

nCoV19+hCOV MIA

donderdag 19 maart 2020 13:57

19-3-2020 Proof of Principle met inhouse corona eiwitten van IDS - Gertjan godeke
Na inventarisatie kleine hoeveelheid (7-10 ug) van OC43, NL63 en HKU1 beschikbaar

Bevat HIS-Tag dus kan koppeling controleren met anti-histag-pe

Te weinig om te dialyseren en te weinig om buffers te vergelijken.
Daarom standaard koppeling in PBS met
Ca 7.5ug per 100ul beads

Regio 29 (van GABY HeB)
Regio 37 (van pneumo)
Regio 64 (van pneumo)

Serum panel + en - voor Humane corona` s

Bestelde eiwitten

Art.code	Description	Packsizes	Quantities
40589-V08B1_100UG	SARS-CoV-2 (2019-nCoV) Spike Protein (S1+S2 ECD, His tag)	100 ug	1
40591-V08H_100UG	2019-nCoV Spike Protein S1 (His Tag)	100 ug	3
40592-V08B_100UG	SARS-CoV-2 (2019-nCoV) Spike Protein (RBD, His Tag)	100 ug	1
40150-V08B1_100ug	Human SARS Coronavirus Spike S1 Subunit Protein (His Tag)	100 ug	1
40588-V08B_100UG	2019-nCoV Nucleocapsid Protein (His tag)	100 ug	1

Verwachte levering volgende week donderdag

20-3-2020

De hoeveelheden hCoV eiwitten laten weinig ruimte voor het uittesten van verschillende buffers en couplings omstandigheden.

Gezien de grote vh eiwit en de aanwezigheid van voldoende lysines, verwacht je op voorhand geen problemen met de koppeling aan gecarboxyleerde beads.

Daarom moeten we uitgaan van een standaard protocol.

Mbt de ids eiwitten is dit maximaal 5-10 ug dit laat weinig ruimte tot dialyseren en ombufferen.

Voor kruisreactie experimenten heb je ook relatief veel eiwit nodig (minimaal 50ug) voor 40-50 samples.

Elutie buffer van IDS his tag eiwitten bevat het volgende

Contains 250 mM Imidazole, 0.3 M sodium chloride, 50 mM sodium phosphate, pH 8.0.

23-3-2020 Conjugation of non-COVID19 human coronavirus S proteins

All proteins are cloned and expressed in house bij IDS and contain a 6x HisTag.

HCoV-OC43 - Beadregio -29

HCoV-NL63- beadregio -37

HCoV-HKU1 -beadregio 64

Ca 10ugr (ca 1mg/ml)

Activatie in PBS aangezien MES buffer nog niet binnen is.

- 120 µl beads (ca 1.5 milj COOH Microspheres, Luminex Corp) per region are collected in 1.5 ml coupling tubes (starlab)
- Spin (= 5 min, 13000rpm) on minispin and wash beads 1x with 200 µl PBS pH 7.4.
- Spin down beads remove supernatant and add 160 µl of PBS, vortex 30 sec and add 20 µl NHS (50mg/ml in AD), mix and subsequently and 20 µl EDC (50mg/ml in AD) and vortex 10 seconds.

Incubate 30 min on rotation in the Dark

Wash twice with 200 µl PBS + 0.05% tw20 (PBST).

Add 10 µl HCOV proteins (1mg/ml) in 200 µl PBS and incubate for 3 hours

Wash with PBST and store in 120 µl storage buffer

24-3-2020

Testen 3x HCoV eiwitten in triplex

Met mouse anti 6xHis-tag_PE (abcam: ab72467) in verdunnings reeks S1 tm S11 2 steps (S1=50x)

Tevens IVIG en gepooled serum (polio ref + RSV ref) in verdunnings reeks S1 tm S12

S1 = 10x V

Anti histag pikt geen signaal op.. Mogelijk heeft het te maken met de c of N terminus conformatie.

IVIG en serum (mixed sera) geven wel signalen, maar er lijkt vrij veel achtergrond in te zitten

(surmodics lijkt beter te zijn dan Assay buffer)

Met name NL63 is beroerd.

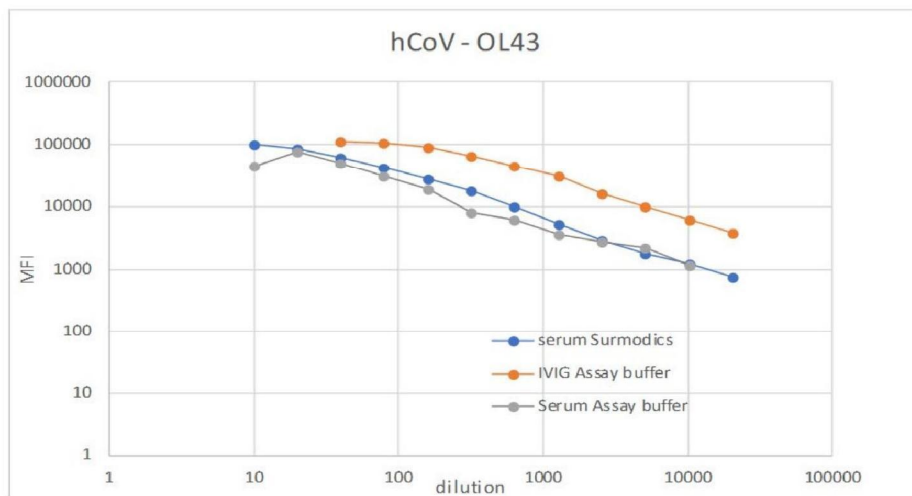
Dit experiment nogmaals herhalen met andere verdunningen en in alles in surmodics.

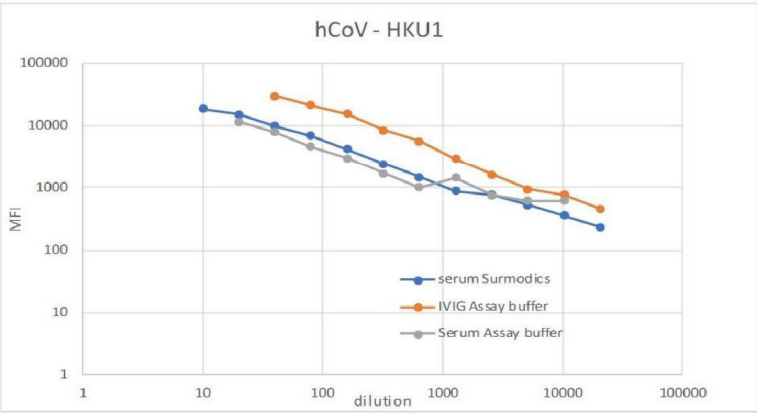
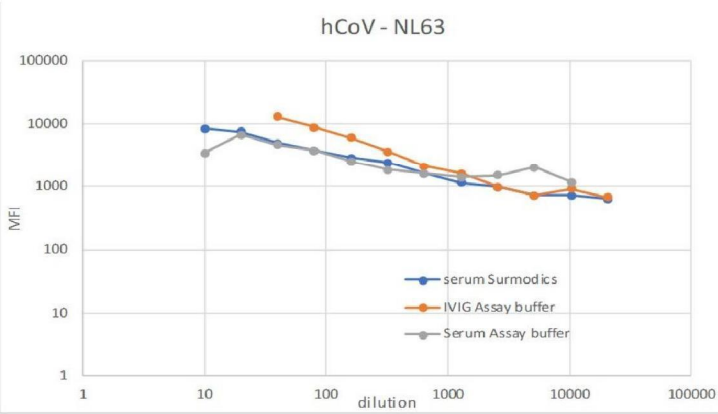
Tevens enkele 0-24m samples testen van uit pienter 3 (daarin verwacht je enkele negatieve of monospecifieke samples) op latere leeftijd is nagenoeg iedereen positief voor alle seizoen corona's

New Section 1

The H-H-H-H-H motif is used as a tag on many recombinant proteins to facilitate purification. The antibody recognizes the His-tag fused to the amino- or carboxy- termini of targeted proteins in transfected or transformed cells

Dus die zou moeten werken volgens mij.





25-3-2020

Conjugation of SARS-CoV-2 proteins

Sars-Cov-2 Spike RBD- histag to bead #23

Sars-CoV-2 Nucleocapsid to bead #75

Sars-CoV-2 Spike protein S1 subunit to bead #73

Spike S1 = 100 μ g in 50 μ l PBS -> add 450 μ l PBS = 500 μ l @ 0.2mg/ml

Nucleocapsid = 100 μ g lyo. (20mM TRIS, 500mM NACL, 10% glycerol pH 8.0, 5% trehalose, 5% mannitol, 0.01% tw80) Dissolved in 500 μ l PBS (0.2mg/ml)

RBD = 100 μ g lyo (from 20 mMPB, 300mM NACL Ph7.0, 10% glycerol, 5% mannitol 5% trehalose, 0.01%tween)

Coniucation

- A) 300 μ l Spike S1 in (500 μ l) PBS ph7.4 to 500 μ l activated beads (60 μ g/ 500ul beads)
- B) 100 μ l Nucleocapsid in 200ul PBS to 200 μ l beads Direct (20ug/200ul beads)
- C) 300yl Nucleocapsid dialysed against PBS (1h RT) and conjugated to 500ul beads (ca 50ug/500ul in PBS)
- D) 300 μ l RBD (in 500ul PBS) to 500ul activated beads (60 μ g/500ul)

Incubation 3h RT

26-3-2020

Run 6 plex panel GJG (5x pos 3x neg) 20/200/600/1800/5400

32 samples P2 0-24m 200 / 4000x

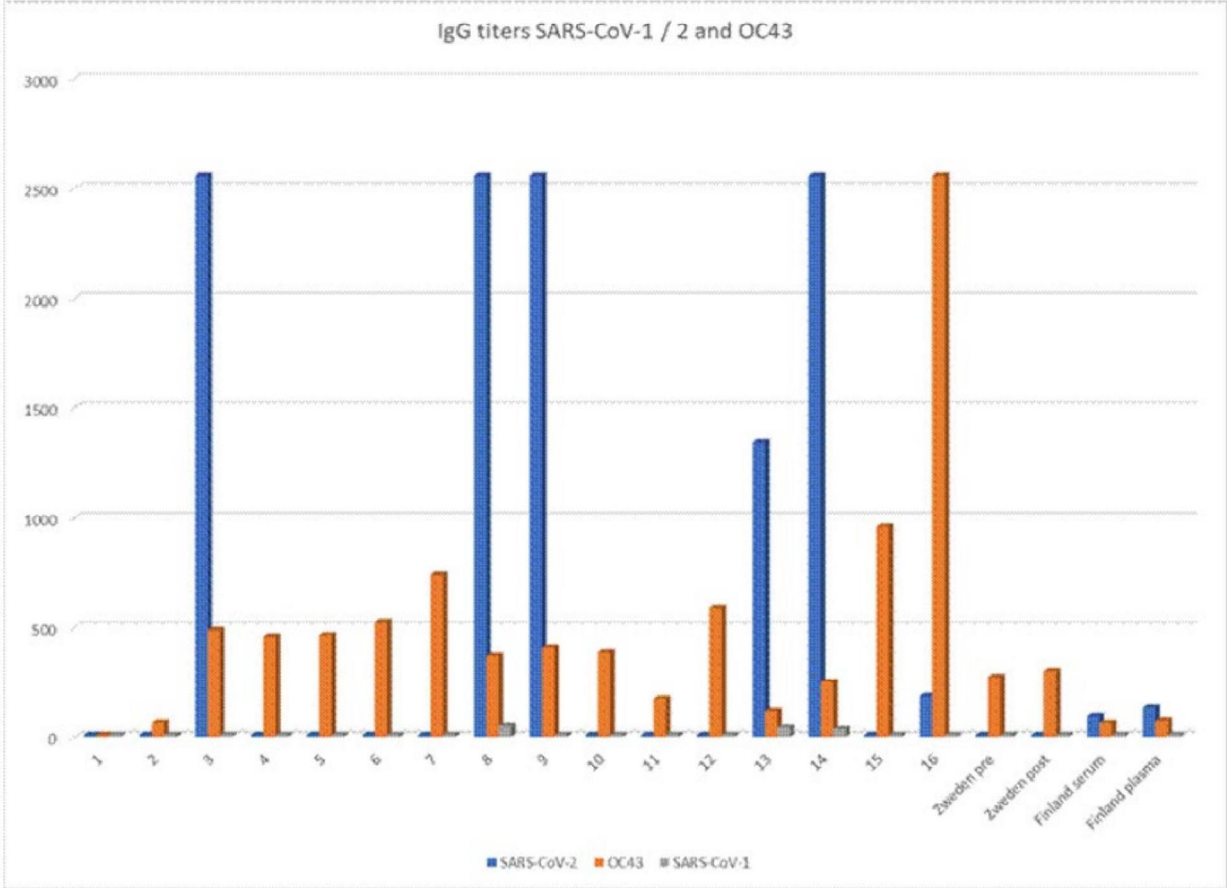
Ref IVIG en Mix serum S1 tm s12 S1 = 50x 3staps serail dilution

Multiplex setup/

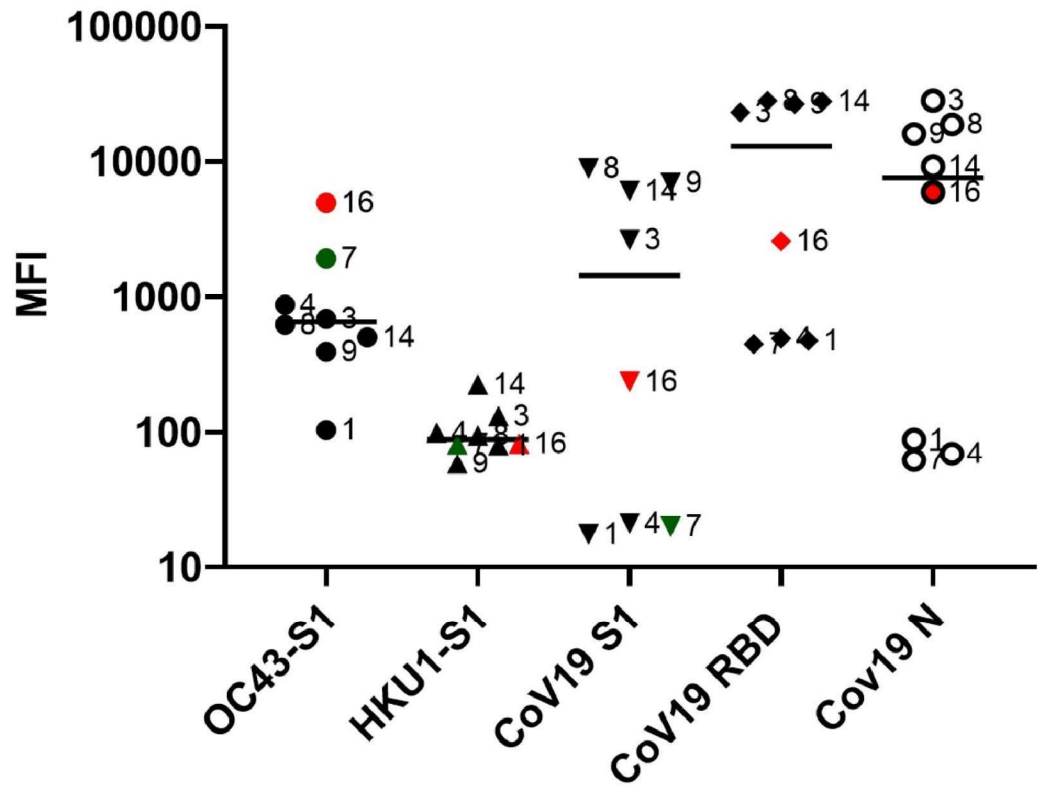
Core N non dialysted test IVIG compare.

