

MG_screening 1_2021-10-21

Collaboration:

Monkam Guy, SARS-CoV-2 screening against Wuhan and Delta virus

Requested compositions to be tested:

Composition 1: d-limonene (40 microg / ml) + Lupeol (40 microg / ml) + B-sitosterol (12.48 microg / ml) + Cinnamaldehyde (2.64 microg / ml).

Composition 2: d-limonene (40 microg / ml) + Lupeol (40 microg / ml) + B-sitosterol (12.48 microg / ml) + Cinnamaldehyde (2.64 microg / ml) + EGCG (18.34 microg / ml)

Composition 3: d-limonene (40 microg / ml) + Lupeol (40 microg / ml) + B-sitosterol (12.48 microg / ml) + Cinnamaldehyde (2.64 microg / ml) + Curcumin (10 microg / ml)

Composition 4: d-limonene (40 microg / ml) + Lupeol (40 microg / ml) + B-sitosterol (12.48 microg / ml) + Cinnamaldehyde (2.64 microg / ml) + EGCG (18.34 microg / ml) + curcumin (10 microg / ml).

Dissolution compounds:

d-limonene	Lupeol	B-sitosterol	Cinnamaldehyde	EGCG	Curcumin
60% DMSO / 40% ETHANOL		100% DMSO		60% DMSO / 40% ETHANOL	

Complete dissolution:

√ √ no √ √ √

B-sitosterol: did not dissolve completely, not in mix and not in pure DMSO > 100% DMSO used

200x stock concentration used for screening:

	µg/ml d-limonene	µg/ml Lupeol	µg/ml B-sitosterol	µg/ml Cinnamaldehyde	µg/ml EGCG	µg/ml Curcumin
Composition 1:	8000	8000	2496	528		
Composition 2:	8000	8000	2496	528	3668	
Composition 3:	8000	8000	2496	528		2000
Composition 4:	8000	8000	2496	528	3668	2000

Compounds were dissolved individually and then equal volumes of each compound was mixed for the compositions as indicated

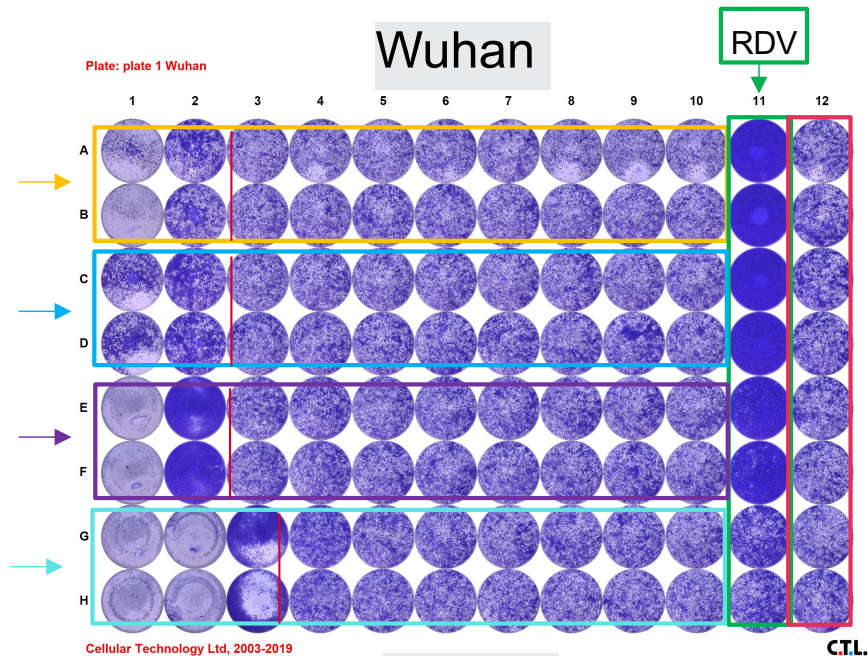
each composition

- > starting final dilution 1:200
- > serial dilution 1:2

1x concentration requested:

	µg/ml d-limonene	µg/ml Lupeol	µg/ml B-sitosterol	µg/ml Cinnamaldehyde	µg/ml EGCG	µg/ml Curcumin
Composition 1:	40	40	12.48	2.64		
Composition 2:	40	40	12.48	2.64	18.34	
Composition 3:	40	40	12.48	2.64		10
Composition 4:	40	40	12.48	2.64	18.34	10

MG_screening 1_2021-10-21



Remdesivir, start 50 μ M final concentration $\rightarrow$ 1:2 serial dilution

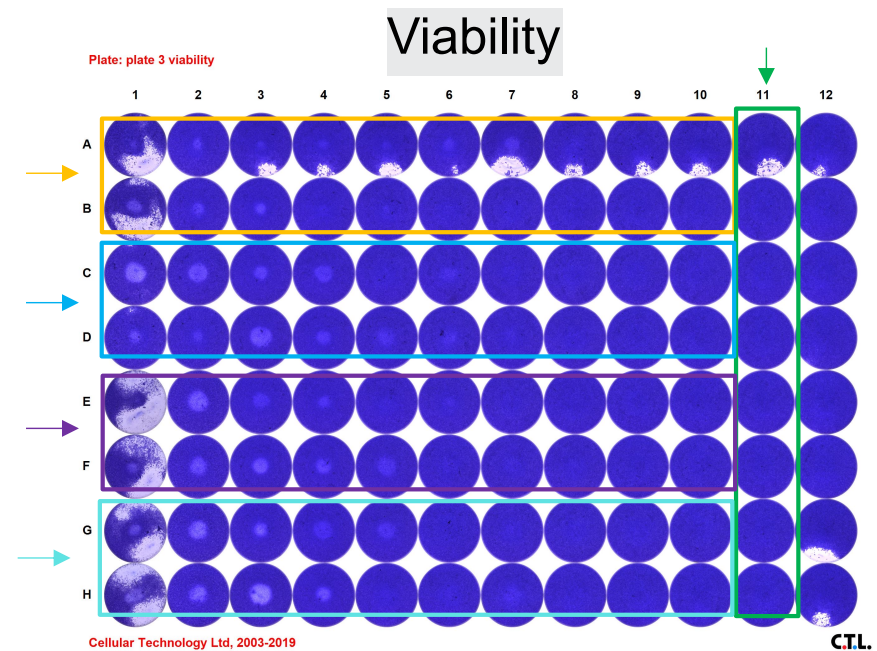
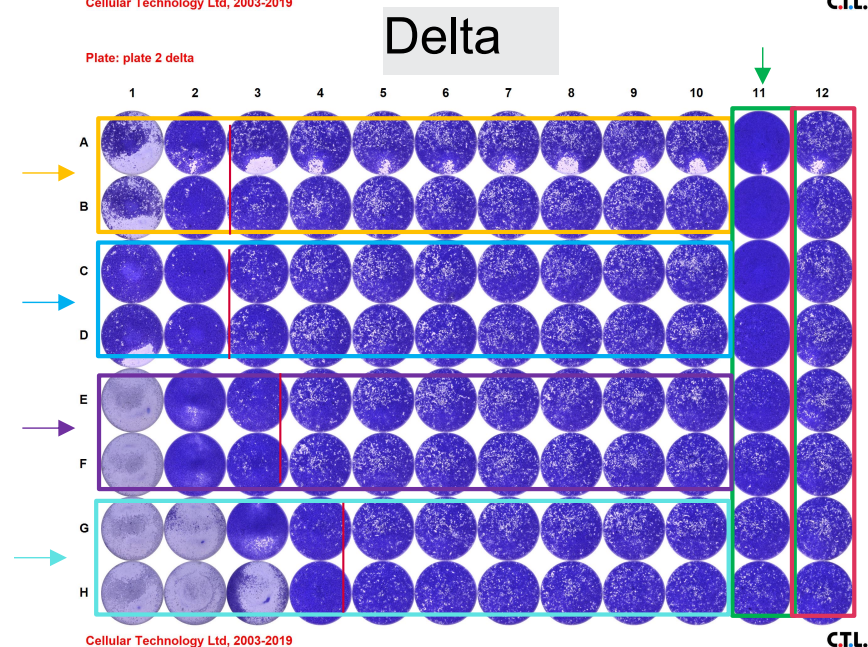
Composition1, start 1x final concentration $\rightarrow$ 1:2 serial dilution

