Target product profiles for identification of yellow fever infection

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Content

Introduction

Yellow fever (YF) is an arboviral disease transmitted predominantly by mosquitoes of the *Aedes* and *Haemagogus genera*. The causative agent, the YF virus (YFV), is found in tropical and subtropical areas of South America and Africa (Monath and Vasconcelos, 2015). Despite the existence of an effective vaccine, a recent study estimated 51,000–380,000 as the number of severe cases and 19,000–180,000 as the number of deaths due to YF in Africa alone in 2013 (Garske et al., 2014).

From 2016 to 2018, the largest YF outbreaks in decades were reported in Africa and South America. Since December 2015, thousands of YF cases and several hundreds of related deaths were reported in Angola, Democratic Republic of the Congo, Uganda, Nigeria, Brazil, Bolivia, Ecuador, Columbia, French Guiana, Peru and Suriname. Some cases of YF have also been reported in China and Europe (Figure 1).

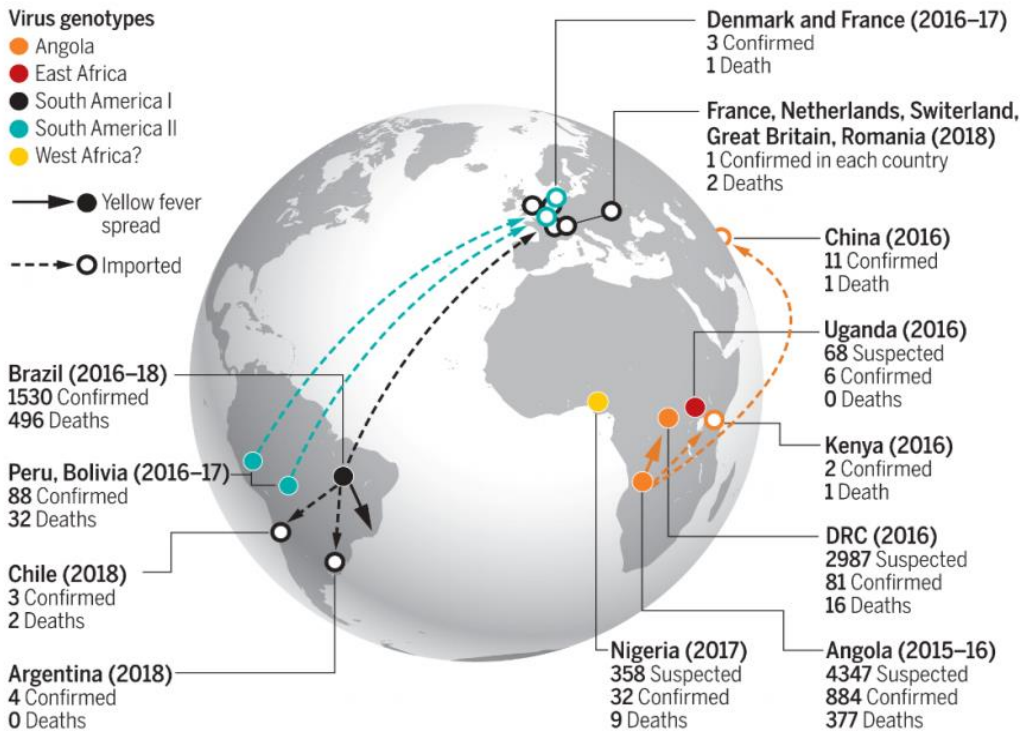

Figure 1: Yellow fever incidence 2016-2018 (Barrett, 2018)

With the availability of a safe and life-long protective vaccine, mass immunization is the most effective preventive measure against YF. However, the success of vaccination campaigns depends on several factors, such as vaccine availability, the proportion of the population that receives the vaccine, and, very importantly, the speed with which a new YF case is confirmed. This last factor is crucial since an outbreak can spread rapidly between the time the first YF case is suspected and the time laboratory confirmation is obtained. In Africa, this can take over one month due to the need for multiple rounds of serologic testing to confirm a suspected YF infection. Samples from suspected YF cases are first sent and tested for presence of YF IgM antibodies at one of the 31 national reference laboratories (NRL) of the YF AFRO laboratory network which comprises 28 countries (Figure 2). If this first test is positive, the sample is then sent for confirmatory testing and differential diagnosis to a regional reference laboratory (RRL).

