Analytical Validation of an Arbovirus Multiplex Serology Assay Using Novel Dried Tube Specimens

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ABSTRACT:

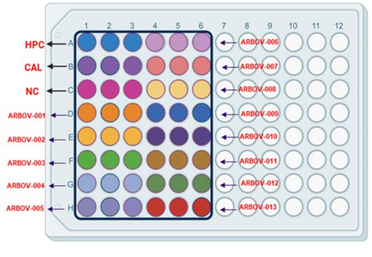
Background: Multiplex serology assays, especially xMAP® microsphere-based immunoassays, have become popular tools for tracking infectious diseases and evaluating large-scale public health interventions. Yet, their full potential is often limited by the lack of validated assays and well-characterized specimen panels.

Methods: In this study, we systematically validated a 16-plex arbovirus IgG test using innovative dried-tube controls and a diverse set of dried test specimens. Our validation process included a negative control, a high-positive control prepared from pooled heat-inactivated samples, and a low-positive sample as a calibrator (CAL). After color-coding and initial screening, samples were carefully aliquoted and dried for stability. The assay used 12 MagPlex® microspheres, each coupled to recombinant proteins from nine viruses, including Zika, four Dengue serotypes, West Nile, Japanese encephalitis, yellow fever, and two Chikungunya virus antigens. These microspheres efficiently captured antibodies, while built-in multiplex assay internal control microspheres ensured reliable performance (Table 1). Dried samples were rehydrated, incubated with the microspheres, and washed, with bound antibodies detected using a fluorescent reporter and analyzed on MAGPIX® instruments (Figure 2) . We used the ratio of the sample Median fluorescence intensity (MFI) to the MFI of CAL to determine the positive cut-off for the samples.

Results and Conclusions: Over a year-long evaluation, from January 2025 to December 2025, involving eleven independent runs by three operators, the assay consistently delivered high precision and repeatability. The inter-assay variances were calculated from true-positive and true-negative signals across 14 plates (Figure 1, Table 2). Extended stability was analyzed through Levey-Jennings chart monitoring (Figure 4). The intra-assay repeatability was calculated as the variance in MFI between replicates within a single plate and was found to be well within the accepted threshold of <20% (Table 3). Altogether, the 16-plex arbovirus assay proved to be a reliable platform, ready to advance integrated sero-surveillance efforts.

Table 1. Composition of the arbovirus multiplex serology assay panel

Arbovirus	Functionality on microspheres
Zika Virus (ZIKV)	Two NS1 antigens and one envelope antigen
Dengue Virus (DENV)	One NS1 antigen for each for DENV 1, 2, 3, & 4
West Nile Virus (WNV)	One NS1 antigen for WNV
Japanese Encephalitis Virus (JEV)	One NS1 antigen for JEV
Yellow Fever Virus (YFV)	One NS1 antigen for YFV
Chikungunya virus (CHIKV)	Two CHIKV E1 envelope antigens
Instrument Control (IC)	Ensures instrument performance
Sample addition control (ScG)	Ensures sample addition
Fluorescent reporter control (FC)	Ensures fluorescence reporter addition
Non-specific binding control (NC)	Monitors non-specific binding



Sample ID	Expected reactivity	Zika virus (ZIKV)	Dengue virus (DENV)	West Nile virus (WNV)	Japanese encephalitis virus (JEV)	Yellow Fever virus (YFV)	Chikungunya virus (CHIKV)
High Positive Control (HPC)	All Positive	POS	POS	POS	POS	POS	POS
Calibrator (CAL)	All Negative	NEG	NEG	NEG	NEG	NEG	NEG
Negative Control (NC)	All Negative	NEG	NEG	NEG	NEG	NEG	NEG
ARBOV-001	DENV & WNV	NEG	POS	POS	NEG	NEG	NEG
ARBOV-002	YFV	NEG	NEG	NEG	NEG	POS	NEG
ARBOV-003	All Negative	NEG	NEG	NEG	NEG	NEG	NEG
ARBOV-004	WNV, YFV	NEG	NEG	POS	NEG	POS	NEG
ARBOV-005	DENV, WNV	NEG	POS	POS	NEG	NEG	NEG
ARBOV-006	ZIKV, DENV, WNV	POS	POS	POS	NEG	NEG	NEG
ARBOV-007	DENV, WNV	NEG	POS	POS	NEG	NEG	NEG
ARBOV-008	ZIKV, DENV, WNV, CHIKV	POS	POS	POS	NEG	NEG	POS
ARBOV-009	ZIKV, DENV, WNV, CHIKV	POS	POS	POS	NEG	NEG	POS
ARBOV-010	ZIKV, DENV, WNV	POS	POS	POS	NEG	NEG	NEG
ARBOV-011	WNV, JEV, YFV	NEG	NEG	POS	POS	POS	NEG
ARBOV-012	WNV, YFV	NEG	NEG	POS	NEG	POS	NEG
ARBOV-013	DENV	NEG	POS	NEG	NEG	NEG	NEG

Figure 1: Plate layout of the 48 wells used for testing in the multi-center validation is depicted in the picture. Expected results for each of the 16 samples is shown in the above table.

