# Target Product Profile on IVD assays for the detection of Yellow Fever in the context of surveillance

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## Contents



# Overview

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; Gaythorpe et al., 2021) .

From 2016 to 2018, the largest YF outbreaks in decades were reported in Africa and South America (Manuel et al., 2024; William et al., 2022; PAHO, 2023). Since December 2015, thousands of YF cases and several hundreds of related deaths were reported in Angola, Democratic Republic of Congo, Uganda, Nigeria, Brazil, Bolivia, Ecuador, Columbia, French Guiana, Peru and Suriname. Some imported cases of YF have also been reported in China and Europe (ECDC, 2019; MMWR, 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, where serological confirmation is the most required, 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-specific 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 testing to a regional reference laboratory (RRL).

Although, turnaround time to case confirmation in the Americas if often quicker thanks to the broad use of molecular testing methods on all suspected cases, the access to quality-assured and recommended commercial assays for this region of the world is often challenging. Despite jointly contribute significantly to the demand, this is often due to high cost of such commodities, and the limited external procurement support available to them.

The Global Strategy to Eliminate Yellow fever Epidemics (EYE) is committed to expanding laboratory capacity within sub-Saharan Africa, including by increasing capacity and resilience of the network and its 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 the Eastern and Southern Africa regions, and by Institut Pasteur du Cameroun which supports the Central Africa region. (Figure 2). This expansion in coverage is expected to support the resilience the network in its confirmatory testing capacity and to also improve the speed of case confirmation in endemic countries, which is key to ensure timely outbreak response measures, including vaccination campaigns.