The Global Strategy to Eliminate Yellow fever Epidemics (EYE) is committed to expanding laboratory capacity within sub-Saharan Africa, including by increasing the number of RRLs. Until recently, the Institut Pasteur de Dakar was the sole RRL, but it has been joined by the Uganda Virus Research Institute which supports Eastern and Southern Africa region. Designation of an additional RRL in Cameroon for the Central Africa region is anticipated in the near future (Figure 2). This expansion is expected to help improve the speed of case confirmation in endemic countries, which is key to ensure timely outbreak response measures, including vaccination campaigns.

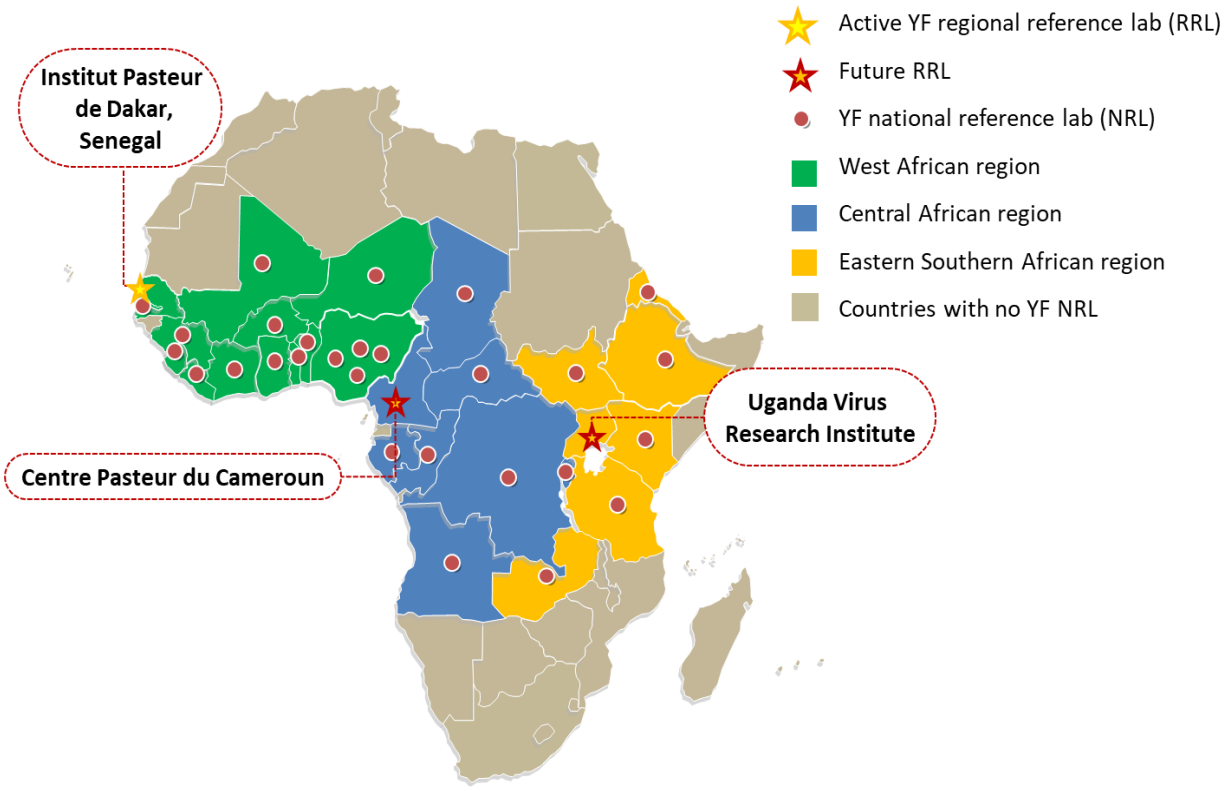

Figure 2: Countries part of the YF AFRO laboratory network with the respective reference laboratories

The availability of standardized, well-performing and validated diagnostic methods is essential to ensure accurate and early confirmation of YF cases.

Laboratory diagnosis of yellow fever

Molecular diagnostics

YFV nucleic acids or RNA can be detected during the first 5–7 days after symptom onset using reverse-transcription polymerase chain reaction (PCR), a fast and specific way of measuring the presence of viral agents in the early phase of illness. Detection of YFV RNA serves as the most rapid and direct confirmation of an active, viremic infection. However, the limitation of this method is that it can only be used for early case confirmation as the virus in the blood rapidly decreases over time.

