DIAGNOSTIC GAPS AND RECOMMENDATIONS FOR HUMAN AFRICAN TRYPANOSOMIASIS

Assessment of user needs, use cases, and the diagnostic landscape

PATH

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Acronyms

CATT	card agglutination test for trypanosomiasis
CTC	capillary tube centrifuge
CSF	cerebral spinal fluid
DRC	Democratic Republic of the Congo
ELISA	enzyme-linked immunosorbent assay
FIND	Foundation for Innovative New Diagnostics
HAT	human African trypanosomiasis
IF	immunofluorescence
IM	intramuscular
IV	intravenous
IgM	immunoglobulin M
ISG	invariant surface glycoprotein
LAMP	loop-mediated isothermal amplification
mAECT	mini anion exchange centrifugation technique
MSF	Médecins Sans Frontières
NASBA	nucleic acid sequence-based amplification
NECT	nifurtimoxeflornithine combination therapy
NSSCP	National Sleeping Sickness Control Program
NTD	neglected tropical disease
PCR	polymerase chain reaction
RDT	rapid diagnostic test
RNA	ribonucleic acid
VSG	variant surface glycoprotein
WBC	white blood cell
WHO	World Health Organization

Executive summary

Human African trypanosomiasis (HAT) is a neglected tropical disease caused by infection with the trypanosome parasite that is spread through the bite of the tsetse fly. HAT, commonly known as sleeping sickness, is a deadly disease found in sub-Saharan Africa, where over 70 million people are at risk of infection. It is almost invariably fatal if patients do not receive treatment. HAT is a focal disease that can be prevented with targeted control measures, but instability and neglect of control efforts have resulted in epidemics. Recently, the number of new cases has declined dramatically. In 2014, 3,796 cases were reported—the lowest number in 75 years.

The World Health Organization (WHO) has targeted HAT for elimination as a public health problem in at least 90 percent of foci by the year 2020. However, current diagnostic tools for HAT may not be sufficient to support elimination. The London Declaration on Neglected Tropical Diseases (NTDs) identified a need to develop and incorporate new diagnostic tools into ongoing elimination efforts in order to achieve the WHO target.

In support of the London Declaration goals, PATH aims to catalyze engagement of the diagnostics industry and product development efforts. As part of this work, PATH conducted a diagnostic landscape analysis to identify gaps and evaluated current and nascent HAT diagnostics that may provide solutions. We conducted literature reviews and interviews with key stakeholders to identify use cases for HAT diagnostics, understand current practices, and analyze progress toward more robust diagnostics across different biomarkers. The decline in prevalence, alongside persistent challenges with disease confirmation and treatment, will have significant implications for development of new diagnostic tools and methods to support elimination goals. Current work to improve screening methods and make available better tools for confirming and managing HAT cases will be instrumental in addressing current diagnostic gaps. Based on the findings of this analysis, PATH developed the following recommendations:

1. **Support strategies to ensure sustainability of HAT surveillance.** Given the declining prevalence, future diagnostic tools should be designed to be appropriate for passive surveillance and used in primary care settings. Research is needed on how best to integrate new tools into policy and practice.
2. **Support development of improved tools for disease confirmation.** Current parasitology methods are limited by sensitivity and reproducibility, and the lumbar puncture method is invasive and discourages patients from seeking or continuing treatment for HAT. Field-friendly, low-cost, sensitive diagnostics that are acceptable to patients are needed.
3. **Support development of tools and other interventions that will reduce barriers to disease staging and treatment monitoring.** New tools and methods are needed to guide treatment in order to achieve improved case management, reduce mortality, and support elimination goals.

