Yellow Fever Surveillance with MAC-HD ELISA Assays

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INTRODUCTION

Yellow fever is an acute viral hemorrhagic disease that is caused by the flavivirus Yellow Fever Virus (YFV). YFV is spread through populations by the bite of mosquitoes (primarily from the *Aedes* genus) and is endemic to countries in Africa, as well as South and Central America. The effect of the disease can range from asymptomatic infections, to infections resulting in a severe toxic phase including high fever, jaundice, and bleeding from the mouth, nose, eyes, or stomach.

Surveillance for acute YFV infections is critically important, as the mosquito vector is expanding due to increased urbanization of rural areas. Early detection of outbreaks is critical for preventing the spread of the virus through unvaccinated and under-vaccinated communities.

Enzyme-linked immunosorbent assays (ELISAs) are an important tool in early detection of outbreaks in communities that would benefit the most from interventions such as vaccination.

METHODS

Overview

The YF MAC-HD ELISA (yellow fever M-Antibody capture half day ELISA) uses anti-human Immunoglobulin M (IgM) to capture IgM in human serum Immunoglobulin is the first protein made by the immune system in response to an infection; if IgM reactive to yellow fever is present it will react with non-infectious yellow fever whole virus antigen, which is detected using a horseradish peroxidase-conjugated flavivirus group-reactive monoclonal antibody. A measurable colorimetric reaction is produced using a 3,3',5,5'-tetramethylbenzidine substrate. The addition of stop solution (H_2SO_4) ceases the reaction and causes a color change from blue to yellow in reactive wells. The plate is then read with a spectrophotometer at 450 nm, and the data produced is evaluated based on absorbance (abs) which indicates the presence or absence of yellow fever reactive IgM.

Kit Production

The Yellow Fever ELISA kit contains microtiter plates (treated in lab for ELISA use) which can test up to 24 samples.

All other components fall into two categories:

- Liquid components which must be dispensed (dH_2O, sample diluent, wash buffer, conjugated antibody, substrate, and stop solution).
- Lyophilized components which must be diluted from source vials and freeze dried (positive and negative controls, yellow fever and normal antigens) conjugate diluent).

ACKNOWLEDGEMENTS

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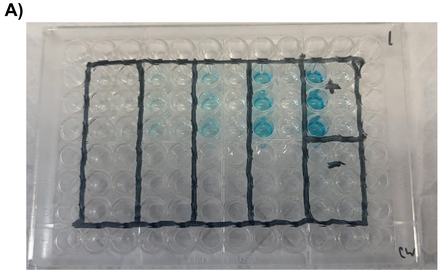
RESULTS

Figure 1: Full MAC-HD ELISA kit contains reagents, plates, and plate seals to run 10 plates with up to 24 samples per plate.

YF-500 kit (**Figure 1**) and quality control plates (**Figure 2**) were considered acceptable if they met suitability criteria.

1. Average blank-subtracted absorbance for the positive assay control with yellow fever antigen was greater than or equal to 1, and less than or equal to 2, with the variability of blank-subtracted absorbances within 0.3 absorbance units.
2. P/N (sample average abs divided by average negative control abs) and NBR (ratio of abs value from YF antigen treated wells to abs value of normal antigen treated wells) were greater than or equal to 2.
3. Average blank-subtracted absorbance for the negative assay control with the YF antigen must have values between 0.02 and 0.12, with variability between samples values of less than 0.025.
4. Test panel samples had P/N values which adhere to the ranges listed in Table 1.

A)



B)

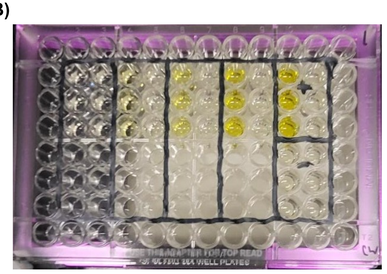


Figure 2: A) QC plate with positive assay control, negative assay control, and low, medium, and high panel samples after substrate addition. **B)** QC plate with positive assay control, negative assay control, and low, medium, and high panel samples after stop solution addition.

Sample	Abs (450 nm)
Negative	≤1.5
Low	2-5
Medium	5-12
High	≥12

Table 1.
P/N value ranges for quality control panel test samples.

RESULTS

Functional shelf life of the kit was demonstrated through continued assessment at specified time points (**Figure 3**). At each time point, 4 plates were tested with test panels at predetermined concentrations and positive controls which indicated the continued viability of all components through 18 months.

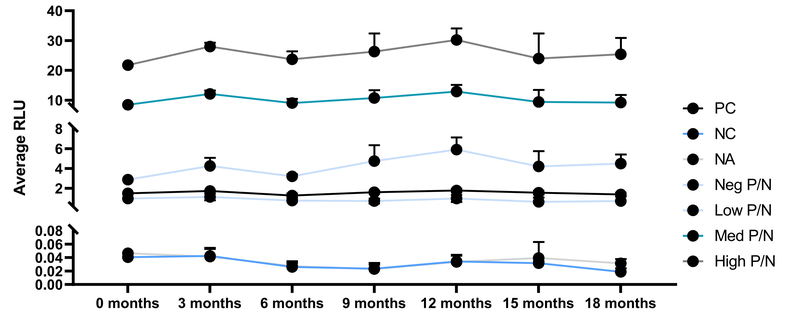
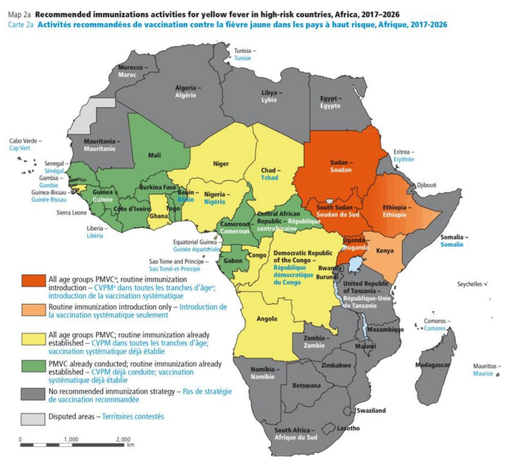


Figure 3: Absorbance values for various panels over 18-month time period.

CONCLUSION

Despite the availability of an effective vaccine which can protect against yellow fever infection, certain regions in Africa and South America remain at a significantly high risk for YFV epidemics. For this reason, the utilization of sensitive surveillance assays which can reliably detect acute infections is essential for detecting and monitoring outbreaks so vaccination efforts can be coordinated effectively.

Important to the field of emerging infectious diseases, the format of the current yellow fever surveillance assay could be easily adapted to detect acute infections caused by other pathogens such as cholera or measles. Therefore, this represents an effective model for the type of detection assays that are required to support future global health initiatives.



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