USAMRIID Serological Testing Summary

Background

The USAMRIID serological assays were performed as a part of a pilot project to investigate distribution of pathogens of concern and evaluate the utility of serologic assays to inform future surveillance needs. Testing results should be considered preliminary and results could change based on follow up testing that is more confirmatory in nature. This testing was not intended to diagnose illness in individual patients and tests results should not be considered definitive. A total of 1,948 PREDICT serum samples were submitted to USAMRIID from Cameroon, DR Congo, Ethiopia, Ghana, Kenya, Senegal, Tanzania, and Uganda in Africa and Laos, Nepal, and Vietnam in Asia. Approximately 78% of samples were collected in clinics from patients meeting our inclusion criteria for acute fever with additional symptoms of interest, while 22% of samples were collected from community participants. All samples were tested for IgG antibodies and clinic samples plus a subset of community samples were tested for IgM antibodies. Results are still pending for 10 samples from Senegal for IgM testing and 1 sample from Ethiopia for IgG testing.

Testing Methodology

The Immunodiagnostic and Biologics Department of USAMRIID's Diagnostic Systems Division utilizes magnetic bead-based immunoassays to test serum samples for the presence of IgM and IgG antibodies against a given pathogen. While historically these assays were performed in a traditional 96-well plate format using inactivated whole pathogen to directly capture antibody, USAMRIID has transitioned these assays to recombinant antigen or VLP based direct detection formats on the MAGPIX instrument to allow the assays to be multiplexable and more sustainable. The current panel utilizes the following capture antigens: panalphavirus (CHIKV VLP), panflavivirus (DENV2 VLP), RVFV (nucleoprotein), MARV (VLP), CCHFV (nucleoprotein), EBOV (nucleoprotein), LASV (nucleoprotein), and HTNV (nucleoprotein). All capture antigens are expected to cross-react with closely related viruses, including novel viruses that may or may not cause illness. Thus for reporting purposes, the testing panel includes the following groups: “Crimean-Congo Hemorrhagic Fever Virus-like”, “Hantaviruses”, “Lassa Fever Virus-like”, “Rift Valley Fever Virus-like”, “Ebolaviruses”, “Marburgvirus-like”, “Alphaviruses”, “Flaviviruses”. Additionally, capture antigens may interact with antibodies generated from immunization; this is a known occurrence for the Flaviviruses assay with yellow fever vaccination but could occur with other assays as well if vaccinations to related pathogens are available.

USAMRIID serology panel virus groups

Virus Group	Genus	Family	Order
Crimean-Congo Hemorrhagic Fever Virus-like	Orthonairovirus	Nairoviridae	Bunyavirales
Hantaviruses	Orthohantavirus	Hantaviridae	Bunyavirales
Lassa Fever Virus-like	Mammarenavirus	Arenaviridae	Bunyavirales
Rift Valley Fever Virus-like	Phlebovirus	Phenuiviridae	Bunyavirales
Ebolaviruses	Ebolavirus	Filoviridae	Mononegavirales
Marburgvirus-like	Marburgvirus	Filoviridae	Mononegavirales
Alphaviruses	Alphavirus	Togaviridae	*not assigned*

Flaviviruses	Flavivirus	Flaviviridae	*not assigned*

For IgG and IgM testing, all samples were run in a direct format at a 1:100 dilution in triplicate, using the appropriate detector conjugate (either anti-human IgG-PE or anti-human IgM-PE, all from commercial sources). The MAGPIX outputs results in median fluorescence intensity (MFI) for all agents in the panel for each sample run and sample triplicates are averaged for interpretation. All plates incorporate both negative and positive controls. The negative control serum is a known negative American serum and the positive control serum is a mixture of serum from known IgG or IgM positive samples for each etiologic agent. The IgM assay excludes EBOV and MARV from the panel as positive control sources were not available for comparison for these pathogens. To improve this assay for future use, USAMRIID aims to identify population relevant negative controls to ensure data analysis is properly tied to the reactivity of naïve serum.

Once the samples were processed, quality control checks were performed to ensure a sufficient number of magnetic beads were read for each sample and to ensure intra-sample variation (within the sample triplicates) were within acceptable limits. Samples failing quality control checks were retested. Data were analyzed by calculating a signal to noise value by dividing the sample MFI value by the negative control MFI value for each agent. A sample was considered IgG or IgM positive if above a designated signal to noise cut-off based on observed variation for the negative and positive controls. A signal/noise cutoff of greater than or equal to 20x the negative control value was identified for positive samples for IgG testing to minimize false positives. A signal/noise cutoff of greater than or equal to 10x the negative control value was identified for positive samples for the IgM testing. Samples were considered negative if the signal/noise value fell below 4x the negative control value for both IgG and IgM. Samples with signal/noise values between the positive and negative cutoffs can be considered suspect positives, but are indeterminant given the limited information we have at this time. For PREDICT teams analyzing serological data in combination with individual risk factor information, the indeterminant values should be excluded from analyses unless there is evidence to indicate that suspect positives had significantly higher levels of exposure to risk factors related to the disease of interest. If suspect positives are deemed at high risk, we ask that this information be shared with other teams evaluating risk so that cutoff values can be revisited as appropriate.

A subset of samples was tested with serial dilutions to confirm linearity of results and address concerns with potential serum stickiness. As funding and resources allow, we propose to utilize neutralization assays or antibody depletion testing to confirm or deny antibody presence in a subset of samples.

Signal/Noise cutoffs for result interpretation

Testing Type	Negative	Suspect/Indeterminant	Positive
IgG	<4x	4x – 20x	>20x
IgM	<4x	4x – 10x	>10x

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