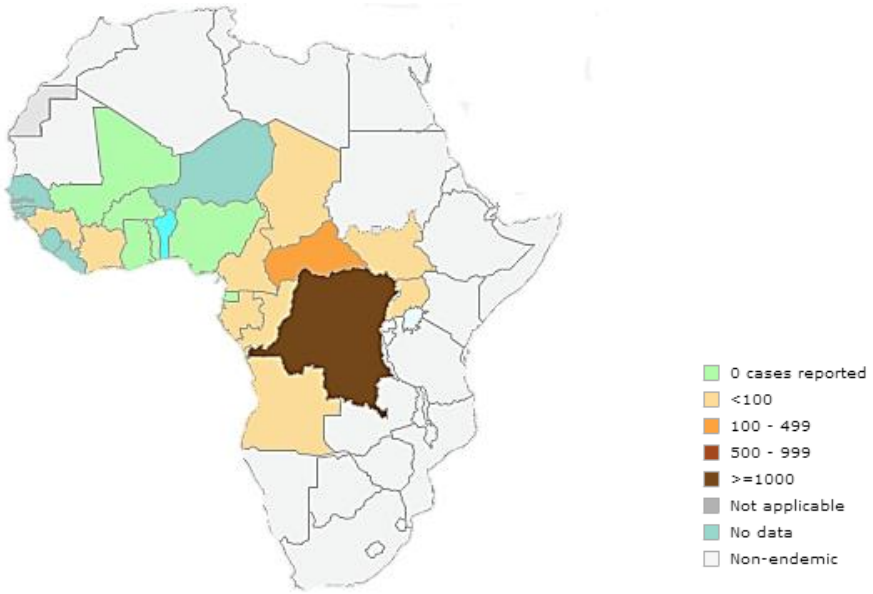
Introduction

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a deadly parasitic disease endemic in 36 countries in rural sub-Saharan Africa. HAT is a complex disease with two causative agents and two distinct clinical manifestations. *Trypanosoma brucei gambiense (T. b. gambiense)* causes a more chronic infection that is responsible for over 90 percent of cases, whereas *Trypanosoma brucei rhodiense, (T. b. rhodiense)* causes an acute infection that is responsible for a smaller proportion of the overall HAT disease burden.[1] Over 70 million people are at risk of contracting HAT, and 21 million people are estimated to live in the highest-risk areas (i.e., where more than one case per 10,000 inhabitants a year is reported).[2,3] New cases of gambiense HAT are found primarily in central Africa, as shown in Figure 1. The Democratic Republic of the Congo (DRC) reports the highest number of cases.[4]

HAT is a focal disease, limited to defined geographic areas categorized into areas of high, moderate, or low intensity of transmission.[2] Within these foci, HAT prevalence has been highly dependent on the intensity of control efforts, which have varied over time. Historically, civic and political instability and a neglect of control efforts have led to devastating epidemics, which in turn have prompted cyclical reinvestments in surveillance and control.[5]

Recent years have seen a dramatic decline in the number of new cases of HAT.[5] In 2014, 3,796 cases were reported (down from 6,314 in 2013), the lowest level of reported cases in 75 years. However, this likely an underestimate due to the fact that HAT foci are very rural and remote, cases may be missed, and there may be limited participation in active screening campaigns, a critical mechanism for diagnosis and treatment.[6,7,8]

Figure 1. Number of new cases of human African trypanosomiasis *T. b. gambiense* reported to the WHO, 2014.[9]



National Sleeping Sickness Control Programs (NSSCPs) in endemic countries, working in coordination with the World Health Organization (WHO), have prioritized HAT for elimination.[10] The 2012 WHO

Neglected Tropical Disease (NTD) Roadmap put forward the initial goal for elimination of HAT as a public health problem, which was subsequently defined as less than 1 new case per 10,000 population in at least 90 percent of HAT foci annually and fewer than 2,000 new cases in Africa by 2020.[11,12] Shortly after the release of the NTD Roadmap, 20 public and private institutions that support global health and international development—including pharmaceutical companies, donors, governments from endemic countries, nonprofit organizations, and others—joined the efforts to reach the 2020 goals for 10 of the 17 diseases, in a document known as the London Declaration on Neglected Tropical Diseases.[6] The London Declaration represents a commitment from these institutions to sustain, expand, and extend programs that ensure the necessary supply of drugs and other interventions to achieve the NTD Roadmap goal for elimination of HAT as a public health problem by 2020.

The London Declaration 3rd Report identified a need to sustain progress by developing and incorporating new diagnostic tools into ongoing elimination efforts.[3] In response to this need, PATH conducted a diagnostic landscape analysis to identify gaps and evaluated current and emerging HAT diagnostics that may provide solutions. This analysis was informed by a review of literature and interviews with stakeholders in the HAT community. The literature review included peer-reviewed publications, policies and guidelines, documents from WHO expert meetings and a review of the technology landscape. Key organizations in the HAT community were identified through their roles in global and country-level programs, academic research, participation in consultative meetings, and through referral from other key stakeholders. Identified stakeholders were interviewed with a semi-structured interview guide focusing on disease progression and treatment, access to care, diagnostic use cases and user needs, and existing technologies and technology gaps. This analysis focused on the chronic form of HAT caused by *T. b. gambiense,* as it contributes to the majority of disease burden. Information from the literature review, product development landscape, and stakeholder interviews was compiled to:

- Identify use cases and understand current diagnostic practices and tools associated with each use case.
- Analyze progress toward robust diagnostics for HAT across different biomarkers.
- Develop recommendations for steps to improve the availability, access, and adoption of HAT diagnostic tools.

