

More MAb testing

Based on suggestions from David, Hot start Taq was prepared as followed together with the anti-bodies as shown in the table. Regular Taq was used as comparison.

	1µg/5U Taq	0.5µg/5U Taq
Taq 40U/µl	10µl	10µl
Mab	88µl	44µl
Final concentration	4UTaq/µl	8UTaq/µl

Complete master mixes with and without enzyme was kept at 4°C overnight. This is to test the effect of Taq on template and primers in the solution. Number of replicates is 32. Template; cDNA, detection; Eva green.

PCR mixes

Fragment:	Covid19 #7	A56C	Date	11.06.2021	
PCR volum, µl	10	# of reactions		32	
Working solutions concentration		Total volume 320 µl Volum		Desired concentration	
H2O		256,40			
10X Thermopol #1	0 mM MgCl	32,00			
MgCl	200 mM	3,20		2,00001 mM	94C-20sec
Primer forward	100 µM	0,96		0,3 µM	A 56C-20sec 45cycles
Primer reverse	100 µM	0,96		0,3 µM	72C-40sec
dNTP	100 mM	1,28		400 µM	Melting
cDNA covid-19	100 ng	3,20		1 ng/µl	
EVA green 20X		12,80		0,8 X	
BSA	100 %	3,20		1 %	
Taq 1µgMab/5U	4 U/µl	6,00		0,075 U/µl	
Fragment:	Covid19 #7	A56C	Date	11.06.2021	
PCR volum, µl	10	# of reactions		32	
Working solutions concentration		Total volume 320 µl Volum		Desired concentration	
H2O		259,40			
10X Thermopol #1	0 mM MgCl	32,00			
MgCl	200 mM	3,20		2,00001 mM	94C-20sec
Primer forward	100 µM	0,96		0,3 µM	A 56C-20sec 45cycles
Primer reverse	100 µM	0,96		0,3 µM	72C-40sec
dNTP	100 mM	1,28		400 µM	Melting
cDNA covid-19	100 ng	3,20		1 ng/µl	
EVA green 20X		12,80		0,8 X	
BSA	100 %	3,20		1 %	
Taq 0.5µgMab/5U	8 U/µl	3,00		0,075 U/µl	
Fragment:	Covid19 #7	A56C	Date	11.06.2021	
PCR volum, µl	10	# of reactions		32	
Working solutions concentration		Total volume 320 µl Volum		Desired concentration	
H2O		261,80			
10X Thermopol #1	0 mM MgCl	32,00			
MgCl	200 mM	3,20		2,00001 mM	94C-20sec
Primer forward	100 µM	0,96		0,3 µM	A 56C-20sec 45cycles
Primer reverse	100 µM	0,96		0,3 µM	72C-40sec
dNTP	100 mM	1,28		400 µM	Melting
cDNA covid-19	100 ng	3,20		1 ng/µl	
EVA green 20X		12,80		0,8 X	
BSA	100 %	3,20		1 %	
Taq	40 U/µl	0,60		0,075 U/µl	

Overnight with enzyme			Overnight without enzyme		
MAb conc.	Mean Cq	1 STD	MAb conc.	Mean Cq	1 STD
0µg	23,8	0,05	0µg	23,9	0,06
0.5µg	24,9	0,14	0.5µg	25,0	0,05
1µg	25,5	0,20	1µg	25,6	0,10

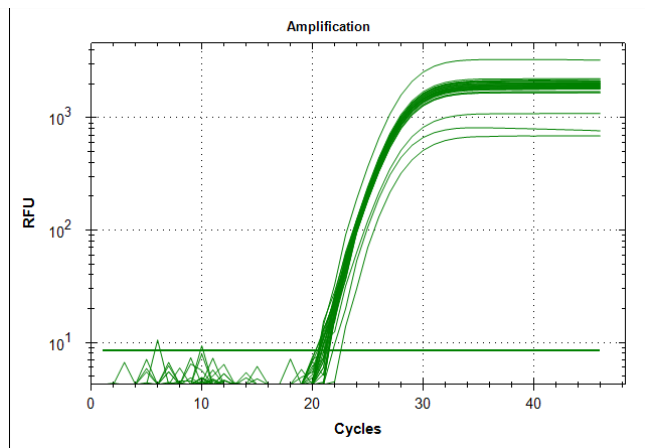
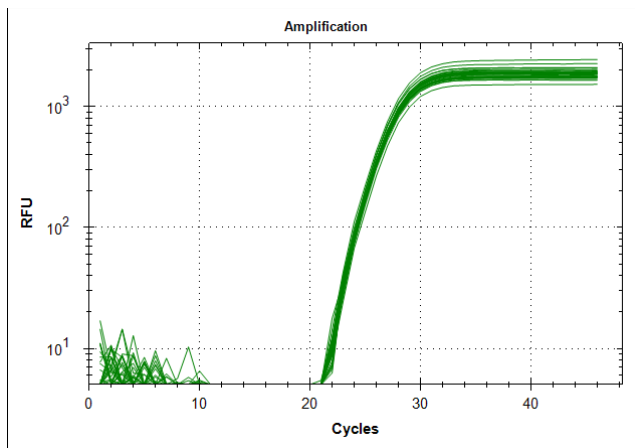
Graphs below is in same order as the table