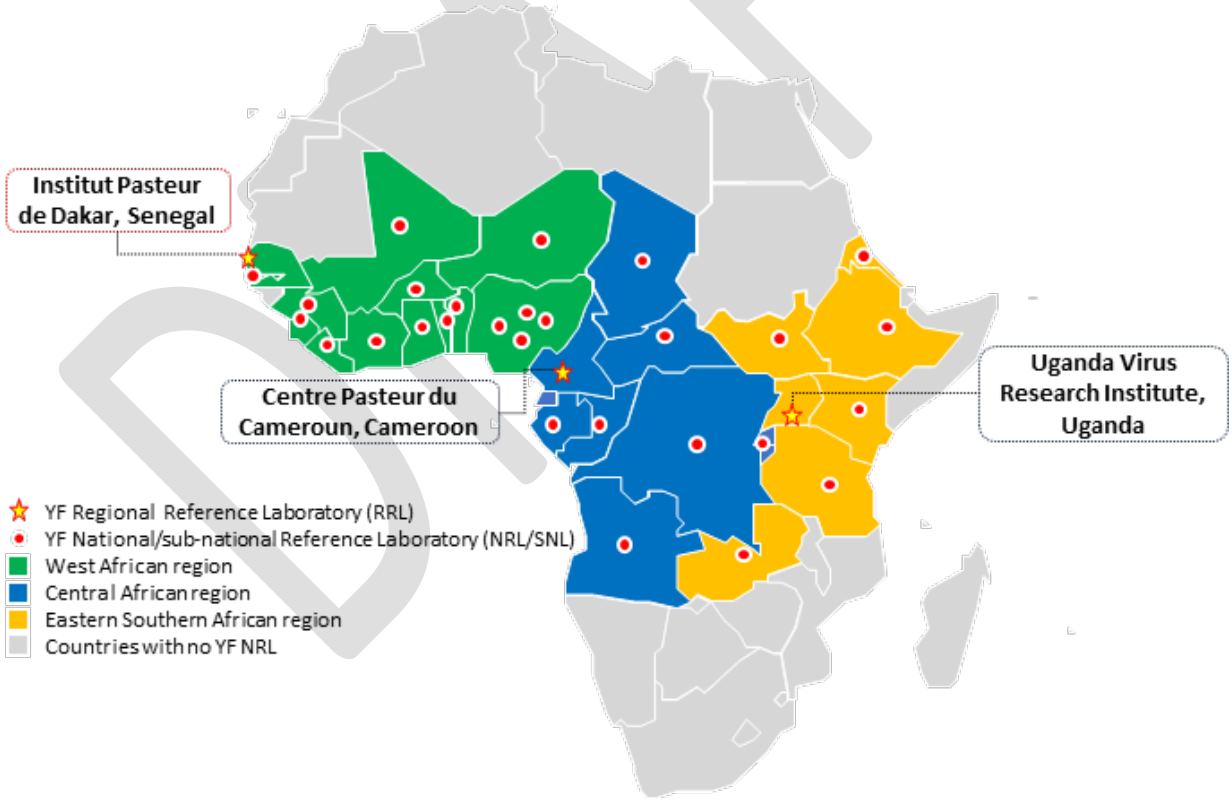


**Figure 2:** Countries part of the YF AFRO laboratory network with the respective reference laboratories

## Purpose

The availability of standardized, well-performing and validated ¨testing methods for surveillance is essential to ensure accurate and early confirmation of YF cases. In response to Gavi's assessment of YF diagnostics needs, a target product profile (TPP) document was developed in 2019 by FIND in consultation with WHO to encourage manufacturers to submit YF assays for evaluation by WHO. Following few rounds of WHO Kit performance evaluations, three commercial assays have been approved for use in the Global Yellow Fever Laboratory Network (GYFLaN) and are now available through UNICEF Supply Division procurement mechanism. The present document is building on the previous TPP document but correspond to a new WHO-led TPP publication replacing the 2019 version, and now including additional assay categories.

## Rationale

Gaps and challenges in the rapid identification of YF outbreaks must be addressed to support effective vaccine campaigns and reduce the spread of the disease. Data and geographical distribution are representative of YF activity and are indispensable for adequate and efficient planning for vaccination campaigns. The addition of newly approved YF assays is needed to ensure the availability of testing methods. Currently, one molecular, one IgM enzyme-linked immunosorbent assay (ELISA) and one rapid serological IgM test has been recommended for use within the GYFLaN and made available for procurement through UNICEF procurement mechanism. While this represents significant improvement over previously used in-house or laboratory-developed assays, further improvements in technology, performance or analytes are desirable. Having several commercial assays across various platform can also help reducing the network vulnerability to any breakdown in manufacturing. The current TPP document is aspirational in nature, highlighting minimal and optimal characteristics, and uses the performance parameters of the current assays as benchmarks for minimum performance of new assays. A section on antigen detection has also been added. The aim of the TPP is to guide on-going and forthcoming efforts in YF assay development to result in products that are fit-for-purpose and addressing current needs observed by the GYFLaN. Future WHO Kit performance evaluations will also use the characteristics mentioned herein to guide the eligibility criteria of the programme.

# Laboratory testing for yellow fever surveillance

## Molecular assays

YFV genomic material (single-stranded RNA) can be detected in blood during the first 10-14 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 as a rule-in test for early case confirmation as the virus in the blood rapidly decreases over time, even to undetectable levels, and viral RNA is easily degraded. Therefore, a negative PCR does not exclude YF infection.

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## Serological assays

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 approximately day five of illness (varies between subjects) 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, or even from previous history of YF vaccination. To confirm active infection following a positive ELISA, a complex serological 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.

## Antigen capture assays

Molecular assays are the ideal laboratory means to confirm YF infection because they allow for direct detection of YFV and do not suffer from cross-reactivity from related viruses and are highly sensitive. Specimen integrity, however, is a major limiting factor in the effectiveness of molecular assays, due to the ease of viral RNA degradation during storage and transport. Viral antigen captured from an acute specimen on the other hand, can be a more stable option, with even a possible longer window period of detectability post onset while still allowing direct detection with the potential for reduced cross-reactivity. It also lends itself to detection via rapid testing which could expand its usefulness. While the sensitivity is likely lower than for molecular testing, there may be a place for antigen capture assays in the YF testing repertoire as has been demonstrated for dengue infections. While the antigenic site(s) for IgM antibodies used in the immunoassays have largely been determined and optimized to include the major immunogenic epitopes (mostly against the E protein), the choice of an antigen for a capture assay has not. The sensitivity of the target antigen would need to be determined, as it has been for the dengue antigen detection assays. A rapid test for the detection of YF antigen would be useful in both laboratory and point-of-care settings, removing the requirement for shipping of specimens thus preserving specimen integrity.

Currently, RRLs and NRLs are involved in the testing of YF suspected cases, along with a few sub-National Laboratories (sub-NL). Lower levels of the health system could be involved in the testing of YF suspected cases if new assays became available that are easier to use, faster and more accurate, and to ensure availability of testing options. To address these gaps this WHO-led target product profile (TPP) development process now covers four testing tools to identify yellow fever infections in the context of surveillance:

- A standardized molecular assay test kit (**Table 1**)
- A standardized IgM immunoassay test kit (**Table 2**)
- Rapid IgM immunoassay test (**Table 3**)
- Rapid antigen test (**Table 4**)

Reminder: 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.

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# Conclusion

These four TPPs will guide assessments of which yellow fever surveillance 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.