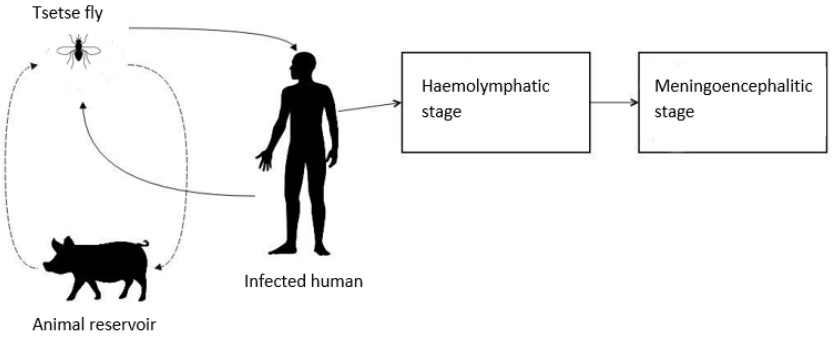
Diagnostic landscape

Disease course and transmission

HAT is caused by *Trypanosoma brucei,* which is transmitted through the tsetse fly vector (see disease course and progression in Figure 2). Humans are considered the epidemiologically important reservoir for *T. b. gambiense*, whereas *T. b. rhodiense* resides in domestic and wild animals.[6] This distinction has important implications for control strategies. Vector control plays an important role for both *T. b. gambiense* and *T. b. rhodiense* but, due to the animal reservoir, *T. b. rhodiense* is not being considered for elimination. [6,13,14]

Once the infected tsetse fly bites a human host, parasites multiply at the site of the bite. Waves of trypanosomes protected by a variant surface glycoprotein (VSG) invade the bloodstream via the lymphatic circulation. The host's immune system recognizes the glycoprotein and produces antibodies, which leads to decreased parasitemia; however, some trypanosomes express a unique VSG type that is not recognized by current host antibodies and therefore are able to evade immune recognition and continue to proliferate.[1,6] It is generally accepted that, given the long duration of infection, humans are the primary reservoir for gambiense HAT. There is some evidence that chronic carriers or asymptomatic individuals infected with trypanosomes may play a role in continued disease transmission, which may explain the reemergence of HAT in foci where the disease was previously thought to be eliminated.[15]

Figure 2. Disease course and progression of human African trypanosomiasis.



Gambiense HAT progresses in two stages, and clinical signs vary depending on the duration of the infection. In stage one—the haemolymphatic stage—an initial asymptomatic period is followed by the onset of nonspecific symptoms such as headache, malaise, joint pain, weight loss, fatigue, and intermittent fever. The parasites spread to the systemic organs, including the spleen, heart, liver, eyes, and endocrine organs.[16] An enlarged spleen or enlarged cervical lymph nodes may be present.[17] In stage two—the meningoencephalitic stage—trypanosomes invade the central nervous system and, as a result, neuropsychiatric symptoms and signs become more prominent. Sleep disturbances, including uncontrollable urges to sleep and a reversal of the normal sleep-wake cycle, typify the second stage of the disease.[16] Other features include psychiatric, motor, and sensory abnormalities.[18]

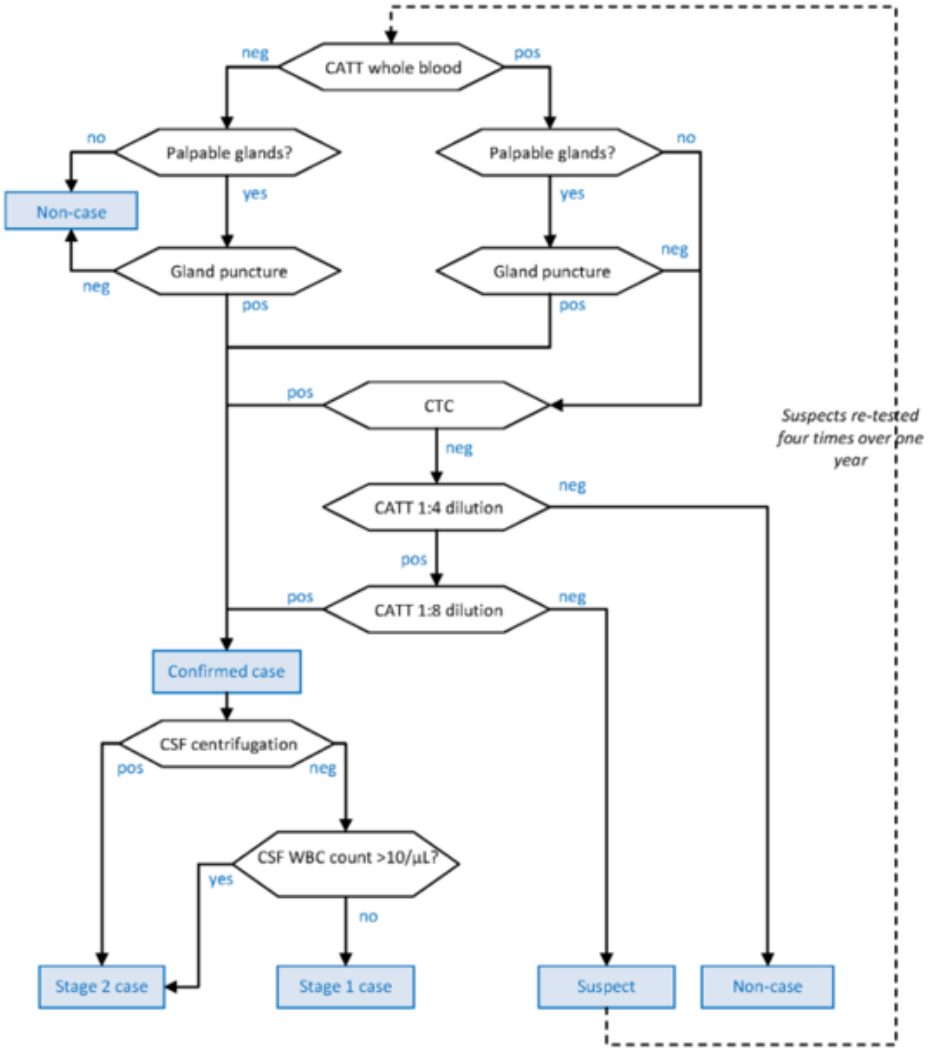
A study of over 2,000 gambiense HAT patients describes the frequency of self-reported symptoms as related to the duration of the infection. It found that adenopathy, headaches, and sleeping disorders are the most common early-stage symptoms, and sleeping disorders and motor weakness are the most common late-stage symptoms.[19] If untreated, patients die from wasting, seizures, organ failure, or dysfunction of the immune system.[17] The duration of both the haemolymphatic and meningoencephalitic stages of the disease are variable and can last from months to years.[20] HAT has a near 100 percent case fatality rate.[1]