Serological diagnostics

Serology is useful for diagnosing yellow fever during the post-viremic phase of the disease. The presence of immunoglobulin M (IgM) detected by an enzyme-linked immunosorbent assay (ELISA) or any other immunoassay (indirect immunofluorescence) in a sample collected after day five of illness is suggestive of a recent YFV infection. However, ELISA testing alone cannot confirm a current YF infection, as antibody response could be due to past YF infection or infection with another flavivirus such as dengue, Zika or West Nile virus. In order to confirm active infection following a positive ELISA, a complex viral culture test (plaque-reduction neutralization test, PRNT) is also required, which can take up to a week to generate results. PRNT requires sophisticated laboratories with high biosafety levels that are rarely available outside of reference laboratories, even in high-income countries.

Lack of diagnostic tools

Gaps and challenges in the rapid identification of YF outbreaks must be addressed in order to support effective vaccine campaigns and reduce the spread of the disease. Currently, there are no commercially available, fully validated diagnostic kits for molecular or serological detection of YFV infections. Research Use Only assays are currently used for patient diagnosis, which means testing, reagents and quality measures are not standardized across laboratories and tests. Laboratories with molecular testing capacities all use non-commercial and non-regulated PCR assays for both surveillance and patient management. Improving case detection through serological testing will require the strengthening and expansion of the current diagnostic testing capacity. It will also rely on the availability of new, easy-to-implement diagnostic tests to simplify the serological component of testing algorithms.

Developing a target product profile

Currently, only RRLs and NRLs are involved in the testing of YF suspected cases. However, lower levels of the health system could be involved in the testing of YF suspected cases if new diagnostic methods became available that are easier to use, faster and more accurate. To address these gaps and support Gavi's assessment of diagnostic needs impacting the availability and use of the yellow fever vaccine, FIND partnered with Gavi and the World Health Organization (WHO) to conduct a consensus target product profile (TPP) development process for three diagnostic tools to identify yellow fever infections:

- A standardized molecular assay test kit (**Table 2**)
- A standardized immunoassay test kit (**Table 2**)
- Rapid diagnostic test (**Table 3**)

The purpose of a TPP is to inform product developers of key characteristics and performance specifications required to meet the end user's needs for a defined use case. TPPs often include an optimal and minimal definition for each performance characteristic. Ideally, products should be designed to achieve as many of the optimal characteristics as are feasible, while still satisfying the minimal criteria for all defined features. An overview of the TPP development process is summarized in Figure 3.