Protein array uitslag

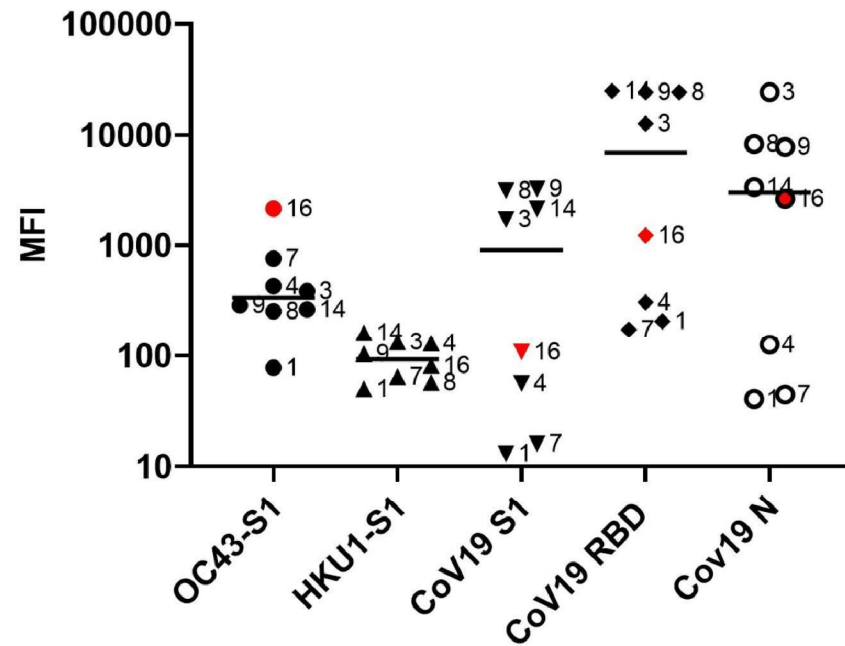
- 1: limsnr 1
- 2: limsnr 3
- 3: limsnr 7
- 4: limsnr 8
- 5: limsnr 9
- 6: limsnr 14
- 7: limsnr 16
- 8: limsnr 4



hCoV MIA vs PA
1/1800

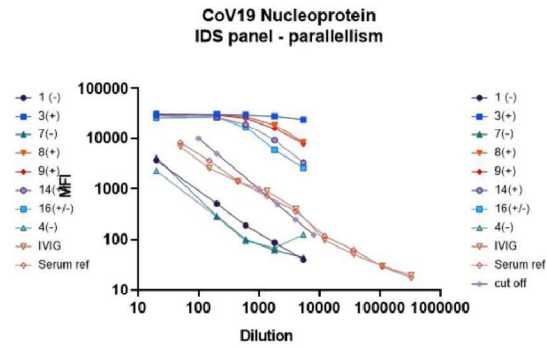
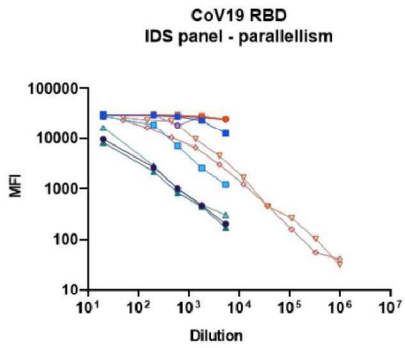
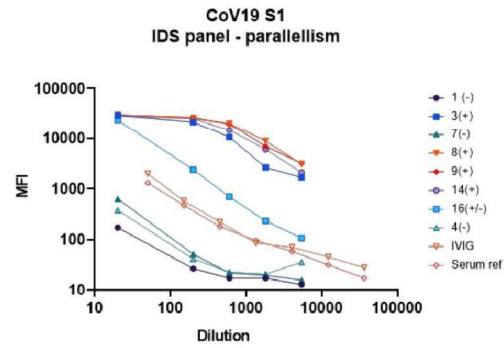
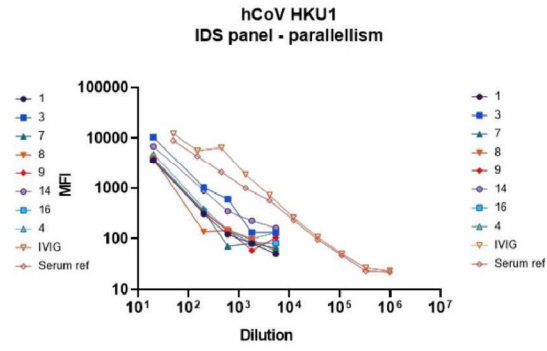
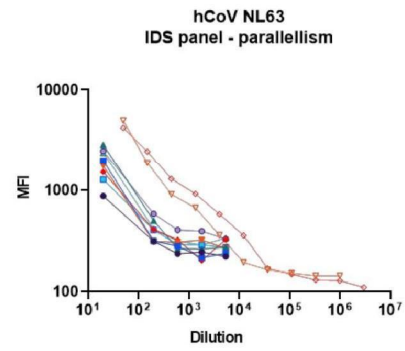
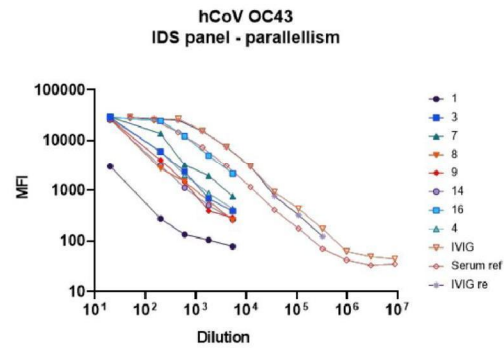


hCoV MIA vs PA 1/5400



Nummer 16 is heel hoog OC43 positief, "laag positief voor S1 maar hoog positief voor cov19 N.
In de array weinig signaal tegen S1

Dit samples is PCR positief getest.

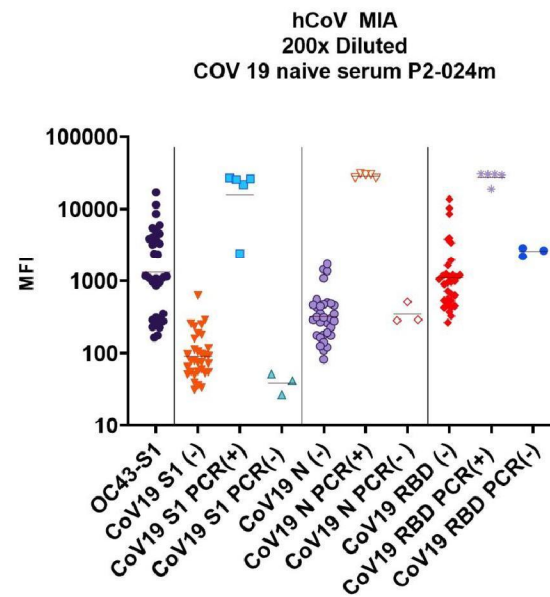
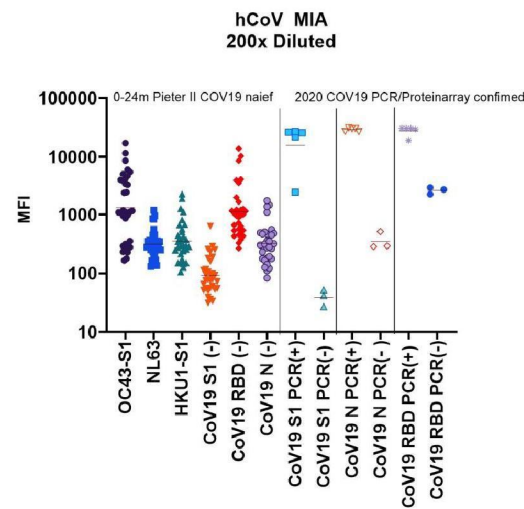


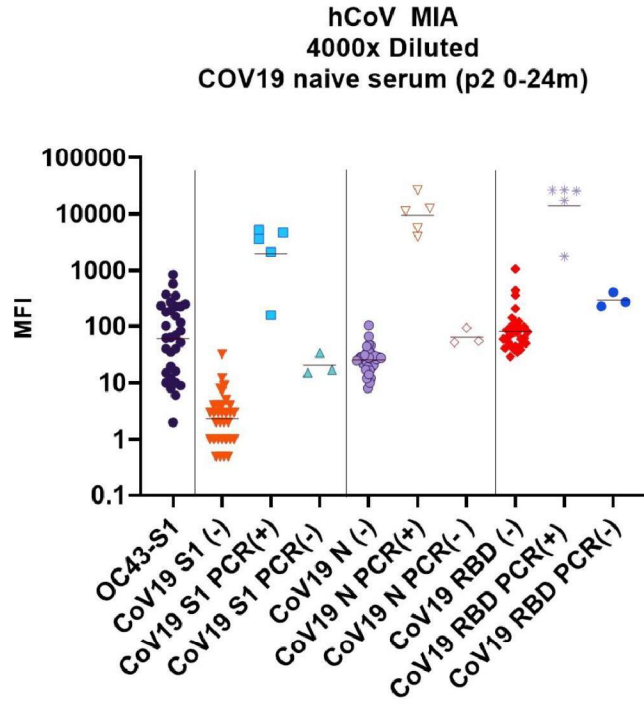
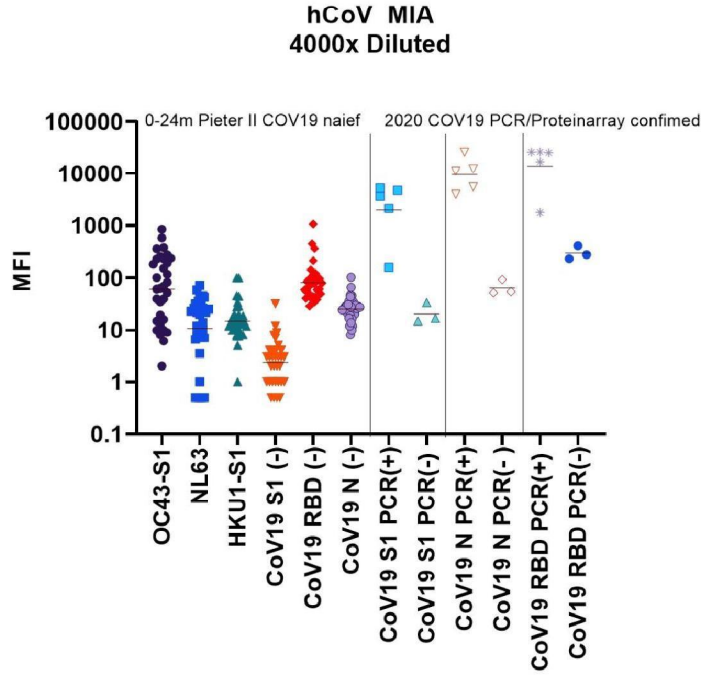
Samples lopen mooi parallel, IVIG en serum pool als referentie meegenomen, mooi signaal tegen OC43, HKU1 en met name NL63 presteren matig, de laagste kruis activiteit is tegen COV19 S1 (IVI/ref is goede indicatie voor te verwachten maximale achtergrond signaal per verdunning) . N en vooral RBD hebben meekruisreactie, maar met name specifieke antistoffen tegen COV19 lijken zeer hoog, waardoor toch goed onderscheid tussen COV19 + en COV19 - samples mogelijk lijkt.

26 maart

Test 32 samples P2 blok 1 0-24m

200 en 4000x in surmodics





Laagste achtergrond met S1 hoogste met RBD

N zit er tussen in, maar er is een beter onderscheid mogelijk tussen de PCR negatieven en de PCR + samples

Metname 1 sample (#16) is heel zwak positief in de prote array (ook laagste in de S1 MIA) maar hoog positief op basis van N.