Diagnosis

The diagnosis of HAT is currently based on complex algorithms involving screening, confirmation, and disease staging that vary by country, see Figure 3 for an example used by Médecins Sans Frontières

(MSF) teams in the Republic of Congo.[14,21] These algorithms all include initial screening using a noninvasive serologic test followed by parasitological confirmation in body fluids such as blood, lymph node aspirate, or chancre aspirate. Once the infection is confirmed though visualization of the parasite, lumbar puncture—an invasive procedure that can be painful and poses risks for complications—is required to obtain a sample of cerebral spinal fluid (CSF) for disease staging. Examination of CSF to determine appropriate stage-specific treatment options may be required if disease relapse occurs after treatment, thus necessitating additional lumbar punctures.

Figure 3. An example of diagnostic algorithm used by MSF in the Republic of Congo.[21]



CATT refers to card agglutination test for trypanosomiasis
CTC refers to capillary tube centrifuge, a parasite concentration method
CSF refers to cerebral spinal fluid, WBC refers to white blood cells

National Sleeping Sickness Control Programs (NSSCPs) have long used active surveillance as a critical component of their HAT control strategies. One of the consequences of declining disease prevalence is that, in some locations, the cost-effectiveness of active screening is decreasing and thus more passive

case-detection strategies are being explored. As the disease approaches elimination targets, vertical approaches to case identification and management may no longer be appropriate or cost-effective.[10,22] Integrating HAT case diagnosis and management into existing health systems and structures will necessitate changes in diagnostic policies, practices, and tools.[23]

Treatment

HAT treatment options are based on the results of disease staging. Stage one gambiense HAT is treated with daily intramuscular (IM) injections of pentamidine for 7 to 10 days.[24] Stage two disease treatment is more complex and involves drugs with greater toxicity and inadequate efficacy, delivered via IM or intravenous (IV) injections over multiple days. Table 1 outlines current treatment options and their associated regimens, efficacy, and fatality rates. High rates of treatment failure and concerns over the development of drug resistance prompted the use of a combination therapy involving nifurtimox and eflornithine (NECT), which was successfully introduced and used to treat over 95 percent of second stage gambiense HAT in 2013.[24,25]

Table 1. Treatment options for second stage gambiense HAT. Adapted from Eperon et al. and Babokhov et al.[24,26]

Drug	Common regimens, drug features, advantages and disadvantages	Percent cure and in-hospital case fatality rates
Pentamidine	• IM injections every day for 7–10 days • Effective for stage one only	-
Suramin	• 5 IV injections every 3–7 days over a period of 4 weeks • Effective for stage one only	-
Melarsoprol	• 10 daily injections, toxic with high rates of treatment failure • Effective at both disease stages • Toxic • Trypanosomal resistance reported to be as high as 30%	Cure rate: 62.9–92.0 In-hospital case fatality rate: 2.2–6.5
Eflornithine	• 4 IV infusions per day for 14 days • Time-consuming	Cure rate: 84.0–95.6 In-hospital case fatality rate: 0.4–3.1
Nifurtimox	• Oral delivery • Variable efficacy	Cure rate: 44.0–88.0 In-hospital case fatality rate: 0.0–6.3
Nifurtimoxeflornithine combination therapy (NECT)	• 7 days of IV eflornithine and 10 days of oral nifurtimox for stage two treatment • High cure rate for both stages and low rate of adverse effects	Cure rate: 93.5–98.4 In-hospital case fatality rate: 0.0–1.6

