Target Product Profile for a gambiense Human African Trypanosomiasis high-throughput test for verification of elimination *(version 0.1 – September 2022)*

PRE-FINAL DOCUMENT – for public consultation **TPP N°4**

Background

Human African Trypanosomiasis (HAT) is a life-threatening parasitic infection transmitted by the tsetse fly, that is endemic in Sub-Saharan Africa. Having caused devastating epidemics during the 20th century, its incidence has now fallen to historically low levels thanks to sustained and coordinated efforts over the past 20 years. Two trypanosome subspecies cause the disease, with distinct epidemiology: *Trypanosoma brucei rhodesiense* (*Tbr*), found in eastern and southern Africa, is harboured by wild and domestic animals which constitute its reservoir, being transmitted occasionally to humans; and *Trypanosoma brucei gambiense* (*Tbg*), in western and central Africa, with humans as the main reservoir, accounting for about 95% of the total caseload.

HAT diagnosis relies on laboratory techniques because clinical signs and symptoms are unspecific. Serodiagnostic tests exist only for *Tbg* and are based on the detection of specific antibodies, thus they are not confirmatory of infection. With the current low disease prevalence, the positive predictive value of serological tests is particularly low. Field-applicable tools include the card agglutination test for trypanosomiasis (CATT) used mainly in active screening by specialized mobile teams, and the rapid diagnostic tests that are more suitable for individual testing at point-of-care. Confirmation of *Tbg* infection requires microscopic examination of body fluids, necessitating specific training. The best performing methods are laborious and reach 85-95% diagnostic sensitivity when performed by skilled personnel. Because trypanosomes are identified visually by their characteristic movement, microscopic examination must be done a short time after sampling (<1 hour).

HAT has been targeted for elimination as a public health problem (PHP) defined as a five years' mean of <1 case/10,000 inhabitants in all endemic districts in a given country. This status has been reached in several countries which have been or will soon be validated by WHO. The next target is the elimination of transmission of *gambiense* HAT, defined as zero autochthonous case for at least five years. Endemic countries reaching either of these goals need to maintain dedicated surveillance because of the persisting risk of re-emergence or re-introduction of HAT.

The progress in HAT elimination is leading to an unintended gradual loss of specialised personnel, while there is clearly need of large-scale testing of populations considered at risk in order to verify the absence of *Tbg* transmission. This calls for feasible methods using non-specialised personnel, because currently available diagnostic tools are too complex and resource-intensive.

Use case: High throughput test for verification of elimination of *Trypanosoma brucei gambiense*

Technical scope

Method for testing in parallel numerous samples collected in remote rural areas. Ideally, possible to be performed in-country, in national or sub-national reference labs. Acceptable at regional reference

labs, knowing that shipping samples to other countries is often complex and subject to strict regulations.

It requires high sensitivity and specificity. Positives may need to be characterised further with additional testing, to discard false positives.

Ideally, the test should be also applicable in animals[1] which could help assessing the parasite circulation in a region. The use in vectors[2] is less important as infection rates in the vector are very low.

Sampling: Ideally non-invasive. Acceptably, finger-prick or venous blood, serum/plasma (stabilised in whatever carrier) with a stability of 4 weeks at 40°C, 12 months at 4°C. It should require a simple specimen collection procedure with no cold chain requirement to transfer samples to reference labs.

After arrival of the specimens in the lab, results -if thousands of specimens are to be analysed- should be available in a relatively short time (high throughput format). Total cost per specimen, when analysed in batches of hundreds or thousands, should remain low.

To aid interpretation, it should be established for how long the test may remain positive in an individual after a *Tbg* infection has cleared: for example, antibody tests may remain positive for years. For molecular tests, the clearance of DNA and in particular RNA from blood is within days. However, persistence of DNA in blood and cerebrospinal fluid (CSF) was observed in around 20% of patients long after treatment considered successful, which remains to be explained. As a consequence, specimens from former HAT patients can be collected and, where applicable, their data should be documented and interpreted in consideration of their HAT history, or alternatively former HAT patients can be excluded from sampling.

Medical need:

The incidence of gambiense-HAT has been strongly declining globally and some historically endemic countries are not reporting new cases for a number of years, either country-wide or in some historical foci. Unfortunately this is often accompanied by a loss of case detection capacities, which are becoming increasingly difficult to maintain.

