WHO public report
for the kit performance evaluation of
*YF MAC-HD 1.0 assay to detect IgM to yellow fever virus*
in the context of surveillance
WHO public report for the kit performance evaluation of YF MAC-HD 1.0 assay to detect IgM to yellow fever virus in the context of surveillance

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Special appreciation to Dr Fien Vanroye and Dr Marjan Van Esbroeck from the Institute of Tropical Medicine in Antwerp (Belgium) for performing the independent laboratory performance testing.

This document was developed in consultation with:

From the World Health Organization: Amal Barakat, Anindya Bose, Maurice Demanou, Jennifer Horton, Jean-François Lemaire, Miguel Mulders, and Jose Rovira Vilaplana.

From the Pan American Health Organization (PAHO) / WHO Regional Office of the Americas (AMRO): Jairo Andres Méndez-Rico.

Assessment Process

Following an Expression of Interest (EoI) call published on January 29th 2021 and subsequent application by manufacturers, WHO underwent a preliminary Dossier Review of all applications during Q3 2021. Dossiers of successful applicants such as ATCC®, underwent a Quality Management System (QMS) documentation and processes review, as well as an independent laboratory performance evaluation between Q3 2021 until Q2 2022. Findings of both the QMS review and independent laboratory evaluation were consolidated into a first draft recommendation document by WHO-HQ VPD Surveillance and Risk Assessment team in Q3 2022, all of which underwent a technical consultation with individual experts in Q4 2022. Such group of individuals located across various regions are Individual experts in the field of arboviruses, and in particular in the field of Yellow Fever diagnostics, as well as experts in IVD regulatory affairs and manufacturing QMS audits, as listed in the below Table. Peer Reviewers were Dr. Barbara W. Johnson and Dr. Eric Mossel, both renown virologists with decades of expertise in yellow fever diagnostics.

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>WHO region</th>
<th>Gender</th>
<th>Expertise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robyn Meurant</td>
<td>ACT-IVD</td>
<td>EURO</td>
<td>Female</td>
<td>IVD regulatory affairs, Manufacturing quality management system audits</td>
</tr>
<tr>
<td>Dragana Milic</td>
<td>MTDV PTY LTD</td>
<td>WIPRO</td>
<td>Female</td>
<td>IVD regulatory affairs, Manufacturing quality management system audits</td>
</tr>
<tr>
<td>Matthias Niedrig</td>
<td>Expert Consultant for Virus Diagnostic GmbH</td>
<td>EURO</td>
<td>Male</td>
<td>Virologist, Yellow Fever laboratory expert and accreditor.</td>
</tr>
<tr>
<td>Abanda Ngu</td>
<td>Centre Pasteur Cameroon</td>
<td>AFRO</td>
<td>Male</td>
<td>Virologist, Yellow Fever laboratory expert</td>
</tr>
<tr>
<td>Ana Bispo</td>
<td>FioCruz</td>
<td>AMRO</td>
<td>Female</td>
<td>Virologist, Yellow Fever laboratory expert</td>
</tr>
<tr>
<td>Cristina Domingo-Carrasco</td>
<td>Robert Koch Institute</td>
<td>EURO</td>
<td>Female</td>
<td>Virologist, Yellow Fever laboratory expert</td>
</tr>
<tr>
<td>Felipe Nevaca</td>
<td>FioCruz</td>
<td>AMRO</td>
<td>Male</td>
<td>Virologist, Yellow Fever laboratory expert</td>
</tr>
<tr>
<td>Gamou Fall</td>
<td>Institut Pasteur de Dakar (IPD)</td>
<td>AFRO</td>
<td>Female</td>
<td>Virologist, Yellow Fever laboratory expert</td>
</tr>
<tr>
<td>Julius Lutwama</td>
<td>Uganda Virus Research Institute (UVRI)</td>
<td>AFRO</td>
<td>Male</td>
<td>Virologist, Yellow Fever laboratory expert</td>
</tr>
<tr>
<td>John Kayiwa</td>
<td>UVRI</td>
<td>AFRO</td>
<td>Male</td>
<td>Virologist, Yellow Fever laboratory expert</td>
</tr>
</tbody>
</table>
All individual experts submitted Declaration of Interest (DoI) and Confidentiality Undertakings (COU) prior to the technical consultation. Assessment and management of conflict of interests from experts was done using WHO’s Individual Expert System (EIS) on the Global Engagement Management platform, and where DoI and COU of WHO experts are reviewed and handled by the Ethics Team (ethicsoffice@who.int). Following this assessment, only experts vetted by the Ethics Team were invited for the consultation.