There are supply-side challenges to successful HAT treatment. NECT is included on the WHO Essential Medicines List, and Bayer and Sanofi pharmaceutical companies have an established agreement with the WHO to donate necessary drugs. However, the donation does not include materials for intramuscular or intravenous delivery, which require additional logistics and procurement costs for national programs. Patients also face barriers to access and successful treatment. Drugs are often administered at treatment centers where patients must remain for the duration of the treatment. Patients and their families incur significant costs, including consultation and hospitalization fees, laboratory tests, travel expenses, and time away from work. A study in the DRC found that the cost of HAT treatment to each household was equivalent to 5 months of income.[27] High rates of treatment failure and relapse mean that treatment follow-up is critical.

Adherence to post-treatment follow-up for stage two gambiense HAT, which involves a lumbar puncture every 6 months for 24 months to assess parasitemia in CSF, is extremely low. One evaluation of an NSSCP found that less than three percent of patients had a recorded outcome after the 24-month follow-up period and less than one percent of patients attended all four follow-up appointments.[28]

As NSSCPs integrate HAT diagnoses and treatment into general health services, less complex, safer and more effective drugs will be needed. Ideally, HAT treatment would be affordable, easily administered through oral delivery, shelf stable for up to three years in target settings, and require a shortened 7–10 day course.[29] New drugs are currently in development. The successful development and introduction of new drugs will have important implications for the future of HAT diagnostic algorithms and tools.[24]

Specifically, a drug that is effective in treating both stages of HAT could eliminate the need for disease staging—thus allowing for treatment initiation following disease confirmation. Removing the painful and invasive lumbar puncture from diagnostic algorithms could also improve compliance with follow-up testing as part of post-treatment monitoring.

Use cases

The declining prevalence of HAT and the integration of HAT diagnosis and care into routine health services is important to support elimination efforts. Any future diagnostic tools must be designed with these primary care settings in mind. This analysis identified four unique use cases for HAT diagnostics (Figure 4): screening of suspected cases, disease confirmation, disease staging, and treatment monitoring.

Figure 4. Use cases for human African trypanosomiasis (HAT) diagnostics.

Screening

Historically, mobile teams have used active screening methods to identify suspected HAT cases and detect seropositive individuals. Mobile teams commonly use the card agglutination test for trypanosomiasis (described below) to identify suspected cases of HAT.[30] However, due to the remote nature of some HAT foci and potential reluctance to participate, active screening may not reach all people. Moreover, due to the declining prevalence of HAT and fewer resources available for vertical HAT programs, the concept of integrating screening into primary care services is becoming increasingly important.[31,32,33] Recently, more suspected cases—nearly half of cases in the period between 2000 and 2012—are being identified through passive screening.[30] As such, the characteristics of diagnostics used for case identification, including field-appropriateness and the flexibility to allow for single use as opposed to batch testing, are increasing in importance, particularly for tools intended for use in passive screening by primary health care providers.

Disease confirmation

Once a person suspected to have HAT has a positive screening test, the disease must be confirmed through observing trypanosomes in a body fluid.[14] MSF diagnostic algorithms recommend systematic gland palpation and, in cases where glands are palpable, a gland puncture. Positive cases are followed by parasitological and serology screening, with CSF screening as the final procedure for diagnosing infection.[21] However, confirmation using body fluids can be challenging particularly when levels of parasitemia are too low for available parasitological methods to detect with sufficient sensitivity. A negative parasitological result after a positive serological test does not necessarily indicate absence of infection, and there is some uncertainly as to whether or not to treat these cases.[34] Tests may have to be repeated over time to achieve an accurate diagnosis. Stakeholders report that confirmation may be done in the field or that suspected cases may be referred to a health facility with laboratory capacity—thus presenting an opportunity for loss to follow-up.

