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Gambiense human African trypanosomiasis test

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Target product profile: Trypanosoma brucei gambiense test to verify elimination

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Abstract

Human African trypanosomiasis is a life-threatening parasitic infection transmitted by the tsetse fly in sub-Saharan Africa. The most common form is caused by Trypanosoma brucei gambiense, with humans as the main reservoir. Diagnosis in the field requires microscopic examination via laborious methods performed by specifically trained personnel. After over two decades of sustained efforts, the incidence of the disease is strongly declining, and some historically endemic countries are no longer detecting cases. The World Health Organization (WHO) has targeted the elimination of transmission of gambiense human African trypanosomiasis by 2030, defined as zero autochthonous cases for at least five consecutive years. Endemic countries reaching this goal must maintain dedicated surveillance to detect re-emergence or re-introduction. With this new agenda, new tools are needed for verification of the absence of transmission. WHO has therefore developed a target product profile calling for development of a method for population-level cross-cutting surveillance of T. b. gambiense transmission. The method needs to be performed in national or sub-national reference laboratories and to test in parallel numerous samples shipped from remote rural areas. Among other characteristics the product profile specifies: (i) a simple specimen collection procedure; (ii) no cold-chain requirement to transfer of specimens to reference laboratories; (iii) high sensitivity and specificity; (iv) high-throughput, substantially automatized; (v) low cost per specimen, when analysed in large batches; and (vi) applicable also in animals.
Introduction

Human African trypanosomiasis, also known as sleeping sickness, is a life-threatening parasitic infection transmitted by the tsetse fly. The disease is endemic in sub-Saharan Africa. Having caused devastating epidemics during the 20th century, the incidence of infection has now fallen to historically low levels due to sustained and coordinated efforts over the past 20 years.\textsuperscript{1} The two trypanosome subspecies that cause the disease have distinct epidemiology. \textit{Trypanosoma brucei rhodesiense}, found in eastern and southern Africa, is harboured by wild and domestic animals which constitute its reservoir and is transmitted occasionally to humans. \textit{T. b. gambiense}, found in western and central Africa, has humans as the main reservoir and accounts for about 95\% of the total caseload between 2011 and 2020 (32 275 out of 34 096 infections).\textsuperscript{1}

The diagnosis of human African trypanosomiasis relies on laboratory techniques because clinical signs and symptoms are unspecific. Field serodiagnostic tests exist only for \textit{T. b. gambiense} 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.\textsuperscript{2} Field-applicable tools include the card agglutination test for trypanosomiasis, 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 \textit{T. b. gambiense} infection requires microscopic examination of body fluids, necessitating specific training for laboratory staff. The best performing methods are laborious and reach 85–95\% diagnostic sensitivity when performed by skilled personnel.\textsuperscript{3} Because trypanosomes are identified visually by their characteristic movement, microscopic examination must be done a short time after sampling (less than 1 hour).

Human African trypanosomiasis has been targeted for elimination as a public health problem, defined as a five years’ mean of less than 1 case per 10 000 inhabitants in all endemic districts in a given country. This status has been reached in several countries and has been or will soon be validated by the World Health Organization (WHO).\textsuperscript{4} The next target is the elimination of transmission of gambiense human African trypanosomiasis, defined as zero autochthonous cases for at least five consecutive years.\textsuperscript{5} Countries where the disease is endemic and who reach either of these goals need to maintain dedicated surveillance because of the persisting risk of re-emergence or re-introduction of human African trypanosomiasis.

An unintended consequence of the progress in human African trypanosomiasis elimination is the gradual loss of specialized personnel. This trend is occurring at a time when there is a greater need for large-scale testing of populations at risk to verify the absence of \textit{T. b. gambiense} transmission. The currently available diagnostic tools are complex and resource-intensive to use.
Here we describe a target product profile to stimulate the development of high-throughput methods of testing for *T. b. gambiense* that can be performed by non-specialized personnel.