30-3-2020

Metten jij panel van OC43/NL63/e229/ HKU1 positieve jij samples

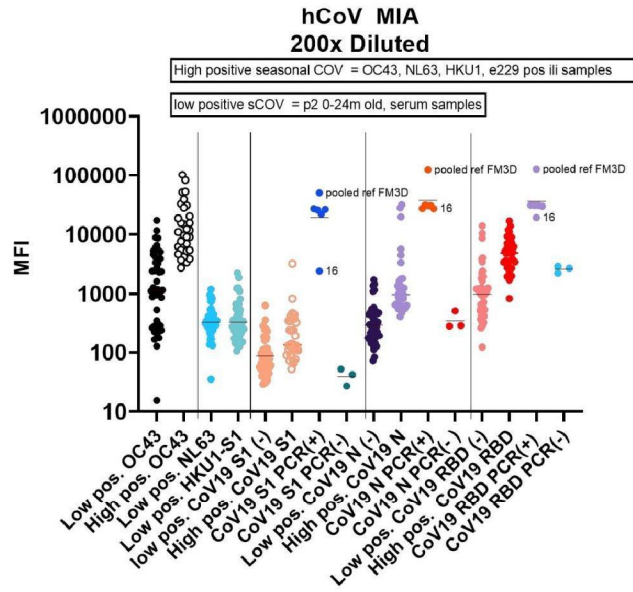
Tevens nog 2 rijtjes p2 0-24 m (rij 5+6 blok1) en 3 rijtjes p3 blok 62 (rij 1,2,3)

Als ref 1/200 gepooled serum gebruikt (de 5 x Pos samples van IDS)

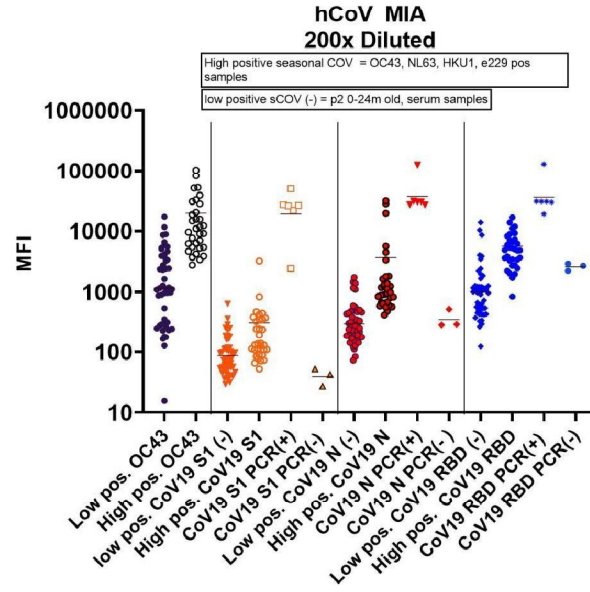
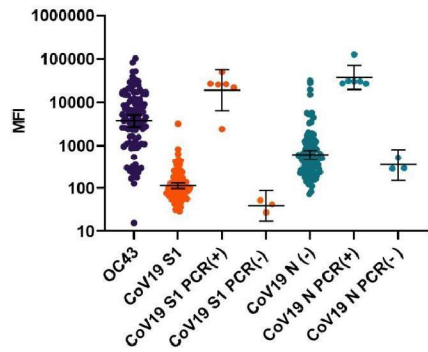
Potency gesteld als MFI ULOL + verdunning / 10e6

NL63 = 663*600/10e5	5 Au/ml
HKU1 = 1000*500/10e5	6 AU/ml
OC43 = 9400 * 600/ 10e5	60 AU/ml
S1 = 14500*1800 / 10e5	260 Au/ml
RBD = 90000*1800/10e5	1620 AU/ml
N= 75000*1800 /10e5	1350 AU/ml

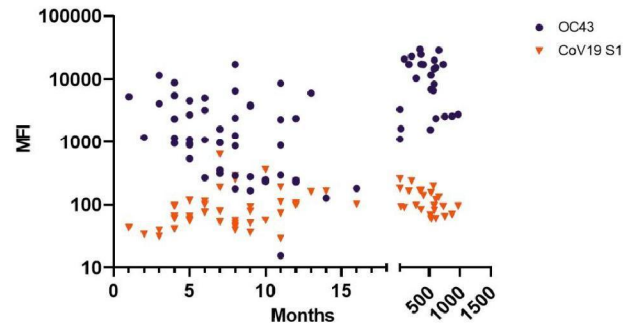
S1 = 200 x

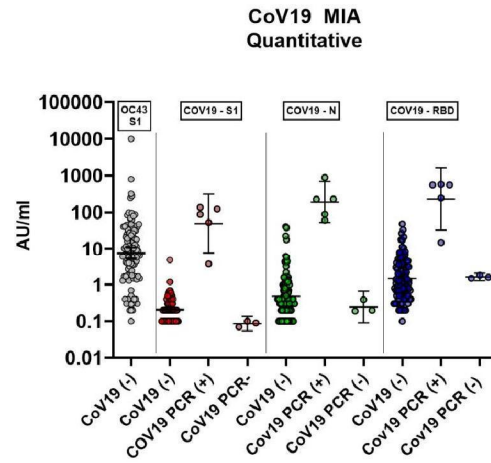
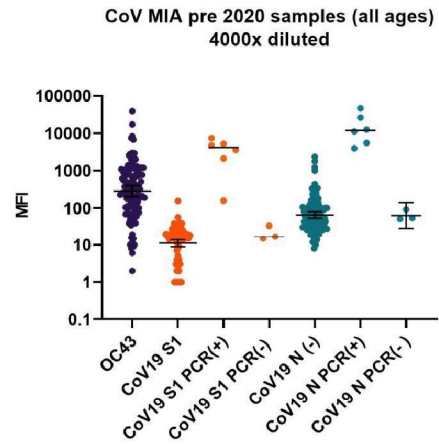
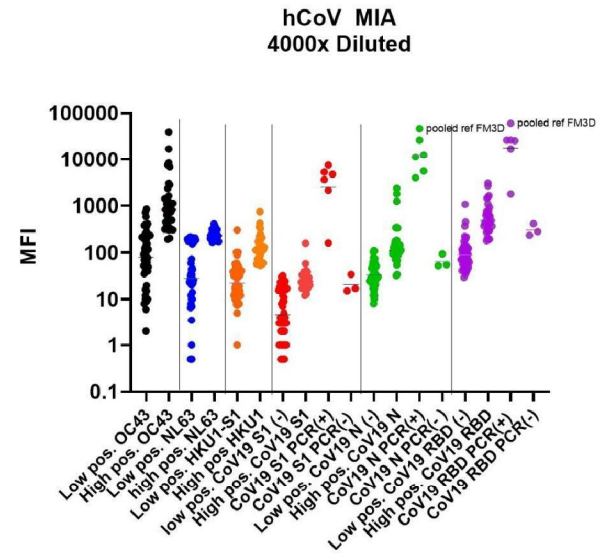
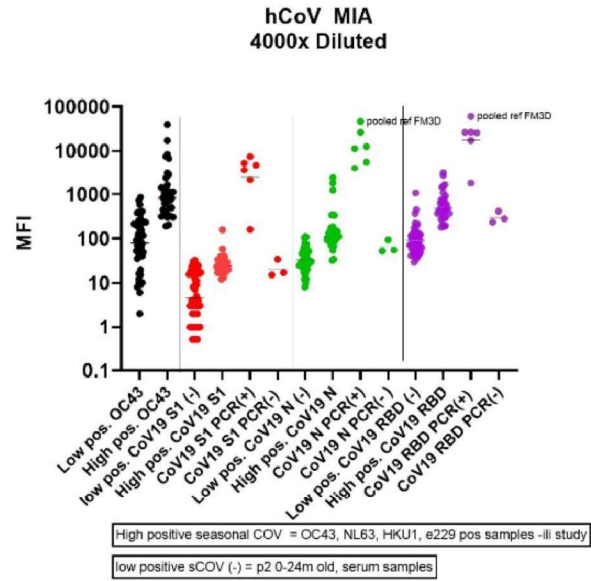


CoV MIA pre 2020 samples (all ages)
200x diluted



hCoV OC43 -S1 vs COV19 S1
age 200x diluted





1-4-2020 15:28

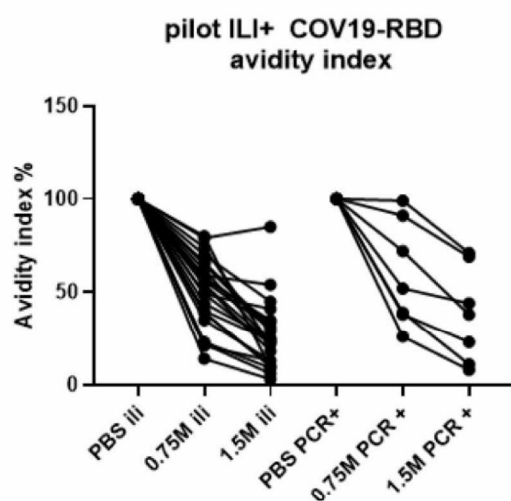
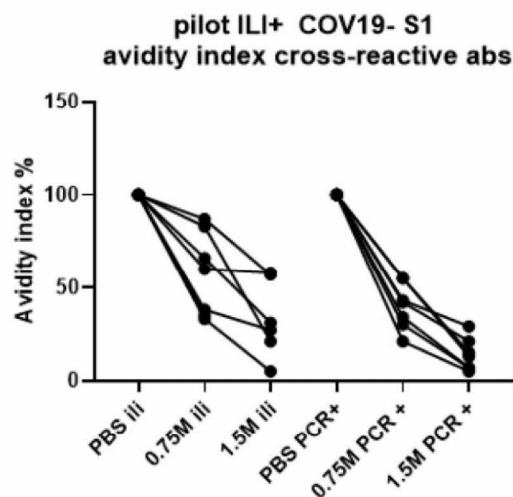
Pilot avidity

5 pcr + samples

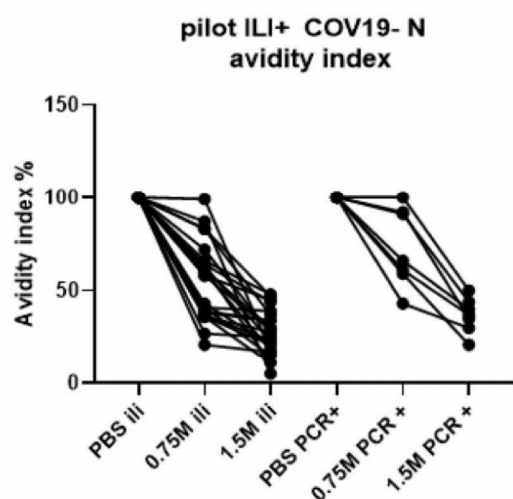
Ig en pooled ref

+ 0.75M Ammonium Thiocyanate

And + 1.0 M NH_4CN

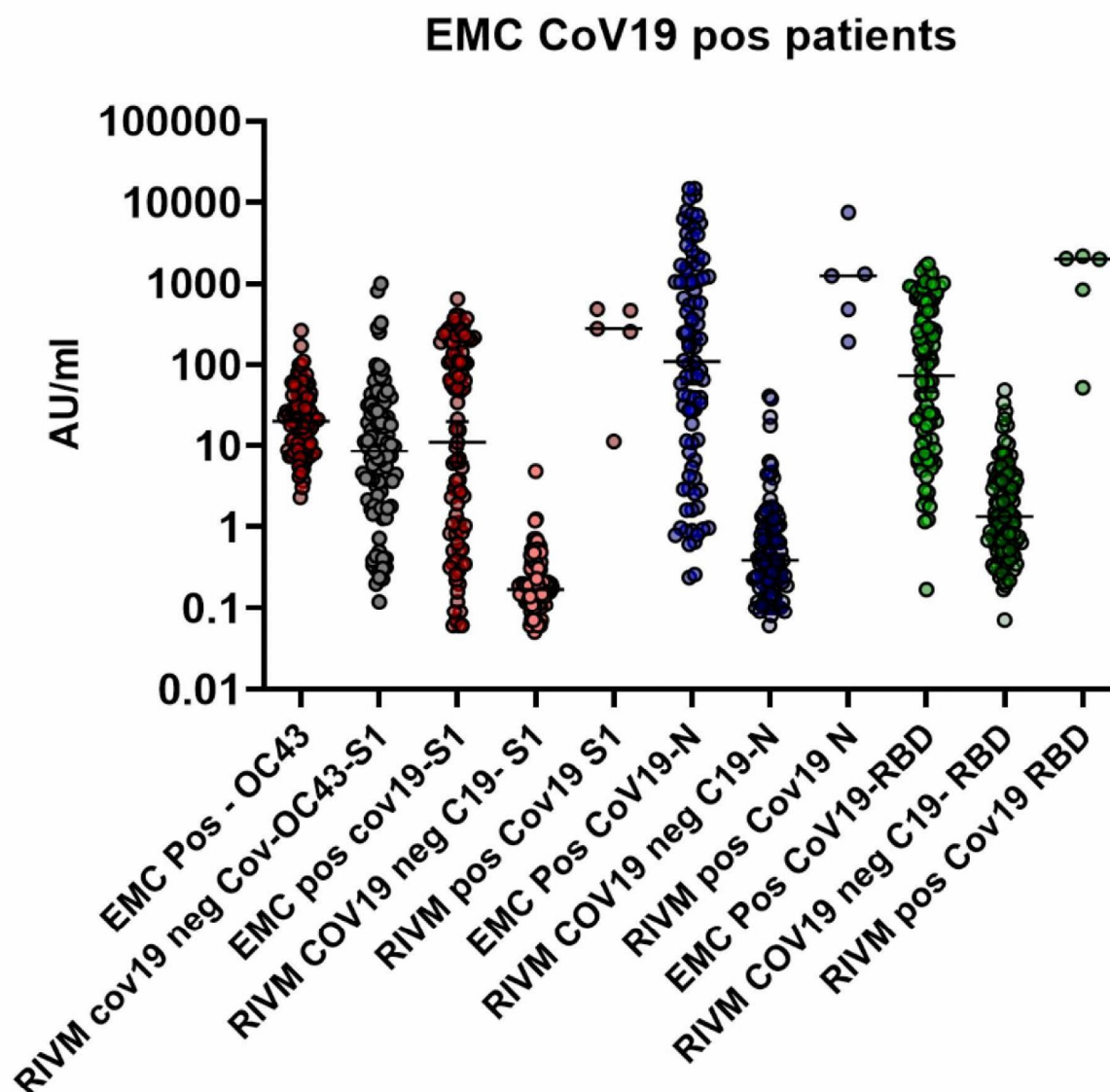


Avidity van die paar positieven is opvallend laag (mogelijk neemt dit toe in de tijd en na meerdere infecties)



2-april 2020
Meten 80 samples EMC Sars patients
200 en 4000x

3 april Redo + 13 laatste Sars patienten
+ IDS panel



6-7 april 2020

Conjugations :

S1 sino 2x 700 ul beads 50 ul per 700ul beads. (#73)

S1 UvU 15ug to 200 ul beads (#40)

OC43 (IDS) 50ug per 700 ul beads (#29)

All in PBS (no dialysis)

80 samples vh cross reactivity panel EMC uitgevuld (20x voor verdund)