Disease staging

Next, in order to inform the treatment strategy, it is critical to determine if trypanosomes have crossed the blood-brain barrier and are present within the CSF. A lumbar puncture is generally performed immediately after disease confirmation through parasitological diagnosis or when severe clinical signs may justify such an invasive procedure.[14] The disease stage is defined by the presence of trypanosomes or the number of white blood cells (WBC) in the CSF. Parasitological methods to demonstrate the presence of trypanosomes in CSF are not sufficiently sensitive. As such, WBC count of CSF is the most commonly used criterion for staging.[14] The WHO guidelines classify patients with fewer than five WBC per microliter in the CSF as first stage and patients with more than five WBC per microliter or trypanosomes in the CSF as second stage; however, there is a lack of consensus on the most appropriate threshold.[14]

Given the risk, complexity, and invasiveness of a lumbar puncture, the development of an improved marker for disease staging was identified as a high priority in HAT diagnostics by global expert stakeholders.[6] However, technical constraints, including the availability of well-validated host

biomarkers, remain a major barrier to the development of next-generation diagnostics for staging. Additionally, considerable effort is underway on the development and evaluation of next-generation drugs that may be effective for both disease stages. Thus, eventually treatment decisions may not require that a staging diagnosis be performed.

Treatment monitoring

As serologic tests can remain positive for up to three years following treatment, a marker of active infection is used to monitor treatment efficacy and disease relapse. This is needed to inform decisions to re-treat or treat with second-line therapies.[35] Although symptoms may recur, detection of parasites is necessary to confirm true disease relapse. As parasite levels may be extremely low, patients may need to be tested multiple times to confirm results, which presents additional opportunities for loss to follow-up. Current treatment outcomes and compliance with treatment follow-up are suboptimal.[28]

Current diagnostic tools

All currently available tools and methods used in screening, case confirmation, staging, and treatment monitoring have strengths and limitations. See Table 2 for an overview of the HAT diagnostic landscape.

Serology

Serologic methods detect the host antibody response to *T. brucei* antigens. Most tests detect antibodies against selected antigen types (LiTat 1.3 and LiTat 1.5) of the variant surface glycoprotein (VSG).[36] Most gambiense HAT patients are reactive against at least one of these types, but these tests are not reliable for rhodiense HAT detection due to a higher level of VSG variation of these parasites. Currently, there are limited serologic test options to screen for rhodiense HAT outside of more complex immunofluorescence techniques. For gambiense HAT, multiple formats of serologic tests are available. The most common tool currently used in screening is the card agglutination test for trypanosomiasis (CATT), as it is simple to use, reliable, and relatively inexpensive.

Additionally, the test is amenable for batch testing used in mass screening by survey teams and has been reported to have good sensitivity and specificity.[6] However, the format of the CATT may become less optimal as the prevalence of HAT decreases to low levels in many endemic areas and surveillance strategies shift to more passive surveillance. For instance, the CATT requires infrastructure including a cold chain and additional equipment, such as rotators, that may not be available in all routine health care facilities in endemic locations where testing is needed. Also, the format of the current kit is not amenable to single use, as it includes 50 tests that once opened must be used within a week if stored cold or within 8 hours if not.[17]

First-generation point-of care rapid diagnostic tests (RDTs) for HAT developed in efforts led by FIND have recently become available and show great promise. A second-generation RDT for HAT that moves away from use of native LiTat antigens to recombinant forms of these antigens is also currently under development to further improve production and sustainability. The sensitivity of the RDTs has been reported to be good (99 percent), but specificity has been more variable (88 to 99 percent) and, while all

RDTs performed fairly well against characterized panels, the reported performance of these tests varied among different evaluation studies.[37,38,39] Reported differences in study outcomes could potentially be due to confounders including reader variability and factors such as regional differences of study populations including disease prevalence, circulating HAT strains, coinfections, and previous exposure to animal trypanosomes. In addition, a multiplex text that includes HAT and malaria is currently under development. Multiplex tests could strengthen HAT screening by leveraging the resources committed to other disease surveillance efforts in endemic areas.

Parasitology

Confirmation of parasites in lymph node aspirate, blood, or CSF is required to confirm a case of HAT. Microscopy on lymph node aspirate and blood is used to visualize the parasite.[40] These methods are complex and labor intensive and may be limited to laboratory environments with skilled microscopists and proper equipment. Due to the often low and intermittent parasite density in chronic infections associated with gambiense HAT, confirmation often requires concentration of parasites in a specimen prior to microscopic examination. Concentration methods include high-speed centrifugation techniques such as the micro-hematocrit centrifugation technique and quantitative buffy coat test.[17]

More recently, kits for performing the mini anion exchange centrifugation technique (mAECT) method, which involves running a whole blood sample over gel filtration column and then transferring the sample to a glass centrifuge tube for low-speed spin to concentrate parasites, have been made available for distribution in HAT-endemic countries to improve access to parasite concentration techniques in confirmatory testing.[41] This method may provide higher sensitivity and is cheaper than molecular tests such as polymerase chain reaction (PCR), reverse transcription PCR, and nucleic acid sequence-based amplification (NASBA), but it still adds additional cost and complexity.[42] Lastly, lysis of red blood cells can also be used improve detection of parasites and does not seem to affect the integrity of the parasites.[43]