Amplification with Taq (0µg MAb)

With enzyme overnight

without enzyme overnight

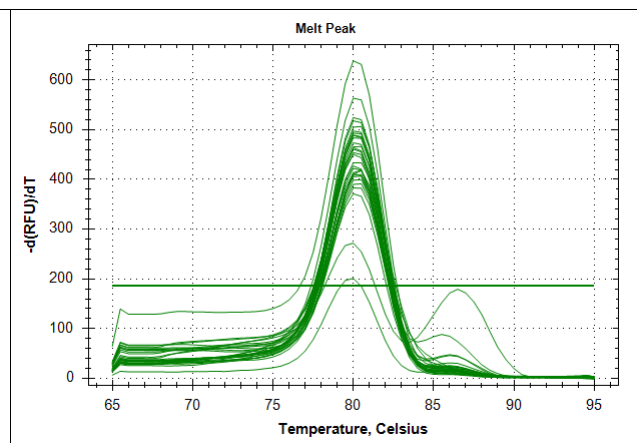
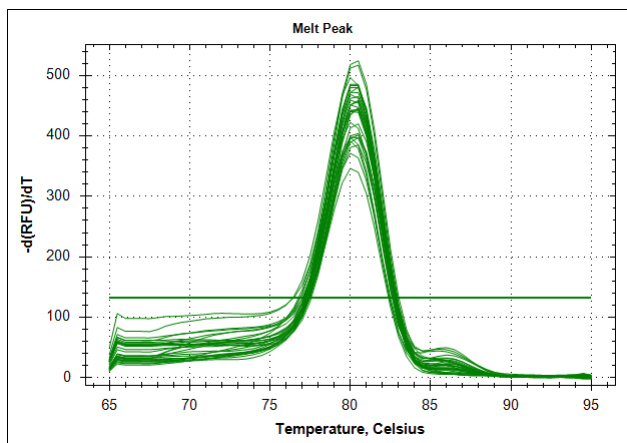
(Enzyme added just before start of PCR)



Derivative of melting curve with Taq (0µg MAb)

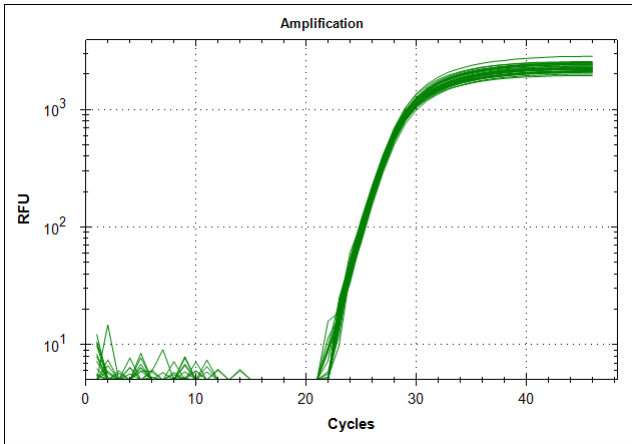
With enzyme overnight

without enzyme overnight

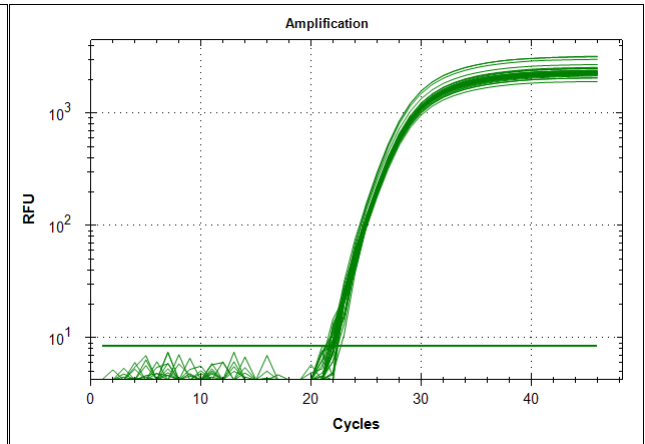


Amplification with Hot-start Taq (0.5µg MAb)