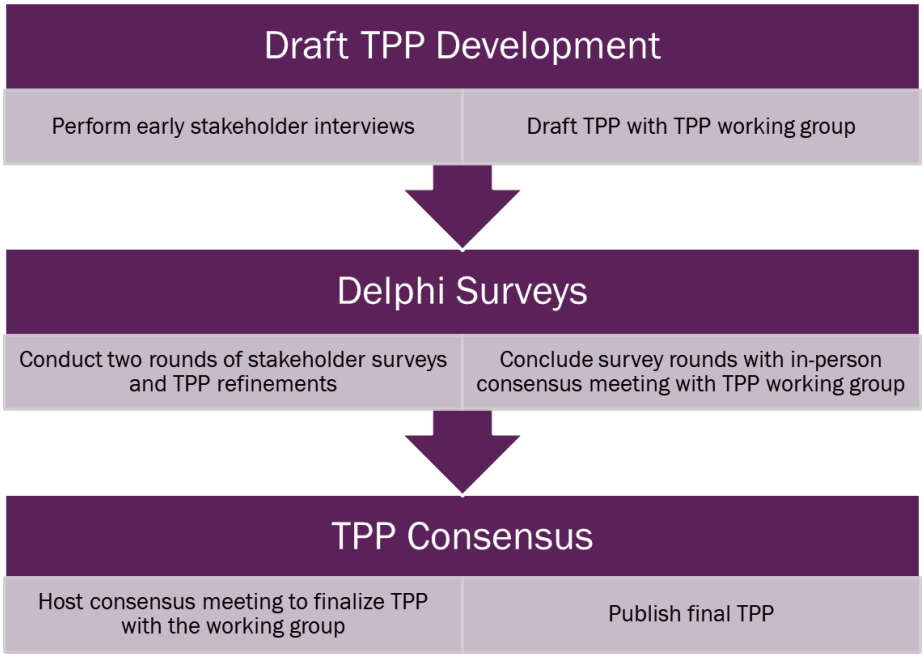

Figure 3: TPP development process

Delphi-like process

To obtain consensus and arrive at three final target product profiles for yellow fever infection, a Delphi-like process was followed enlisting stakeholder input from over 50 content authorities. Stakeholders were surveyed electronically to obtain input on all three TPPs in the first survey round. Survey participants were asked to rank their level of agreement based on a Likert scale ranging from 1 to 5 (1-disagree, 2-mostly disagree, 3-don't agree or disagree, 4-mostly agree, 5-fully agree). Individuals were asked to provide comments when they scored a characteristic at 3 or lower. Consensus was pre-specified as >50% of responders agreeing with the proposed characteristics (Likert score of 4 or 5). A second level of consensus was evaluated at >75% agreement. Responses were analysed separately for industry and non-industry responses. Responses were collated, and revisions were talked over by the TPP working group to address survey respondent concerns for those characteristics with lower levels of agreement. The revised TPP was sent for a second Delphi survey round and the process was repeated, but the rapid diagnostic test TPP was left out of the second survey since it had shown high levels of agreement, and for the sake of brevity.

A TPP consensus meeting, co-hosted by WHO and Gavi, was held on 25 June 2019, in Rotterdam, Netherlands. This consensus meeting included a select group of experts who are part of the EYE laboratory technical working group and who have extensive and relevant field experience in yellow fever. Essential characteristics from each TPP were discussed (intended use, sensitivity, specificity and cost) as well as those with lower levels of agreement (two characteristics). Survey comments were deliberated and revisions to the TPP were drafted during the meeting and agreed upon by voting participants (n=13). Voting was based on a super majority, with a 70% threshold. During the consensus meeting, revisions to the TPP were completed and full consensus was achieved on all characteristics for all three TPPs.

Conclusion

These three TPPs will guide assessments of which yellow fever diagnostic tests perform well enough to warrant use in the laboratory network and facilitate outreach to manufacturers to encourage them to develop test kits that demonstrate the specified level of performance. The full TPPs are listed in the tables below.

Table 1: Standardized molecular assay test kit

Target product profile for a standardized molecular assay test kit to identify yellow fever infection		
Characteristic	**Minimal**	**Optimal**
SCOPE		
1. Intended use	A standardized molecular assay test kit for confirmation of yellow fever (YF) infection	
2. Target population	Testing of specimens collected from individuals suspected of yellow fever infection[1] or individuals living in close geographic distance to a confirmed or suspected YF case/outbreak	
3. Target use setting	National reference laboratory (level 3[2]) or above	District Hospitals (Level 2) or above
4. Target users	Laboratorians with training in immuno- and/or molecular diagnostics	
MOLECULAR ASSAY KIT CHARACTERISTICS		
5. Test kit format	A standardized kit that contains all materials required for the procedure in a self-contained kit that includes controls, reagents and needed consumables (e.g., reagent grade water) to perform the assay	Same, plus lyophilized master mix with all reagents required to be aliquoted by user
6. Test kit stability and storage conditions	12 months, stable between 2-4°C, 70% humidity, up to 2000 meter altitude; Indicator of instability or expiration	24 months, stable between 2-40°C, 90% humidity, up to 3000 meter altitude; Indicator of instability or expiration
7. Specimen capacity & throughput	96 reaction molecular assay test kit with flexibility to run smaller numbers of samples with individual components packaged to enable smaller usage of reagents	
8. Target analytes for yellow fever	Yellow fever RNA	Able to distinguish YF vaccine RNA from wild type yellow fever RNA
9. Stability of the kit once opened	6 months; Storage of aliquoted master mix by freezing[3] with at least 1 freeze thaw cycle tolerated	12 months or more; Storage of aliquoted master mix by freezing with at least 3 freeze thaw cycles tolerated
10. Clinical sensitivity[4]	≥90%	≥95%
11. Clinical specificity	≥99%	

[1] Case definition of suspected yellow fever as defined by the Eliminate Yellow fever Epidemics (EYE) laboratory technical working group

[2] Ghani AC, Burgess DH, Reynolds A, Rousseau C (2015). Expanding the role of diagnostic and prognostic tools for infectious diseases in resource-poor settings. *Nature* 528: S50-52

[3] Freezing defined as -15-25ºC at a minimum for cold storage

[4] The analytic limit of detection for the molecular assay will be determined by the EYE laboratory technical working group.