WHO continues to monitor the fulfillment of the commitments agreed upon by the manufacturer, or any changes made to the product evaluated that may affect this recommendation for use. Should any factors change, WHO will issue a further update.
WHO public report for the kit performance evaluation of YF MAC-HD 1.0 assay to detect IgM to yellow fever virus in the context of surveillance

Product: YF MAC-HD 1.0 Kit to detect IgM to Yellow Fever Virus (RUO)

On 31 January 2023, YF MAC-HD 1.0 Kit to detect IgM to Yellow Fever Virus (henceforth referred to as the YF MAC-HD 1.0 assay) with product code YF-500, was provisionally recommended for use by WHO in the context of surveillance of yellow fever virus (YF).

Note: this is a provisional recommendation for use based on the fact that the original submission to WHO was undertaken by the American Type Culture Collection (ATCC®). At time of acceptance, the product was undergoing comprehensive technical transfer from the United States Centers for Disease Control and Prevention (CDC) to ATCC®, the latter to take on the role of legal manufacturer. CDC is monitoring the transfer to ensure that the standard of manufacturing expected for this product when transfer is complete will be acceptable, and that the performance will not be adversely impacted by the transfer of manufacturing.

Intended use:
According to the claim of intended use provided with the assay, the YF MAC-HD 1.0 assay is for research use only (RUO) and is intended for use in the detection of anti-YF IgM in human serum, for surveillance purposes. It should be used in a laboratory setting by trained laboratory personnel. The results of this assay are qualitative and should be used in the context of laboratory surveillance detection of anti-YF IgM in human serum where IgM results should be confirmed according to applicable laboratory guidelines.

This assay has not been validated for any clinical claims e.g., for diagnosis of yellow fever.

Assay principle:
The ATCC® YF MAC-HD 1.0 assay is a qualitative assay, the design being based on the CDC MAC-ELISA which detects IgM to YF. The YF MAC-HD 1.0 assay uses anti-human IgM to capture IgM in human serum. If IgM reactive to yellow fever is present, this reacts with non-infectious YF whole virus antigen, which is detected using a horseradish peroxidase-conjugated flavivirus group-reactive monoclonal antibody, and a measurable colorimetric reaction is produced using a 3,3',5,5'-tetramethylbenzidine substrate.

Test kit contents:

1. Wash buffer concentrate (liquid, 10X concentration) (clear bottle) (YF-1)
2. Conjugate concentrate (liquid in stabilizer) (amber vial with white lid) (YF-2)
3. Conjugate diluent (lyophilized) (glass vial with ●--marked silver seal) (YF-3)
4. Sterile water for reconstitution (opaque bottle with green sticker) (YF-4)
5. Negative serum control (lyophilized in buffer) (glass vial with blue seal) (YF-5)
6. Normal antigen – mock-inactivated Vero cell culture supernatant (lyophilized in buffer) (glass vial with green seal) (YF-6)
7. One IgM coated and stabilized 96-well microtiter plate (YF-7)
8. YF IgM positive control (humanized monoclonal antibody) (lyophilized in buffer) (glass vial with red seal) (YF-8)
9. Sample diluent (liquid, at working dilution) (opaque bottle with orange sticker) (YF-9)
10. Stop solution (liquid, at working dilution) (opaque bottle) (YF-10)
11. Substrate (liquid, at working dilution) (brown bottle) (YF-11)
12. YF antigen – inactivated YF 17D Vero cell culture supernatant (lyophilized in buffer) (glass vial with silver seal) (YF-12)
13. Three (3) plate sealers