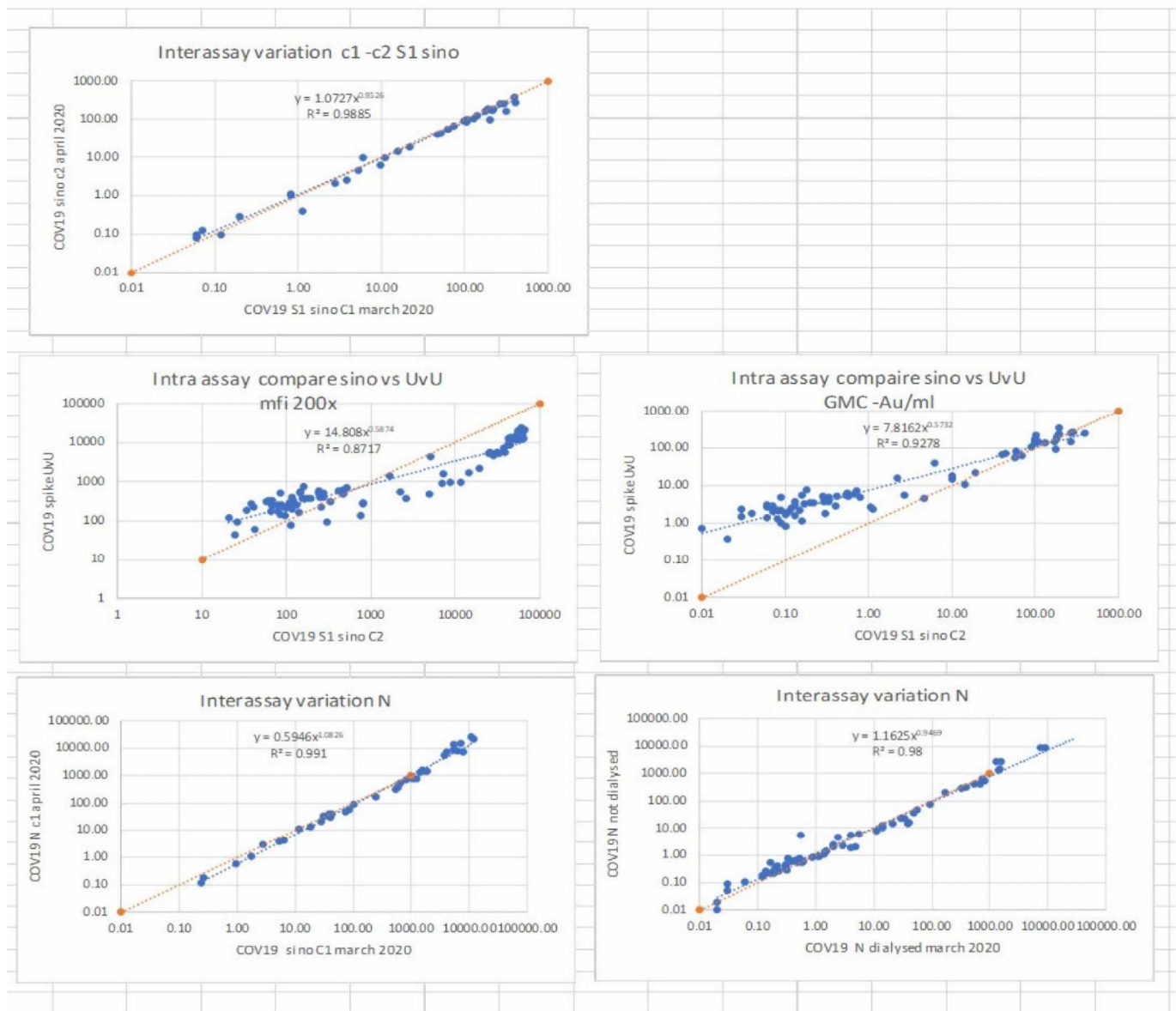
Test and compare

40 samples cross reactive panel 200 and 4000 x dilution (row 2, 4, 8,9,10)

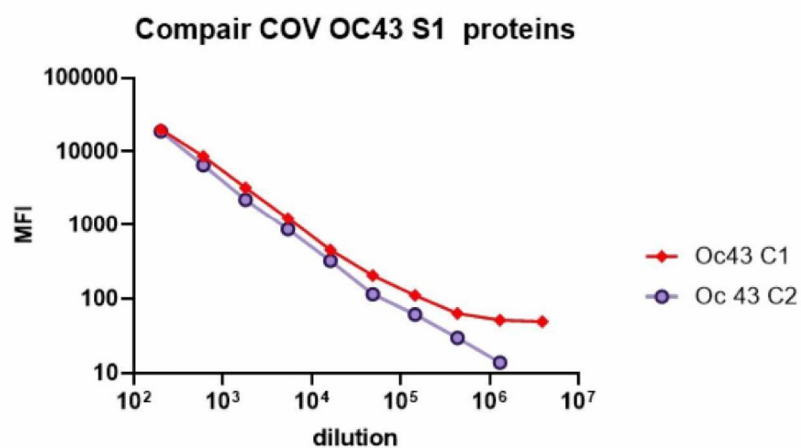
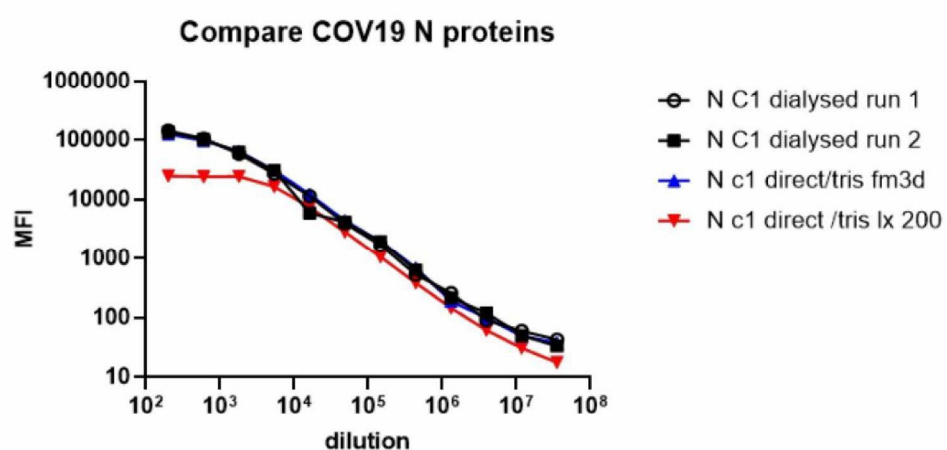
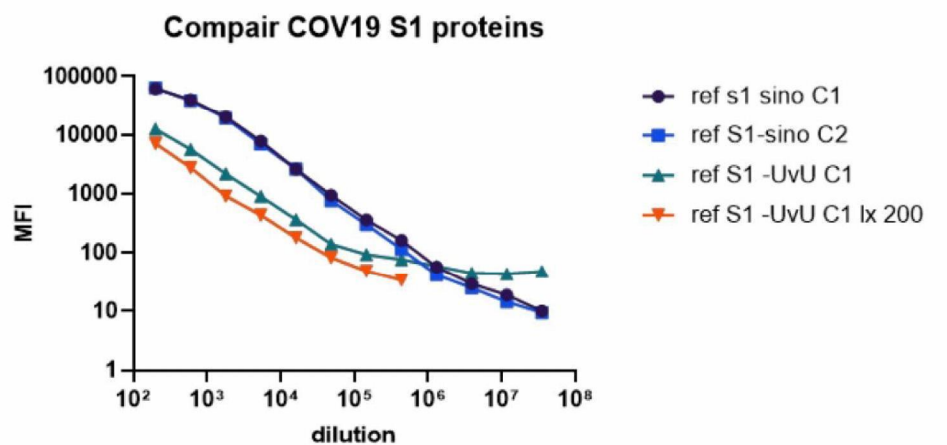
40 samples Sars panel 200x and 4000x dilution (row 1 to 5)

Oc43- c2/ N-c1/S1-c2/RBD-c1 vs S1-uvu-C1/ N-c1 dialysed

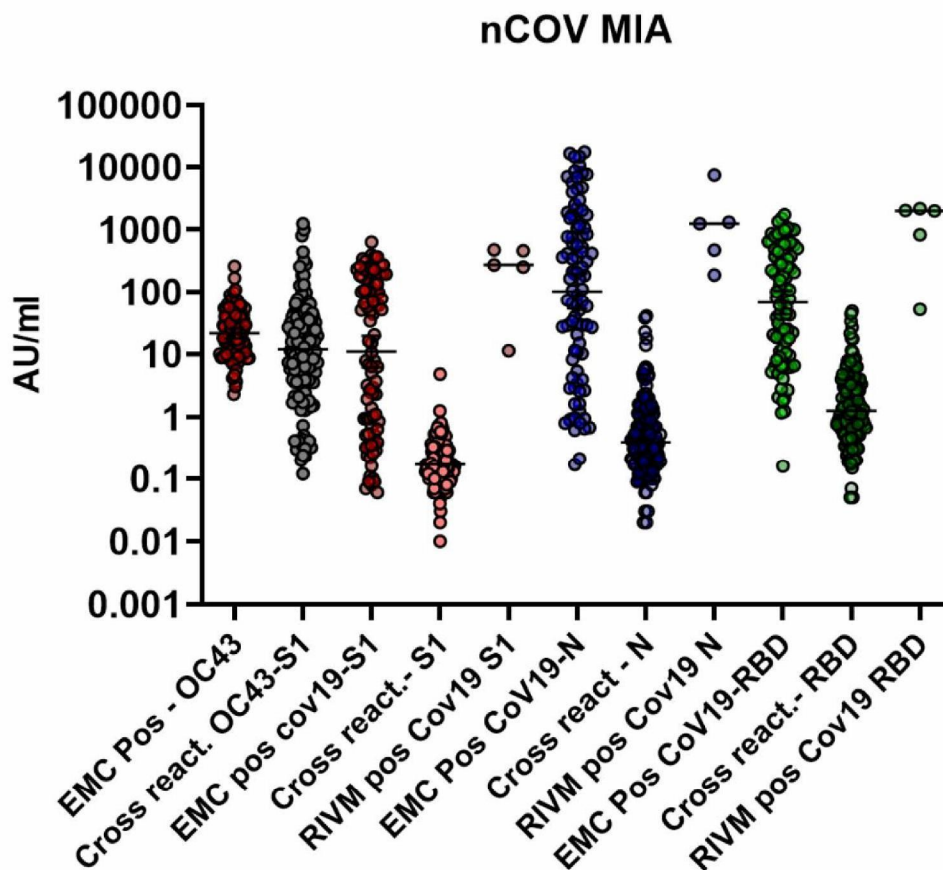
Ref is pooled sera (5) 1/200 3 fold serial dilution.



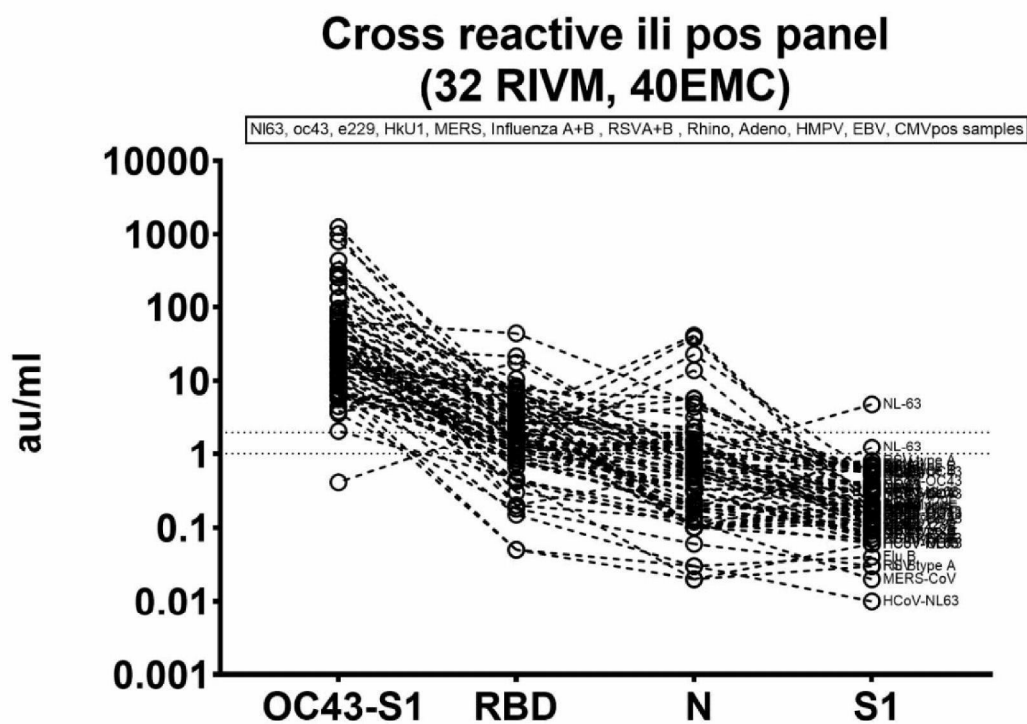
Nieuwe koppelingen zijn er goed ondanks wat minder eiwit, voor N maakt dialyseren niet veel uit, daarom volgende keer verdunnen in PBS (0.1mg/ml) = 100ug + 1ml pbs en 500 ul koppelen aan 700 ul activated beads



Tevens extra samples gemeten van het EMC cross reactive panel (40) en toegevoegd aan bestaande data



Alleen ILI+ samples RIVM + EMC.