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## Table 1: Standardized YF molecular test kit

| Target product profile for a standardized molecular assay test kit to identify yellow fever infection | | |
|---|---|---|
| **Characteristic** | **Minimal** | **Optimal** |
| ***SCOPE*** | | |
| **1. Intended use** | Confirmation of yellow fever (YF) infection in human specimens | Same, plus ability to exclude or distinguish from circulating remnant material from YF vaccination |
| **2. Target test type** | A standardized reverse transcription molecular assay test kit to specifically detect YFV genomic material (or fragments of) | Same as minimal and to allow minimally trained laboratorians to conduct the assay |
| **3. 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 | |
| **4. Target use setting** | National reference laboratory (level 3[2]) or above | District Hospitals (Level 2) or above |
| **5. Target users** | Laboratorians with training in molecular diagnostics | |
| **6. Target analytes** | Yellow fever genomic material (RNA) from all strains of YF | |
| **7. Target kit format** | A standardized kit that contains all materials required for the procedure including controls, reagents and needed consumables (e.g., reagent grade water) | Same, plus lyophilized master mix with all reagents required to be aliquoted by user |
| ***SPECIMEN REQUIREMENTS*** | | |

[1] Case definition of suspected yellow fever as defined WHO Surveillance Standards for Yellow Fever, https://www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-yellow-fever

[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

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| **8. Specimen types** | Serum and plasma | Serum, plasma, whole blood, urine, and blood collection tubes or cards that stabilize nucleic acids without the need for cold storage |
| **9. Specimen volume** | Extracted RNA volume 10 µl or less per reaction | |
| **10. Specimen transport conditions tolerated by test** | ≥3 days on cold packs | ≥4 days at 10-35°C where stabilized specimens that require no cold chain are compatible with the test |
| ***OPERATIONAL CHARACTERISTICS*** | | |
| **11. Ease of use** | No more than 10 steps needed to perform test. Clear and complete instructions provided with the kit. | No more than 5 steps needed to perform test. No preparation of Master Mix (except for reconstitution of lyophilized Master Mix) or reagent dilution required. Clear and complete instructions provided with the kit. |
| **12. Quality control** | Internal (housekeeping gene to control for RT-qPCR inhibition/correct amplification) and positive target (YFV RNA) assay controls provided with test kit | Same as minimal plus an extraction control (or the recommended use, as per the instruction, of the internal control to act as both the extraction and amplification control) |
| **13. Time to result** | Excluding RNA extraction, <3h | |
| **14. Stability of valid result** | N/A | |
| **15. Specimen capacity and throughput** | Flexible set-up to run few or many samples up to 96 reactions and maintain kit shelf-life regardless of number of uses | |
| **16. Patient ID capacity** | Ability to track electronic identification number of the patient either manually or via barcode | |
| **17. Result type** | Qualitative | Quantitative |
| **18. Result output** | Real-time curve, Ct value | Same as minimal plus controls validity check |
| **19. Result interpretation** | Manual where interpretation parameters are included in the instructions | Automatic |
| **20. Data export** | Manual | Automatic |

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| **21. Platform considerations** | Validated by manufacturer for the five most commonly available thermocyclers with thermocycler-specific Ct cut off values for assay determined (see Appendix A) | |
| **22. Waste disposal** | Small environmental footprint; recyclable or compostable plastics for test cartridges and other materials. Ease of disposal after decontamination through general waste, no incineration required | |
| **23. Safety precautions** | No further biosafety requirements beyond what is currently state of practice for regional and national labs | |
| ***ENVIRONMENTAL CONSIDERATIONS*** | | |
| **24. Operating conditions** | Operation between 10°C and 35°C; Ability to tolerate humidity from 30-85% up to 2500 m altitude | Operation between 10°C and 45°C, between 15% and 95% non-condensing humidity, and altitude of at least 3500m |
| **25. Test kit storage conditions** | -20°C, 30-85% humidity, up to 2500-meter altitude. Kit should include indicator of instability or early expiration | 2-50°C, 10-90% humidity, up to 3500-meter altitude.<br>Indicator of instability or early expiration |
| **26. Test kit stability (unopened)** | 15 months | 24 months |
| **27. Test kit stability (opened)** | 6 months<br>Storage of aliquoted master mix by freezing[3] with at least 2 freeze thaw cycle tolerated | 12 months or more<br>Storage of aliquoted master mix by freezing with at least 3 freeze thaw cycles tolerated |
| **28. Test shipping conditions** | Cold packs with ability to tolerate 72 hours with fluctuations between 2°C and 45°C and 10-95% humidity | Ambient temperature with ability to tolerate 72 hours with fluctuations between 2°C and 55°C and humidity of 10-95% |
| ***PERFORMANCE CHARACTERISTICS*** | | |
| **29. Analytical sensitivity** | Limit of detection ≤25 RNA copies/reaction | Limit of detection ≤10 RNA copies/reaction |

