**Robo-TCID50**

*Walker Orr, 02/26/2024*

**Required Equipment**

**Required Supplies**

3 plates of cells for TCID50.

1 deep well 96-well block for diluting and distributing the virus.

OpenTrons tip racks: 2 racks per 12-plate run (you’ll add a new rack halfway through).

**Required Reagents**

**Sample Workflow**

Total time: About 2 hours per run (4 samples)

Active time: About 20 minutes per run, not including plating.

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| **Steps** |
| 1. The day before the assay, plate and label three 96-well plates of 5000 cells for each sample. |
| 1. Setup:    1. About an hour before starting, remove virus samples from the freezer. You need 500 ul of each sample.    2. 10 minutes before starting, turn on the air circulation for the BioBubble (200-250 setting). Also turn on the OpenTrons air circulation system.    3. Prepare a deep well 96-well plate. Add 1.5 mL serum-free media (from the fridge is fine) to each well. Use the repeat pipettor. Do not overfill the wells and be very precise with this step! Place the tip into the base of the well and pipette slowly to avoid splashout.    4. Place the virus samples in position A1, A2, A3, and A4 on the tube rack in the OpenTrons. |
| 1. Running the robot:    1. Select the appropriate program (I use the “technical triplicate” program). Follow the setup directions, making sure the pipette tips and the cell plates are in the correct location.    2. After starting, set a 70 minute timer. You’ll need to pause the program and change plates halfway through the run.    3. When the timer goes off, **pause the program**. remove the cell plates to the (virus) incubator and replace with fresh plates. Make sure the lids are off. Run the program. |
| 1. Between runs: empty the trash bin to biohazard waste bag (handle inside the biobubble). |
| 1. After you’re done: clean with microchem and ethanol, and turn off the robot. |