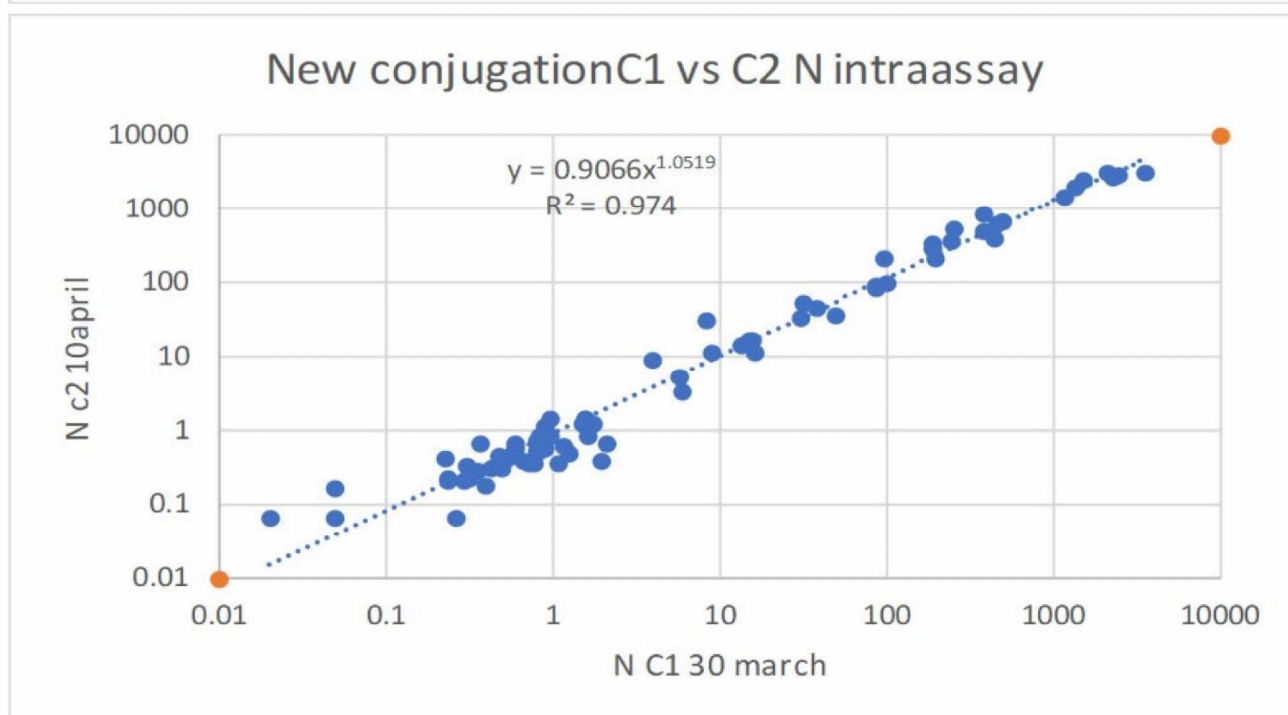
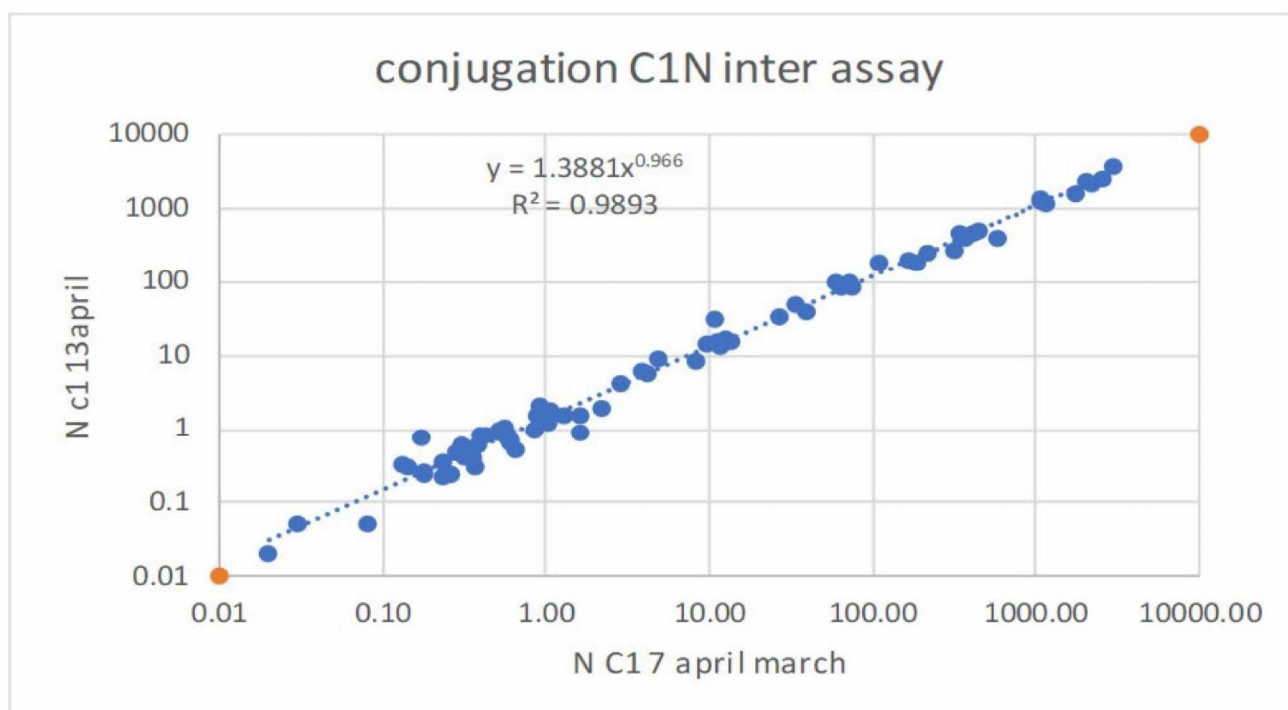
10-4-2020

Conjugation OC43 (IDS) and N (SINO)

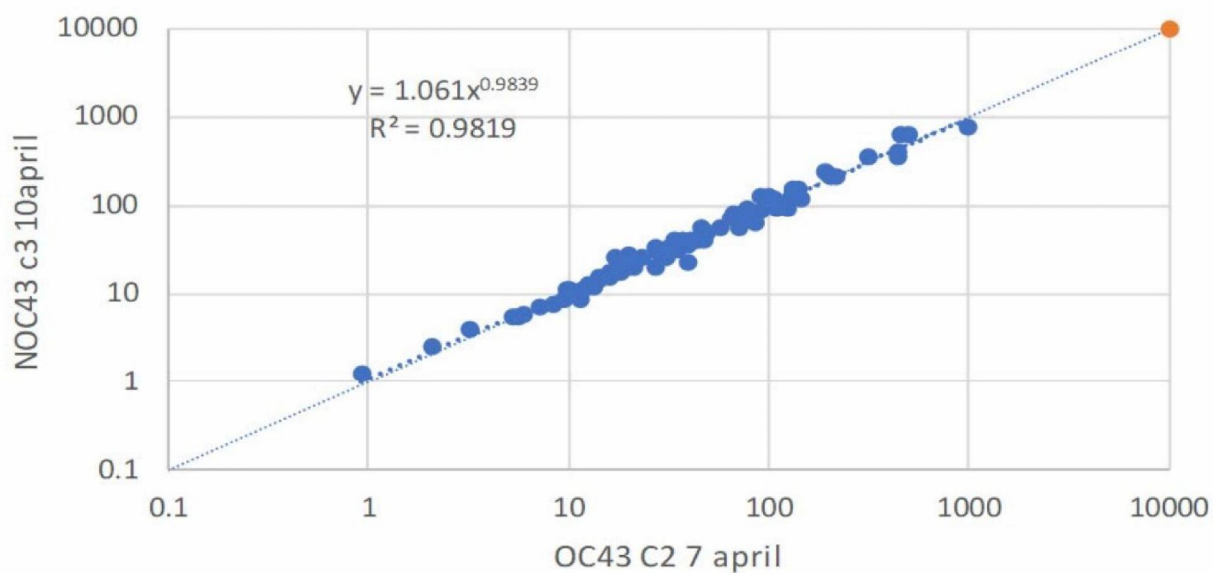
OC43 ca 40 ug to 500ul activated beads (40 ul in 500 ul PBS) activation in 50 mM MES pH5.5 and conjugation in PBS (#29)
 N 100 ug dissolved in 1 ml PBS ph 7.4 conjugation to 2x 700 ul beads (#75)

12 april

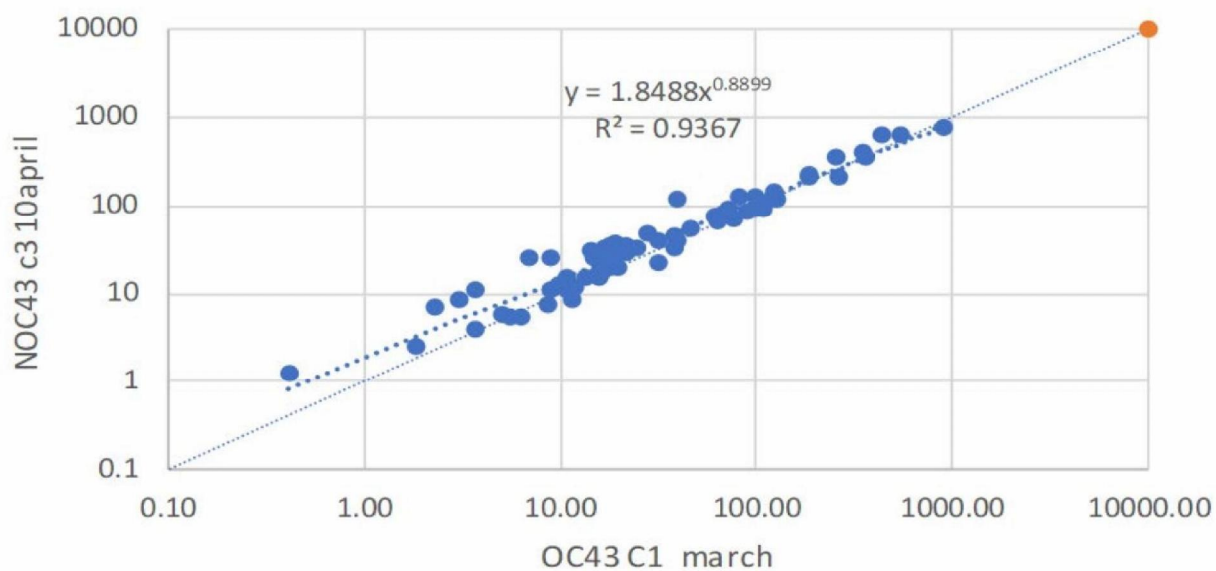
Validation Conjugation c3 oc43 and c2 N (not dialysed)



New conjugation OC 43 intraassay



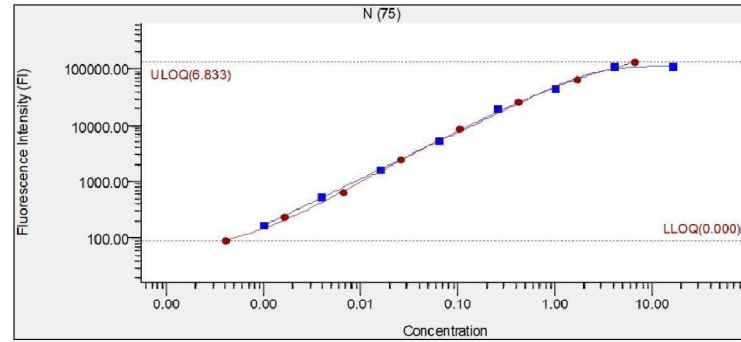
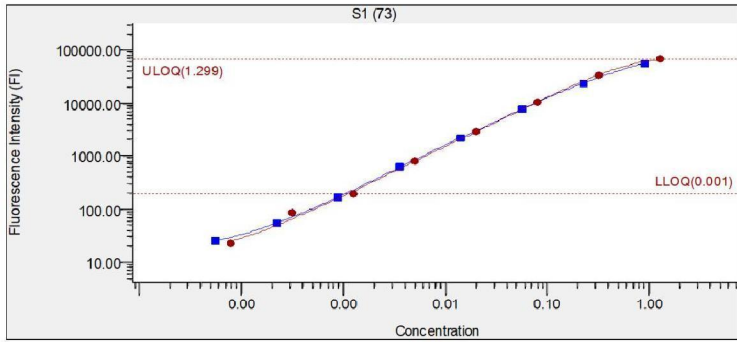
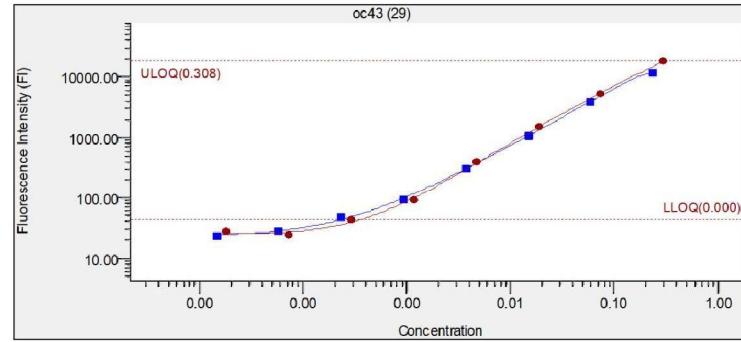
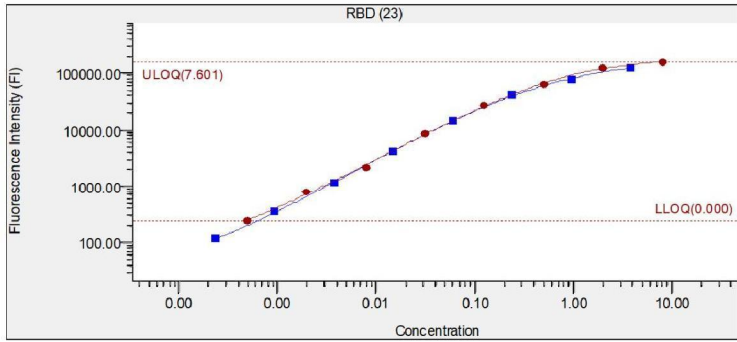
New conjugation OC 43 interassay

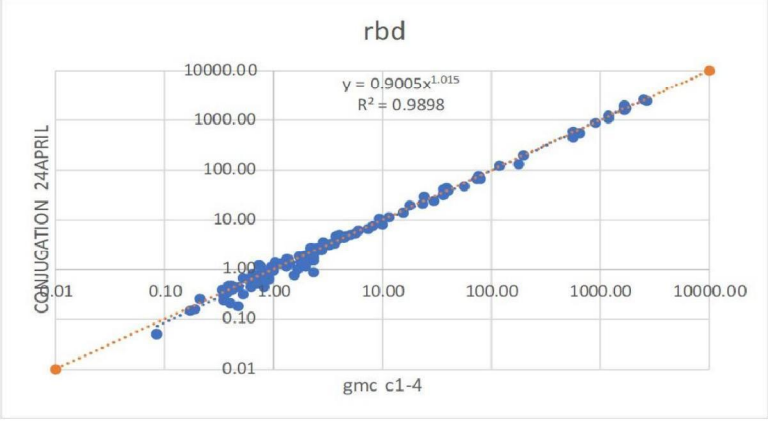
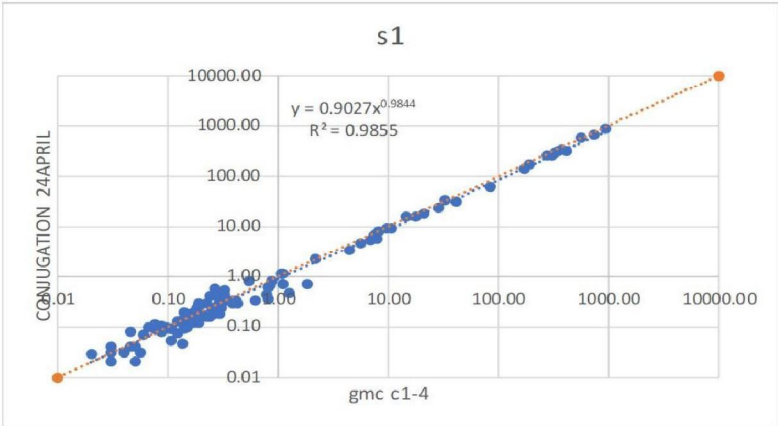
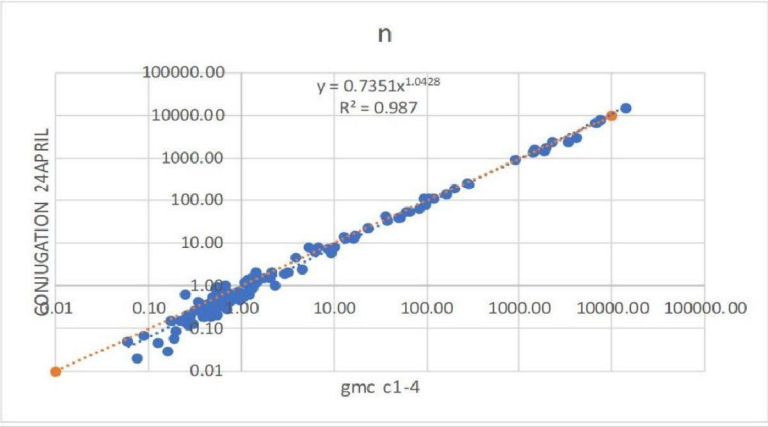
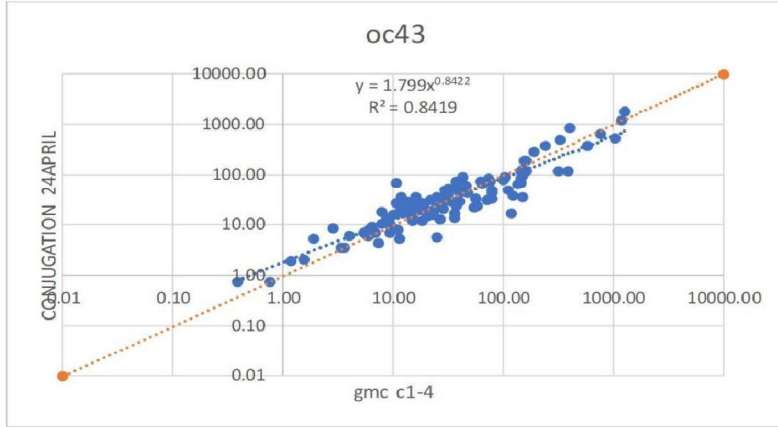