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

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| **30. Analytical specificity** | 100% demonstrated in non-YF flaviviruses and diseases in YF differential, and minimally including Plasmodium falciparum (Malaria), Leptospira, HIV, Hepatitis B virus, Hepatitis E virus, Zika virus, Dengue 1 virus, Dengue 2 virus, Dengue 3 virus, Dengue 4 virus, Japanese encephalitis virus, West Nile virus, and Chikungunya virus; plus any other related viruses or agents relevant for differential etiology in regions at-risk of yellow fever | |
| **31. Analytical inclusivity** | Assay detects at least 9 geographically and genetically diverse yellow fever viral strains | |
| **32. Interfering substances** | Assay demonstrates no interference from 1) Endogenous substances: triglycerides, bilirubin, and haemoglobin and 2) Exogenous substances: paracetomol, EDTA, citrate | Same as minimal plus 1) Endogenous substances: malaria, human genomic DNA, albumin |
| **33. Clinical sensitivity** | ≥95% positive percent agreement | ≥99% positive percent agreement |
| **34. Clinical specificity** | ≥95% negative predictive value | ≥99% negative predictive value |
| **35. Lot-to-lot consistency** | No change in Ct cut-off from lot to lot (CV <1) | |
| ***PRICING AND ACCESSIBILITY*** | | |
| **36. Target list price** | <$10 USD per sample tested | <$5 USD per sample tested |
| **37. Regulatory requirements** | Successfully evaluated bv WHO and/or approved by stringent regulatory body | |
| **38. Reference samples used to evaluate test performance** | Samples from:<br>• Representative strains from African and Latin American lineages<br>• Samples from individuals with confirmed yellow fever (not vaccine) viremia by validated molecular assay<br>• Samples from individuals with confirmed viremia with other | Same as minimal plus:<br>• Samples from individuals with confirmed acute yellow fever infection with varying time points up to resolution of infection/disease<br>• Samples from individuals in both acute and toxic phase of disease |

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| | flaviviruses and pathogens, including Zika, Dengue, West Nile, Chikungunya and others as appropriate)<br>• Samples from individuals with recent yellow fever vaccination<br>• Defined dilution of known negative sera spiked with genomic material from well-characterized YF strains,<br>• Confirmed non-arboviruses | |

| Appendix A: Example of 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 |

*Note that these instruments and numbers will change over time

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## Table 2: Standardized YF immunoglobulin M (IgM) ELISA test kit

| Target product profile for a standardized serological assay test kit to identify yellow fever infection | | |
|---|---|---|
| **Characteristic** | **Minimal** | **Optimal** |
| ***SCOPE*** | | |
| **1. Intended use** | Presumptive identification of yellow fever infection for surveillance purposes | Same as minimal plus distinguish between natural infection and vaccination |
| **2. Target test type** | YF IgM ELISA assay | |
| **3. Target population** | Testing of specimens collected from individuals suspected of yellow fever infection[4] or in the context of a documented outbreak, also specimens collected from individuals with fever and an epidemiological link to a confirmed case/outbreak | |
| **4. Target use setting** | National reference laboratory (Level 3[5]) or above | District Hospitals (Level 2) or above |
| **5. Target users** | Laboratorians with training in immunodiagnostics | |
| **6. Target analytes** | No additional target analytes beyond IgM to YF | YF plus IgM to the following pathogens in descending level of priority:<br>• Dengue 1-4<br>• Zika<br>• West Nile |
| **7. Target kit format** | 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 for wash buffers) to perform the assay | |
| ***SPECIMEN REQUIREMENTS*** | | |
| **8. Specimen types** | Serum or plasma | Serum, plasma, whole blood, dried blood spots |
| **9. Specimen volume** | ≤50 µL | |
| **10. Specimen transport conditions required by test** | ≥3 days on cold packs | ≥4 days at 10-35°C where stabilized specimens that require no cold chain are compatible with the test |
| ***OPERATIONAL CHARACTERISTICS*** | | |

[4] Case definition of suspected yellow fever as defined by the WHO Surveillance Standards for Yellow Fever, https://www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-yellow-fever

[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

[5] Test performance for yellow fever is required to be the same for a multiplex test as specified for a monoplex test