Composition2, start 1x final concentration $\rightarrow$ 1:2 serial dilution

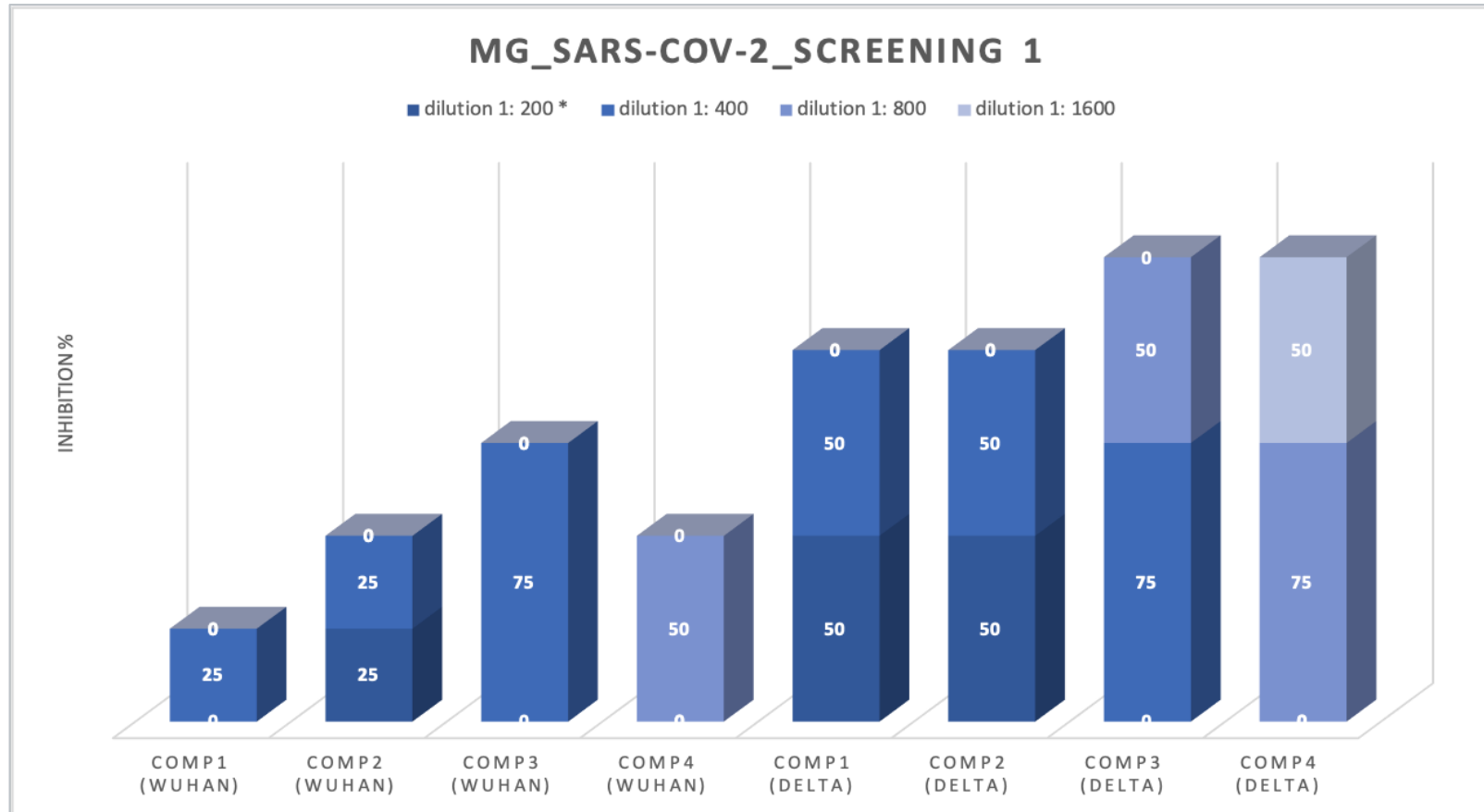
Composition3, start 1x final concentration $\rightarrow$ 1:2 serial dilution