12:52 23-4-2020 12:52

Testen parralellism . 3x ffx samples 5x Stoof samples ref oud en ref nieuw , refs onafhankelijke reeks met marjan B

	oc43 (29)	S1 (73)	N (75)	RBD (23)					
Pienter C biopl	45.60	180.80	2958.00						
pienter C r	48.80	176.20	4000.00			bij n de 4000 waarde niet meegenomen\			
Ref in ijk MB	46.11	183.58	3337.68	770.12					
Ref in ijk RS	49.40	191.25	3358.67	775.01					
gmc	47.45	182.88	3212.70	772.56		0.24	0.91	16.06	3.86
	46	182	3200	775		0.23	0.9	16	3.9
1e ref terug berekend									
potency gesteld	60	260	1360.00	1650					
MB	62.12	239.91	1275.24	1504.59					
RS	60.62	276.71	1378.21	1647.61					



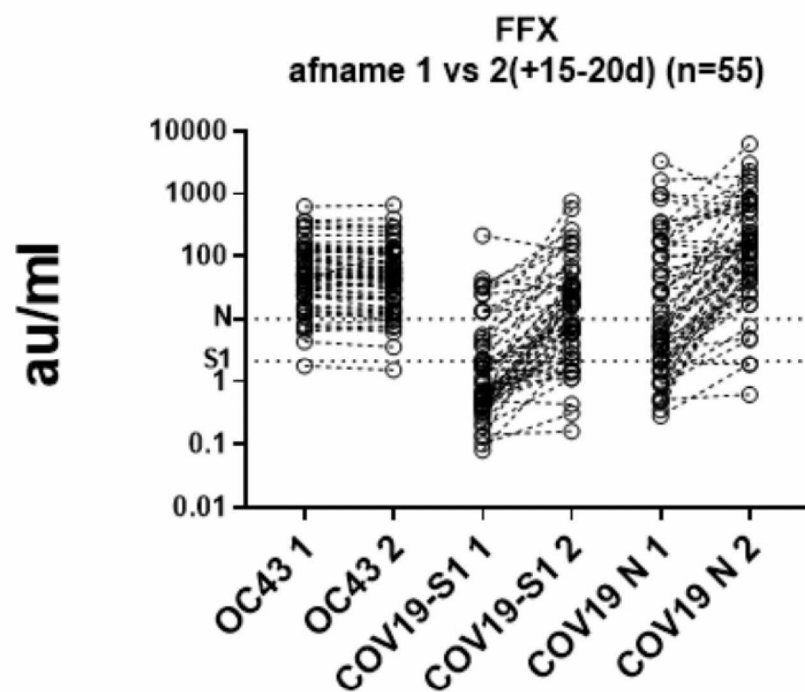


30-4-2020

Testen FFX 2 blokken (100 samples)

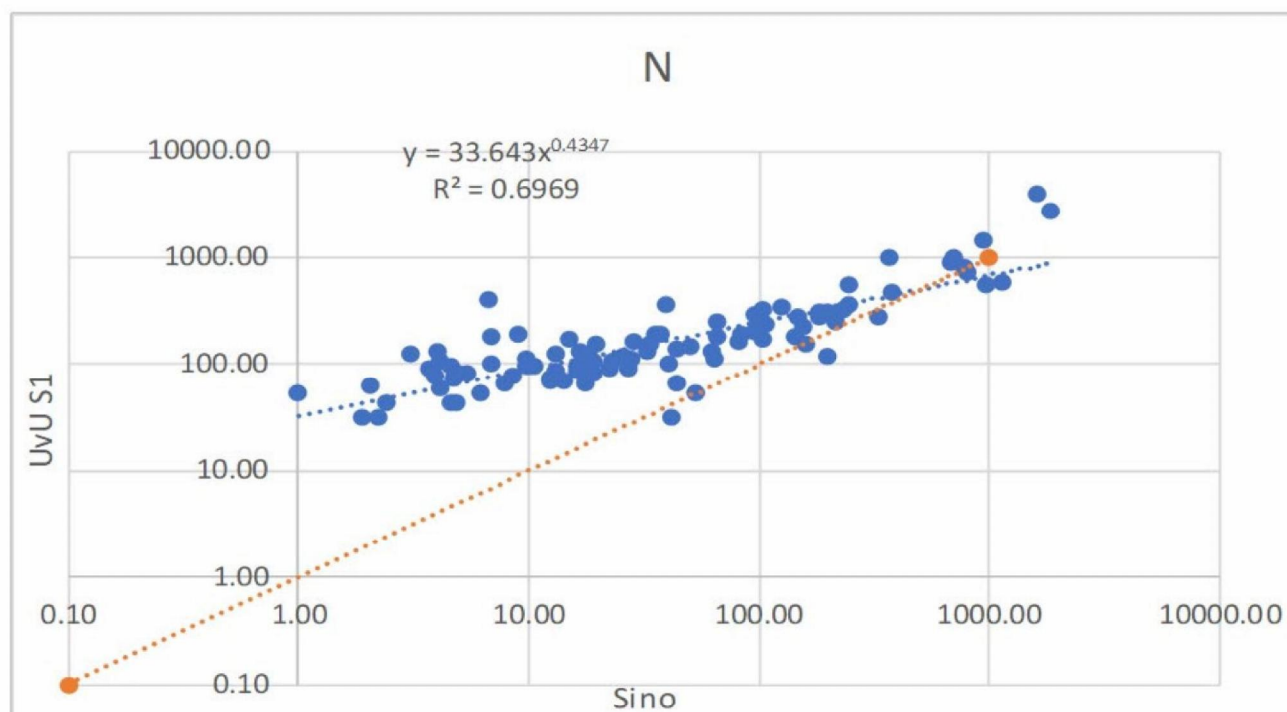
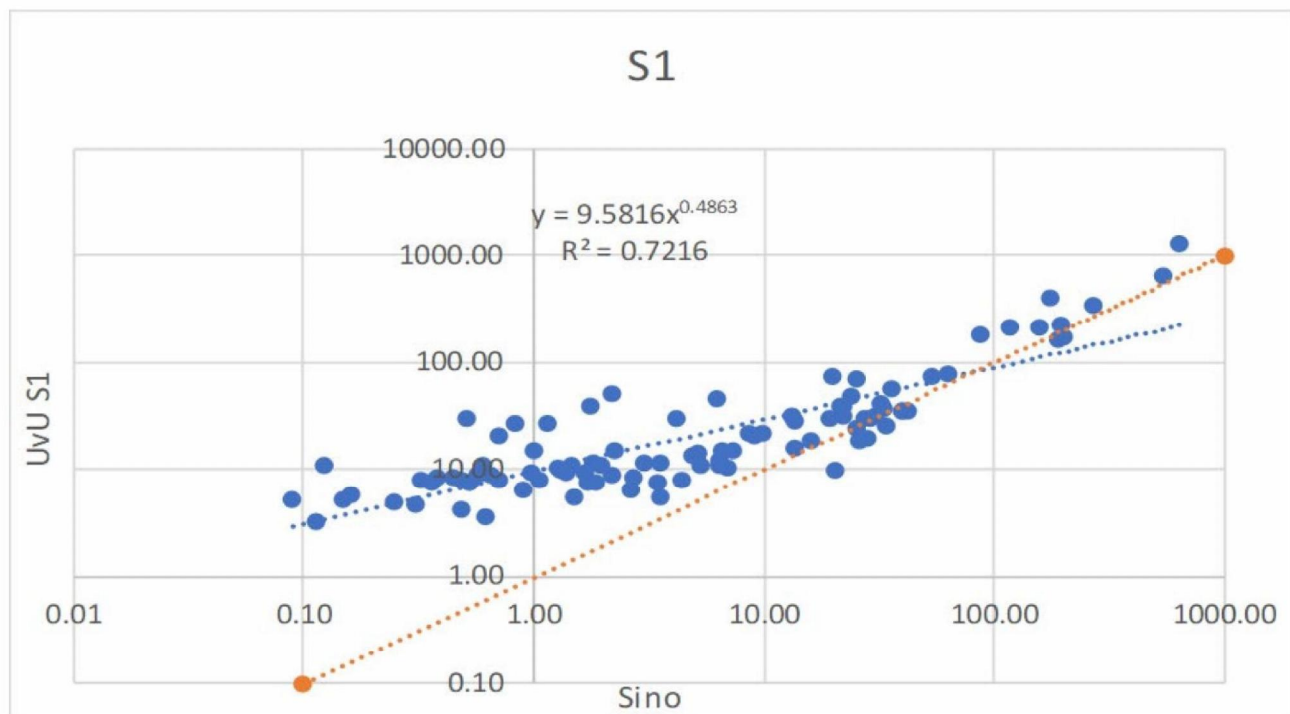
400 en 10.000x

Met nieuwe ref en beads



2mei : koppelen UvU en Sino S1/S2

4-5-2020 ffx panel blok 1 en 2 400 en 1000x v vergelijk beads S1/RBD/N sino met UvU S1 + RBD en SINO S1+S2



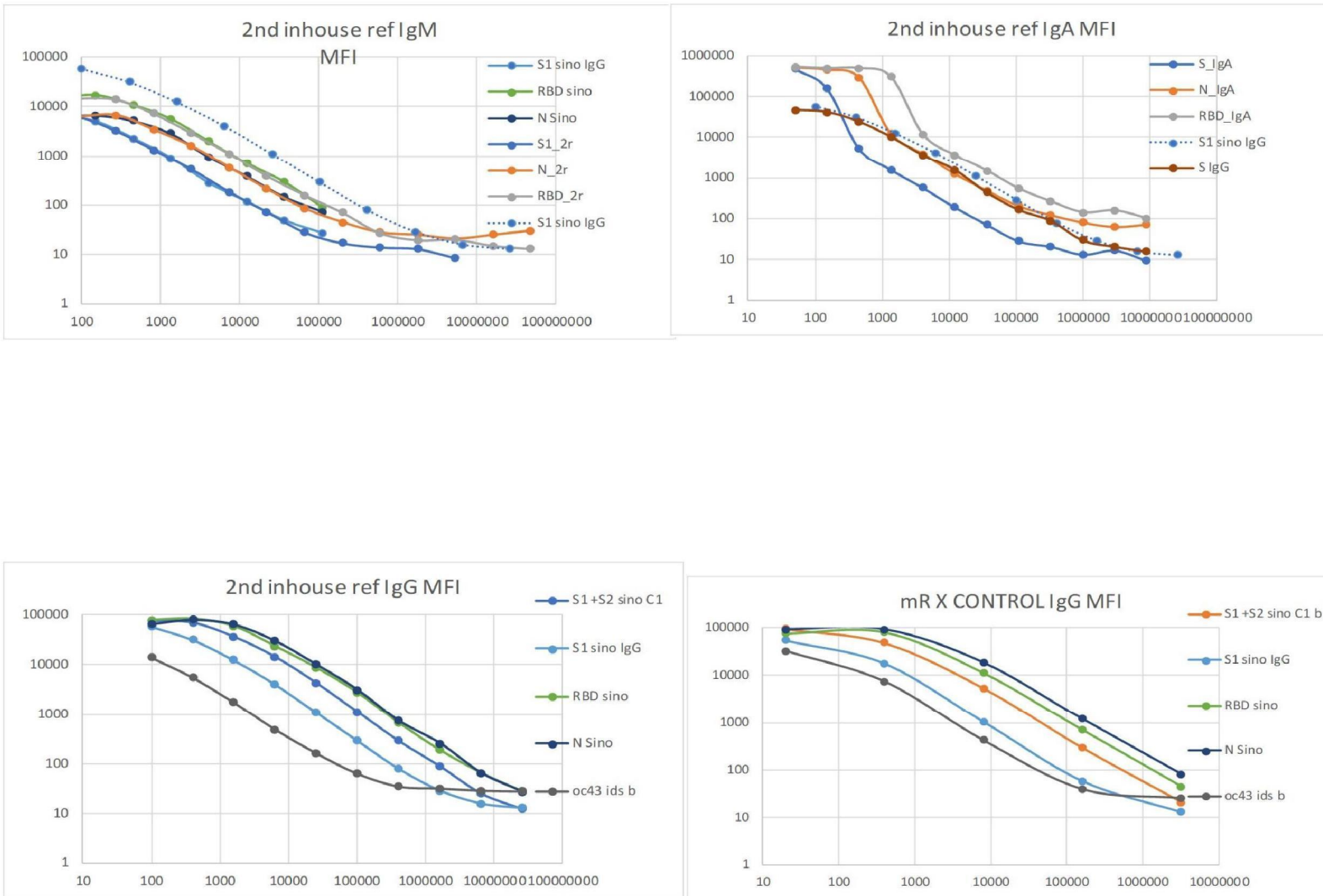
25mei

Oxford panel selectie 51 samples.