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| **11. Ease of use** | Clear and complete instructions for use; some dilution or reconstitution of reagents required | Clear and complete instructions for use; no dilution or reconstitution of reagents required (with the exception of reagents lyophilized for stability) |
| **12. Quality control** | All assay controls provided with test kit | |
| **13. Time to result** | < 6 hours (i.e., same day result) | < 3 hours |
| **14. Stability of valid result** | >10 min | >30 min |
| **15. Specimen capacity and throughput** | 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. Plate frame must be provided. | |
| **16. Patient ID capacity** | N/A | |
| **17. Result type** | Qualitative | Semi-quantitative (comparative to a standard YF IgM specimen) |
| **18. Result output** | Optical density | Same as minimal or allow for other chemistries or visual evaluation |
| **19. Result interpretation** | Manual if using visual evaluation; otherwise, automated | |
| **20. Data export** | Manual | Automated |
| **21. Platform considerations** | Kit and instructions compatible with manual plate washing and standard automated plate washers (both "row" and 96-well) and standard plate readers[6] | |
| **22. Waste disposal** | Small environmental footprint: recyclable or compostable plastics for test cartridges and other materials after decontamination, no incineration required | |
| **23. Safety precautions** | No further biosafety requirements beyond what is currently state of practice for regional and national labs | |
| ***ENVIRONMENTAL CONSIDERATIONS*** | | |
| **24. Operating conditions** | Operation between 10°C and 35°C; Ability to tolerate humidity from 30-85% up to 2500 m altitude | Operation between 10°C and 45°C, between 15% and 95% non-condensing humidity, and altitude of at least 3500m |
| **25. Test kit storage conditions** | 2-8°C, 30-85% humidity, up to 2500-meter altitude. Kit should include indicator of | 2-50°C, 10-90% humidity, up to 3500-meter altitude; Indicator of instability or early expiration |

[6] 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.

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| | instability or early expiration | |
| **26. Test kit stability (unopened)** | 15 months | 24 months |
| **27. Test kit stability (opened)** | N/A for single use kit; 3 months for multiple use formats such as 8-well strips | N/A for single use kit; 12 months for multiple use formats such as 8-well strips |
| **28. Test shipping conditions** | Cold packs with ability to tolerate 72 hours with fluctuations between 2°C and 45°C and 10-95% humidity | Ambient temperatures with ability to tolerate 72 hours with fluctuations between 2°C and 55°C and 10-95% humidity |
| ***PERFORMANCE CHARACTERISTICS*** | | |
| **29. Analytical sensitivity** | N/A | |
| **30. Analytical specificity** | Assay demonstrates negative results for samples containing IgM to non-flavivirus arboviruses in the YF differential, and non-arboviruses malaria, hepatitis C, Leptospira, and Epstein-Barr virus. | Same as minimal plus non-YF flaviviruses, and YF vaccine |
| **31. Analytical inclusivity** | Assay detects IgM immune response to geographically and genetically diverse yellow fever viral strains | Same as minimal plus detection of IgM immune response to geographically and genetically diverse strains of the other pathogen target analytes |
| **32. Interfering substances** | Assay demonstrates no interference of results when 1) Endogenous substances: hemolytic samples, samples containing rheumatoid factor, | Same as minimal plus no interference of results when 1) Endogenous substances: lipemic samples are used |

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| | or samples containing anti-nuclear antibodies, are used, 2) Exogenous substances : EDTA and citrate, are present | |
| **33. Clinical sensitivity** | ≥90% positive agreement with results from a reference assay | ≥95% positive agreement with results from a reference assay |
| **34. Clinical specificity** | ≥90% negative agreement with results from a reference assay | ≥98% negative agreement with results from a reference assay |
| **35. Lot-to-lot consistency** | No recalibration or change in cut-off from lot to lot | |
| ***PRICING AND ACCESSIBILITY*** | | |
| **36. Target list price** | <$10 USD / sample for a full 96-well plate including the required controls | <$3.3 USD per sample for a full 96-well plate including the required controls |
| **37. Regulatory requirements** | WHO PQ or other stringent regulatory body | |
| **38. Reference samples used to evaluate test performance** | Samples from:<br>• individuals with proven past YF infection (PRNT or lab-based IgM)<br>• individuals with known flavivirus exposure and no evidence of YF IgM<br>• individuals with proven previous infection with other flaviviruses (Zika, dengue, West Nile)<br>• individuals with prior YF vaccination<br>• Confirmed non-arboviruses | Samples from a well-characterized cohort:<br>• individuals with virological confirmation of acute YF infection, with varying time points after resolution of acute infection<br>• individuals with no known flavivirus exposure and no evidence of YF IgM<br>• asymptomatic individuals with proven past YF infection (PRNT or lab-based IgM)<br>• individuals with proven previous infection with other flaviviruses, with varying time points after resolution of infection<br>• individuals with previous infection of both YF and other flaviviruses<br>individuals with prior and recent YF vaccination |

