**Microneutralization Assay (IC50)**

*Walker Orr, 02/26/2024*

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| **Steps** |
| Day One |
| 1. Plate as for a TCID50: 5,000 cells/well in a 96 well plate. You need approx. one plate per serum tested, plus an extra plate for back-titration of virus. |
| Day Two |
| 1. Dilute virus to 2000 TCID50/ml in serum-free DMEM. You need 5 ml virus per plate. |
| 1. Perform an initial dilution of antibody in serum-free DMEM if desired. |
| 1. Dispense 100 ul antibody into the starting positions on your antibody dilution plate, and 50 ul serum-free DMEM everywhere else on the plate. |
| 1. Perform two-fold serial dilutions of antibody. Change tips after every dilution. Remove the 50 ul diluted antibody that remains in the final position of the plate. |
| 1. Dispense 50 ul virus to each well on the antibody dilution plate. |
| 1. Incubate for 1-2 hours at 37C. |
| 1. Transfer to cells, starting with the most dilute sample. |
| 1. Incubate 3-5 days at 37C, then score as a TCID50. |