Meten IgM 1/400 , conjugaat 1/1000

En IgA 1/400 conjugaat 1/400

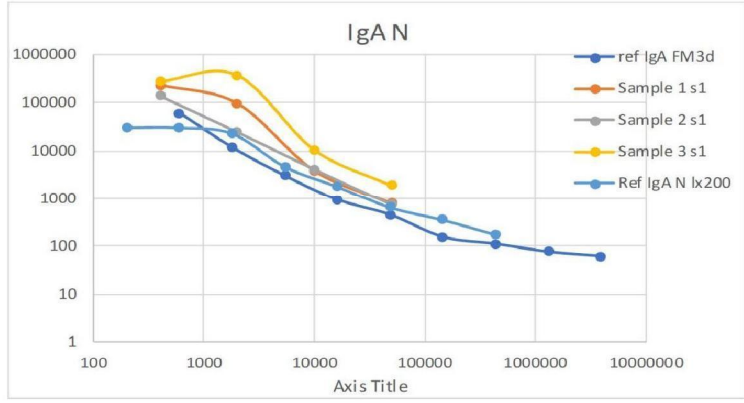
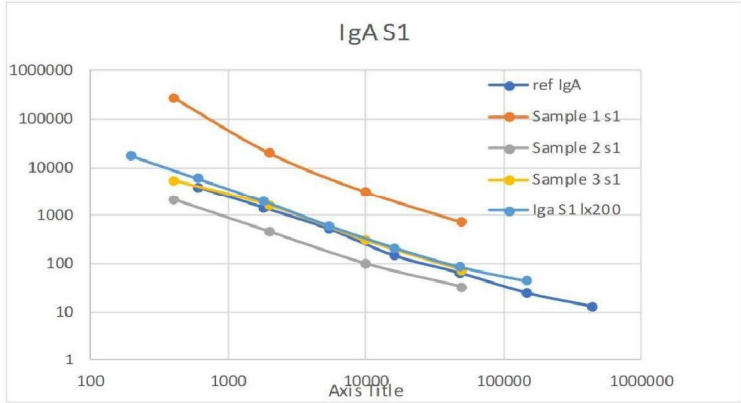
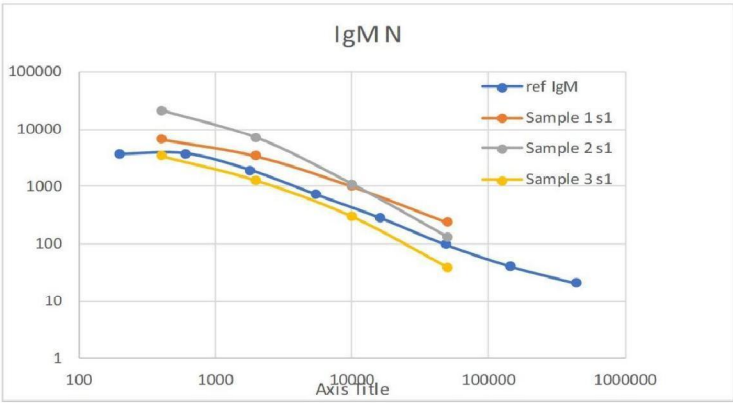
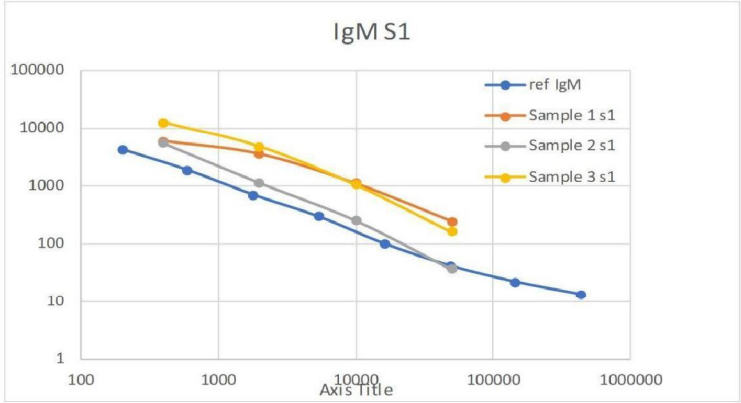
Redelijk veel signaal in ref (s1 1/50) en ook in samples



25-28 mei

Optimaliseren IgA en IgM, tevens kort reeksje van paar hoge samples

Met name voor IgA op het N eiwit is veel aspecifiek signaal curves lopen ook niet mooi lineair en parrallel
Voor S1 minder probleem. Ook moeten de samples genoeg worden verdund op ongewenste effecten weg te verdunnen



29 mei

Refs gesteld opnieuw oxford panel meten in de juiste verdunningen met de gesteld ref
 Vervolgens deze waarden aanhouden

2nd inhouse ref CoV19	oc43 (29)	S1 (73)	N (75)	RBD bac /hek	S1+S2 (64)
IgG (27april)	46	182	3050	750 /200	750
IgM (28mei20)		15	30	60/?	
IgA(28MEI20)		30	200	500/?	
S1; 1/200 IgG	0.23	0.91	15.25	3.75	3.75
S1: 1/200 IgM		0.075	0.30	0.60	
S1/IgA 1/400 IgA		0.075	0.5	1.25	
W beads per plate	13	15	20	20	20
Control: Mr X 10000x	58	62	2260	285	184
Control: hCOV Low; 200x	48	1.2	4.7	9.3	15.0

IgG Samples 1000/ 2000x verdunnen

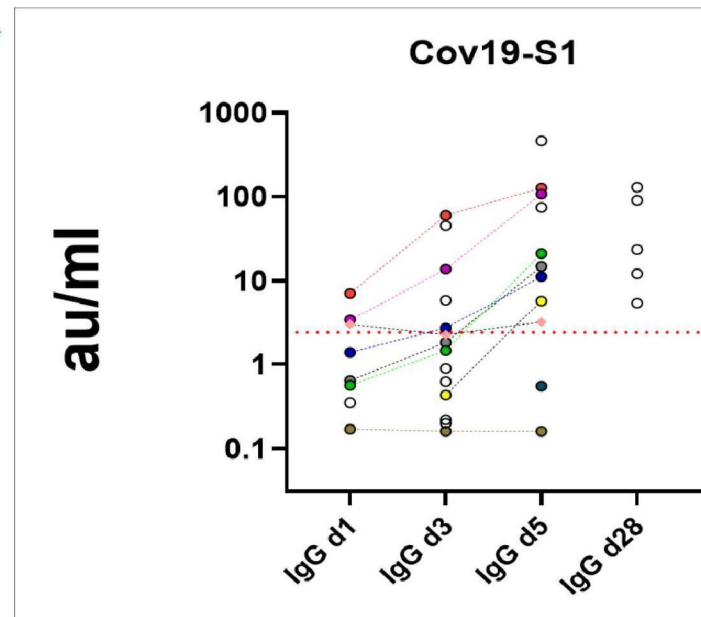
IgM 200/400 verdunnen

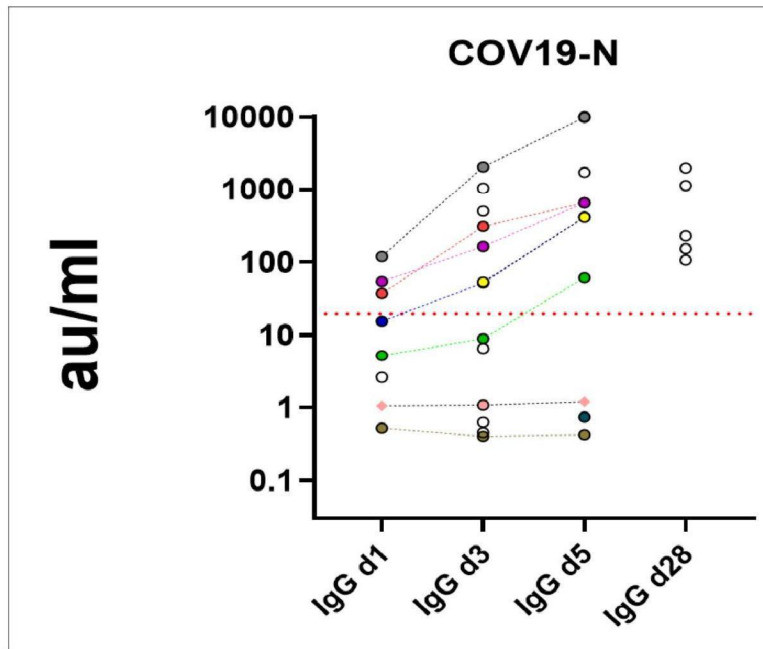
Meten op 1x 200 starten S1 bij 1/200

Conjugaat IgA 1/500

IgM 1/1000, 1/500 (maakt niet veel uit)

Meting van 24 mei herhalen met juiste waarden voor ref.!



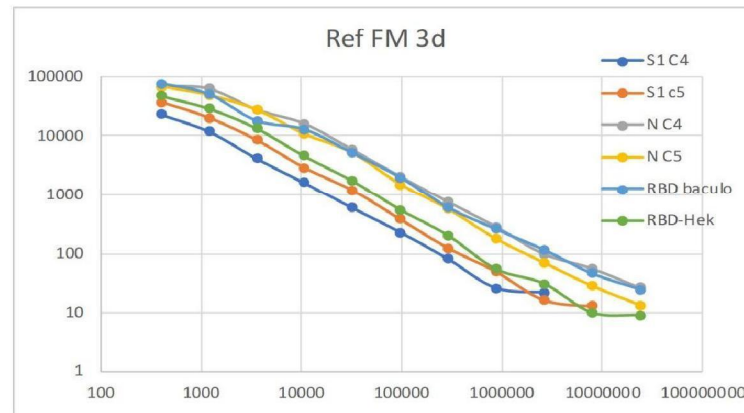
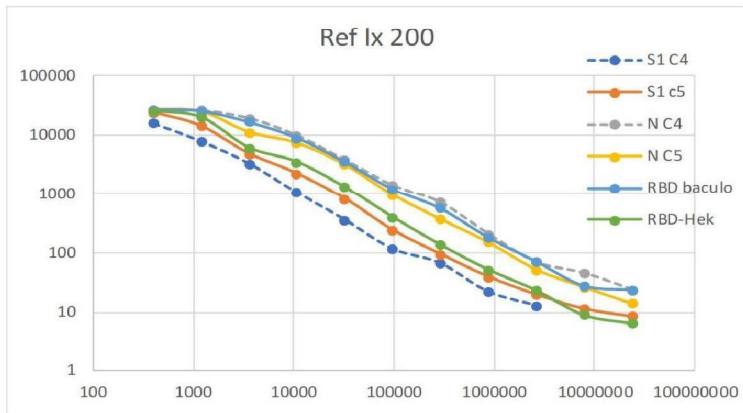


20 juni Koppeling C5 S1/RBD/N

RBD is nieuwe batch uit HEK cellen ipv baculo

S1 is in oplossing (PBS) ipv gevriesdroogt

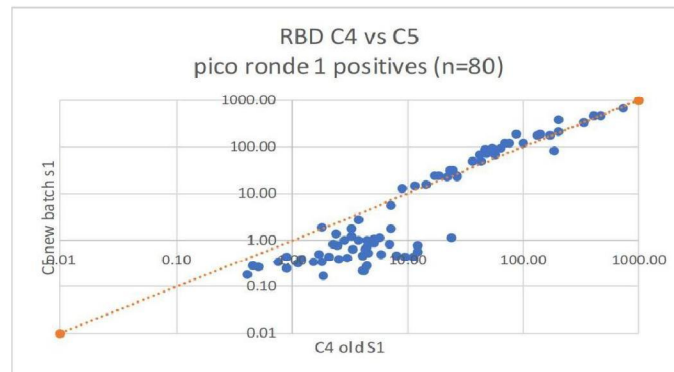
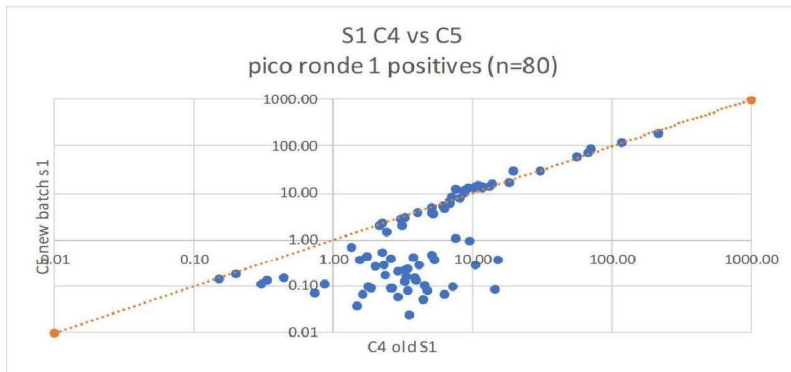
Koppeling lijkt goed op basis vd ref curves



Echter er zit duidelijk verschil in een aantal (vnl vals positieven samples)

De hoog positieven correleren erg goed echt een hoop zijn van een concentratie tussen 3-10 volledig negatief geworden

Dit S1 lijkt anders (meer specifiek) tov de eerdere gevriesdroogde S1



Ook het nieuwe RBD lijkt nu sterk op het S1

26 juni (30 juni nieuwe IgM en IgA waarden voor ref gebruikt)

