

## Sample report

Experiment 10/07/20 filter paper containing 1% of antiviral agent

Objective: Evaluate the effect of anti-viral agent in filter paper on SARS-CoV-2, perform IF (immunofluorescence) assay without freezing of the recovered virus

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

- 1) Filter-paper for antiviral testing
  - a. Paper containing 1% of antiviral agent
  - b. Paper without antiviral agent as control
- 2) 20  $\mu$ L of SARS-CoV-2 (approx. 10e6 IU/mL) was added on filter paper sitting in 12-well dish, solution containing virus was absorbed in the paper
- 3) Papers with virus were incubated for 10 and 20 minutes
- 4) After incubation virus was washed out from the paper with 180  $\mu$ L 1x PBS and the wash was used directly for VERO-E6 cells infection
- 5) Recovered virus was titered in IF assay in VERO-E6 cells with 1:2 serial dilution, starting from 10  $\mu$ L. VERO-E6 cells were incubated for 72 hours in CO<sub>2</sub> incubator set to 37°C. After incubation IF assay was performed. Briefly, medium was washed out, cells were fixed using 4% PFA, cell membranes were perforated with 0.2% Triton-X100 and SARS-CoV-2 virus was labeled with 1<sup>st</sup> mouse anti-SARS-CoV-2 antibody. 2<sup>nd</sup> anti-mouse antibody was conjugated with Cy3 fluorophore and for signal detection was used fluorescent microscope.
- 6) Titer was counted by Reed-Muench 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 - 10min/filter paper with 1% antiviral agent

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	49 411	494	Red	Red	Red	0%	100%
1/2	19 764	198	Red	Red	Red	0%	100%
1/4	7 906	79	Red	Red	Red	0%	100%
1/8	3 162	32	Green	Red	Red	33.3%	66.6%
1/16	1 265	13	Green	Green	Red	66.6%	33.3%
1/32	506	5.1	Green	Green	Green	100%	0%
1/64	202	2.0	Green	Green	Green	100%	0%
1/128	81	0.8	Green	Green	Green	100%	0%
1/256	32	0.32	Green	Green	Green	100%	0%
1/512	13	0.13	Green	Green	Green	100%	0%
1/1024	5	0.05	Green	Green	Green	100%	0%

SARS CoV-2 - 10min/ control filter paper without antiviral agent

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	173 660	1 737				0%	100%
1/2	69 464	695				0%	100%
1/4	27 786	278				0%	100%
1/8	11 114	111				0%	100%
1/16	4 446	44.5				0%	100%
1/32	1 778	17.8				33.3%	66.6%
1/64	711	7.11				100%	0%
1/128	285	2.85				100%	0%
1/256	114	1.138				100%	0%
1/512	46	0.455				100%	0%
1/1024	18	0.182				100%	0%

SARS CoV-2 - 20min/ filter paper with 1% antiviral agent

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	19 764	198				0%	100%
1/2	7 906	79				0%	100%
1/4	3 162	32				33.3%	66.6%
1/8	1 265	13				66.6%	33.3%
1/16	506	5.1				100%	0%
1/32	202	2.0				100%	0%
1/64	81	0.8				100%	0%
1/128	32	0.32				100%	0%
1/256	13	0.13				100%	0%
1/512	5	0.05				100%	0%
1/1024	2	0.02				100%	0%

SARS CoV-2 - 20min/ control filter paper without antiviral agent

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	65 890	659				0%	100%
1/2	26 356	264				0%	100%
1/4	10 542	105				33.3%	66.6%
1/8	4 217	42				33.3%	66.6%
1/16	1 687	16.9				33.3%	66.6%
1/32	675	6.7				100%	0%
1/64	270	2.7				100%	0%
1/128	108	1.08				100%	0%
1/256	43	0.43				100%	0%
1/512	17	0.17				100%	0%
1/1024	7	0.07				100%	0%

Exposure time [min]	Titer control [IU/mL]	Titer anti-viral 1% agent [IU/mL]	Virus yield reduction [%]
10	173 660.10	49 410.59	71.55
20	65 890.08	19 764.24	70.00

Conclusion:

We observed SARS-CoV-2 yield reduction at the level approx. 70% after 10 and 20 minutes exposure to filter paper with 1% antiviral agent.

However, we were not able to inactivate virus completely in the span of 20 minutes. The prolongation of exposure time to 30 minutes led to the complete virus inactivation in control filter paper and in filter paper with 1% antiviral agent as well.