Composition4, start 1x final concentration $\rightarrow$ 1:2 serial dilution

Reference wells, virus w/o compound



MG_screening 1 (DMSO)_2021-10-21_result



Testing of compositions (comp1-4) according to request

% Inhibition	dilution 1:200 *	dilution 1:400	dilution 1:800	dilution 1:1600	dilution 1:3200	dilution 1:6400
Comp1 (Wuhan)	CT	25	0	0	0	0
Comp2 (Wuhan)	25	25	0	0	0	0
Comp3 (Wuhan)	CT	75	0	0	0	0
Comp4 (Wuhan)	CT	CT	50	0	0	0
Comp1 (Delta)	50	50	0	0	0	0
Comp2 (Delta)	50	50	0	0	0	0
Comp3 (Delta)	CT	75	50	0	0	0
Comp4 (Delta)	CT	CT	75	50	0	0

Cytotoxicity (CT)

* diution 1:200 = 1x concentrated

Result:

→ All tested compositions inhibit both SARS-CoV-2 variants at least partially at the highest tested concentration.

→ Highest activity was observed with composition 3

MG_screening 2_2021-10-28

Collaboration:

Monkam Guy, SARS-CoV-2 screening against Wuhan and Delta virus

Requested compositions to be tested:

Composition 1: d-limonene (40 microg / ml) + Lupeol (40 microg / ml) + B-sitosterol (12.48 microg / ml) + Cinnamaldehyde (2.64 microg / ml).

Composition 2: d-limonene (40 microg / ml) + Lupeol (40 microg / ml) + B-sitosterol (12.48 microg / ml) + Cinnamaldehyde (2.64 microg / ml) + EGCG (18.34 microg / ml)

Composition 3: d-limonene (40 microg / ml) + Lupeol (40 microg / ml) + B-sitosterol (12.48 microg / ml) + Cinnamaldehyde (2.64 microg / ml) + Curcumin (10 microg / ml)

Composition 4: d-limonene (40 microg / ml) + Lupeol (40 microg / ml) + B-sitosterol (12.48 microg / ml) + Cinnamaldehyde (2.64 microg / ml) + EGCG (18.34 microg / ml) + curcumin (10 microg / ml).

Dissolution compounds:

d-limonene	Lupeol	B-sitosterol*	Cinnamaldehyde	EGCG	Curcumin
60% DMSO / 40% Ethanol		100% Ethanol		60% DMSO / 40% Ethanol	

Complete dissolution:

✓ ✓ ✓ ✓ ✓ ✓

* B-sitosterol: dissolved completely after few hours

200x stock concentration used for screening:

	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
	d-limonene	Lupeol	B-sitosterol	Cinnamaldehyde	EGCG	Curcumin
Composition 1:	8000	8000	2496	528		
Composition 2:	8000	8000	2496	528	3668	
Composition 3:	8000	8000	2496	528		2000
Composition 4:	8000	8000	2496	528	3668	2000

