jamonneau, P. Truc, F. Doua, N. Djé, F. X. N'Siesi, S. Bisser, E. Magnus, I. Wouters, J. Konings, T. Vervoort, F. Sultan, and P. Büscher, "IgM quantification in the cerebrospinal fluid of sleeping sickness patients by a latex card agglutination test.," Trop. Med. Int. Health, vol. 7, no. 8, pp. 685–92, 2002.

[44] WHO, "Parallel evaluation of serological tests applied in african trypanosomiasis: a WHO collaborative study.," Bull. World Health Organ., vol. 54, no. 2, pp. 141–7, Jan. 1976.

[45] B. J. and R. E. J. Knapen F. V., "Enzyme linked immunosorbent assay (ELISA) and its application in the serodiagnosis of African Trypanosomiasis," Ann. Soc. belge Med trop, vol. 57, no. 4–5, pp. 281–292, 1977.

[46] Voller, D. Bidwell, and A. Bartlett, "A serological study on human Trypanosoma rhodesiense infections using a micro-scale enzyme linked immunosorbent assay.," Tropenmed. Parasitol., vol. 26, no. 2, pp. 247–51, Jun. 1975.

[47] E. Komba, M. Odiit, D. B. Mbulamberi, E. C. Chimfwembe, and V. M. Nantulya, "Multicentre evaluation of an antigen-detection ELISA for the diagnosis of Trypanosoma brucei rhodesiense sleeping sickness.," Bull. World Health Organ., vol. 70, no. 1, pp. 57–61, 1992.

[48] V. M. Nantulya, "An antigen detection enzyme immunoassay for the diagnosis of rhodesiense sleeping sickness," Parasite Immunol., vol. 11, no. 1, pp. 69–75, Jan. 1989.

[49] N. Van Meirvenne, E. Magnus, and P. Büscher, "Evaluation of variant specific trypanolysis tests for serodiagnosis of human infections with Trypanosoma brucei gambiense," Acta Trop., vol. 60, no. 3, pp. 189–199, 1995.

[50] V. Jamonneau, B. Bucheton, J. Kaboré, H. Ilboudo, O. Camara, F. Courtin, P. Solano, D. Kaba, R. Kambire, K. Lingue, M. Camara, R. Baelmans, V. Lejon, and P. Buscher, "Revisiting the immune trypanolysis test to optimise epidemiological surveillance and control of sleeping sickness in West Africa," PLoS Negl. Trop. Dis., vol. 4, no. 12, pp. 1–8, 2010.

[51] W. K. Isharaza and N. Van Meirvenne, "Variant-specific trypanolytic antibodies in sera from patients infected with Trypanosoma brucei rhodesiense.," Bull. World Health Organ., vol. 68, no. 1, pp. 33–7, 1990.

[52] O. Camara, M. Camara, V. Lejon, H. Ilboudo, H. Sakande, M. Léno, P. Büscher, B. Bucheton, and V. Jamonneau, "Immune trypanolysis test with blood spotted on filter paper for epidemiological surveillance of sleeping sickness," Trop. Med. Int. Heal., vol. 19, no. 7, pp. 828–831, 2014.

[53] WHO, "Report on African trypanosomiasis (sleeping sickness)," no. June, pp. 4–8, 2001.

[54] M. Wery, P. Van Wettere, S. Wery-Paskoff, M. N. Van, and M. M, "The diagnosis of human african trypanosomiasis (T. garnbiense) by the use of the fluorescent antibody test. 2. First results of field application," Ann. Soc. beige Med. trop, vol. 50, no. 6, pp. 711–730, 1970.

[55] S. Rogé, L. Van Nieuwenhove, M. Meul, A. Heykers, A. Brouwer de Koning, N. Bebronne, Y. Guisez, and P. Büscher, "Recombinant Antigens Expressed in Pichia pastoris for the Diagnosis of Sleeping Sickness Caused by Trypanosoma brucei gambiense," PLoS Negl. Trop. Dis., vol. 8, no. 7, pp. 1–9, 2014.

[56] P. Büscher, Q. Gilleman, and V. Lejon, "Rapid diagnostic test for sleeping sickness.," N. Engl. J. Med., vol. 368, no. 11, pp. 1069–70, 2013.

[57] P. Büscher, P. Mertens, T. Leclipteux, Q. Gilleman, D. Jacquet, D. Mumba-Ngoyi, P. P. Pyana, M. Boelaert, and V. Lejon, "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. Heal., vol. 2, no. 6, pp. 359–363, 2014.

[58] L. Sullivan, J. Fleming, L. Sastry, A. Mehlert, S. J. Wall, and M. a J. Ferguson, "Identification of sVSG117 as an Immunodiagnostic Antigen and Evaluation of a Dual-Antigen Lateral Flow Test for the Diagnosis of Human African Trypanosomiasis," PLoS Negl. Trop. Dis., vol. 8, no. 7, 2014.

[59] M. Sternberg, M. Gierliński, S. Biéler, M. a. J. Ferguson, and J. M. Ndung'u, "Evaluation of the Diagnostic Accuracy of Prototype Rapid Tests for Human African Trypanosomiasis," PLoS Negl. Trop. Dis., vol. 8, no. 12, p. e3373, 2014.

[60] HAT and OND, "developing new diagnostic tests for HAT.," FIND Communications, 2013. [Online]. Available: http://www.finddiagnostics.org/export/sites/default/resource-centre/find_reports/pdfs/developing_new_ diagnostic_ tests_for_hat_may2013.pdf. [Accessed: 11-Aug-2015].