12. Specimen type	Serum and plasma	Serum, plasma, whole blood, and blood collection tubes that stabilize nucleic acids without the need for cold storage, urine ideal
13. Specimen volume	0.2 mL or less per reaction	
14. Analytical inclusivity	Assay detects all geographically and genetically diverse yellow fever viral strains	
15. Platform considerations	Compatible and validated by manufacturer for at least the two most commonly available thermocyclers[5] with thermocycler-specific Ct cut off values for assay determined	Validated by manufacturer for the five most commonly available thermocyclers with thermocycler-specific Ct cut off values for assay determined
16. Lot–to–lot stability	No change in Ct cut-off from lot to lot	
OPERATIONAL CHARACTERISTICS		
17. Shipping conditions	Ability to tolerate transport stress (48 hours with short term fluctuations up to 50°C and down to 0°C) and exposures between 2°C and 45°C including condensing humidity	
18. Operating conditions	Operation between 20°C and 25°C; Ability to tolerate extremely low relative humidity to condensing humidity	Same, plus operation between 10°C and 35°C
19. Time to result	< 6 hours (i.e., same day result)	< 3 hours
20. Sample transport	< 2 days transport under cold chain (e.g., cold packs only)	Sample types and preparation that stabilize specimens so that no cold chain is required
21. Safety precautions	No further biosafety requirements beyond what is currently state of practice for regional and national labs	
22. Waste/disposal requirements	Standard biohazardous waste disposal or incineration of consumables, no high temperature incineration required	Small environmental footprint; recyclable or compostable plastics for test cartridges and other materials after decontamination, no incineration required
23. Quality control	All internal assay controls provided with test kits; External controls should be made available[6] to evaluate longitudinal test performance and inter-lot variation	
24. Patient identification capability	Ability to track electronic identification number of the patient either manually or via barcode	

[5] Assay performance can vary with different equipment and evaluation on platforms currently available for use in laboratories is essential to speed introduction and uptake in these settings. A list of equipment available in national public health yellow fever laboratories in Africa is provided in Appendix A.

[6] Not required by test manufacturer and could be provided by a validated secondary party as established by the EYE laboratory technical working group

25. Data export to user/result interpretation	Manual by operator	Automated
26. Result readout	Qualitative result	
PRICING AND ACCESSIBILITY		
27. Target list price[7] per test price includes the cost of all reagents in the standardized test kit[8]	<$15 USD per sample tested	<$10 USD per sample tested
28. Regulatory requirements	WHO PQ or other stringent regulatory body (e.g., FDA or CE mark)	
29. Reference samples used to evaluate the performance of test kit	Samples from: • Representative strains from African and Latin American lineages • Samples from individuals with confirmed yellow fever (not vaccine) viremia by validated molecular assay • Samples from individuals with confirmed viremia with other flaviviruses and pathogens, including Zika, Dengue, West Nile, Chikungunya and others as appropriate) • Samples from individuals with recent yellow fever vaccination	Same as minimal plus: • Samples from individuals with confirmed acute yellow fever infection with varying time points up to resolution of infection/disease • Samples from individuals in both acute and toxic phase of disease

Appendix A: Molecular test platforms in use in at least two yellow fever national public health laboratories in Africa in 2019	
Platform type	**Number of laboratories known to have platforms**
– ABI 7500 Real-Time PCR	9
– ABI 7500 Fast Real-Time PCR	5
– Qiagen Rotor-Gene Q	3
– Cepheid SmartCycler	3
– ABI 2720 Thermal Cycler	2

[7] List Price – the price the manufacturer has arrived at for the product, taking into account the cost of goods and other factors (e.g., margin); the list price does not include any volume or other discounts or potential markup for distribution or other costs, including freight, taxes, etc. This cost is assumed at volume production and the prices listed in the TPP are considered for public health preferential pricing in low- and middle-income countries only. Note this price includes the entire kit including sample preparation reagents (see 5. Test kit format)