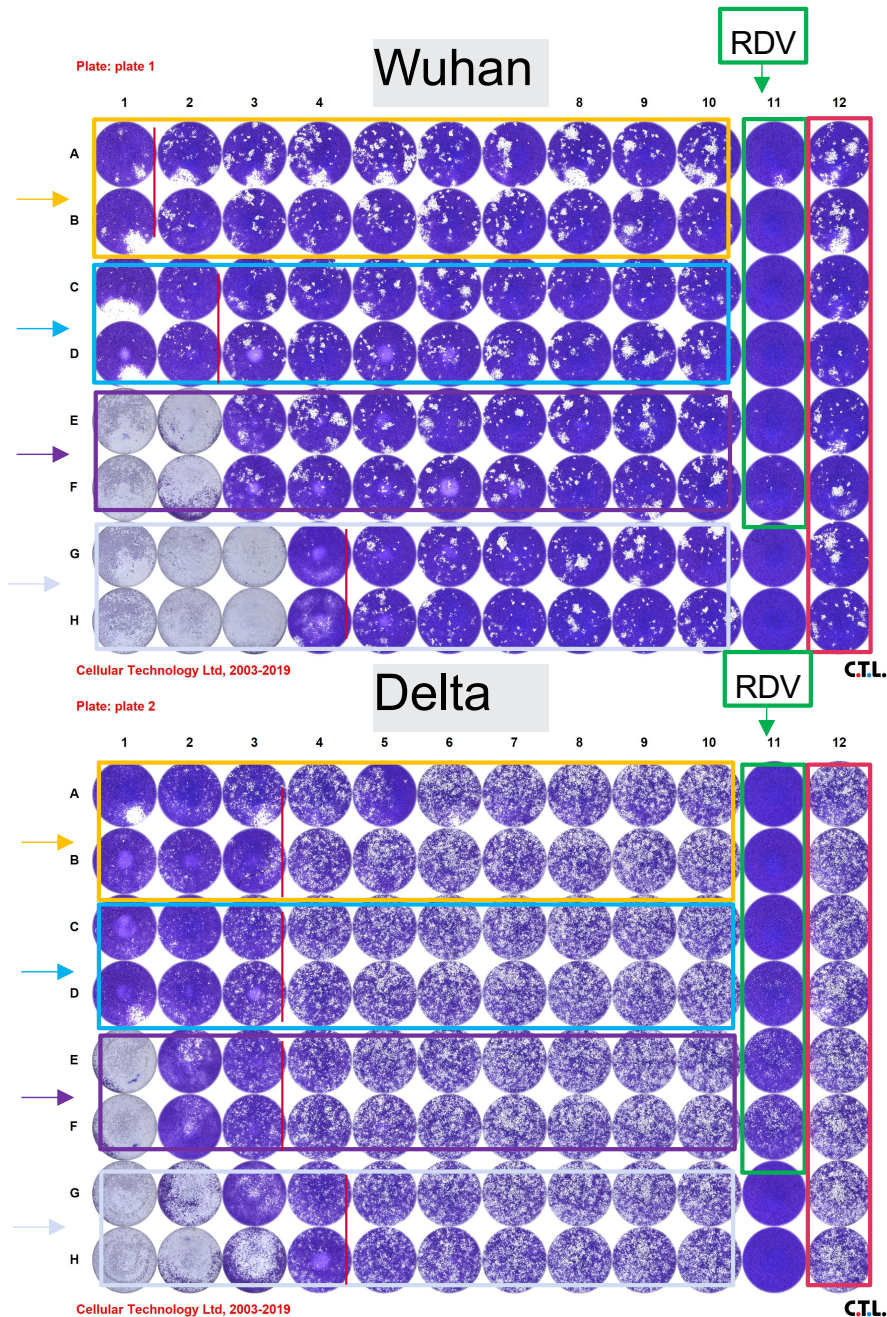
1x concentration requested:

	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
	d-limonene	Lupeol	B-sitosterol	Cinnamaldehyde	EGCG	Curcumin
Composition 1:	40	40	12.48	2.64		
Composition 2:	40	40	12.48	2.64	18.34	
Composition 3:	40	40	12.48	2.64		10
Composition 4:	40	40	12.48	2.64	18.34	10

each composition:

- > starting final dilution 1:200
- > serial dilution 1:2

Monkam Guy_screening 2_2021-10-28



Remdesivir, start 50 μ M final concentration $\rightarrow$ 1:2 serial dilution

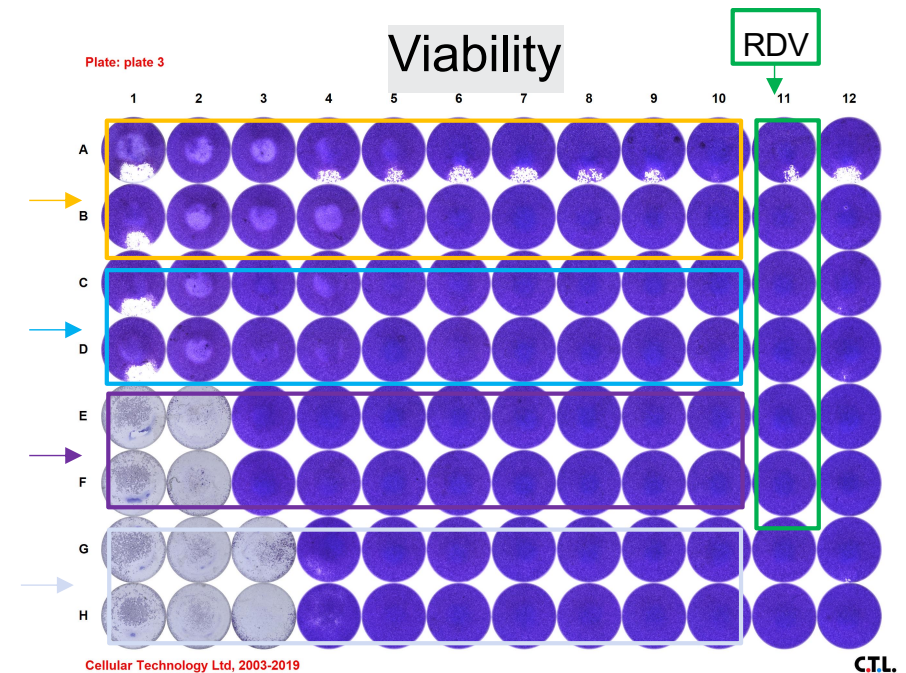
Composition1, start 1x final concentration $\rightarrow$ 1:2 serial dilution