Items required to perform the assay but not included:
1. Calibrated pipettors [(P-10, P-200, P-1000, multichannel (200 µL)]
2. Biosafety cabinet for manipulation of potentially infectious samples
3. Reagent reservoirs (minimum of 2) – required for use with multichannel pipette
4. Deionized (DI) water for wash buffer
5. 1L PETG bottle or similar container for mixing DI water and wash buffer
6. Plate reader (450 nm filter)
7. Refrigerator (2°C to 8°C)
8. Permanent marker
9. Sample dilution tubes (e.g., Polypropylene; 1 mL tubes; 1 per sample tested) and stoppers

Recommended items not included in the kit:
1. In-house YF virus IgM positive control – strongly recommended to assure testing consistency
2. Plate washer (preferred over washing plates by hand)
3. Vortexer (useful but not required)
4. Incubator set at 28°C (preferred)
5. Scissors
6. Pipet aid (100 mL into 900 mL) or you may pour the pre-measured 100 mL 10X wash buffer into the premeasured 900 mL DI water.

Storage and Transport
The YF MAC-HD 1.0 assay should be stored at 4°C until the expiration date after which time the kit should be disposed of. The transport temperature should be maintained at 4°C. Limited exposure to room temperature (20-25°C) will not cause deterioration of the kit, but exposure to higher temperatures (e.g., 37°C) will cause the substrate and controls to degrade. A Tempilabel® is affixed to the substrate bottle. If the dot is black, this indicates that the kit may have been compromised due to high temperature and the kit should be discarded.
WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL This kit contains reagents made with human serum. The serum is commercially-sourced and tested negative for antibodies against HIV-1, HIV-2, and HCV; and for antigen detection of HIV, HBs, and RPR by FDA approved methods. Handle all sera and kits as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.
Shelf-life upon manufacture:
12 months from date of manufacture

Warnings/limitations:
Refer to the latest version of instructions for use. Research use only; not validated for clinical purposes. Results should be confirmed according to applicable laboratory guidelines.

Product dossier assessment:
In accordance with the WHO procedure for evaluation of yellow fever antibody tests, technical documentation was submitted in support of the safety and performance of the assay. Notwithstanding, certain aspects of the technical documentation were considered lacking during technical review of this documentation. The commitments listed below (refer to section Commitments to WHO Recommendation) must be fulfilled for this product to remain recommended on the WHO website.

Quality Management System (QMS) audit:
A desk review of the quality management system documentation including specific manufacturing documentation of American Type Culture Collection – ATCC®, located at 10801 University Boulevard Manassas, VA 20110-2209 USA, was undertaken by WHO. The quality management system appeared to be well established, however, there are ongoing efforts to bring the design and production transfer of the YF MAC-HD 1.0 assay into the scope of the QMS according to ISO 13485.

ATCC® must commit to providing WHO with required documentation, as requested in section below (refer to Commitments to WHO Recommendation), for the QMS to be considered satisfactory in accordance with WHO requirements and the intended use (surveillance) of this product.

WHO Product Performance Evaluation:
American Type Culture Collection (ATCC®) YF MAC-HD 1.0 assay was evaluated for WHO by the Institute of Tropical Medicine, Antwerp, Belgium. The laboratory evaluation was conducted according to the “WHO PROTOCOL FOR THE PERFORMANCE EVALUATION OF YELLOW FEVER VIRUS (YFV) SEROLOGY ASSAYS”.