With enzyme overnight

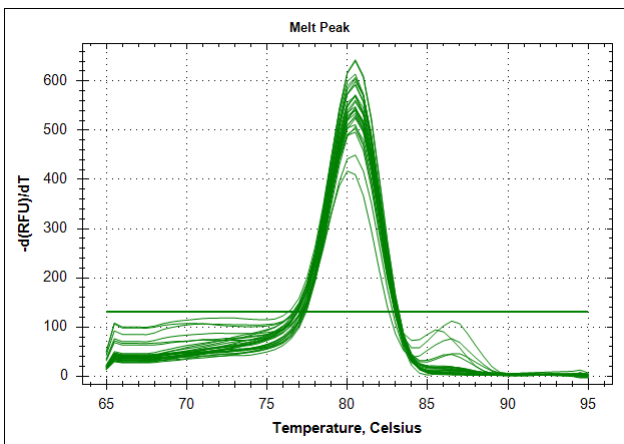


without enzyme overnight

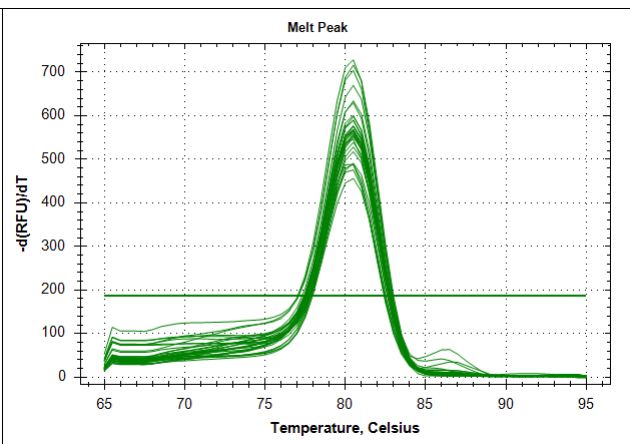


Derivative of melting curve with Hot-start Taq (0.5µg MAb)

With enzyme overnight

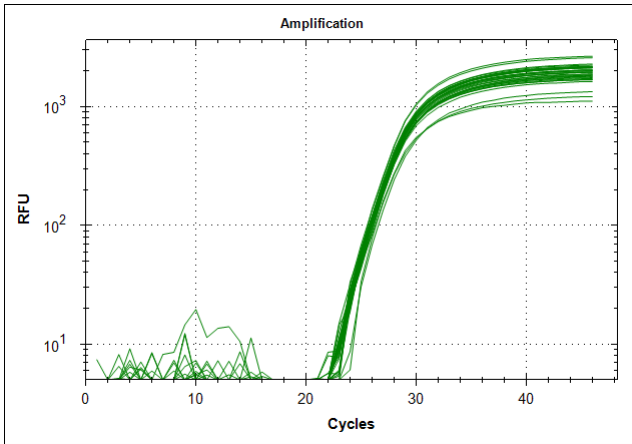


without enzyme overnight

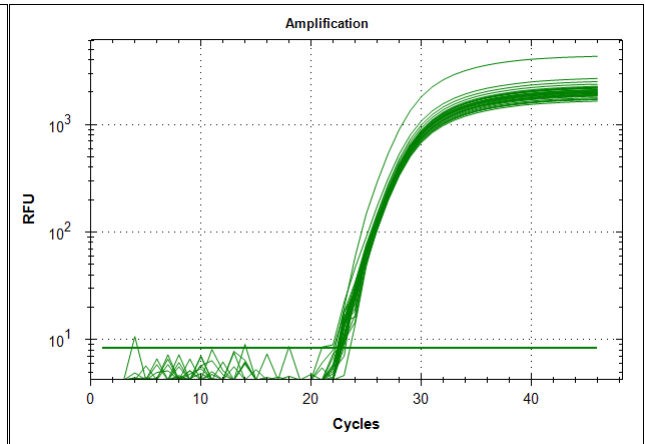


Amplification with Hot-start Taq (1 μ g MAb)