[8] Including extraction kits and reagent controls

Table 2: Standardized immunoassay test kit

Target product profile for a standardized immunoassay test kit to identify yellow fever infection		
Characteristic	**Minimal**	**Optimal**
SCOPE		
1. Intended use	An IgM immunoassay for the identification of yellow fever infection	Same as minimal except if performance of immunoassay enables case confirmation of yellow fever infection
2. Target population	Testing of specimens collected from individuals suspected of yellow fever infection[9] or individuals living in close geographic distance to a confirmed or suspected YF case/outbreak	
3. Target use setting	National reference laboratory (Level 3[10]) or above	District Hospitals (Level 2) or above
4. Target users	Laboratorians with training in immuno- and/or molecular diagnostics	
IMMUNOASSAY TEST KIT CHARACTERISTICS		
5. Test format / test kit	A standardized, self-contained kit that contains all materials required for the procedure including controls, reagents and needed consumables (e.g., reagent grade water for rehydration of kit components, excluding wash buffers) to perform the assay	
6. Test kit stability and storage conditions	12 months, stable between 2-4°C, 70% humidity, up to 2000 meter altitude; Indicator of instability or expiration	24 months, stable between 2-40°C, 90% humidity, up to 3000 meter altitude; Indicator of instability or expiration
7. Specimen capacity and throughput	96-well standardized immunoassay	Immunoassay test kit in 8-well individualized strips to enable flexibility to run fewer samples with all reagents and controls included in sufficient amounts to allow for running partial plates
8. Target analytes for yellow fever detection	Individual immunoassay test kit for the detection of IgM specific to yellow fever	
9. Target analytes for additional pathogens[11]	No additional analytes beyond yellow fever	A single standardized immunoassay test kit capable of multiplex detection of IgM to the following pathogens in descending level of priority: • Dengue • Zika • West Nile

[9] Case definition of suspected yellow fever as defined by the WHO Eliminating Yellow Fever Epidemics (EYE) working group
[10] Ghani AC, Burgess DH, Reynolds A, Rousseau C (2015). Expanding the role of diagnostic and prognostic tools for infectious diseases in resource-poor settings. *Nature* 528: S50-52
[11] Test performance for yellow fever is required to be the same for a multiplex test as specified for a monoplex test

10. Stability of the kit once opened	3 months[12]	6 months
11. Clinical sensitivity	≥90%	≥95%
12. Clinical specificity	≥90%	≥98%
13. Specimen type	Serum or plasma	Serum, plasma, dried blood spots
14. Specimen volume	<0.5 mL	< 0.3 mL
15. Analytical inclusivity	Assay detects IgM immune response to all geographically and genetically diverse yellow fever viral strains	Same as minimal plus detection of IgM immune response to all geographically and genetically diverse strains of the other pathogen target analytes
16. Platform considerations	Kit and instructions compatible with manual and standard plate-washers (both "row" and 96-well) and standard plate readers[13]	
17. Lot–to–lot stability	No recalibration or change in cut-off from lot to lot	
OPERATIONAL CHARACTERISTICS		
18. Shipping conditions	Ability to tolerate transport stress (48 hours with short term fluctuations up to 50°C and down to 0°C) and exposures between 2°C and 45°C including condensing humidity	
19. Operating conditions	Operation between 20°C and 25°C; Ability to tolerate extremely low relative humidity to condensing humidity	Same, plus operation between 10°C and 35°C
20. Time to result	< 6 hours (i.e., same day result)	< 3 hours
21. Sample transport	< 2 days transport under cold chain (e.g., cold packs only)	Sample types and preparation that stabilize specimens so that no cold chain is required
22. Safety precautions	No further biosafety requirements beyond what is currently state of practice for regional and national labs	
23. Waste/disposal requirements	Standard biohazardous waste disposal or incineration of consumables, no high temperature incineration required	Small environmental footprint; recyclable or compostable plastics for test cartridges and other materials after decontamination, no incineration required
24. Quality control	All internal assay controls provided with test kits; External controls should be made available[14] to evaluate longitudinal test performance and inter-lot variation	

[12] Applies to 8-well strip format only, since use of a 96-well format would require the use of the entire kit once opened for use

[13] Equipment commonly in use in yellow fever national public health laboratories is preferred. For example, Thermo Scientific Wellwash, BioTek ELx50, and BioTek ELx508 washers are the only types of ELISA plate-washers that are each in use in at least two yellow fever national public health laboratories in Africa. Thermo Scientific Multiskan and BioTek ELx800 plate readers are the only types of ELISA plate readers that are each in use in at least two yellow fever national public health laboratories in Africa.