Methods

**Development process**

The development of this target product profile was led by the WHO Department of Control of Neglected Tropical Diseases following standard WHO guidance for target product profile development. To identify and prioritize diagnostic needs, a WHO neglected tropical disease diagnostics technical advisory group was formed, and different subgroups were created to advise on specific neglected tropical diseases, including a subgroup working on the need for innovations in diagnosis of human African trypanosomiasis. This group of independent experts included leading scientists, public health officials and representatives from trypanosomiasis-endemic countries. Standard WHO declaration of interest procedures were followed. To identify unmet needs the subgroup conducted a landscape analysis of the test that were currently available and those currently being developed. Through meetings and remote consultations, the subgroup developed use cases for hypothetical tools that would fill the main gaps in requirement for testing and gave the uses an order of priority. The subgroup agreed on a template adapted to the context of human African trypanosomiasis for use in the development of the target product profile. The draft of this target product profile (rated as priority no. 4) underwent several rounds of review by the subgroup members. The diagnostics technical advisory group members reviewed the resulting version. Draft version 0.1 and a proforma comment form were posted on the WHO website for public consultation over 28 days. WHO then released the current version of the target product profile.6

**Use case**

The use case was defined as a high-throughput test for verification of elimination of *T. b. gambiense*.

**Technical scope**

The technical scope described a method for testing in parallel numerous samples collected in remote rural areas. Ideally, testing should be possible to perform within the country, in national or subnational reference laboratories. At a minimum, testing could be carried out at regional reference laboratories, bearing in mind that shipping samples to other countries is often complex and subject to strict regulations. The test would need to have high sensitivity and specificity. Positive results might need to be characterized further with additional testing, to discard false positives. Ideally, the test would be also applicable in animals, which could help with assessing the circulation of *T. b. gambiense* parasites in a region. The use of the test in vectors would be less important as infection
rates in the vector are very low. (In this document, animals refer to non-human vertebrates; vectors refers to tsetse flies.)

Sampling
Ideally, sampling should be non-invasive. Acceptable sampling methods would be finger-prick or venous blood, serum or plasma (stabilized in whatever carrier), with a stability of 4 weeks at 40 °C, 12 months at 4 °C. Sampling should require a simple specimen collection procedure with no cold-chain requirement to transfer samples to reference laboratories.

After arrival of the specimens in the laboratory, the results should be available in a relatively short time even if thousands of specimens are to be analysed (that is, a high-throughput method). The 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 an individual can test positive after a *T. b. gambiense* infection has cleared. For example, antibody testing may show a positive result for years after infection, whereas for molecular tests the clearance of DNA (DNA) and in particular ribonucleic acid (RNA) from blood is within days. However, persistence of DNA in blood and cerebrospinal fluid has been observed in around one-fifth of patients 2 years or more after treatment was considered successful, an effect which remains to be explained. Specimens should therefore be collected from individuals who have recovered from human African trypanosomiasis and, where applicable, the data should be documented and interpreted in view of the patients’ disease history. Alternatively, former patients could be excluded from sampling.

Medical need
The incidence of gambiense human African trypanosomiasis has been strongly declining globally, and some countries historically endemic for the disease have not reported new cases for several years, either countrywide or in some previous foci of disease. Unfortunately, the decline in incidence is often accompanied by a loss of capacity for testing so that case detection becomes increasingly difficult to maintain. There is therefore an increasing need 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 *T. b. gambiense* 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 and where absence of transmission needs verification.
Target product profile

1. Intended use

Target taxonomy, species, subspecies and type

Minimal
Trypanozoon.

Optimal
T. b. gambiense.

Notes
Specificity of subspecies is particularly important if vectors or animals are tested.

Target population

Minimal
Populations (human) at risk of gambiense human African trypanosomiasis.

Optimal
Populations (human, animal or vector) at risk of being infected with T. b. gambiense.

Use of information obtained

Minimal
Establish recent circulation of T. b. gambiense in humans.

Optimal
Establish current circulation of T. b. gambiense in humans, animals or vectors.

Type of specimen collected

Minimal
Minimally invasive specimen.

Optimal
Non-invasively collected specimen.

Notes
Minimally invasive specimens include finger-prick or venous blood. Non-invasive specimens include saliva, urine or tears. In animals, easy collection avoids the need to capture the animal (faeces) or involves limited discomfort to animal and collector. Invasiveness is not applicable in vectors. Room-temperature storage or shipment is needed.
Analyte to be detected

**Minimal**
Antibodies, antigens, whole parasite or nucleic acids.

**Optimal**
Antigens, whole parasite or RNA.

**Notes**
Antibodies may persist in a previously infected and cured patient. RNA is a better marker for current infection than DNA.

