Qualification of Microneutralization Assays for HMPV and HPIV-3: Robust Tools for Clinical Vaccine Evaluation

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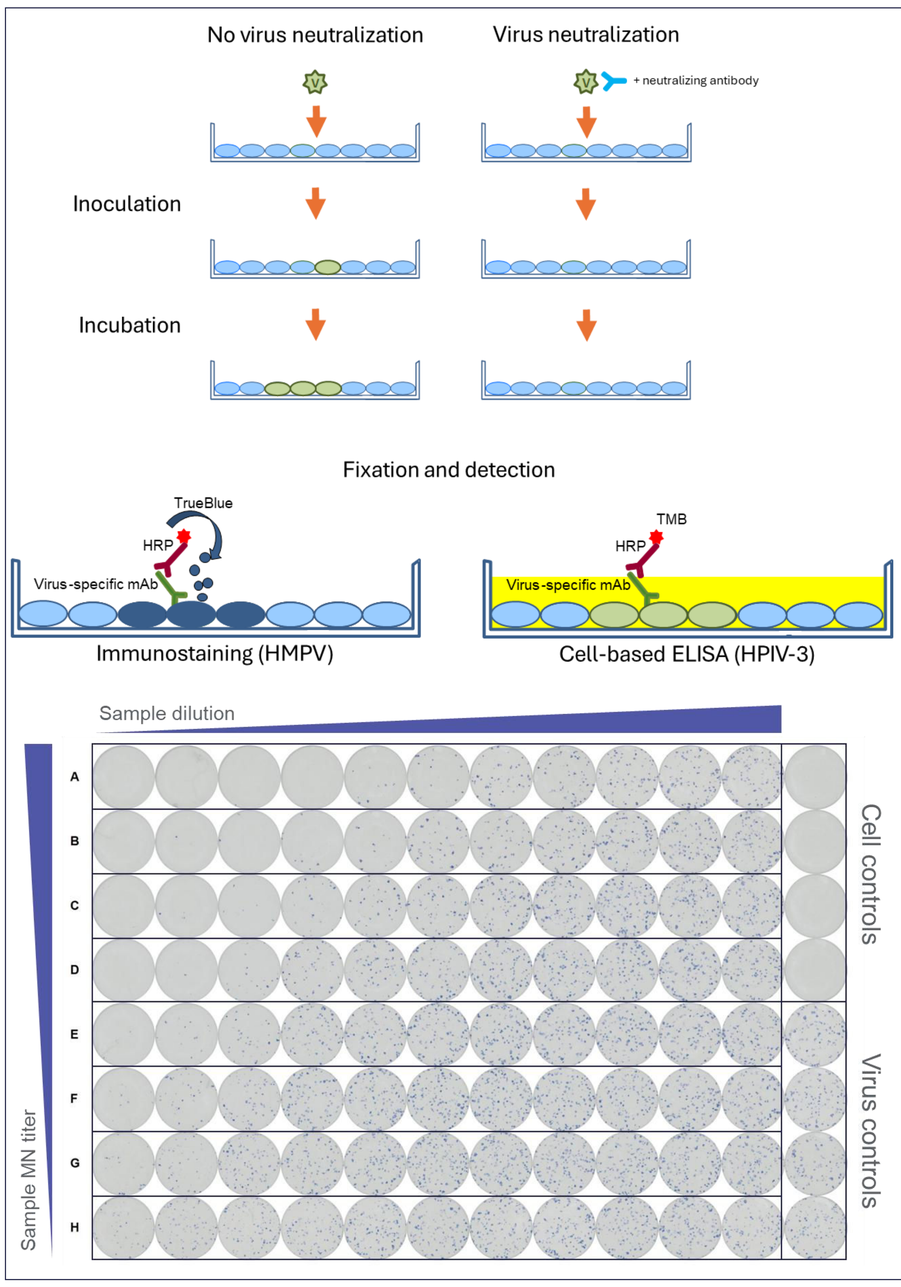
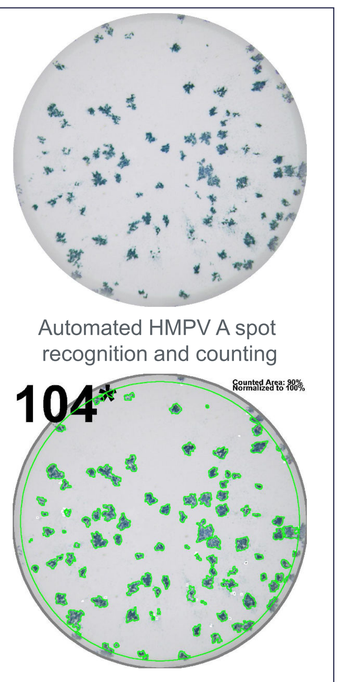
Introduction

