

Report

Experiment 21/05/20 - Virucidal effect of treated textile and paper

Objective: to evaluate virucidal effect of the treated textiles and papers on SARS-CoV-2 virus, perform IF (immunofluorescence) assay without freezing of the recovered virus

Experiment was performed in triplicates in 96-well plates using VERO-E6 cell line

Used samples and workflow:

- 1) Treated textiles and papers for anti-viral testing
 - a. treated textile (samples 1/1; 1/2; 1/4; 1/7; 1/9)
 - b. textile as a control (sample 1/0)
 - c. treated paper (sample 2/1)
 - d. paper as a control (sample 2/0)
- 2) 20 μ L of SARS-CoV-2 virus (approx. 10^6 IU/mL) was added on textile or paper sitting in 12-well dish
- 3) Tested textiles and papers were incubated with virus for 10 and 60 minutes
- 4) After incubation virus was washed out with 180 μ L 1x PBS and the wash was used directly for VERO-E6 cells infection
- 5) Recovered virus was tittered in IF assay in VERO-E6 cells with 1:5 serial dilution, starting from 10 μ L. VERO-E6 cells were incubated for 48 hours in CO₂ incubator set to 37°C. After incubation IF assay was performed. Briefly, medium was washed out, cells were fixed using 4% paraformaldehyde, cell membranes were perforated with 0.2% Triton-X100 and SARS-CoV-2 virus was labeled with 1st mouse anti-SARS-CoV-2 antibody. 2nd anti-mouse antibody was conjugated with Cy3 fluorophore and for signal detection was used fluorescent microscope.
- 6) Titer was counted by Spearman-Karber method, compared to results from untreated control and results were expressed as virus titer reduction in percentage.

Results: (RED = infected, GREEN = uninfected)

SARS CoV-2 virus - 10min/control textile sample 1/0

Dilution	SARS-CoV-2 titer IU/mL	SARS-CoV-2 IU in well	IF assay			% of wells uninfected	% of wells infected
			well 1	well 2	well 3		
0	38236.66	38.24	RED	RED	RED	0%	100%
1/5	7647.33	7.65	RED	GREEN	GREEN	66.6%	33.3%
1/25	1529.47	1.53	GREEN	GREEN	GREEN	100%	0%
1/125	305.89	0.31	GREEN	GREEN	GREEN	100%	0%
1/625	61.18	0.061	GREEN	GREEN	GREEN	100%	0%
1/3 125	12.24	0.012	GREEN	GREEN	GREEN	100%	0%
1/15 625	2.45	0.0024	GREEN	GREEN	GREEN	100%	0%
1/78 125	0.49	0.00049	GREEN	GREEN	GREEN	100%	0%

SARS CoV-2 virus - 10min/ textile sample 1/1

Dilution	SARS-CoV-2 titer IU/mL	SARS-CoV-2 IU in well	IF assay			% of wells uninfected	% of wells infected
			well 1	well 2	well 3		
0	0	0				100%	0%
1/5	0	0				100%	0%
1/25	0	0				100%	0%
1/125	0	0				100%	0%
1/625	0	0				100%	0%
1/3 125	0	0				100%	0%
1/15 625	0	0				100%	0%
1/78 125	0	0				100%	0%

SARS CoV-2 virus - 10min/ textile sample 1/2

Dilution	SARS-CoV-2 titer IU/mL	SARS-CoV-2 IU in well	IF assay			% of wells uninfected	% of wells infected
			well 1	well 2	well 3		
0	0	0				100%	0%
1/5	0	0				100%	0%
1/25	0	0				100%	0%
1/125	0	0				100%	0%
1/625	0	0				100%	0%
1/3 125	0	0				100%	0%
1/15 625	0	0				100%	0%
1/78 125	0	0				100%	0%