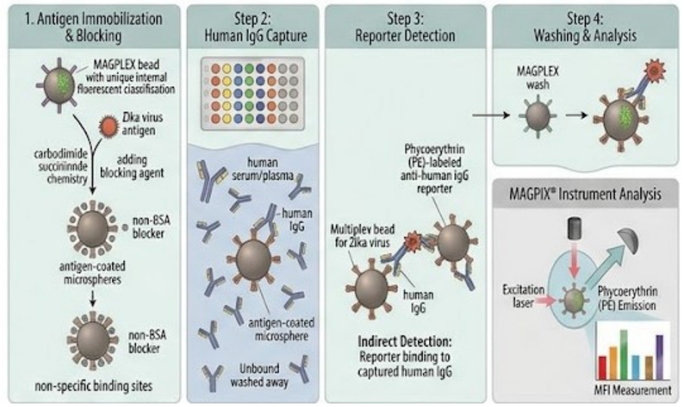


Figure 2: Multiplex assay work flow for measuring IgG antibodies to arboviruses using xMAP technology

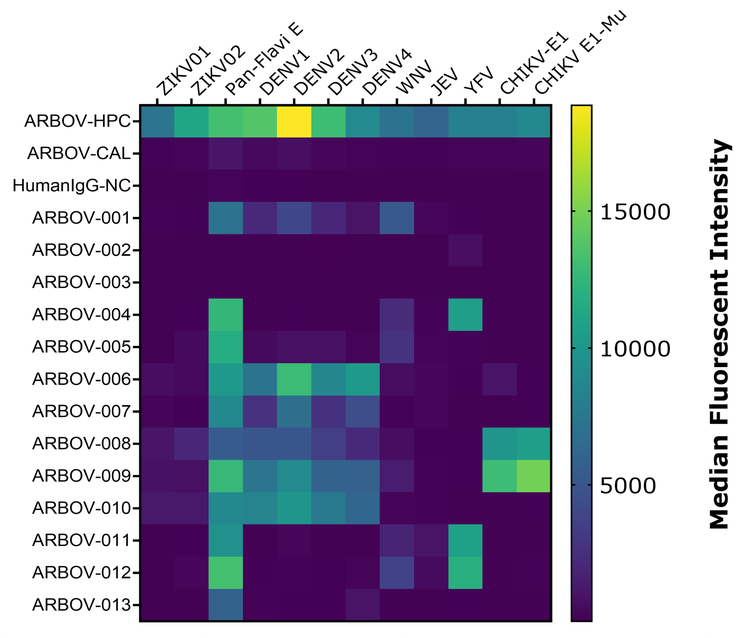


Figure 3: Pattern of IgG reactivity in the 13 samples, HPC, CAL and NC to different arboviral antigens in the panel

Table 2: Pattern of IgG reactivity in the 13 samples, HPC, CAL and NC to different arboviral antigens in the panel

Description	ZIKV	DENV	WNV	JEV	YFV	CHIKV
True Positives	197	377	411	84	210	123
False Positives	0	5	5	5	0	3
True Negatives	462	289	247	583	462	543
False Negatives	13	1	9	0	0	3
Total	672	672	672	672	672	672
Sensitivity	94%	100%	98%	100%	100%	98%
Specificity	100%	98%	98%	99%	100%	99%
Accuracy	98%	99%	98%	99%	100%	99%

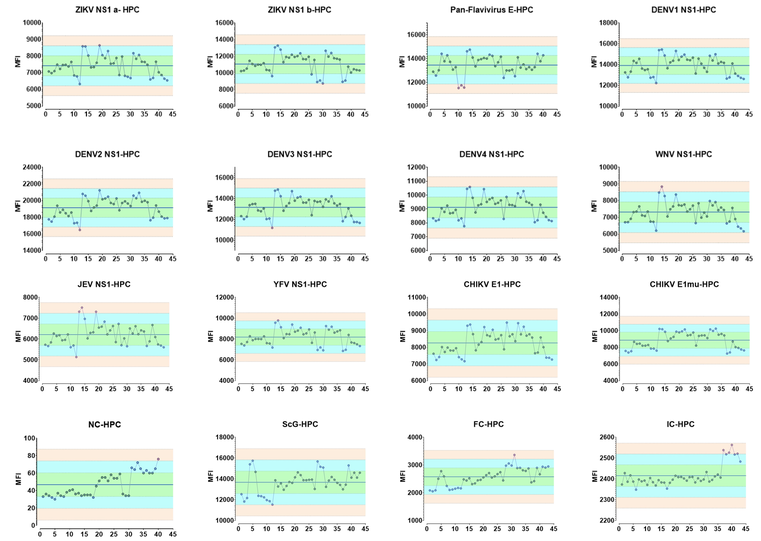


Figure 4: Levey Jennings Plots of High Positive Control (HPC) show monitoring of MFI values for one year from January 2025 to December 2025.

Table 3: Coefficient of variation in raw MFI values of HPC sample

Antigens	Average MFI (N=42)	SD (N=42)	%CV
ZIKV	7421.9	600.4	8.09%
ZIKV	11073.6	1170.3	10.57%
PanFlavi-E	13414.9	812.9	6.06%
DENV1	13919.1	862.0	6.19%
DENV2	19157.8	1152.5	6.02%
DENV3	13166.3	920.6	6.99%
DENV4	9118.1	739.9	8.11%
WNV	7314.5	612.2	8.37%
JEV	6208.5	512.5	8.26%
YFV	8179.9	781.7	9.56%
CHIKV-E1	8259.7	686.3	8.31%
CHIKV-E1 mu	8854.3	959.0	10.83%
NC	46.7	13.6	29.06%
ScG	13671.2	1077.6	7.88%
FC	2574.0	318.1	12.36%
IC	2415.5	52.0	2.15%

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References:

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2.N Venkateswaran, J Sarwar, N Parameswaran, et al; Development and testing of a novel multiplex Sero diagnostic assay for Zika and other arboviruses; J Immunol. May 1, 2017, 198 (1 Supplement) 81.26