22Feb19: Neutralization assays against WT Perth/2009 + WSN NA GFP virus with Vietnam serum samples

"A-H" will be referred to as columns, and "1-12" as rows. I had the plate with column A-H going left to right, and rows 12-1 from top to bottom.

ROW	*COLUMN* A	B	C	D	E	F	G	H
1	NAM only	virus only	virus + cells only	serum dilution	serum dilution	serum dilution	virus + cells only	NAM only

I used the "Perth/2009 HA + WSN NA GFP virus" that Juhye rescued on 20180905. Based on the MOI test Juhye conducted, I will use an MOI of 0.5

Serum Sample	Vol of Ab for serial dil	Time start virus + Ab	Time start virus+Ab+cells
16 (HC080048)	40 uL (3-fold dilutions)	2:07	3:44
17 (HC080043)	40 uL (3-fold dilutions)	2:09	3:47
18 (HC060106)	40 uL (3-fold dilutions)	2:11	3:50
19 (HC140010)	40 uL (3-fold dilutions)	2:13	3:52
20 (HC070072)	40 uL (3-fold dilutions)	2:15	3:55
21 (HC070041)	40 uL (3-fold dilutions)	2:17	3:57
22 (HC120043)	40 uL (3-fold dilutions)	2:19	4:00
23 (HC150036)	40 uL (3-fold dilutions)	2:20	4:03

For multichannel, used Fisher brand blue box/green wafer tips, #02-707-431

1. Thawed sera and made dilutions to working stock solutions using NAM
    - **Diluted 16.67 uL of RDE-treated serum in 133.33 ul NAM for 150 uL total volume. Note that the RDE treatment of the serum involves a 1:4 dilution, so the overall dilution of the serum in the first row after the 1:3 dilution in step (2) is (1 / 4) * (16.67 / 150) * (1 / 3)**
2. Added 80 uL NAM to all wells of all 8 plates (~65 mL of NAM). Also began thawing viruses
3. Added 40 uL of the diluted serum to the top row (row 12) of the plate for columns D, E, and F. Did this for each plate
4. Using the multichannel with three tips attached, mixed the top row D/E/F and transferred 40 uL the second row, mixed, etc. Removed 40 uL from the last row. Did not change tips in between rows (not necessary here)
5. Added 40 uL NAM to column B of all plates (virus only column) to make up for no cells being added to these columns.
6. Prepared virus inoculum. Diluted Perth/09 HA + WSN NA GFP 48 hpt virus to 500 IP/uL. (~3.5 mL per plate= 28 mL total)
    - Titer = 2419 IP/uL. Make MM of virus (5.79 mL virus + 22.2 mL NAM)— need 6 aliquots of virus
7. Using a reservoir and multichannel added 40 uL of virus to columns B-G. Used new tips for each row, but did not mix virus and Ab (no need to mix). Wrote down time once virus was added to all wells of a plate, and incubated at 37°C.
8. While virus + antibody mixtures were incubating, started preparing cells by trypsinizing MDCK-SIAT1-PB1-TMPRSS2 cells, neutralizing with D10, spinning at 1200 rpm for 4 min, then resuspending in 20 mL NAM
9. Made a master mix of cells at 1e6 cells/mL, making a 28 mL master mix (~3.5 mL per plate)
    - Counted 2.29e6 cells/mL =>12.23 mL cell culture + 15.77 mL NAM
10. After ~1.5 hr of virus + Ab incubation, took each plate out of the incubator in order, and added 40 uL cells to columns C-G, using fresh tips for each row. Used a fresh reservoir for each plate, re-mixed cell suspension before adding to reservoir. Wrote down time cells were added to the wells of a plate
11. At ~18 hpi (9:30 AM), read on plate reader with "Neutralization assay 96 well" program.