Molecular

Molecular tests indirectly assess infection through detection of parasite nucleic acids. Molecular tests offer possible alternatives to parasitological assays for disease confirmation, staging, and test for cure. These methods are not currently recommended by the WHO, as they may detect other non-pathogenic trypanosome infections and inactive infections, which could limit the ability to use these tests for informing treatment decisions. However, these methods still may have value as an initial confirmation of serologic screening results prior to use of more complex and labor-intensive parasitological methods. The average diagnostic sensitivity and specificity are 98.7 and 97.4 percent, respectively, but most PCR assays have only been evaluated in phase one trials.[44] Isothermal assays such as loop-mediated isothermal amplification (LAMP) are also in development and evaluation and could potentially be used as a simpler alternative to conventional molecular methods such as PCR to improve access in HAT-endemic settings. Follow-on field studies will further assess the feasibility of these new molecular tests in use for diagnosis of HAT in target settings.[45]

Other platforms

Other diagnostic platforms for HAT have been explored but, given the need to prioritize high-impact diagnostics and due to possible performance issues and technical complexities, not all possible platforms have been pursued. For instance, prior efforts have explored the use of new antigen detection tests to differentiate between active and cured infection to support better treatment monitoring. An evaluation of an antigen-based CATT test, the TrypTectCIATT, showed high sensitivity but questionable specificity.[17] For disease staging as well as treatment follow-up, the use of host biomarkers including neopterin have been evaluated, including some in point-of-care RDT formats. While initial assessments were promising, the tests have not advanced as quickly as other prioritized tests for HAT, including the serologic RDTs. This may be due in part the fact that to results using human biomarkers are less specific to HAT infection; therefore, using host biomarkers may not be straightforward for informing case management decisions.[46,47]

Table 2. Overview of human African trypanosomiasis (HAT) diagnostic landscape.

	Diagnostic platform					
	Clinical	**Serology**	**Parasitology**	**Antigen**	**Molecular**	**Host biomarker**
Biomarker	Clinical disease	Host antibody to *T. brucei* antigens	Whole parasite	*T. brucei* antigens	DNA, RNA	Host proteins
Formats	Clinical examination	RDT, CATT, IF, ELISA [#], immune trypanolysis	Microscopy	RDT, TrypTectCIATT, ELISA [#],	PCR, isothermal	Immunoassay
Diagnostic measure	Disease	Exposure	Infection	Infection	Infection	Clinical
Stage of product development	N/A	Available	Research and development	Research and development	Research and development	Research and development
Example products	N/A	• CATT (ITM) • SD Bioline HAT (SD) • HAT Sero-K-Set (Coris BioConcept)	• mAECT (INRB/ITM) • Primostar iLED microscope (Zeiss/FIND)	• Nanobodies (FIND/ U. Brussels)	• LAMP (FIND/Eiken)	• FIND/U. Geneva
Description	• Clinical presentation depends on parasite species, stage, and host • Common symptoms include headache, intermittent fever, sleep disorder; neurologic, and death	• Detects host antibodies against parasite antigens • Antibodies usually detectable within a few days of infection and can persist for life • Biomarkers include: selected antigen types (LiTat 1.3 and LiTat 1.5) of the variant surface glycoprotein (VSG); invariant surface glycoprotein (ISG) 75, ISG65, ISG64	• Directly detects the presence of parasite in patient's blood or biopsy • Provides a definitive diagnosis of infection or treatment failure • Sensitivity can be parasite confirmation	• Detects parasite antigens in blood • Development remains limited compared to other platforms • Biomarkers include: paraflagellar rod protein, VSG	• Detects parasite nucleic acid in blood or biopsy • PCR, LAMP, and NASBA based assays under development • Biomarkers include: satellite DNA, rRNA, *T. b. gamiense*–specific target (single-copy TGSGP gene), *T. b. rhodiense*–specific target (SRA gene), spliced leader RNA	• Still in early discovery phase • Detects and quantitates the levels of host proteins in CSF • Biomarker candidates include: white blood cells, IgM, neopterin, CXCL10, CXCL13, ICAM-1, • VCAM-1, MMP-9