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## Table 3: Rapid YF immunoglobulin M test

| Target product profile for a standardized rapid immunoassay test kit to identify yellow fever infection | | |
|---|---|---|
| **Characteristic** | **Minimal** | **Optimal** |
| ***SCOPE*** | | |
| **1. Intended use** | Qualitative detection of IgM antibodies against yellow fever virus (YF) in human for the presumptive identification of syndromic YF infection for surveillance purposes | Case confirmation of YF infection |
| **2. Target test type** | Rapid immunoassay (e.g. lateral flow assay) | |
| **3. Target population** | Suspected YF cases[7] | |
| **4. Target use setting** | For use at primary health care settings including health posts (Level 1[8]) and above | |
| **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** | IgM antibodies specific to YFV | Same as minimal plus multiplexing with IgM detection of dengue, Zika, and West Nile viruses and/or with YF antigen detection |
| **7. Target kit format** | A single use disposable assay, housed in a plastic cassette or on individual strip card | |
| ***SPECIMEN REQUIREMENTS*** | | |
| **8. Specimen types** | Capillary blood, whole blood and serum. | Same as minimal plus samples extracted from protein saver cards or dried blood spots |
| **9. Specimen volume** | ≤50 µl | <10 µl |
| **10. Specimen transport conditions required by test** | ≥3 days on cold packs [9] | ≥4 days at 10-35°C where stabilized specimens that require no cold chain are compatible with the test |
| ***OPERATIONAL CHARACTERISTICS*** | | |

[7] Case definition of suspected yellow fever as defined by WHO Surveillance Standards for Yellow Fever

[8] 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

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

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| **11. Ease of use** | No more than two non-labour-intensive operator steps, none of which requires a fixed period of incubation (excluding assay run time), and excluding waste disposal | One non-labour-intensive operator step (excluding assay run time and waste disposal) |
| **12. Quality control** | Procedural (reagent/specimen-addition) control internalized for each individual test run | Procedural (reagent/specimen-addition) control internalized for each individual test run; at least a positive control and if possible a negative control for quality control testing provided in each box of test kits |
| **13. Time to result** | ≤20 minutes | ≤10 minutes |
| **14. Stability of valid result** | A valid result reading period of at least 30 minutes (after which results may be false or invalid)<br>Clear language in the instructions for use regarding test reading | A valid result reading period of ≥1 hour (after which results give invalid rather than false results); Clear language in the instructions for use regarding test reading[10] |
| **15. Specimen capacity and throughput** | 1 specimen per test | |
| **16. Patient ID capacity** | Simple, self-contained way to indicate a patient identifier | |
| **17. Result type** | Qualitative | |
| **18. Result output** | Visible control line(s) or checkmarks to verify that the assay has not been compromised and the result is valid, and a visible line or checkmark for positive specimen result | |
| **19. Result interpretation** | It must be possible for result to be read with the naked eye including in low light settings with minimal instructions for interpretation required by user, without excluding the possibility to be read using an external and portable reader | |
| **20. Data export** | None | 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)[11] |

[10] 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.)

[11] Reader requirements have been previously defined through a TPP consensus process (https://iris.who.int/handle/10665/365980)

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| **21. Platform considerations** | N/A | |
| **22. Waste disposal** | Small environmental footprint: recyclable or compostable plastics for test cartridges and other materials after decontamination, no incineration required | |
| **23. Safety precautions** | No further biosafety requirements beyond what is currently state of practice for healthcare testing facilities | |
| ***ENVIRONMENTAL CONSIDERATIONS*** | | |
| **24. Operating conditions** | Operation between 10°C and 35°C; Ability to tolerate humidity from 30-85% up to 2500 m altitude | Operation between 10°C and 45°C, between 15% and 95% non-condensing humidity, and altitude of at least 3500m |
| **25. Test kit storage conditions** | Ambient temperature between 2-35C, 30-85% humidity, up to 2500 meters altitude, no cold chain required at any point Kit should include indicator of instability or early expiration | Ambient temperature between 0-50°C, 10-90% humidity, up to 3500 meters altitude, no cold chain required at any point; Kit should include indicator of instability or early expiration |
| **26. Test kit stability (unopened)** | 15 months | 24 months |
| **27. Test kit stability (opened)** | 3 months | 12 months |
| **28. Test shipping conditions** | Ambient temperatures with ability to tolerate 72 hours with fluctuations between 2°C and 45°C and 10-95% humidity | Ambient temperatures with ability to tolerate 72 hours with fluctuations between 2°C and 55°C and 10-95% humidity |
| ***PERFORMANCE CHARACTERISTICS*** | | |
| **29. Analytical sensitivity** | N/A | |
| **30. Analytical specificity** | Assay demonstrates negative results for samples containing | Same as minimal plus non-YF flaviviruses. |