SARS CoV-2 virus - 10min/ textile sample 1/4

Dilution	SARS-CoV-2 titer IU/mL	SARS-CoV-2 IU in well	IF assay			% of wells uninfected	% of wells infected
			well 1	well 2	well 3		
0	7647.16	7.65				66.6%	33.3%
1/5	1529.43	1.53				100%	0%
1/25	305.89	0.31				100%	0%
1/125	61.18	0.061				100%	0%
1/625	12.24	0.012				100%	0%
1/3 125	2.45	0.0024				100%	0%
1/15 625	0.49	0.00049				100%	0%
1/78 125	0.10	0.00010				100%	0%

SARS CoV-2 virus - 10min/ textile sample 1/7

Dilution	SARS-CoV-2 titer IU/mL	SARS-CoV-2 IU in well	IF assay			% of wells uninfected	% of wells infected
			well 1	well 2	well 3		
0	0	0				100%	0%
1/5	0	0				100%	0%
1/25	0	0				100%	0%
1/125	0	0				100%	0%
1/625	0	0				100%	0%
1/3 125	0	0				100%	0%
1/15 625	0	0				100%	0%
1/78 125	0	0				100%	0%

SARS CoV-2 virus - 10min/ textile sample 1/9

Dilution	SARS-CoV-2 titer IU/mL	SARS-CoV-2 IU in well	IF assay			% of wells uninfected	% of wells infected
			well 1	well 2	well 3		
0	7647.16	7.65				66.6%	33.3%
1/5	1529.43	1.53				100%	0%
1/25	305.89	0.31				100%	0%
1/125	61.18	0.061				100%	0%
1/625	12.24	0.012				100%	0%
1/3 125	2.45	0.0024				100%	0%
1/15 625	0.49	0.00049				100%	0%
1/78 125	0.10	0.00010				100%	0%

SARS CoV-2 virus - 10min/control paper sample 2/0

Dilution	SARS-CoV-2 titer IU/mL	SARS-CoV-2 IU in well	IF assay			% of wells uninfected	% of wells infected
			well 1	well 2	well 3		
0	65383.78	65.38				0%	100%
1/5	13076.76	13.08				33.3%	66.6%
1/25	2615.35	2.62				100%	0%
1/125	523.07	0.52				100%	0%
1/625	104.61	0.10				100%	0%
1/3 125	20.92	0.021				100%	0%
1/15 625	4.18	0.0042				100%	0%
1/78 125	0.84	0.00084				100%	0%

SARS CoV-2 virus - 10min/paper sample 2/1

Dilution	SARS-CoV-2 titer IU/mL	SARS-CoV-2 IU in well	IF assay			% of wells uninfected	% of wells infected
			well 1	well 2	well 3		
0	13076.76	13.08				66.6%	33.3%
1/5	2615.35	2.62				66.6%	33.3%
1/25	523.07	0.52				100%	0%
1/125	104.61	0.10				100%	0%
1/625	20.92	0.021				100%	0%
1/3 125	4.18	0.0042				100%	0%
1/15 625	0.84	0.00084				100%	0%
1/78 125	0.17	0.00017				100%	0%

Sample name	Exposure time [min]	Titer control [IU/mL]	Titer sample [IU/mL]	Virus yield reduction [%]
Textile 1/1	10	38236.66	0.00	100.00
Textile 1/2	10	38236.66	0.00	100.00
Textile 1/4	10	38236.66	7647.16	20.00
Textile 1/7	10	38236.66	0.00	100.00
Textile 1/9	10	38236.66	7647.16	20.00
Paper 2/1	10	65383.78	13076.76	20.00

Conclusion:

For textile samples 1/1, 1/2 and 1/7, we have observed 100% virucidal effect on SARS-CoV-2 virus after 10 minutes incubation. Textile samples 1/4 and 1/9 show 20% virucidal effect on SARS-CoV-2 virus after 10 minutes incubation.

For paper sample 2/1, we observed 20% virucidal effect on SARS-CoV-2 virus after 10 minutes incubation.

Results for 60 minutes incubation could not be correctly evaluated as the virus was completely inactivated also on at least one of the "control" textile/paper).