	Clinical	Serology	Parasitology	Antigen	Molecular	Host biomarker
Proposed Target use	• Screening	• Screening	• Disease confirmation • Disease staging • Treatment monitoring	• Disease confirmation • Treatment monitoring	• Disease confirmation • Disease staging • Treatment monitoring	• Disease staging • Treatment monitoring
Advantages	• Low cost • Accessibility • Potential use in conjunction with diagnostic to inform care	• CATT is approved for use by WHO • Field-deployable formats • Sensitivity 91%–99% • Inexpensive RDT format shows promise for use in both active and passive detection • CATT is amenable to mass screening	• Provides definitive diagnosis of infection • Sensitive for acute phase • Sensitive for *T. b. rhodiense*	• Provides definitive diagnosis of infection • Potential for field-deployable platforms • Nanobodies could be used for detection of non-surface proteins to improve performance (potential commercial interest w/Standard Diagnostics)	• Potentially easier use and higher throughput than traditional parasitology • High analytical sensitivity possible (>1 parasite/mL reported) • Good sensitivity/specificity possible (>97%) • May allow for further speciation	• Potential to move away from parasite detection with limited sensitivity for staging or treatment monitoring • Standard Diagnostics engaged to help develop test
Limitations	• Case may be asymptomatic • Clinical signs and symptoms are unspecific and vary • Two disease stages, but clinical symptoms often overlap	• Limited use in rhodiense HAT • CATT is the only WHO-approved test, but is costly and requires cold chain and equipment • Low PPV in low prevalence areas • Potential for strain selection by test • Variable specificity (88%–99%)	• Sampling • Limited sensitivity during chronic phase or post-treatment • Expensive • Complex and labor intensive	• Limited sensitivity • Variation in parasite surface antigens	• Sampling • Not WHO-recommended • Restricted to reference labs • Complex and labor intensive • Limited commercial availability • Expensive • No consensus on use of DNA as measure of infection	• Invasive sampling • Early research phase with limited progression; no definitive marker • Biomarker is only suggestive (limited specificity)

Conclusions

Current diagnostic tools and practices may not be sufficient to achieve the goals for the elimination of HAT set forth in the London Declaration and WHO NTD Roadmap. Existing tools and methods may be inadequate to satisfy critical use cases, and they continue to rely on invasive and painful procedures to distinguish between the haemolymphatic and meningoencephalitic stages of the disease. Moreover, as control efforts move toward elimination, existing diagnostic use cases may change and require new or adapted tools. As described, the continued efforts of the HAT diagnostics research and development community has the potential to improve screening with promising new rapid tests, offer better microscopy tools, and introduce new diagnostic platforms using molecular methods. These innovations—along with related operations research and advocacy—will be instrumental in addressing current diagnostic needs. To support this work and other elimination efforts, PATH offers the following recommendations to the HAT research community.

1. **Support strategies to ensure sustainability of HAT surveillance.**

In the face of declining prevalence, cost-effective strategies are critical to sustain progress toward elimination goals. Current active case finding should be continued. In addition, new strategies may also be needed to support passive surveillance. Screening tools should be low cost and low complexity in order to improve access by mobile surveillance teams and usage in low-level health facilities. The sustainability of current surveillance efforts in endemic areas may also benefit from multiplex tests that include other diseases such as malaria. Further operational research will likely be needed to determine how to integrate new tools and help generate evidence for new guidelines to support the uptake and use of those new tools.

2. **Support the development of improved tools for disease confirmation.**

Field-appropriate, sensitive diagnostics to confirm active infection remains a critical gap in both public health and clinical interventions. Due to the risk and complexity of HAT treatment options, screening for seropositivity is insufficient for case management. Current parasitology-based methods are costly and complex, and they have limited sensitivity and reproducibility. Confirmation of active infection will likely remain a requirement whether or not disease-stage-independent drugs are developed and introduced. A low-cost, rapid, point-of-care tool that does not require invasive sampling would improve access to HAT confirmatory testing and provide more immediate linkage to care and treatment.

3. **Support the development of tools and other interventions that will reduce barriers to disease staging and treatment monitoring.**

Sampling to test for disease staging and treatment monitoring currently requires an invasive lumbar puncture. To obviate the need for lumbar punctures, new tools and other interventions should be developed and introduced. This could be achieved with better tools for diagnosing central nervous system infection, including those exploring new blood-based biomarkers. This could also be achieved through new treatment strategies and follow-up policies that are less reliant on disease staging and samples of

CSF. Overcoming current barriers to disease staging and treatment monitoring would improve case management and has the potential to reduce HAT mortality and limit disease transmission.

Table 3. Product attributes of needed diagnostic tools for human African trypanosomiasis (HAT).

Use case	Disease confirmation	Disease staging	Treatment monitoring
Marker	Active infection; antigen or molecular	Host marker that indicates whether the parasite has crossed the blood-brain barrier	Active infection; antigen or molecular
Specimen	Blood	Blood	Blood
Context of use	Tier 2	Tier 2–3	Tier 2–3
Value proposition	A point-of-care tool that would confirm the disease case with a higher level of certainty	A diagnostic tool that could inform treatment strategy based on the disease stage without requiring invasive lumbar punctures	A point-of-care tool that could monitor the risk of disease relapse and improve treatment compliance

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