The clinical performance evaluation of the YF MAC-HD 1.0 assay demonstrated acceptable levels of agreement of this assay against the Reference testing method broadly used for routine surveillance in WHO yellow fever network laboratories (in-house YF MAC-ELISA method developed by CDC) 1,2,3.

1 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC86599/
2 https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0009417
3 https://stacks.cdc.gov/view/cdc/36321
This evaluation was performed on human serum samples only. The performance of the test on plasma and whole blood samples was not evaluated.

Equivocal results were repeat-tested using the overnight incubation method recommended for specimens with initial equivocal results, as described in the instructions for use. The repeat-testing equivocal and positive results were defined as Positive.

All testing runs were valid (i.e., quality controls showed expected results).

**Clinical Performance Results:**

**Percentage Agreement with Reference Assay:**

The evaluation of the YF MAC-HD 1.0 assay demonstrated acceptable levels of agreement of this assay against the Reference method. NOTE: The negative sera were sampled from different WHO regions, most frequently from Asia (21 of the 50 negative sera).

<table>
<thead>
<tr>
<th>Results of YF MAC-HD 1.0 assay (ATCC®)</th>
<th>Reference method results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YF-positive</td>
</tr>
<tr>
<td>Reactive</td>
<td>28</td>
</tr>
<tr>
<td>Non-reactive</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

Percentage positive agreement against Reference method YF positive samples: 93.3% (95% CI: 78.7% - 98.2%)

Percentage negative agreement against Reference method YF negative samples: 98.0% (95% CI: 89.5% - 99.6%)

No difference was found in percentage agreement against Reference method YF positive samples when calculating the initial and final results.

**Analytical Performance results:**

**Precision**

Precision, as determined by intra-run and inter-run variability of the assay was deemed acceptable.

**Analytical Sensitivity panel**

Analytical sensitivity was demonstrated using five distinct sera positive for YF IgM that were analysed in seven dilution steps from 1:1 to 1:64 (35 samples total). The level of reactivity to specific antibodies, as tested by the reference method, varied among the five non-diluted sera, where concentration was High in one serum, Medium/High in two sera, and Medium in two sera. IgM antibodies against yellow fever were detected in all five sera using the ATCC® YF MAC-HD 1.0 assay, at least in the undiluted specimens. The results indicated a linear decline of the P/N value with increasing dilutions in the positive testing range.

**Analytical Specificity panel**

The analytical specificity panel composed of 90 specimens. Not all samples had been tested previously with the Reference method (the in-house YF MAC-ELISA method developed by CDC) therefore all samples with discrepant results to the expected results were further tested using
the alternative Reference Method, the Euroimmun yellow fever IgM Immunofluorescence (IFA) assay. This assay has proven high levels of performance.

The ATCC® YF MAC-HD 1.0 assay produced false positive results in a total of 29 specimens (all confirmed negative for YF IgM antibodies), as follows:

<table>
<thead>
<tr>
<th>Analytical Specificity - Cross-Reactivity - other Flaviviruses</th>
<th>N° of samples of given marker tested</th>
<th>No Samples reactive in the ATCC® YF MAC-HD 1.0 assay (False Positive YF IgM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Dengue 1, 2, 3, 4 virus (DENV) IgM</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Anti-Zika virus (ZIKAV) IgM</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Anti-West Nile virus (WNV) IgM</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Powassan virus (POWV) IgM</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Anti-Japanese encephalitis virus (JEV) IgM</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Anti-Saint Louis encephalitis virus (SLEV) IgM</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Anti-Hepatitis C virus (HCV)</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analytical Specificity - Cross-Reactivity - other organisms</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Chikungunya virus (CHIKV) IgM</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Anti-Cytomegalovirus (CMV)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Anti-Leptospira (LEP) Ig</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Anti-Eastern equine encephalitis virus (EEEV) IgM</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Anti-Ross River virus (RRV) IgM</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Anti-Epstein Barr Virus IgM (EBV)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Malaria PCR-positive sera (MAL)</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analytical Specificity - Interfering substances - endogenous</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Haemolytic sera (HMS)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Rheumatoid Factor (RF)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Anti-Nuclear antibodies (NA)</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

