Inactivation of viruses by dry heat for re-use of respirator masks

What temperature-time is required for complete inactivation of the virus studied?
What is the maximum level of inactivation shown and is that relevant?

Pathogens

1. SARS-COV-2: 1 mL high conc stock: number and titer:
2. Influenza H1N1: 1 mL high conc stock: number and titer:
3. poliovirus model for non-enveloped (as in rhinovirus): 1 mL high conc stock: number and titer:
4.

Approach: Spike mask material with virus suspension, allow to dry, pack in sterilization bag, and expose to designated temperature and time, elute virus in culture medium and titrate.

Materials

Masks (define)
A: 3M 9925
B: IMG

Puncher
Petri dishes
Sterilization bags/ rol + sealer
Forceps
Kweek medium DMEM/EMEM?
2 mL cups
Pipets
microscope
vortex
label printer
clock/stopwatch
96 well plates

Woensdag 1 april
Eerst 70 C en RT, in de middag de 58 C

protocol: Influenza and PV SI1

1 Punch samples out of base material; collect in a petri dish, n >64
2 Label 2 mL cups and add 1 mL culture medium, keep on ice or 4-8 C.
3

In a BSC

3 In a petri dish: spread the punched (n=22 filter A and 22 filters B) filters and spike each filter separately with 10 uL virus
4 Take 2 spiked filters and put directly in a 2 mL ep with 1 mL cold medium (Yield: FLU-A-1/2 and FLU-B-1/2 : SL1-A-1/2 and SL1-B-1/2 AND FLU-A-23/24 and FLU-B-23/24 : SL1-A-23/24 and SL1-B-23/24
5 allow the rest to air dry 45 min, RT in BSC
6 take (t= 0 min cont)
7 Pack 8x2 filters Flu-A, 8x2 filters Flu-B, 8x2 filters SL1-A and 8x2 filters SL1-B in a sterilization bag and seal
8 transfer to the dry heat oven at 70 C or leave at RT
9 Expose to heat as described in Tables in dry-heat inactivation.xlsx
10 At designated time: Remove from the incubator and transfer to BSC
11 Open the sterilization bag

12 Put the samples (1 filter per cup) directly in a labeled 2 mL ep with 1 mL cold medium / keep on ice or in refrigerator.
13 set the incubator to 58 C
14 Spike filters A (n=10)and B (n=10) with 10 uL Flu and PV
15 allow to air dry 45 min, RT in BSC
16 take (t= 0 min cont) and....
17 take t= 20 min.....etc

If all samples are collected / ie filters are in medium
18 Vortex each cup for 5 min and make 10 fold dilution series
A: undiluted 300 uL from labeled ep
B: 10-1 30 uL A + 270 uL medium
C:10-2 30 uL B + 270 uL medium
.....
13 Bereid een celsuspensie zoals beschreven in SOP IDS/VIR/M099 en verdun tot 2*10e5 cellen per mL.
14 Add per well in 100 uL cells (2*10e4 per well)
15 Add 100 uL virus dilution A-H to the cells as in dry-heat-titration.xlsx
16 Seal the plate and incubate at 35/37 C?
17 Read CPE at day 5?
18 determine the CCID50
19 Determine inactivation rates for the treatments

RT-PCR analysis
q-RT-PCR will be used to determine extraction efficiency of this method.
For RNA extraction and q-RT-PCR the samples for all three viruses can be pooled.

Pool 100 uL each: CoV-A-1 + Flu-A-1 + SL1-A-1
Make 1:100 dilution of the stocks (10 +990 uL medium)
Pool 100 uL of each 1:100 stock dilution: CoV + Flu + SL1
Pool 100 uL of each 1:100 stock dilution: CoV + Flu + SL1

Add 200 uL of the pooled samples to 250 uL maganpure lysisbuffer with EAV
Analyse by RT-PCR:

- Corona Sarbeco-E PCR
- Influenzavirus A PCR
- Sabin PCR