Aerosolisation

1. Set working conditions (temperature, humidity level) in the isolator
2. Prepare the viral suspension in the nebulizer's liquid reservoir in a BSL3 BSC :
    - MEM
    - 1x Penicillin-Streptomycin
    - Tricin
    - 1 mg/mL rhodamine B
    - 10% FBS
    - 0.005% antifoam A
    - 150 mM NaCl
    - Influenza virus at 10^3 to 10^7 $TCID_{50}$/mL
3. Transfer the suspension to the isolator using an hermetic box and the chapelle de transfert
4. Connect nebulizer to the pressurized nitrogen bottle and to aerosol outlet
5. Connect the liquid reservoir filled with 250 mL of the aerosol solution :
6. Open the pressurized nitrogen bottle (pressure should be 200 bar)
7. Set the low pressure regulator to 3 bar
8. Turn the nebulizer on
9. Adjust the nebulizer pressure minimizer to 2 bar
10. Keep generator running for approx. 1 min
11. Turn the nebulizer off
12. Close the pressurized nitrogen bottle
13. Wait for the nitrogen to be evacuated from the regulator
14. Turn the low pressure regulator off
15. At the end of the experiment, take the liquid reservoir back to the BSC using the hermetic box and the chapelle de transfert
16. Autoclave and clean it

Exposure to parameters

17. Set the parameter(s) in the isolator (UV, AirWasher, …) for the different amounts of time
18. Proceed to air samplings according to the experimental design

Sampling

19. Before the beginning of the experiment, make sure that the adapter for gelatine filter unit is in place at the air inlet of the MD8 Airscan
20. Fix a gelatine filter unit to the adapter without touching the gelatine part
21. Turn the MD8 Airscan on
22. Check (and change if necessary) the sampling parameters :
    - Sampling time : 1 to 2 min.
    - Air flow rate : 2.0 m^3/h
    - Filter class : 3 µm
23. Start the sampling by pressing the Start button
24. After sampling, take the gelatine filter unit out of the MD8 Airscan
25. Detach the top part of the filter unit by turning it counterclockwise
26. With plastic clamps, take the filter membrane and dissolve it in a stool box with 5 mL of MEM
27. Store the stool box in the isolator until the end of the experiment
28. Proceed to other sampling the same way
29. At the end of the experiment, take the stool box to the BSC using the hermetic box and the chapelle de transfert