WHO Provisional Recommendation and Risk Benefit Analysis

WHO has taken into consideration the limitations of the ATCC® YF MAC-HD 1.0 assay. At time of writing of this report, the product is not yet produced fully within an ISO 13485 compliant quality system, and in addition, in analytical studies, it exhibits cross-reactivity to a number of pathogens found commonly in settings where yellow Fever virus can be found. Also, there appears to be potential for false positive results due to the presence of endogenous substances e.g., rheumatoid factor. These risks are considered of relevance to WHO in its decision to provisionally recommend the product.

In contrast to the risks identified above, the setting of use of the test must be considered in greater detail. The current standard of care is a set of reagents (the Reference method referred to in this report) provided to the WHO Global YF Laboratory network list of accredited laboratories. From a regulatory perspective, the use of this test is defined as an in-house laboratory developed test (LDT) with testing undertaken via WHO issued standard operating procedures. The ongoing quality of results of this LDT is monitored based on the results of External Quality Assessment Scheme testing, with the overall findings that the LDT is generally well performed across the Global Yellow Fever Laboratory Network (GYFLaN). However, the probability of variability of quality is much greater with an LDT than with a commercial assay such as the ATCC® YF MAC-HD 1.0 assay, where manufacturing controls should ensure that variability is minimised. Provided that the ATCC® YF MAC-HD 1.0 assay can be made under an appropriate management system, a product less subject to variability in quality and results compared to an LDT, should be assured.

In addition, it is important to note that the test is not used as a clinical tool on patients, but to map the footprint of outbreaks epidemiologically. As such, it is acceptable that the product has Research Use Only status. However, so that precious resources are not diverted due to false results, generated either by design or by manufacturing quality, WHO requires that manufacturing is undertaken to a quality standard similar to that described in ISO 13485:2016 “Medical devices — Quality management systems — Requirements for regulatory purposes”. Published data supports the accuracy of the ATCC® YF MAC-HD 1.0 assay when used in yellow fever-endemic regions⁵.

As a holistic multi-test approach based on the detection of various target analytes and as a countermeasure to performance limitations across assays, the WHO laboratory manual for YF provides a comprehensive testing algorithm including confirmatory and differential testing required to inform final case classification. This measure can account and help reduce misdiagnosis due to documented cross-reactivity of YF IgM testing. Additional warnings have also been added to the product Instructions for Use. Training in understanding the potential for false positives will be given by WHO. ATCC® has agreed to fulfil the commitments below and when WHO is satisfied that ATCC® has fulfilled these commitments, the recommendation will no longer be provisional.

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⁵ https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0009417
Commitments to WHO Recommendation

ATCC® has agreed to the following actions, to ensure ongoing WHO recommendation for use. Should WHO be satisfied with the fulfilment of these actions, this recommendation will no longer be provisional. However, as with any other recommendation for use, should the standard of the test at any point no longer meet WHO requirements, WHO reserves the right to withdraw the recommendation for use.

- Testing of HIV positive specimens: The potential for cross-reactivity to HIV should be investigated, given the high rates of this disease in some areas susceptible to YF outbreaks.
- Transport studies for assay stability: It is understood that these assays must be stored within a critical temperature range and that temperature indicators are provided with the kits. However, claims are made in the IFU that there is a tolerance to fluctuations in shipping temperature. Studies must be undertaken to support these claims. Packaging robustness studies also must be undertaken.
- ATCC® must provide, on request from WHO, specific QMS documentation to demonstrate effectiveness of the QMS as legal manufacturer of the YF MAC-HD 1.0 assay.
- ATCC® must amend its procedures for the management of recalls and regulatory notification to include provisions for reporting/notification of WHO.