Nature of the result

**Minimal**
Qualitative.

**Optimal**
Qualitative.

Infrastructure level and operating environment

**Minimal**
Laboratory at national level, or even international reference laboratory.

**Optimal**
Laboratory at sub-national or national level.

**Notes**
There may be a trade-off between the difficulties of international shipment of many samples and the set-up of capacity to perform this test in endemic countries.

Intended user

**Minimal**
Trained laboratory technician.

**Optimal**
Trained laboratory technician.

**2. Assay performance characteristics**

Assay performance characteristics are relevant to individual patients or population needs.

**Clinical sensitivity**

**Minimal**
> 95%.

**Optimal**

> 99%.

**Notes**

Clinical sensitivity should be at least equal to the most sensitive parasitological tests currently used.

**Clinical specificity**

**Minimal**

> 99%.

**Optimal**

> 99.5%.

In case of a positive result, the test might be combined with confirmatory testing.

**Analytical specificity or cross reactivity**

**Minimal**

*Trypanozoon*-specific for humans, *T. b. gambiense*-specific for animals or vectors.

**Optimal**

*T. b. gambiense* type 1.

**Notes**

Specificity should be to *T. b. gambiense* type 1 if applied in animals. For human testing *Trypanozoon* might be sufficient to raise concern, yet only infections with *T. b. gambiense* type 1 are a threat to gambiense human African trypanosomiasis elimination.

**Analytical sensitivity**

**Minimal**

Corresponding to ≤ 50 parasites/mL.

**Optimal**

Corresponding to ≤ 10 parasites/mL.

**Notes**

Tests detecting antigens or nucleic acid sequences may reach lower detection thresholds than those detecting whole parasites.

**Repeatability**

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Repeatability is the intra-reader agreement (different tests, same instruments or environment, same
sample, same reader).

**Minimal**

\[ K > 0.8. \]

**Optimal**

\[ K > 0.9. \]

**Reproducibility**

Reproducibility is the inter-reader agreement (different tests, other instruments or environment,
same sample, same reader or different readers).

**Minimal**

\[ K > 0.8. \]

**Optimal**

\[ K > 0.9. \]

**Notes**

Given the importance of this test in verification of human African trypanosomiasis elimination,
repeatability and reproducibility should be as high as possible.

**Quality control**

**Minimal**

Control of functionality, positive and negative controls for batch testing and per run.

**Optimal**

Control of functionality, positive and negative controls for batch testing and per run.

**Notes**

A proficiency panel would be useful.

**3. Regulatory and normative needs**

**Regulatory approvals and standards**

**Minimal**

Test components manufactured according to GMP (ISO 13 485:2016).

**Optimal**

CE marking or other comparable regulatory approval. QMS ISO 13 485:2016.

**Notes**
New, more demanding CE marking rules may entail unrealistic production costs. Alternative registration (e.g. Australian Therapeutic Goods Administration) may be considered. The quality management system should be defined. Dependence on commercial availability.

**Promotional and marketing material**

**Minimal**
Not applicable.

**Optimal**
Not applicable.

**4. Health-care system needs**

**4.1. Environment description**

**Operating environment**

**Minimal**
Can be operated at 10–30 °C at 40–70% relative humidity.

**Optimal**
Can be operated at 10–40 °C at 10–88% relative humidity.

**Notes**
The test will be applied in laboratories where temperature and humidity will be well controlled.

**Workflow requirements**

**Minimal**
Specimen preparation in the field in < five 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 fairly well automatized, with < five manual steps; > 100 specimens tested daily.

**Optimal**
Specimen preparation in the field in < two 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 < two manual steps. No need for precision liquid handling; > 500 specimens tested daily.

