How to use the Sartorius Virus Counter
EB, 5/7/21

Sample Preparation

Check that you have the CORRECT sample dilution buffer for the stain that you want to use: AB buffer is used for BSBV and VSVG, DY is used for DY

*You need to have 200 uL diluted and stained sample per vial.

-Do serial dilutions of both your virus sample and conditioned medium blank from 1:10 to 1:10,000 in provided sample dilution buffer, "SBD". For example 30 uL virus into 270 uL SBD.

-Take 195 uL diluted sample into a new Eppendorf tube, and add 5 uL dye (either "DY", the non- specific protein/DNA dye stored at RT, or "BCVB", the baculovirus antibody stored at 4*C).

-Incubate for at least 30 minutes, protected from light, before running the sample. Can incubate up to 8 hours.

Instrument Initialization

-Open the software on the laptop.

-Turn on instrument with black power switch on the front.

-The software will prompt you to check the fluidics bay reservoirs: fill the back bottle with provided sheath fluid, the middle bottles with wash fluid, and empty the front waste bottle. Check the box on the software.

-Make sure the "SSR" (startup shutdown rinse) vial is attached to the instrument, it has a blue cap, then check the box on the software.

-Turn on the nitrogen gas tank (just open the tank, don't adjust the regulator), and check the box on the software.

-Click "start", and a startup wash cycle will begin, using the soapy solution in the SSR vial.

Instrument Test

*purpose is to make sure instrument is clean and has no air bubbles

-Remove the SSR vial and attach the red-capped "ISW" (inter-sample wash) vial.

-Run AutoPrime with 70% ethanol after the ISW. This will take about 10 minutes.

-Click "ISW" on the software, for a wash step that takes a few minutes.

-Remove the ISW vial and attached the green capped-capped "CVF" (cleanliness verification fluid) vial.

-Click “CVF” on the software and it will try to count particles (should be much lower than the detection limit: “Result: <IQL”). If particles above the 5E5 threshold are detected, more washing is necessary until the CVF gets a negative result.

-Remove the CVF vial and attached the yellow-capped “PVS” (performance validation standard) vial.

-Click “PVS” on the software and it will try to count particles. This is the positive control, there are fluorescently tagged beads inside.

-The PVS will “pass” if more than 3E8 particles are detected, where channel 1 peak heights are above 0.5V and channel 2 peaks heights are above 0.25V. If the PVS fails, the standard may have aggregated (obvious if peak heights are very high), or more likely there are air bubbles trapped inside, blocking detection.

(-Troubleshooting: fill a vial with 70% EtOH and attach to the system. Run autoprime, a ~10 minute wash. Then repeat CVF and PVS again to see if it passes.)

-After final PVS, attach red-capped ISW vial to wash out the positive control, so that this does not contaminate your virus sample.

-Verify cleanliness with green-capped CVF.

Sample Titration

*Now you can run your samples from most to least dilute.

-Transfer 1:10,000 conditioned medium sample into a sartorius vial and attach to system.

-Click “start analysis”, the software will prompt you to name the sample, select the dye you used, and indicate the dilution. Select “save results only”, you don’t need the trace files, then “run”.

-Click the yellow “save” button when the run is complete, then do the rest of the medium samples.

-Prepare the system for the virus sample by running ISW and CVF.

-Collect virus sample data from most to least dilute.

Analyzing Results

-Go to the “results” tab in the software.

-Click on the virus sample files, right click, and select screening > sample.

-Click on the medium sample files, right click, and select screening > matrix

-Go to “screening” tab in software.

-Click on the calculator and it will plot the dilution series, and give you an ideal dilution for obtaining the most accurate titer.

-Delete points not in the linear range (i.e. below the 5E5 detection limit or above 1E9).

-The software will give you a corrected particle on this page, which you can convert to titer with the dilution factor.

*Now you are supposed to go back and prepare at least three replicates of the virus sample and medium at the recommended dilution, run those samples, and obtain an accurate titer, but this is probably not necessary for our purposes.

Shutdown

-Run ISW followed by CVF to confirm that particles are flushed out.

-Attach the white-capped “DSW” (daily system wash) vial.

-Click “shutdown” in the software and confirm that the DSW vial is attached.

-It will prompt you to then attach the SSR vial and click “ready”.

-Then it will prompt you to turn off the instrument and air, and click “OK” to exit the software.

-Empty the waste bottle into the sink.