NATIONAL CENTER FOR IMMUNIZATION AND RESPIRATORY DISEASES

Development of a Multiplex Bead-Based Assay to Assess Enterovirus Immunity in Acute Flaccid Myelitis Patient Samples

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Development of an assay for the detection of EV-D68-specific IgM and IgG antibodies could have a significant impact in the outcome of patients afflicted by AFM by pinpointing the etiologic agent of the illness.

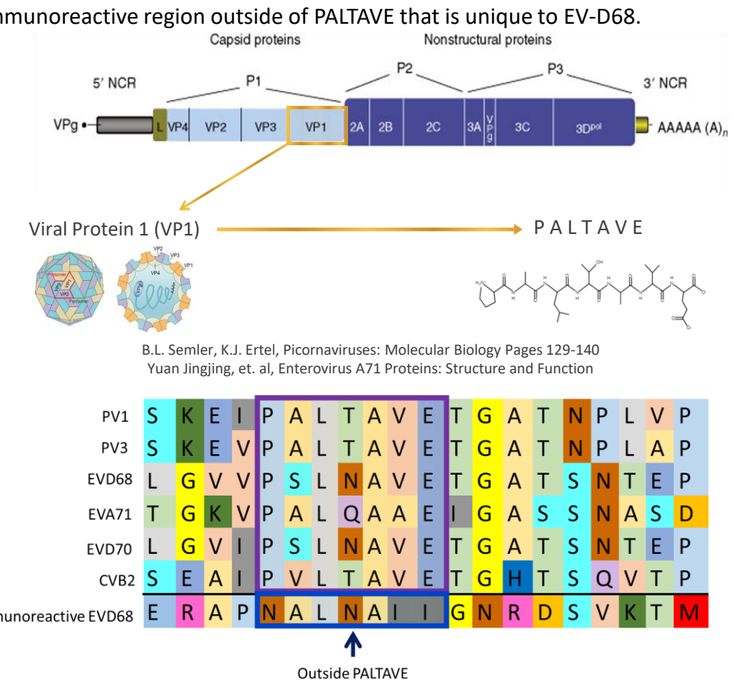
BACKGROUND

Acute Flaccid Myelitis (AFM) is a poliomyelitis-like neurological illness characterized by sudden onset limb weakness that is observed primarily in children. While several pathogens have been linked to AFM, Enterovirus D68 (EV-D68) is the most common agent detected in AFM patient samples. Following the establishment of AFM surveillance in 2014, peaks in AFM cases were observed in 2014, 2016, and 2018, concordant with outbreaks of EV-D68. Although molecular, PCR-based, assays can detect the presence of EV-D68 in patient samples, there is a need to develop diagnostic and screening methods to determine if individuals have been exposed to EV-D68 and to better assess the immune response to EV-D68 infection (Figure 1). Using Luminex xMAP® technology, we developed a multiplex bead-based assay to test for the presence of IgG and IgM antibodies against various enteroviruses, including EV-D68, in patient samples. Detection of enterovirus-specific IgM antibodies suggests a recent enterovirus exposure, whereas detection of enterovirus-specific IgG antibodies suggests an enterovirus exposure.

METHODS

Peptide Design:

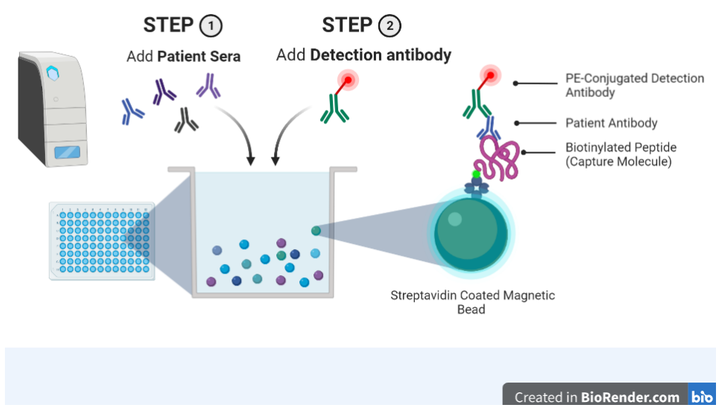
Enterovirus-specific peptides were designed targeting a conserved region within the immunogenic PALTAVE viral protein 1 (VP1) and an immunoreactive region outside of PALTAVE that is unique to EV-D68.



- **Six PALTAVE enterovirus peptides (Enterovirus screening)**
- **One immunoreactive EVD68 peptide (EVD68 specificity)**
- **Three tandem peptide sequences (Increased signal)**
- **One scrambled peptide sequence (Control)**

Indirect Immunoassay Method:

Paramagnetic beads pre-coated in streptavidin (Luminex MagPlex) were coupled to a biotinylated peptide. A master mix of each bead region was prepared and added to a 96-well plate with AFM patient sample and incubated at room temperature. Secondary antibody was added, and Mean Fluorescence Intensity (MFI) was calculated using a Luminex MagPix instrument and normalized according to the signal from the scrambled peptide.