With enzyme overnight

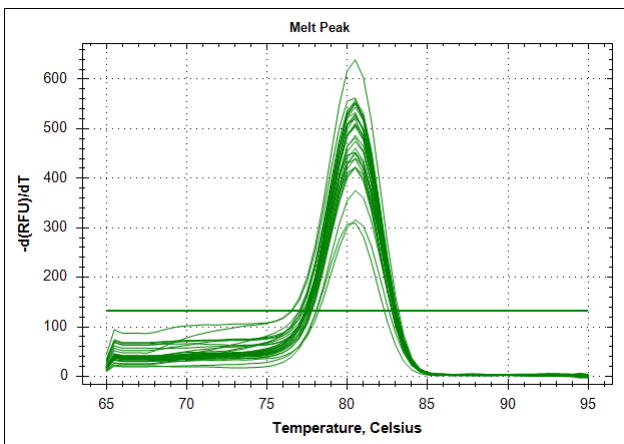


without enzyme overnight

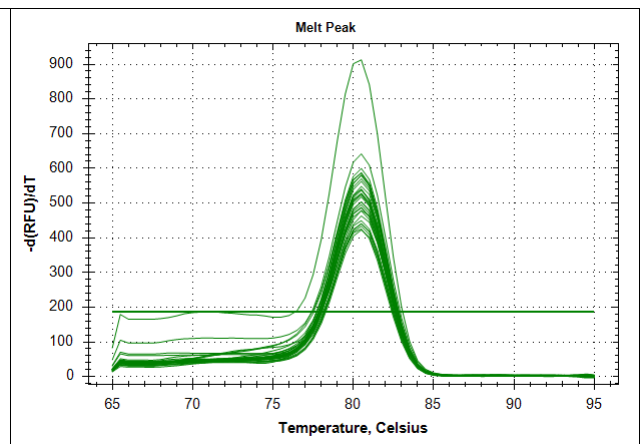


Derivative of melting curve with Hot-start Taq (1 μ g MAb)

With enzyme overnight



without enzyme overnight



Test of Hot-start Taq on full Covid-19 testing chemistry.

Data below is for hot-start Taq experiments using Taqman probes and RNA at template. Complete master mixes with everything was kept for 2.5 hour at 4°C and compared with fresh mix. (No melting curve is available when using Taqman probes).

PCR mix

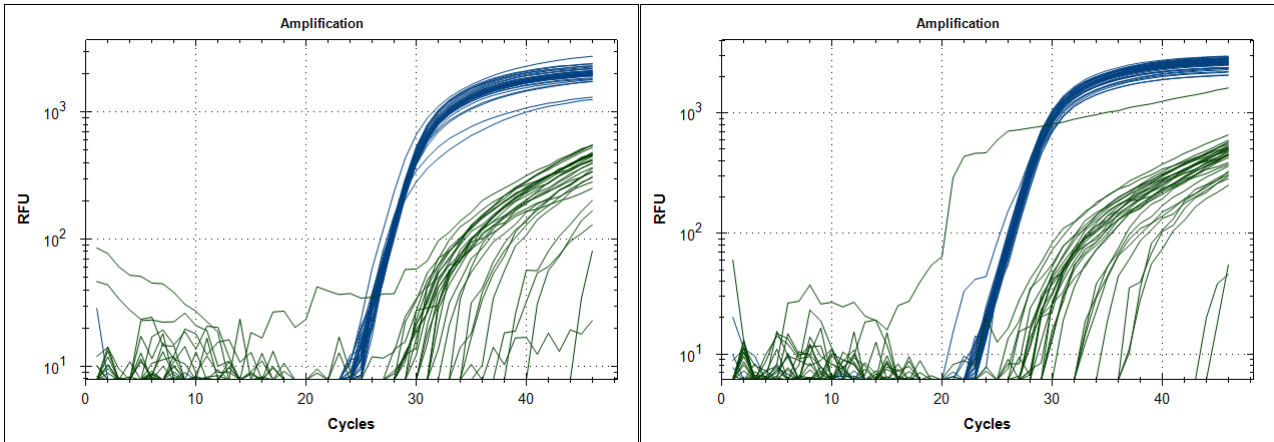
Fragment:	Covid19 Sarbeco	A56C	Date	11.06.2021			
PCR volum, µl	20	# of reactions		200			
	Working solutions		Total volume 4000 µl	Desired			
	concentration		Volum	concentration		42°C 2.5 min	cDNA sythesis
H2O			2392,00			50°C 2.5 min	cDNA sythesis
10X Thermopol #1	0 mM MgCl	400,00				94°C 1min	
MgCl	200 mM	40,00		2,00001 mM		94°C 20 sec	
Primer forward	100 µM	12,00		0,3 µM		60°C 1min	45 cycles
Primer reverse	100 µM	12,00		0,3 µM		read plate	
Probe	100 µM	12,00		0,3 µM			
Primer forward STD	100 µM	12,00		0,3 µM			
Primer reverse STD	100 µM	12,00		0,3 µM			
Probe STD	100 µM	12,00		0,3 µM			
dNTP	100 mM	16,00		400 µM			
RNA covid-19, pool	4 ng	1000,00		1 ng/µl			
EVA green 20X		0,00		0 X			
BSA	100 %	40,00		1 %			
Taq	40 U/µl	0,00		0 U/µl			
MashUp	7,5 U/µl	40,00		0,075 U/µl			

