Human serum IgG antibody profiling for Mpox using a multiplex serology panel

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Background: Mpox is a zoonotic disease caused by Monkey Pox Virus (MPXV), a double-stranded DNA virus of the Orthopoxvirus genus in Poxviridae. MPXV is one of the four orthopoxvirus (OPXV) species causing disease in humans; the other three are smallpox-causing variola major virus (VARV), variola minor virus, and cowpox virus (CPXV). In 1980, the World Health Assembly declared that the smallpox was eradicated. Smallpox was eliminated due to a very successful global vaccination program. This vaccine also provided cross-protection against other OPXV; there have been no naturally occurring cases of smallpox reported since. Historically, the pox disease was rarely seen outside West and Central Africa. The first case of mpox was identified in 1970, in a 9-year-old boy from the Democratic Republic of Congo (DRC) two years after the smallpox vaccination was discontinued. Since then, it has been reported in 11 African countries – Benin, Cameroon, the Central African Republic, the Democratic Republic of the Congo, Gabon, Cote d'Ivoire, Liberia, Nigeria, the Republic of the Congo, Sierra Leone, and South Sudan. Currently, the actual burden of mpox is not known. In 2003, the first mpox outbreak outside of Africa was in the USA and was linked to contact with infected pet prairie dogs. Mpox has also been reported in travelers from Nigeria to Israel in September 2018, to the United Kingdom in September 2018, December 2019, May 2021, and May 2022, to Singapore in May 2019, and to the USA in July and November 2021. In May 2022, multiple cases of mpox were identified in several non-endemic countries. The herd immunity has waned since 1978 when the smallpox vaccination was discontinued. As of October 26th, 2023, a total number of 31,010 cases and 55 deaths have been reported from the US (https://www.cdc.gov/poxvirus/mpox/response/2022/us-map.html accessed 0n 10/27/2023). The recent 2022-2023 mpox outbreak has generated renewed interest in the scientific community to understand the level of OPXV-specific immunity in the various geographic regions, among different age groups, and especially in the risk groups and its impact on future outbreaks. We developed a novel multiplex assay for the detection of human IgG antibodies using Luminex® xMAP® technology for the detection of the human IgG profiles in the general population that has received childhood vaccination for smallpox, exposed individuals, healthy unvaccinated population, and mpox vaccinated population.

Methods: We have developed a 15-plex novel multiplex assay for detecting human IgG antibodies to OPXV using Luminex® xMAP® technology. We have covalently coupled optically coded magnetic microspheres to three recombinant proteins from VARV, three from VACV, and five from MPXV to interrogate IgG responses in healthy, vaccinated, or exposed serum or plasma samples (Figure 1). This 15-plex assay also includes four internal controls that assure the assay and reagent performance (Table 1). In this preliminary evaluation, we have tested 84 de-identified serum and plasma from healthy subjects, of which 39 samples are from subjects presumed negative, and 45 are assumed to be positive for OPXV antibodies based on age.

Results and conclusions: We have tested 11 different antigens and in this study with healthy subjects. The subjects 57 years and older were considered to have been vaccinated for smallpox and all of those subjects showed substantial reactivity with the membrane proteins, A35R of MPXV; A36R of VARV, A33R of VACV, the homologous proteins[1] in the three species. There was some reactivity with B6R of MPXV protein.

Table 1: Three immobilized proteins from OPXV in 15-plex assay

#	Microsphere coupling functionality	ID
1	Variola major virus M1R recombinant protein	VARV M1R
2	Variola major virus B6R recombinant protein	VARV B6R
3	Variola major virus A36R recombinant protein	VARV A36R
4	Vaccinia virus WR A33R recombinant protein	VACV A33R
5	Vaccinia virus WR A27L recombinant protein	VACV A27L
6	Vaccinia virus WR L1R recombinant protein	VACV L1R
7	Monkeypox virus M1R recombinant protein	MPXV M1R
8	Monkeypox virus B6R recombinant protein	MPXVB6R
9	Monkeypox virus A35 recombinant protein	MPXV A35
10	Monkeypox virus A29 recombinant protein	MPXV A29
11	Monkeypox virus E8L recombinant protein	MPXV E8L
12	IC (Instrument control)	IC
13	NC (Non-specific binding control)	NC
14	ScG (Human IgG Sample control)	ScG
15	FC (Fluorescent Reporter control)	FC