Composition2, start 1x final concentration $\rightarrow$ 1:2 serial dilution

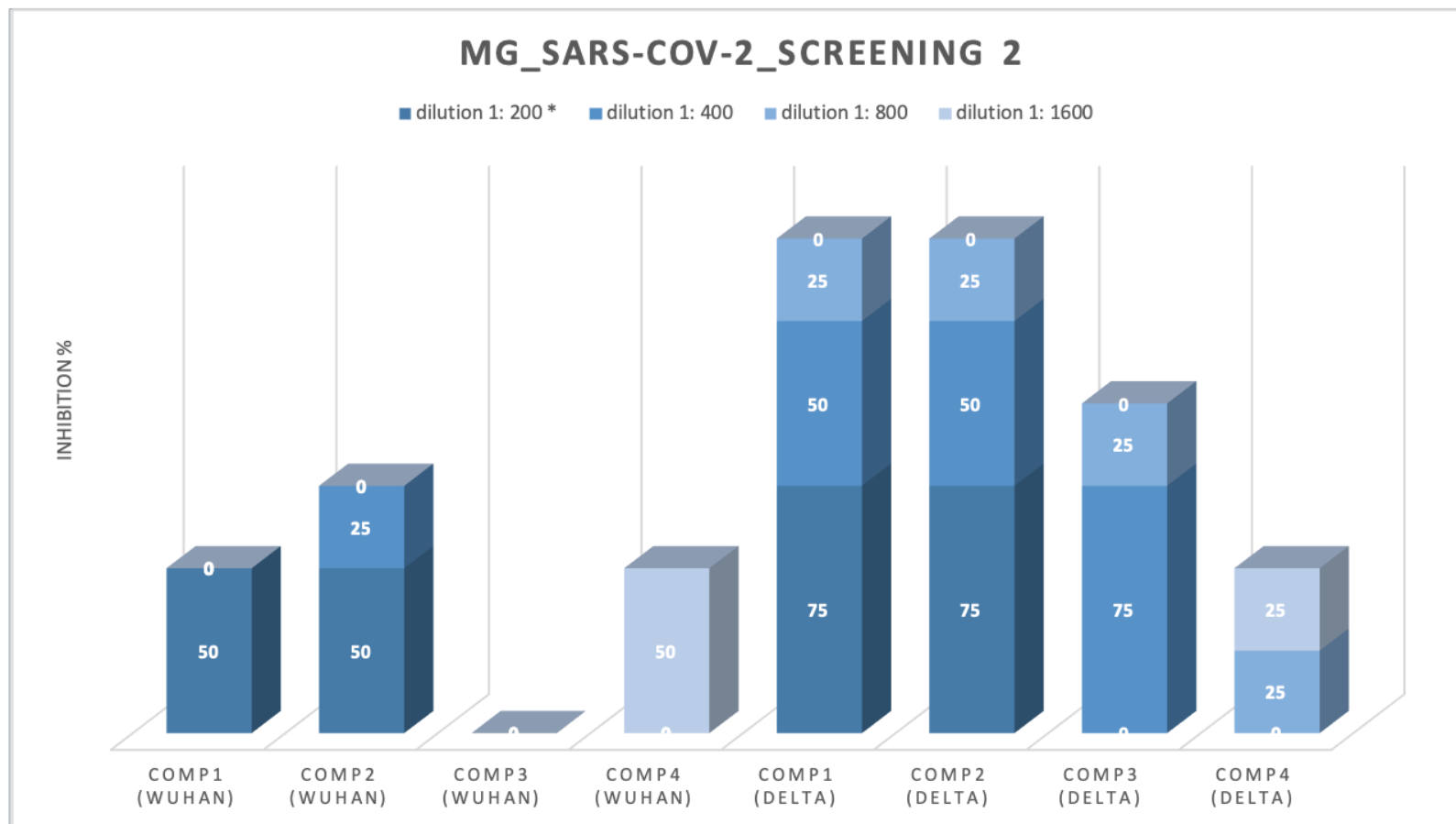
Composition3, start 1x final concentration $\rightarrow$ 1:2 serial dilution

Composition4, start 1x final concentration $\rightarrow$ 1:2 serial dilution

Reference wells, virus w/o compound



Monkam Guy_screening 2 (EtOH)_2021-10-28_result



Testing of compositions (comp1-4) according to request

% Inhibition	dilution 1:200 *	dilution 1:400	dilution 1:800	dilution 1:1600	dilution 1:3200	dilution 1:6400
Comp1 (Wuhan)	50	0	0	0	0	0
Comp2 (Wuhan)	50	25	0	0	0	0
Comp3 (Wuhan)	CT	CT	0	0	0	0
Comp4 (Wuhan)	CT	CT	CT	50	0	0
Comp1 (Delta)	75	50	25	0	0	0
Comp2 (Delta)	75	50	25	0	0	0
Comp3 (Delta)	CT	75	25	0	0	0
Comp4 (Delta)	CT	CT	25	25	0	0

Cytotoxicity (CT)

* dilution 1:200 = 1x concentrated

Result:

→ Most tested compositions inhibit SARS-CoV-2 at least partially and at the highest concentration.

→ For C3 no effect against the Wuhan strain was observed; this could be due to the toxicity of C3 at higher concentrations.