**Notes**
Analysing pooled samples instead of individual ones could also be considered.
4.2. Instrument and device characteristics

*Instrumentation needed*

**Minimal**
Requiring instrumentation and devices that can be implemented at laboratories at national level.

**Optimal**
Requiring instrumentation and devices usually present at laboratories at national or subnational level.

4.3. Information and communication technology

*Test result*

**Minimal**
Test results scored visually or by read-out of a device. Test result stable for at least 15 minutes.

**Optimal**
Test results scored by read-out of a device. Test result stable for at least 30 minutes.

*Recording of results and data capture*

**Minimal**
Results are recorded in a computer, either automatically or manually.

**Optimal**
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, electronic image or video). Automatic interpretation of result (positive or negative).

*Notes*
Data should include results and demographics or other information. Data should be exportable to any database if needed. Storage needs may vary per programme.

*Transmission*

**Minimal**
Test results transmitted electronically.

**Optimal**
Data automatically integrated in server databases without need of additional equipment.

*Notes*
Transmission should be adaptable to connectivity. Data format should be compatible with health-care databases (JSON, DHIS2) supporting seamless transmission to them if required.
4.4 Reagent and control handling

Reagents, storage and packaging

Minimal
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 minutes with maximum 5 additional steps.

Optimal
Reagents stable at 4–45 °C and 40–88% relative humidity for ≥ 24 months. One-week transport stress at 50 °C. Transport not needing cold chain. Operating instructions and bench aids available. Reagents ready to use or maximum two additional steps needed.

Notes
The stability should consider the time-frame for distribution from manufacturer, passage through customs and local distribution.

4.5. Sample handling

Sample volumes

Minimal
Depending on the type of specimen. For blood (or serum or plasma) ≤ 5 mL.

Optimal
Depending on the type of specimen. For blood ≤ 0.07 mL (finger-prick, capillary tube).

Notes
Extra specimen material can be collected at the same time for repeat or remote testing if needed. For other tissues or body fluids, volumes can be specified later.

Specimen collection and processing

Minimal
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).

Optimal
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**

**Minimal**
Amenable to standard biosafety measures for handling potentially infectious materials. Waste disposal in biosafety bin and sharps containers following standard guidelines.

**Optimal**
Amenable to standard biosafety measures for handling potentially infectious materials. Waste disposal in biosafety bin and sharps containers following standard guidelines.

### 4.6. Distribution, training and support

**Training (sampling)**

**Minimal**
Specific training needed (< 4 hours).

**Optimal**
Basic training needed (< 1 hour).

**Training (laboratory testing)**

**Minimal**
Extended specific training needed (7 days).

**Optimal**
Specific training needed (max 1–2 days).

**Instrument and test supply reliability**

**Minimal**
Supply guaranteed for ≥ 5 years after marketing. Manufacturer should replace non-functioning tests or instruments.

**Optimal**
Supply guaranteed for ≥ 7 years after marketing. Manufacturer should replace non-functioning tests or instruments.

**Service and support response time**

**Minimal**
External support available. Support response within 1 week.
5. Commercial and sustainability aspects

Sustainability

Minimal
Sustainable production.

Optimal
Sustainable production.

Notes
As it is a non-profitable area, sustainable funding and a production or access innovative model is needed, with donors ensuring affordability. Advocacy is needed.

Pricing per sample collected

Minimal
≤ 0.5 United States dollars (US$).

Optimal
≤ 0.1 US$.

Notes
Costs of hardware, shipment of material and human resources are not included here.

Pricing per sample tested

Minimal
≤ 5 US$.

Optimal
≤ 0.5 US$.

Notes
All logistics, operational laboratory costs, investments, hardware, shipment of material and salaries, are not included here. Molecular methods cost is a trade-off with clinical sensitivity.

Conclusion
This target product profile was developed by a WHO advisory group of independent experts working on gambiense human African trypanosomiasis, comprising leading scientists and public health officials international and endemic-countries. The product profile is intended to promote the development of a
new test that would be most useful in the agenda of human African trypanosomiasis elimination, including in the post-elimination phase. Among other characteristics the product profile specifies: (i) a simple specimen collection procedure; (ii) no cold-chain requirement to transfer of specimens to reference laboratories; (iii) high sensitivity and specificity; (iv) high-throughput, substantially automatized; (v) low cost per specimen, when analysed in large batches; and (vi) applicable also in animals.

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Competing interests:
None declared.

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