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|---|---|---|
| | IgM to other viruses in the YF differential (e.g. viral hepatitis, Chikungunya, Rift Valley Fever), and samples positive for malaria and Leptospira | |
| **31. Analytical inclusivity** | Assay detects IgM immune response to geographically and genetically diverse yellow fever virus strains | Same as minimal plus detection of IgM immune response to geographically and genetically diverse strains of the other pathogen target analytes |
| **32. Interfering substances** | Assay demonstrates no interference of results when 1) Endogenous substances: hemolytic samples, samples containing rheumatoid factor, or samples containing anti-nuclear antibodies, are used and 2) Exogenous substances EDTA and citrate, are present | Same as minimal plus no interference of results when 1) Endogenous substances: lipemic samples are used |
| **33. Clinical sensitivity** | >90% positive percent agreement (PPA) with reference method | ≥95% PPA with reference method |
| **34. Clinical specificity** | ≥90% Negative percent agreement (NPA) with reference method | ≥98% NPA with reference method |
| **35. Lot-to-lot consistency** | No clear visual difference in band intensity of positive control between lots | No clear visual difference in the positive control band intensity as compared to a validated external control sample |
| ***PRICING AND ACCESSIBILITY*** | | |
| **36. Target list price** | <$3.5 USD | <$1 USD |

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|---|---|---|
| **37. Regulatory requirements** | WHO PQ or other stringent regulatory body | |
| **38. Reference samples used to evaluate test performance** | Samples from:<br>• Individuals with proven past YF infection (positive PRNT or PCR result)<br>• Individuals with known flavivirus exposure and no evidence of YF IgM<br>• Individuals with proven previous infection with other flaviviruses (Zika, dengue, West Nile)<br>• Individuals with prior YF vaccination<br>• Confirmed non-arboviruses | Samples from a well-characterized cohort:<br>• Individuals with virological confirmation of acute YF infection, with varying time points after resolution of acute infection<br>• Individuals with no known flavivirus exposure and no evidence of YF IgM<br>• Asymptomatic individuals with proven past YF infection (PRNT or lab-based IgM)<br>• Individuals with proven previous infection with other flaviviruses, with varying time points after resolution of infection<br>• Individuals with previous infection of both YF and other flaviviruses<br>Individuals with prior and recent YF vaccination |

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## Table 4: Rapid YF antigen test

| Target product profile for a standardized rapid antigen assay test kit to identify yellow fever infection | | |
|---|---|---|
| **Characteristic** | **Minimal** | **Optimal** |
| ***SCOPE*** | | |
| **1. Intended use** | Qualitative detection of yellow fever virus (YF) antigen particles in human for the presumptive identification of YF infection for surveillance purposes | Case confirmation of YF infection |
| **2. Target test type** | Rapid antigen detection assay | Rapid lateral flow antigen detection assay or self-contained assay |
| **3. Target population** | Individuals suspected of YF infection[12] or individuals with an epidemiological link to a confirmed case or an outbreak[13] | |
| **4. Target use setting** | For use at primary health care settings including health posts (Level 1[14]) and above | For use at primary health care settings including health posts (Level 1[15]) and above |
| **5. Target users** | Community health workers with minimal training and any health worker or laboratorian with a similar or superior training level | |
| **6. Target analytes** | YF antigen | Same as minimal plus YF IgM test or DEN NS1 antigen test |
| **7. Target kit format** | A single use disposable, rapid self-contained assay housed in a test cassette | |
| ***SPECIMEN REQUIREMENTS*** | | |
| **8. Specimen types** | Capillary blood, whole blood, plasma and serum | Same as minimal plus urine, saliva and samples extracted from dried blood spots |
| **9. Specimen volume** | ≤100 µl | ≤10 µl |

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

[13] Examples of an epidemiological link to a confirmed case or an outbreak include household members or persons in close proximity to case through work, residence in past month), as described in the WHO Surveillance Standards for Yellow Fever.

[14] 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

[15] 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

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|---|---|---|
| **10. Specimen transport conditions required by test** | ≥3 days on cold packs | ≥4 days at 10-35°C where stabilized specimens that require no cold chain are compatible with the test |
| ***OPERATIONAL CHARACTERISTICS*** | | |
| **11. Ease of use** | No more than two non-labour-intensive operator steps, none of which requires a fixed period of incubation (excluding assay run time), and excluding waste disposal | One non-labour-intensive operator step (excluding assay run time and waste disposal) |
| **12. Quality control** | Procedural (reagent/specimen-addition) control internalized for each individual test run | Procedural (reagent/specimen-addition) control internalized for each individual test run; at least a positive control and if possible a negative control for quality control testing provided in each box of test kits |
| **13. Time to result** | ≤60 minutes | ≤10 minutes |
| **14. Stability of valid result** | A valid result reading period of at least 20 minutes (after which results may be false or invalid)<br>Clear language in the instructions for use regarding test reading | A valid result reading period of ≥1 hour (after which results give invalid rather than false results); Clear language in the instructions for use regarding test reading[16] |
| **15. Specimen capacity and throughput** | 1 specimen per test | |
| **16. Patient ID capacity** | Simple, self-contained way to indicate a patient identifier | |
| **17. Result type** | Qualitative | |
| **18. Result output** | Visible line or checkmark for positive specimen result | |
| **19. Result interpretation** | It must be possible for result to be read with the naked eye including in low light settings with minimal instructions for interpretation required by user, | |