[14] Not required by test manufacturer and could be provided by a validated secondary party as established by the EYE laboratory technical working group

25. Patient identification tracking	Ability to track electronic identification number of the patient either manually or via barcode a-	
26. Data export to user/ result interpretation	Manual by operator	Automated
27. Result readout	Qualitative result	
PRICING AND ACCESSIBILITY		
28. Target list price[15] per test (price includes the cost of all reagents in the standardized test kit)	<$10 USD / sample for a full 96-well plate including the required controls	<$5 USD per sample for a full 96-well plate including the required controls
29. Regulatory requirements	WHO PQ or other stringent regulatory body (e.g., FDA or CE mark)	
30. Reference samples used to evaluate the performance of the test kit	Samples from: • individuals with proven past YF infection (PRNT or lab-based IgG) • individuals with known flavivirus exposure and no evidence of YF IgG • individuals with proven previous infection with other flaviviruses (Zika, dengue, West Nile) • individuals with prior YF vaccination	Samples from a well-characterized cohort: • individuals with virological confirmation of acute YF infection, with varying time points after resolution of acute infection • individuals with no known flavivirus exposure and no evidence of YF IgG • asymptomatic individuals with proven past YF infection (PRNT or lab-based IgG) • individuals with proven previous infection with other flaviviruses, with varying time points after resolution of infection • individuals with previous infection of both YF and other flaviviruses • individuals with prior and recent YF vaccination

[15] List Price – the price the manufacturer has arrived at for the product, taking into account the cost of goods and other factors (e.g., margin); the list price does not include any volume or other discounts or potential markup for distribution or other costs, including freight, taxes, etc. This cost is assumed at volume production and the prices listed in the TPP are considered for public health preferential pricing in low- and middle-income countries only. Pricing cost is the same regardless of test format (8-well strips of 96-well plates)

Table 3: Rapid diagnostic test

Target product profile for a rapid diagnostic test to identify yellow fever infection		
Characteristic	**Minimal**	**Optimal**
SCOPE		
1. Intended use	Screening for yellow fever (YF) IgM antibody to rule out YF infections in patients presenting with fever and jaundice	Same as minimum, plus equivalent performance to immunoassay test kit target product profile[16]
2. Target population	Suspected yellow fever cases	
3. Target use setting	For use at primary health care settings including health posts (Level 1[17]) and above	
4. Test format	A single use disposable, rapid diagnostic test (RDT) housed in a test cassette	
5. Target users	Target users include community health workers with minimal training and any health worker or laboratorian with a similar or superior training level	
6. Target analytes	Detection of IgM antibodies specific to yellow fever required	
PERFORMANCE CHARACTERISTICS		
7. Clinical sensitivity	≥85% required to achieve the minimal intended use; ≥90% required to achieve the optimal intended use	>90% required to achieve the minimal intended use; ≥95% required to achieve the optimal intended use
8. Clinical specificity[18]	≥90% required to achieve either the minimal or optimal intended use	≥98% required to achieve the either the minimal or optimal intended use
9. Specimen	Fingerstick blood, whole blood and serum; Ability to collect serum from venipuncture required if positive result	
10. Sample transport (if required)	< 2 days transport under cold chain (e.g., cold packs only)[19]	Sample types and preparation that stabilize specimens so that no cold chain is required
11. Analytical inclusivity	Assay detects IgM immune response to all geographically and genetically divers yellow fever virus trains; if antigen assay, detects antigens for all yellow fever viral strains	
OPERATIONAL CHARACTERISTICS		

[16] The assay performance characteristics of sensitivity and specificty are defined in the target product profile for a standardized immunoassay test kit to identify yellow fever infection. If a rapid diagnostic test could be developed with equivalent performance to these specifications, it could in theory be used in place of immunoassays performed in regional and national laboratories for yellow fever identification.