	2.5h at 4°C			Instant		
	MAB conc.	Mean Cq	1 STD	MAB conc.	Mean Cq	1 STD
Cq Covid	0µg	27,1	0,48	0µg	23,9	0,06
	0.5µg	27,2	0,28	0.5µg	25,0	0,05
	1µg	27,2	0,30	1µg	25,6	0,10
Cq internal Std	MAB conc.	Mean Cq	1 STD	MAB conc.	Mean Cq	1 STD
	0µg	30,6	4,64	0µg	31,0	6,14
	0.5µg	31,5	4,27	0.5µg	31,3	5,18
	1µg	33,4	3,81	1µg	30,9	5,04

Amplification with Taq (0 μ g MAb) (blue Covid signal, green internal standard)

With enzyme in fridge for 2.5 hours

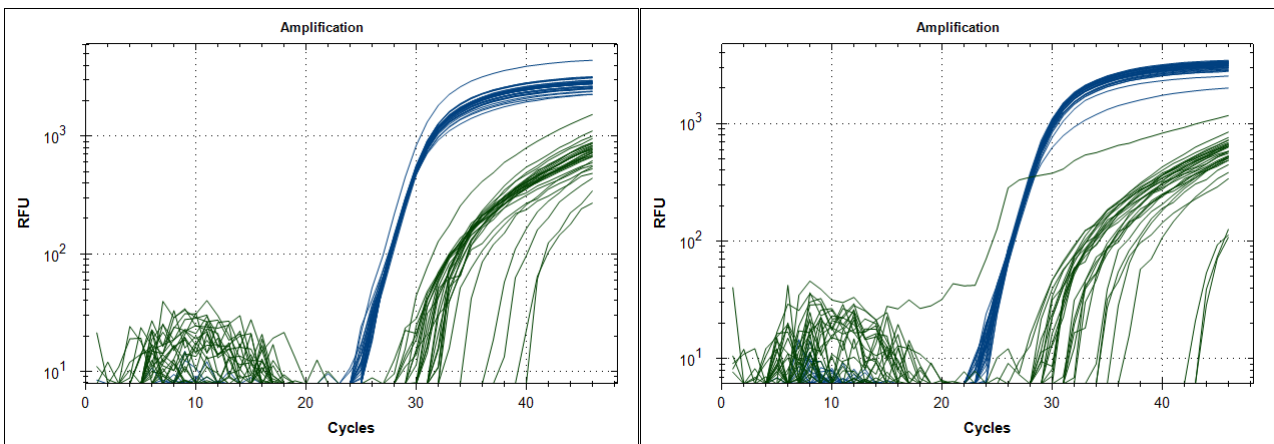
instant analysis



Amplification with Taq (0.5 μ g MAb) (blue Covid signal, green internal standard)

With enzyme in fridge for 2.5 hours

instant analysis



Amplification with Taq (1 μ g MAb) (blue Covid signal, green internal standard)

With enzyme in fridge for 2.5 hours

instant analysis