[16] 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.)

| | | |
|---|---|---|
| | without excluding the possibility to be read using an external and portable reader | |
| **20. Data export** | None positioning system (GPS) tags)[17] | 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 positioning system (GPS) tags[18] |
| **21. Platform considerations** | N/A | |
| **22. Waste disposal** | Small environmental footprint: recyclable or compostable plastics for test cartridges and other materials after decontamination, no incineration required | |
| **23. Safety precautions** | No further biosafety requirements beyond what is currently state of practice for healthcare testing facilities | |
| ***ENVIRONMENTAL CONSIDERATIONS*** | | |
| **24. Operating conditions** | Operation between 10°C and 35°C; Ability to tolerate humidity from 30-85% up to 2500 m altitude | Operation between 10°C and 45°C, between 15% and 95% non-condensing humidity, and altitude of at least 3500m |
| **25. Test kit storage conditions** | Ambient temperature between 2-35°C, 30-85% humidity, up to 2500 meters altitude, no cold chain required at any point. Kit should include indicator of instability or early expiration | Ambient temperature between 0-50°C, 10-90% humidity, up to 3500 meters altitude, no cold chain required at any point. Kit should include indicator of instability or early expiration |
| **26. Test kit stability (unopened)** | 15 months | 24 months |
| **27. Test kit stability (opened)** | 3 months | 12 months |
| **28. Test shipping conditions** | Ambient temperatures with ability to tolerate 72 hours with fluctuations between 2°C and | Ambient temperatures with ability to tolerate 72 hours with fluctuations between 2°C and 55°C and 10-95% humidity |

[17] Reader requirements have been previously defined through a TPP consensus process (https://iris.who.int/handle/10665/365980)

[18] Reader requirements have been previously defined through a TPP consensus process (https://iris.who.int/handle/10665/365980)

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|---|---|---|
| | 45°C and 10-95% humidity | |
| ***PERFORMANCE CHARACTERISTICS*** | | |
| **29. Analytical sensitivity** | N/A | |
| **30. Analytical specificity** | Assay demonstrates negative results for samples containing antigen to other flaviviruses and non-flavivirus arboviruses in the YF differential | Same as minimal plus Leptospira and hepatitis C |
| **31. Analytical inclusivity** | Assay detects antigen response to geographically and genetically diverse yellow fever virus strains | Same as minimal plus YF vaccine strains |
| **32. Interfering substances** | Assay demonstrates no interference of results when 1) Endogenous substances: hemolytic samples, samples containing rheumatoid factor, or samples containing anti-nuclear antibodies, are used, and 2) EDTA and citrate, are present | Same as minimal plus no interference of results when 1) Endogenous substances: lipemic samples are used |
| **33. Clinical sensitivity** | ≥90% positive percent agreement compared to a reference method | ≥95% positive percent agreement compared to a reference method |
| **34. Clinical specificity** | ≥95% negative percent agreement with reference method | ≥99% negative percent agreement with reference method |
| **35. Lot-to-lot consistency** | No clear visual difference in reactivity of positive control between lots | No clear visual difference in the positive control band intensity as compared to a validated external control sample |

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| ***PRICING AND ACCESSIBILITY*** | | |
|---|---|---|
| **36. Target list price** | <$3.5 USD per sample tested | <$1 USD per sample tested |
| **37. Regulatory requirements** | WHO PQ or other stringent regulatory body | |
| **38. Reference samples used to evaluate test performance** | Samples from:<br>• Characterized lysates from representative strains from African and Latin American lineages<br>• Individuals with confirmed yellow fever (not vaccine) viremia by validated molecular assay<br>• Individuals with confirmed viremia with other flaviviruses and pathogens, including Zika, Dengue, West Nile, Chikungunya and others as appropriate)<br>• Individuals with recent yellow fever vaccination<br>• Confirmed non-arboviruses | Same as minimal plus:<br>• Samples from individuals with confirmed acute yellow fever infection with varying time points up to resolution of infection/disease<br>• Samples from individuals in both acute and toxic phase of disease |

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