Assay Development:

Multiple parameters were established for the assay:

- Assay Buffer (1% BSA-PBS)
- Identification of controls and standards
- Peptide concentration coupled to beads
- Ratio of bead master mix to patient sera needed per reaction
- Total beads needed per bead region per reaction
- Use of rheumatoid factor (RF)-absorbent to remove IgG in sera samples for use in enterovirus-specific IgM assay.

Results

A limit of detection was established using a human monoclonal antibody (EV68-219) from Dr. James Crowe, Vanderbilt University Medical Center (**Figure 2**). EV-D68-positive samples from AFM-confirmed patients were screened for the presence of anti-EV-D68 IgM antibodies. A positive control was selected based on specificity for Immunoreactive EV-D68 and EV-D68 peptides (**Figure 3**).

FINDINGS

Preliminary data suggest the EV-D68 immunoreactive peptide allows for the identification of EV-D68-specific antibodies. Future work will define the specificity and sensitivity of the PALTAVE and the EV-D68 immunoreactive peptides for the identification of EV-D68 and enterovirus-specific antibodies as well as validation of our procedure for future use with AFM patient samples.

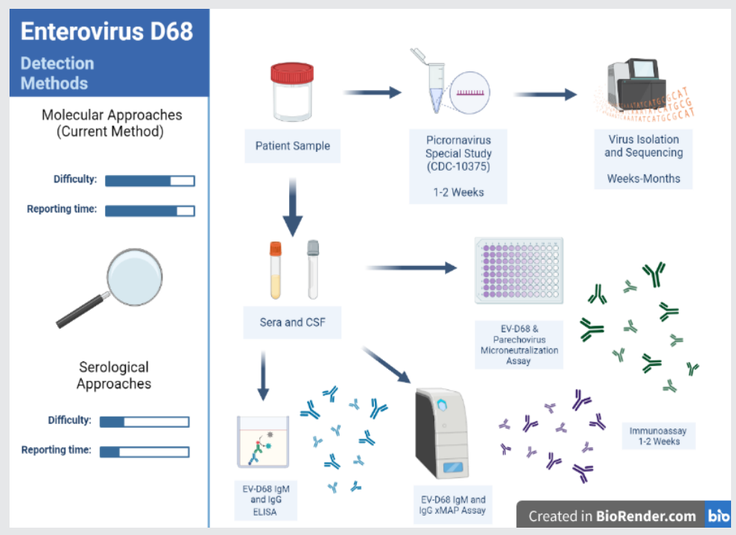


Figure 1: Overview of Enterovirus D68 methods used in PPLB.

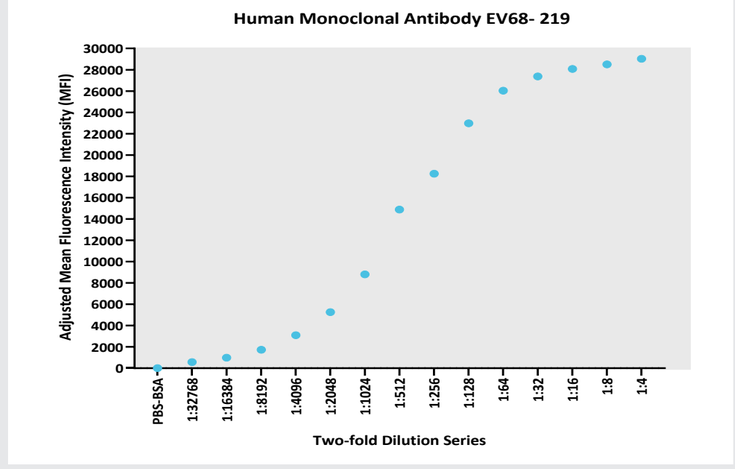


Figure 2: Limit of detection for enterovirus-specific IgG assay using human mAb EV68-219 and the EV-D68 Immunoreactive peptide.

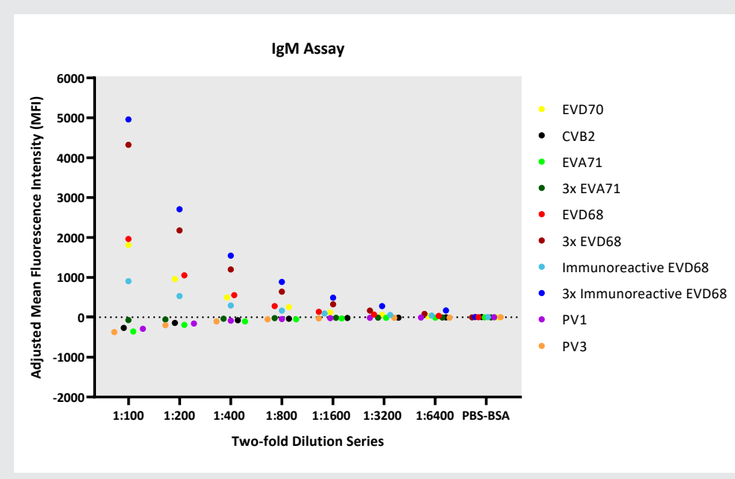


Figure 3: Detection of EV-D68-specific IgM. Enterovirus-specific peptides were used to detect presence of IgM in an EV-D68+ human sera sample. 3x delineates tandem peptides.

SCAN HERE FOR MORE INFORMATION

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