Therefore the need is increasing for high-throughput methods that can complement the classic strategies of passive and active screening, each with its own limitations, with appropriate tools for population-level cross-cutting surveillance of *Tbg* transmission.

These tools and methods would allow for testing with more comprehensive coverage of populations considered at risk, and particularly of populations thought to have become risk-free where absence of transmission needs verification.

[1] In this document, “animals” refers to non-human vertebrates
[2] In this document, “vectors” refers to tsetse flies

Target product profile (TPP) - TPP N°4

Diagnostic test attribute	Minimally acceptable	Desirable	Annotations
1. Intended use			
Target taxon/ species / subspecies/type	*Trypanozoon*	*T. b. gambiense (Tbg)*	Specificity of subspecies is important in particular if vectors or animals are tested
Target population	Populations (human) at risk of g-HAT	Populations (human, animal or vector) at risk of being infected with *Tbg*	
Use of information obtained	Establish recent circulation of Tbg in humans	Establish current circulation of Tbg in humans, animals and/or vectors	
Type of specimen collected	Minimally invasive specimen (finger-prick or venous blood)	non-invasively collected specimen Room-temperature storage/shipment	Minimally invasive: e.g. finger-prick, venous blood; non-invasive: e.g. saliva, urine, tears. In animals: easy collection (no need to capture animal, e.g. faeces) or limited discomfort to animal and collector. Invasiveness not applicable in vectors.
Analyte to be detected	Antibodies, antigens, whole parasite or nucleic acids	Antigens, whole parasite or RNA	Antibodies may persist in a previously infected and cured patient. RNA is a better marker for current infection than DNA.
Nature of the result	Qualitative	Qualitative	
Infrastructure level and operating environment	Laboratory at national level, or even international reference lab.	Laboratory at sub-national or national level.	There may be a trade-off between international shipment of many samples and setup of capacity to perform this test in endemic countries.
Intended user	Trained lab technician	Trained lab technician	
2. Assay performance characteristics (individual (patient) or population needs)			
Clinical sensitivity	>95%	> 99%	It should be at least equal to the most sensitive parasitological tests currently used.
Clinical specificity	>99%	>99.5%	In case of positive result, might be combined with confirmatory testing.
Analytical specificity / cross reactivity	*Trypanozoon* specific for humans, *Tbg* specific for animals/vectors	*T.b. gambiense* type 1	Should be *T.b. gambiense* Type 1 if applied in animals. For human testing *Trypanozoon* might be sufficient to raise concern, yet only infections with *Tbg1* are a threat to g-HAT elimination.

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Diagnostic test attribute	Minimally acceptable	Desirable	Annotations
Analytical sensitivity	Corresponding to ≤50 parasites/mL	Corresponding to ≤10 parasites/mL	Tests detecting antigens or nucleic acid sequences may reach lower detection thresholds than those detecting whole parasites.
Repeatability Intra-reader agreement (different tests, same instruments/environment, same sample, same reader)	Kappa > 0.8	Kappa > 0.9	
Reproducibility Inter-reader agreement (different tests, other instruments/environment, same sample, same reader or different readers)	Kappa > 0.8	Kappa > 0.9	Given the importance of this test in verification of HAT elimination, repeatability and reproducibility should be as high as possible.
Quality control	Control of functionality, positive and negative controls for batch testing and per run.	Control of functionality, positive and negative controls for batch testing and per run.	A proficiency panel would be useful.
3. Regulatory and normative needs			
Regulatory approvals and standards	Test components manufactured according to GMP (ISO13485:2016)	CE marking or other comparable regulatory approval. QMS ISO13485:2016	New, more demanding CE marking rules, may entail unrealistic production costs. Alternative registration (e.g. Australian TGA) may be considered. Quality management system should be defined. Dependence on commercial availability.
Promotional and marketing material	not applicable	not applicable	
4. Healthcare system needs			
4.1. Environment description			
Operating environment	Can be operated at 10-30°C at 40-70% relative humidity	Can be operated at 10-40°C at 10-88% relative humidity	This test will be applied in labs where temperature and humidity will be rather controlled.