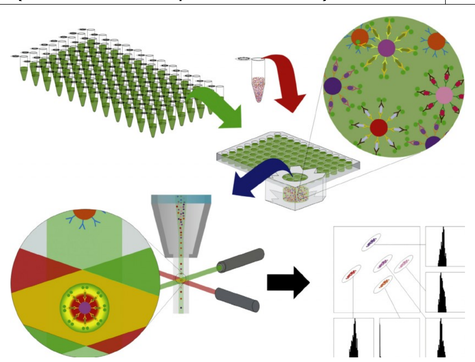

Figure 1. Overview of the Luminex xMAP technology.[2] Samples are prepared and transferred to a 96 well microtiter plate (top left) and a mixture of antigens conjugated to the microsphere sets is added (top middle). During the incubation step, the antibodies from the sample bind to the antigens and then visualized with a fluorescent reporter, (top right). Samples are measured in a Luminex analyzer (bottom left) and the results are graphically presented (bottom right). If no antibodies are present no fluorescent reporter will be detected, resulting in no signal (as depicted in the lower middle panel). Positive samples are represented in the other 4 panels.

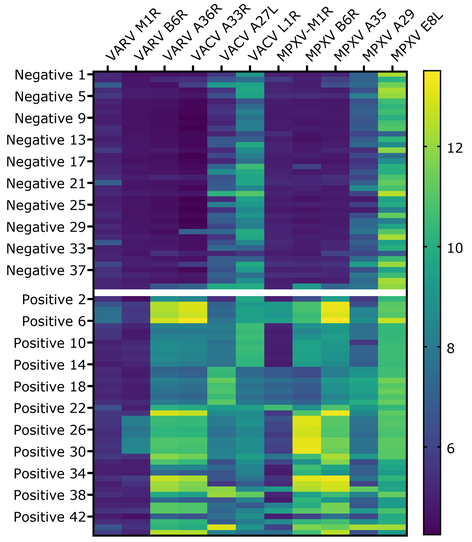

Figure 2: Comparison of the median florescence intensity (MFI) as a measure of IgG response to eleven different antigens in 15-plex assays in subjects presumed to be OPXV antibody positive and negative based on age.

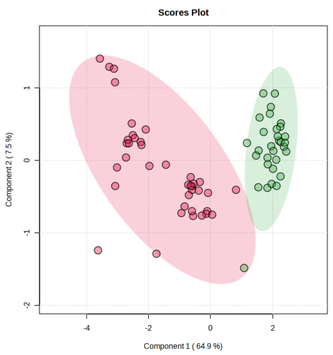

Figure 3: Principal component analysis (PCA) shows that four of eleven analytes used in this panel can sufficiently differentiate positive from negative in this dataset.

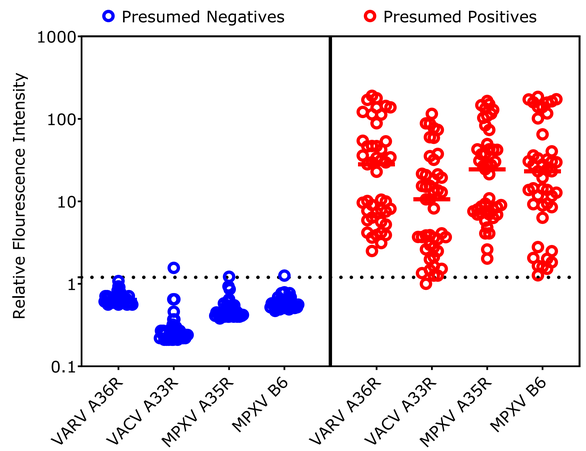

Figure 4: The distribution of positive and negative samples for the four analytes selected by PCA. Relative fluorescence intensity (RFI) was calculated as a ratio of MFI of each sample to the average + 3 Standard deviation of all 39 negative samples.

Results and Discussion:

We observed a antibody responses in individuals after decades of childhood vaccination which is distinct from unvaccinated individuals younger than 55 (figures 2, 3, & 4). A longitudinal study conducted in 2008 reported that immunity from smallpox vaccine can persist for decades[3].

Acknowledgement:

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References:

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