- Human metapneumovirus (HMPV) and human parainfluenza virus type 3 (HPIV-3) are respiratory pathogens in the *Pneumoviridae* and *Paramyxoviridae* family, respectively
- Cause upper and lower respiratory tract infections (bronchiolitis, pneumonia)
- High disease burden: major cause of pediatric hospitalizations, also impacts elderly and immunocompromised
- Clinical presentation overlaps with RSV and influenza, reinfections are common
- No licensed vaccines or antivirals available
- Microneutralization (MN) assays:
  - Measure functional, virus-neutralizing antibodies
  - Can provide biologically relevant correlates of protection
  - Essential tools for vaccine evaluation

MN assay principles

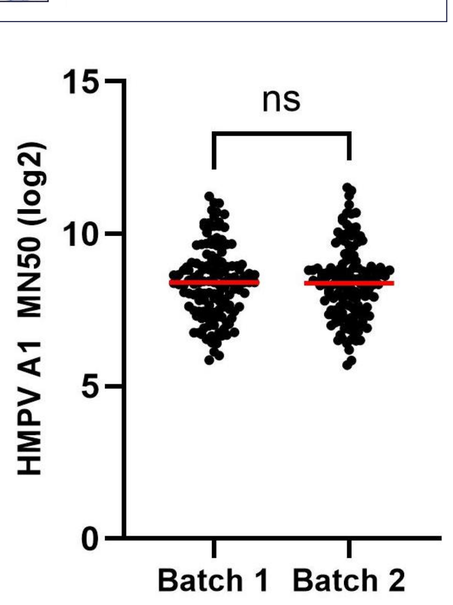
- Prepare serial 2-fold serum dilutions (horizontal, starting in column 1 up to column 11) in infection medium (IM) and incubate with virus working dilution
- After incubation, transfer serum/virus mixture to Vero cells on triplicate plates
  - For HMPV only: remove inoculum and add IM containing carboxymethyl cellulose
- After an incubation period, fix the cells
- Virus detection:
  - "ViroSpot" immunostaining[1], suitable for slower-replicating virus such as HMPV
  - Cell-based TMB ELISA, suitable for faster-replicating virus such as HPIV-3
- Scan the plates to quantify viral antigen level
- Compute sample neutralization titer (MN50) based on sample dilution showing 50% reduction of signal, relative to virus control wells (=100% signal)[2]





Virus batch release

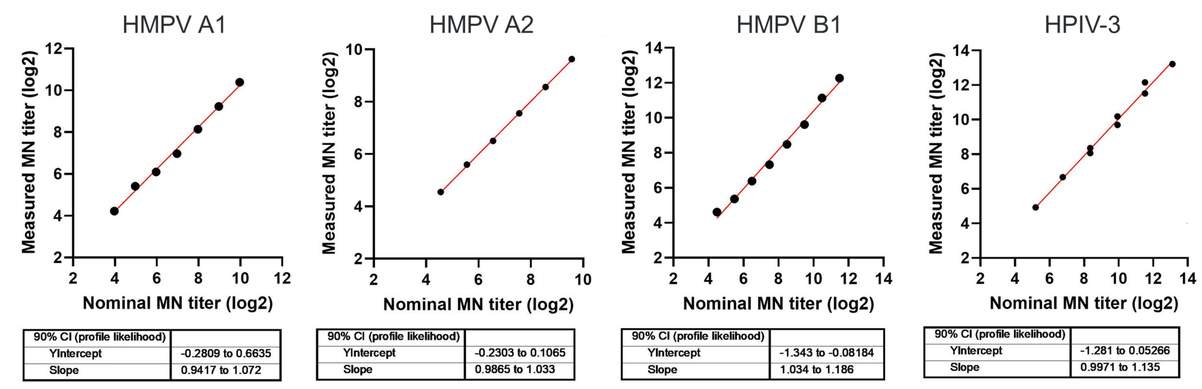
- The purpose of the procedure is to ensure that every new virus batch performs comparably to the current batch in MN assays, maintaining assay reliability and historical continuity before the new lot is approved for sample testing
- The new and current virus batches are tested head-to-head in at least six independent runs using a minimum of 20 serum samples spanning the assay range. This generates at least 120 paired data points for statistical comparison
- A paired t-test is applied to the $\log_2$-transformed MN titers to assess whether the mean difference between batches is statistically significant and within the acceptance limit (L = 0.25 $\log_2$). The candidate batch is accepted only if the difference does not exceed this limit