[17] Ghani AC, Burgess DH, Reynolds A, Rousseau C (2015). Expanding the role of diagnostic and prognostic tools for infectious diseases in resource-poor settings. *Nature* 528: S50-52

[18] Specificity for yellow fever as evaluated against other flaviviruses known to cross-react with yellow fever

[19] If sample transport is required (e.g. to National laboratories) for testing with the yellow fever RDT

12. Ease of use	No more than three operator steps, none of which is timed (excluding assay run time) or labour intensive	One operator step (none of which has a timed interval, excluding assay run time), excluding waste disposal
13. Test kit	One test should be packed individually with all materials required to run a single test procedure including: sample collection devices/equipment (e.g. lancets), reagents, reagent grade water or other consumables; A test kit box should include a number of individually packaged tests (e.g. 20, 50 or 100 tests) and any other required consumables and or controls that are required	
14. Test kit stability and storage conditions	12 months, stable between 2-35°C, 70% humidity, up to 3000 meter altitude, no cold chain required at any point	24 months, stable between 0-50°C, 90% humidity, up to 3000 meter altitude, no cold chain required at any point
15. Shipping conditions	Transport stress (48 hours with fluctuations up to 50°C and down to 0°C); Ability to tolerate exposures between 2°C and 45°C at an altitude up to 3000 meters, up to and including condensing humidity	
16. Operating conditions	Operation between 15°C and 40°C at an altitude up to 2000 meters; Ability to tolerate extremely low relative humidity to condensing humidity	Same, plus operation between 10°C and 45°C at an altitude up to 3000 meter preferred
17. Time to result	≤20 minutes	≤10 minutes
18. Stability of valid result (read window)	At least 30 minutes (after which results may be false or invalid); Clear language in the instructions for use regarding test reading	≥1 hour (after which results give invalid rather than false results); Clear language in the instructions for use regarding test reading[20]
19. Waste/disposal requirements	Inactivation of potentially infectious samples required; Standard biohazardous waste disposal or incineration of consumables; No high temperature incineration required	Same as minimal, plus small environmental footprint; recyclable or compostable plastics for test cartridges and other materials after decontamination, no incineration required
20. Reagent controls	Procedural (reagent-addition) control internalized for each individual test run; positive and/or negative control for quality control provided in each box of test kits	Procedural (specimen-addition/sample adequacy) control internalized in test for each individual test run; positive and/or negative control for quality control provided in each box of test kits
21. Kit controls	Indicator of instability or expiration	Same as minimal plus indicator of inadequate sample and incorrect procedure and/or use

[20] If long-term stability of the test result is required for surveillance, an image of the test result and patient identification is acceptable (reader, cell phone, etc.)

22. Patient identification capability	Simple, self-contained way to indicate a patient identifier	
23. Result display and interpretation	Result can be read with the naked eye including in low light settings with minimal instructions for interpretation required by user, or with an external and portable reader	
24. Data export	If data export is required, inclusion of a portable and battery-operated reader (e.g. cell phone with an App or other dedicated reader device) for data export to enable image acquisition of the test result and/or global positioning system (GPS) tags)[21]	
PRICING AND ACCESSIBILITY		
25. Target list price[22] per test[23]	<$10 USD	<$5 USD
26. Regulatory requirements	WHO PQ or other stringent regulatory body (e.g., FDA or CE mark)	
27. Reference samples used to evaluate the performance of a test	Samples from: • Individuals with proven past YF infection (PRNT or lab-based IgG) • Individuals with known flavivirus exposure and no evidence of YF IgG • Individuals with proven previous infection with other flaviviruses (Zika, dengue, West Nile) • Individuals with prior YF vaccination	Samples from a well-characterised cohort: • Individuals with virological confirmation of acute YF infection, with varying time points after resolution of acute infection • Individuals with no known flavivirus exposure and no evidence of YF IgG • Asymptomatic individuals with proven past YF infection (PRNT or lab-based IgG) • Individuals with proven previous infection with other flaviviruses, with varying time points after resolution of infection • Individuals with previous infection of both YF and other flaviviruses • Individuals with prior and recent YF vaccination

[21] Reader requirements have been previously defined through a TPP consensus process (pending publication)

[22] List Price– the price the manufacturer has arrived at for the product, taking into account the cost of goods and other factors (e.g., margin); the list price does not include any volume or other discounts or potential markup for distribution or other costs, including freight, taxes, etc. This cost is assumed a volume production and the prices listed in the TPP are considered for public health preferential pricing in low- and middle-income countries only.

[23] List pricing excludes the cost of a reusable reader, if required