



Fourth report on Shimyatech Ltd samples: Virucidal assays (last samples)

Samples:

- uncoated carbon fabric
- 1
- 2
- Uncoated foam
- 3
- Regular cotton
- 4
- 5

Virus: recombinant 229E human coronavirus expressing GFP (HCoV-229E/GFP)

Cell line: Huh-7 human hepatoma cell line.

Test procedure: For each experimental condition, a fixed volume of a coronavirus preparation (approximate infectious titre of 10^6 TCID₅₀/mL) were deposited dropwise on 2×2 cm squares of coated or uncoated fabric samples placed inside small tissue culture dishes (35×10 mm). After 3h-incubation at room temperature, the virus was recovered from the fabric by adding DMEM culture medium supplemented with 2% foetal bovine serum over the samples followed by incubation at 4°C for 30 min in a shaker.

The viral suspensions obtained from both fabric uncoated controls and coated test samples were titrated by the Reed and Muench method of endpoint dilution, to determine residual virus infectivity (TCID₅₀/mL) after exposure to the coated fabrics compared to the paired uncoated control. Dilutions from 10^{-1} to 10^{-6} of each sample were used in sextuplicate to infect 96-well culture plates seeded with confluent Huh-7 cells. For the assessment of the infection outcome, cultures that showed at least one green-fluorescent cell, indicative of recombinant coronavirus infection, were considered positive.

The virucidal activity values (see Figure) were calculated by subtracting the logarithm of the infective titre associated to test sample from the logarithm of viral titres recovered from uncoated sample.

Results:

The following table shows the virus infectious titres recovered upon exposure to uncoated controls and coated samples, as well as their log₁₀, and virucidal activity values from two independent experiments.

No cytotoxicity was observed in the cell monolayers at any dilution during titration of recovered virus after exposure to these samples.

rep1				rep2			mean virucidal activity
sample	titer	log	virucidal activity	titer	log	virucidal activity	
uncoated fabric	1,00E+06	6,00		6,81E+05	5,83		
sample 1	3,98E+01	1,60	4,40	3,65E+02	2,56	3,27	3,84
sample 2	3,83E+02	2,58	3,42	6,81E+02	2,83	3,00	3,21
uncoated foam	3,16E+05	5,50		6,81E+05	5,83		
sample 3	3,16E+01	1,50	4,00	3,16E+01	1,50	4,33	4,17
regular cotton	2,15E+05	5,33		1,47E+05	5,17		
sample 4	3,16E+01	1,50	3,83	3,16E+01	1,50	3,67	3,75
sample 5	3,16E+01	1,50	3,83	3,16E+01	1,50	3,67	3,75

In general, samples showing a reduction in virus titre below 1 log₁₀ are considered inactive (no virucidal activity) as this is equivalent to less than 90% of virions being inactivated.

Samples with a reduction of virus titre between 1 and 2 log₁₀ are generally considered to exert mild virucidal effect (>90% of viral particles being inactivated upon treatment).

Samples reducing virus titres by more than 2 log₁₀ are considered to possess a strong virucidal activity (>99% of viral particles being inactivated upon treatment). All samples from this batch fell within this category, with reduction indexes greater than 3 log₁₀ (>99.9 % of viral particles being inactivated upon treatment). The lowest virucidal activity was recorded for sample 2 (though still strong reduction index >3 logs) (Tukey's multiple comparison post-hoc test, $p=0.0492$). No significant differences were detected for samples 1, 3, 4 and 5; Tukey's post-hoc test, $p>0.05$).

Samples 3, 4 and 5 completely abolished virus replication since no positive well was observed even at the lowest dilution used for titration (10^{-1}). In these conditions, the Reed and Muench titration method cannot provide accurate titre values because a 100% positive wells at the lowest dilution is a prerequisite. Thus, for these samples, the infectious titres (3.16×10^1 TCID₅₀/mL) were determined by assuming 100% positive wells in the undiluted sample (10^0), which might not necessarily be true (as we did not generally inoculate the undiluted sample). However, the undiluted sample could also be 100% negative as well, in which case the recovered virus titre might be even lower than that. Overall, it means that the virucidal activity for these samples could be underestimated, and might be even higher than the values shown in the table.