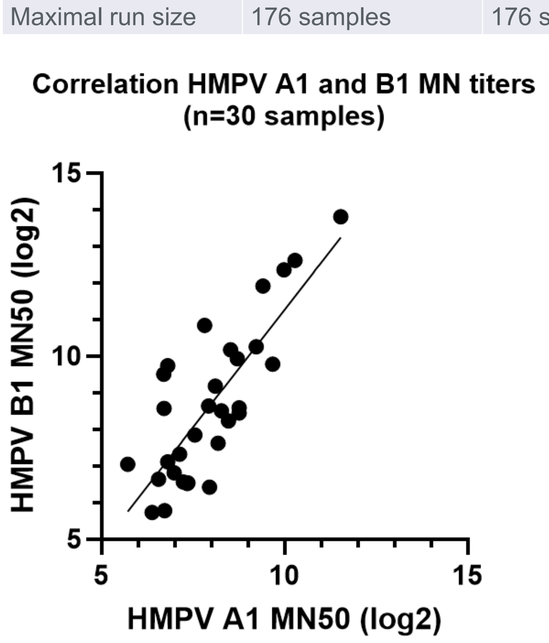
Assay qualification setup

- Purpose: Assess assay performance
- Reference materials used: Commercially available sera of non-vaccinated adults
- Parameters assessed:
  - Linearity & accuracy
    - ≥3 independent serum dilution series of high-positive sera diluted in antibody depleted serum matrix
    - Used to define the assay range
  - Repeatability & intermediate precision
    - ≥34 serum samples tested
    - Assessed across operators, days, and assay runs
  - Specificity
    - Antibody-depleted sera tested; expected negative response
- Study design:
  - Each sample was tested 3 times per run, across ≥5 independent runs

Results



Parameter	Results HMPV A1	Results HMPV A2	Results HMPV B1	Results HPIV-3
Specificity	100% of the MN titers of the antibody depleted sera were <LLOQ	100% of the MN titers of the antibody depleted sera were <LLOQ	98% of the MN titers of the antibody depleted sera were <LLOQ	100% of the MN titers of the antibody depleted sera were <LOD
Relative accuracy	The difference between the measured and nominal HMPV A1 MN titers was ≤0.45log2	The difference between the measured and nominal HMPV A2 MN titers was ≤0.27log2	The difference between the measured and nominal HMPV A1 MN titers was ≤0.83log2	The difference between the measured and nominal HPIV-3 MN titers was ≤0.30log2
Repeatability	Overall GCVr was 20.2% (16x sample starting dilution)	Overall GCVr was 18.7% (16x sample starting dilution)	Overall GCVr was 29.6% (16x sample starting dilution)	At the 16x sample starting dilution, the overall GCVr was 25.7%. At the 128x sample starting dilution, the overall GCVr was 21.3%.
Intermediate precision	Overall GCVi was 24.2% (16x sample starting dilution)	Overall GCVi was 21.8% (16x sample starting dilution)	Overall GCVi was 32.0% (16x sample starting dilution)	At the 16x sample starting dilution, the overall GCVi was 29.2%. At the 128x sample starting dilution, the overall GCVi was 25.4%.
Linearity	The 90% confidence interval of the slope of the regression lines (n=3 dilution series) were: • 0.92 to 1.00 • 0.93 to 1.07 • 0.94 to 1.07	The 90% confidence interval of the slope of the regression lines (n=4 dilution series) were: • 0.91 to 0.95 • 0.99 to 1.03 • 0.93 to 1.00 • 0.93 to 0.99	The 90% confidence interval of the slope of the regression lines (n=4 dilution series) were: • 1.03 to 1.19 • 1.02 to 1.12 • 0.94 to 1.14	The 90% confidence interval of the slope of the regression lines (n=3 dilution series) were: • 1.00 to 1.14 • 1.00 to 1.103 • 0.99 to 1.09
LLOQ	19	27	20	31
ULOQ	1,344	793	4,891	9,471
Maximal run size	176 samples	176 samples	144 samples	176 samples



Conclusions

- We developed and successfully qualified robust MN assays for HMPV and HPIV-3
- The virus batch release procedure ensures comparability between virus stocks which are used in the MN assays
- HMPV and HPIV-3 assays were successfully qualified and are fit-for-purpose to support vaccine immunogenicity testing in Phase 1 and Phase 2 clinical studies
- Assay range can be extended once higher-titer post-vaccination samples become available

[1] van Baalen, Carel A et al. "ViroSpot microneutralization assay for antigenic characterization of human influenza viruses." Vaccine vol. 35,1 (2017): 46-52.
[2] Zielinska, Edyta et al. "Development of an improved microneutralization assay for respiratory syncytial virus by automated plaque counting using imaging analysis." Virology journal vol. 2 84. 9 Nov. 2005