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Diagnostic test attribute	Minimally acceptable	Desirable	Annotations
Workflow requirements	Specimen preparation in the field in <5 steps, minimal need for precision liquid handling, and minimal need for specialized material (generally available or provided in a specimen collection kit). Specimen shipment needs minimal security measures (minimal infection risk) and no or limited cold chain. Testing is much automatized, with < 5 manual steps. >100 specimens tested daily.	Specimen preparation in the field in <2 steps, no need for precision liquid handling, and no need for specialized material. Specimen shipment needs no special security measures (no infection risk) nor cold chain. Testing is substantially automatized, with < 2 manual steps. No need for precision liquid handling. >500 specimens tested daily.	Analysing pooled samples instead of individual ones could also be considered.
4.2. Instrument & device characteristics			
Instrumentation needed	Requiring instrumentation and devices that can be implemented at laboratories at national level	Requiring instrumentation and devices usually present at laboratories at national or sub-national level	
4.3. Information & communication technology			
Test result	Test results scored visually or by read-out of a device. Test result stable for at least 15 minutes	Test results scored by read-out of a device. Test result stable for at least 30 minutes	
Recording of results and data capture	Results are recorded in a computer, either automatically or manually.	Results recorded in a computer. Integrable into national data and reporting. Test results can be stored for retrospective interpretation (e.g. electronic result, optical density or intensity, etc, electronic image or video). Automatic interpretation of result (positive/negative)	Data should include results and demographics/other information. Data should be exportable to any database if needed. Storage needs may vary per program.
Transmission	Test results transmitted electronically	Data automatically integrated in server databases without need of additional equipment	Transmission should be adaptable to connectivity. Data format should be compatible with healthcare databases (JSON, DHIS2) supporting seamless transmission to them if required.
4.4 Reagent and control handling			

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Diagnostic test attribute	Minimally acceptable	Desirable	Annotations
Reagents, storage and packaging	Reagents stable at 4-8°C and 40-88% relative humidity for at least 12 months. Operating instructions and bench aids available. Reagents ready to use, or within 15 mins with max 5 additional steps.	Reagents stable at 4-45°C and 40-88% relative humidity for ≥ 24 months. 1 week transport stress at 50 °C. Transport not needing cold chain Operating instructions and bench aids available. Reagents ready to use or max 2 additional steps needed.	The stability should consider the time frame for distribution from manufacturer, passage through customs and local distribution.
4.5. Sample handling			
Sample volumes	Depending on the type of specimen. For blood (or serum or plasma) ≤5 mL.	Depending on the type of specimen. For blood ≤0.07 mL (finger prick, capillary tube).	Extra specimen material can be collected at the same time for repeat and/or remote testing if needed. For other tissues or body fluids, volumes can be specified later on.
Specimen collection and processing	Specific collecting devices provided as a kit. Some specimen processing. Transfer of samples within 1 week. Cold chain recommended but not strict. Thousands of samples can be managed in a reasonable time. Specimen shipment needs minimal security measures (minimal infection risk)	Routinely used collecting devices, minimal or no specimen processing. Transfer of samples not urgent (e.g. 4 weeks) and not requiring cold chain Thousands of samples can be managed quickly. Specimen shipment needs no special security measures (no infection risk).	Occasionally, left-over specimens could be preserved and transported under certain conditions
Waste management and biosafety	Amenable to standard biosafety measures for handling potentially infectious materials. Waste disposal in biosafety bin and sharps containers following standard guidelines.	Same as minimal.	
4.6. Distribution, training and support			
Training (sampling)	Specific training needed (<4 hours)	Basic training needed (<1 hour)	
Training (lab testing)	Extended specific training needed (7 days)	Specific training needed (max 1-2 days)	

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Diagnostic test attribute	Minimally acceptable	Desirable	Annotations
Instrument and test supply reliability	Supply guaranteed for ≥ 5 years after marketing. Manufacturer should replace non-functioning tests or instruments	Supply guaranteed for ≥ 7 years after marketing. Manufacturer should replace non-functioning tests or instruments	
Service and support response time	External support available. Support response within 1 week.	External support available. Support response within 1 day.	
5. Commercial and sustainability aspects			
Sustainability	Sustainable production	Sustainable production	As it is a non-profitable area, sustainable funding and a production/access innovative model is needed, with donors ensuring affordability. Advocacy needed.
Pricing per sample collected	≤0.5 USD	≤0.1 USD	Costs of hardware, shipment of material, and human resources, are not included here.
Pricing per sample tested	≤5USD	≤0.5 USD	All logistics, operational lab costs, investments, hardware, shipment of material, and salaries, are not included here. Molecular methods cost is a trade-off with clinical sensitivity

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