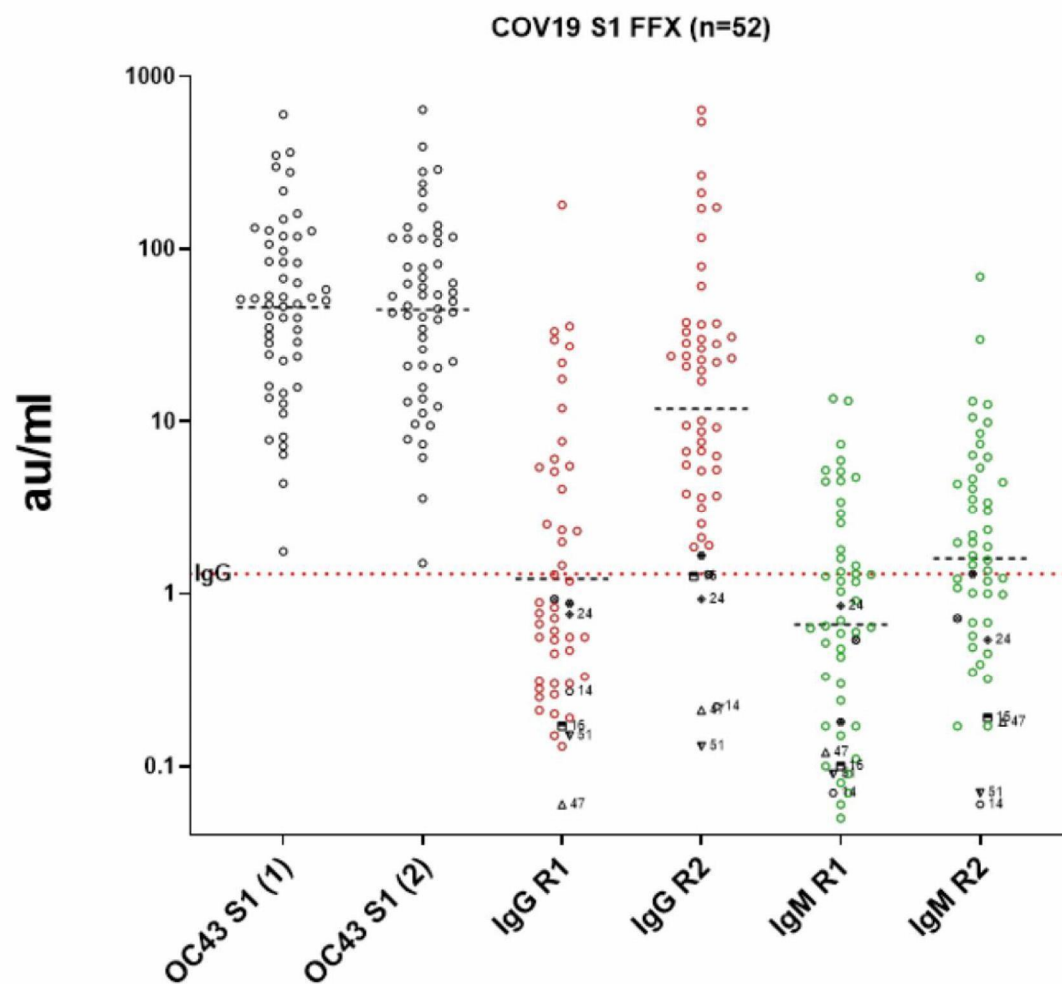
IGM FFX panel (54 pairs)

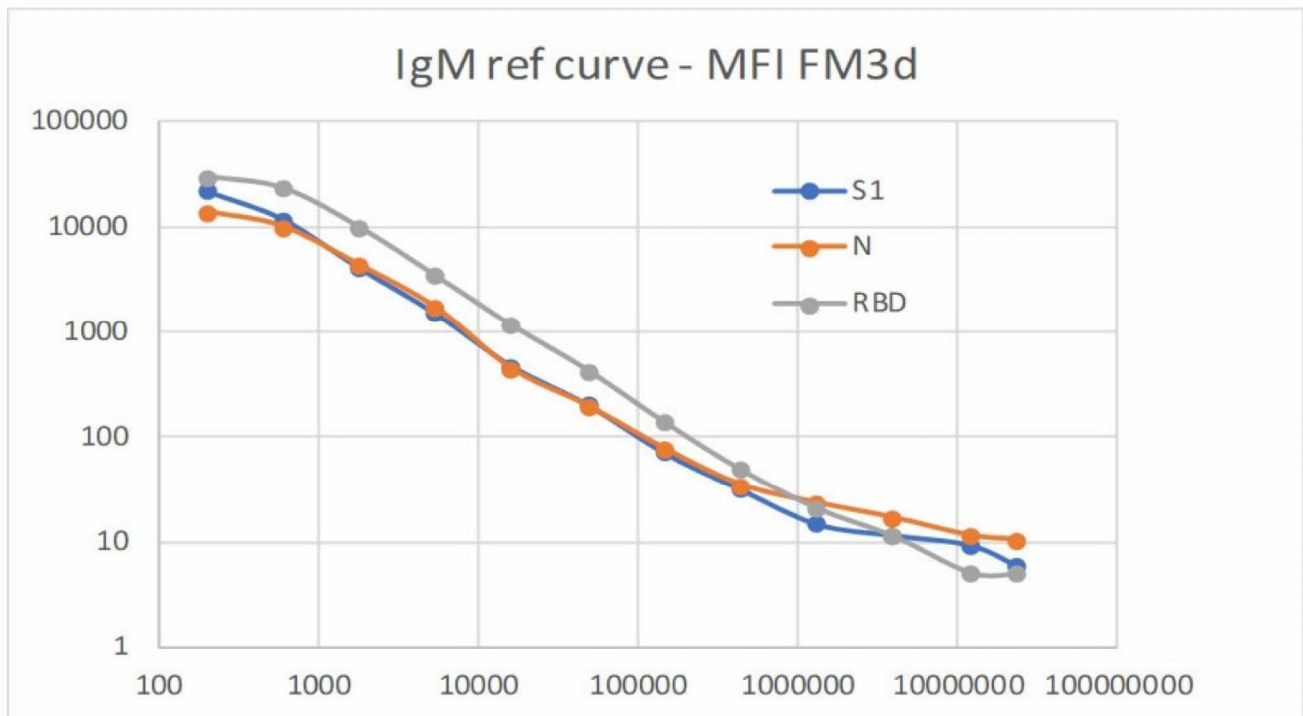
Samples 1/400

Ref s1 = 1/200

Ref waarden S1/N/RBD = 15/20/30 au/ml

Conjugaat IgM; 1/500





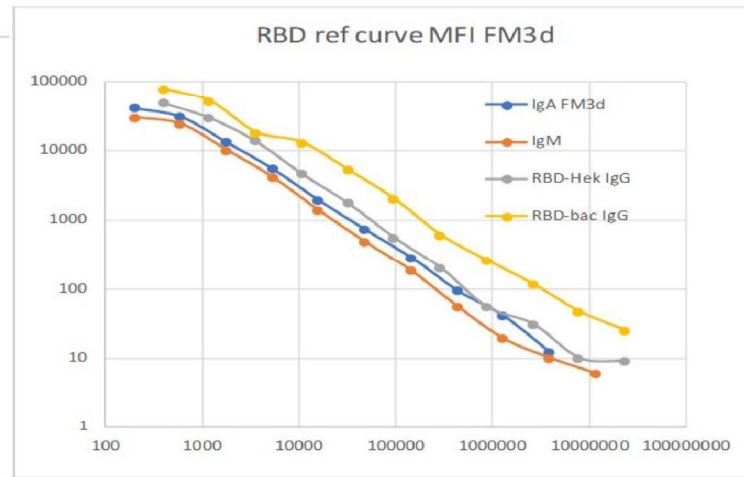
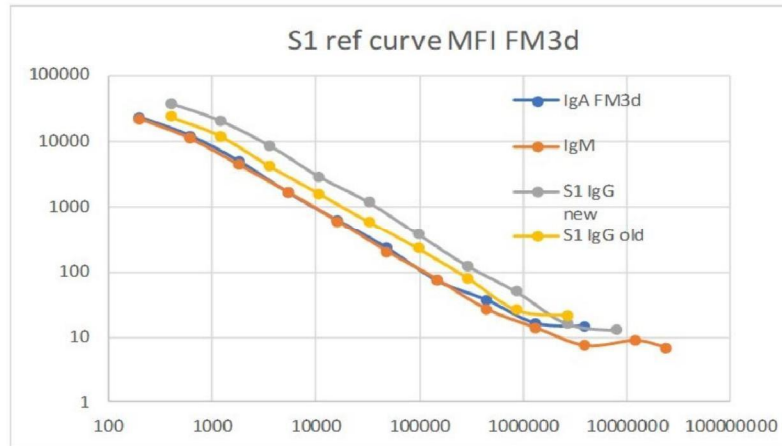
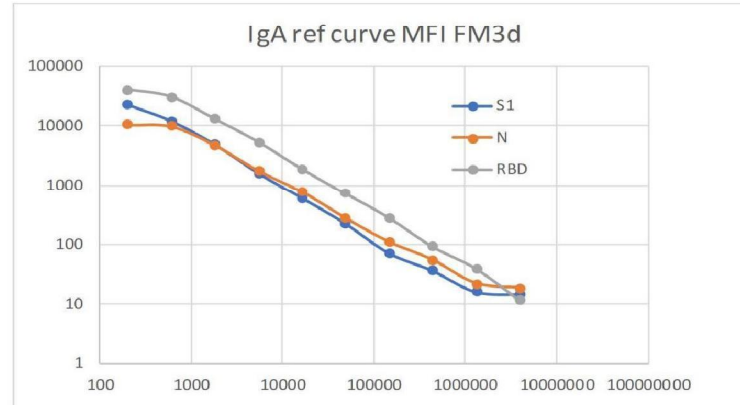
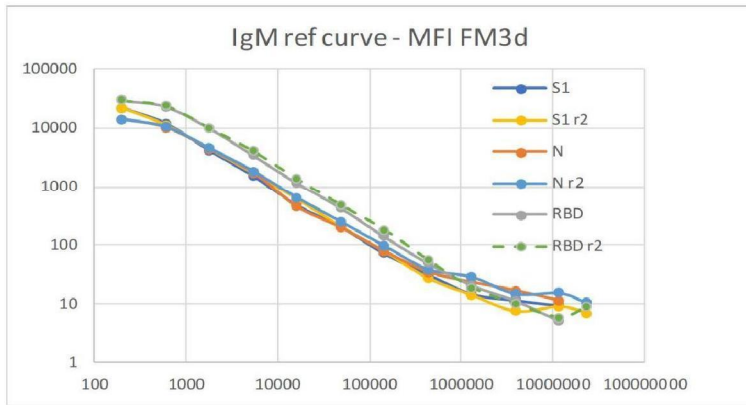
29jun20

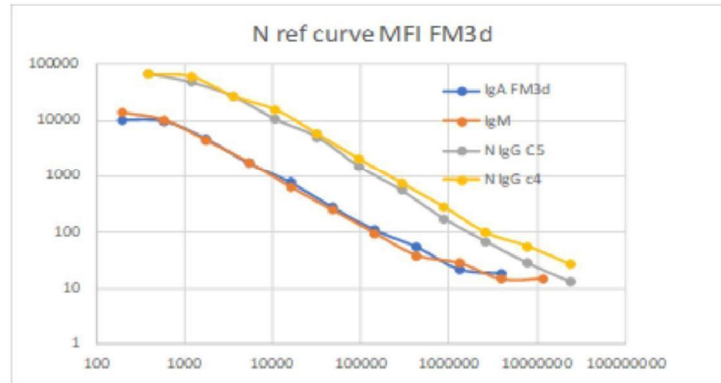
Testen P3 bl 26 IgA en IgM

Ref: 2nd inhouse ref 1/200
IgA en IgM conjugaat 1/500

Dit keer geen problemen met de fm3d dus beter Iga en Igm Mfi's te vergelijken en in te ijken.

Dit keer cleane data en ref curves





Ref waarden opnieuw instellen voor IgA en IgM

2nd inhouse ref CoV19	oc43 (29)	S1 (73)	N (75)	RBD Hek (23)	S1+S2 (64)
IgG	46	182	3050	200	750
IgM		40	100	60	
IgA		40	100	150	
S1; 1/200 IgG	0.23	0.91	15.25	1.0	3.75
S1; 1/400 IgG	X	0.456	7.63	0.5	1.88
S1; 1/200 IgM		0.2	0.5	0.3	ND
S1; 1/200 IgA		0.2	0.5	0.75	ND

2-7-2020

FFX bl1 + 2 IgA

S1; 1/200

Samples 1/400

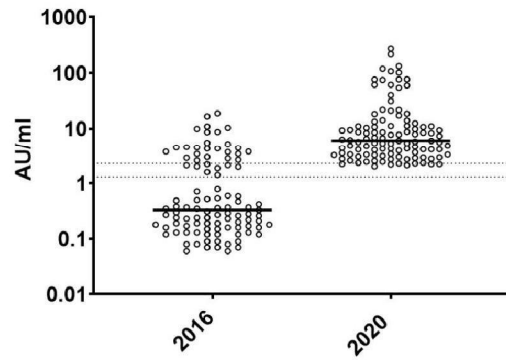
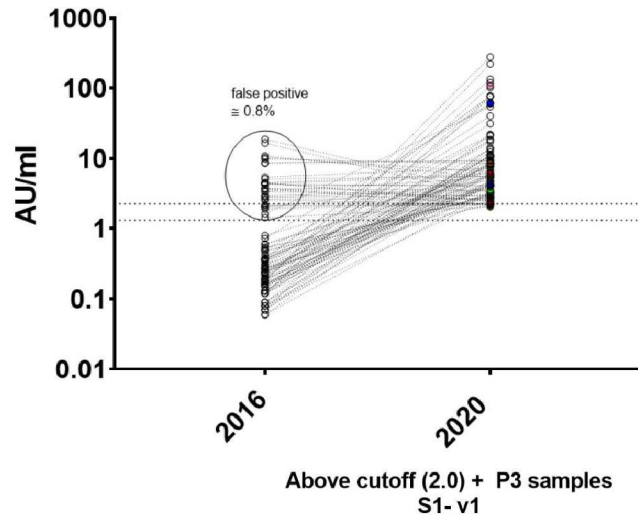
14-7-2020

Conjugation 7 100 ug 1600ul beads, geen bijzonderheden

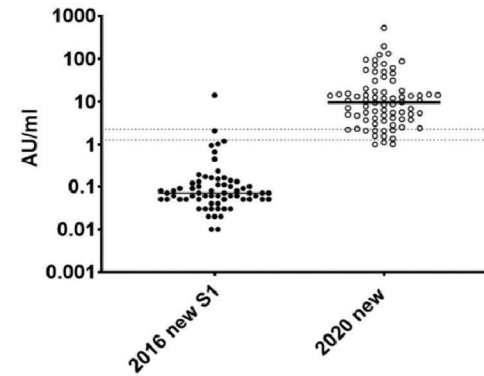
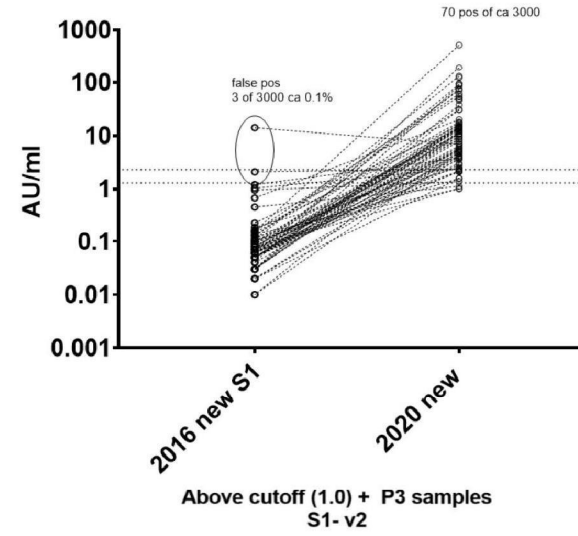
15-7-2020

Metten P3 Pre samples van cov19 positieven RONDE 1 met nieuwe S1 en RBD

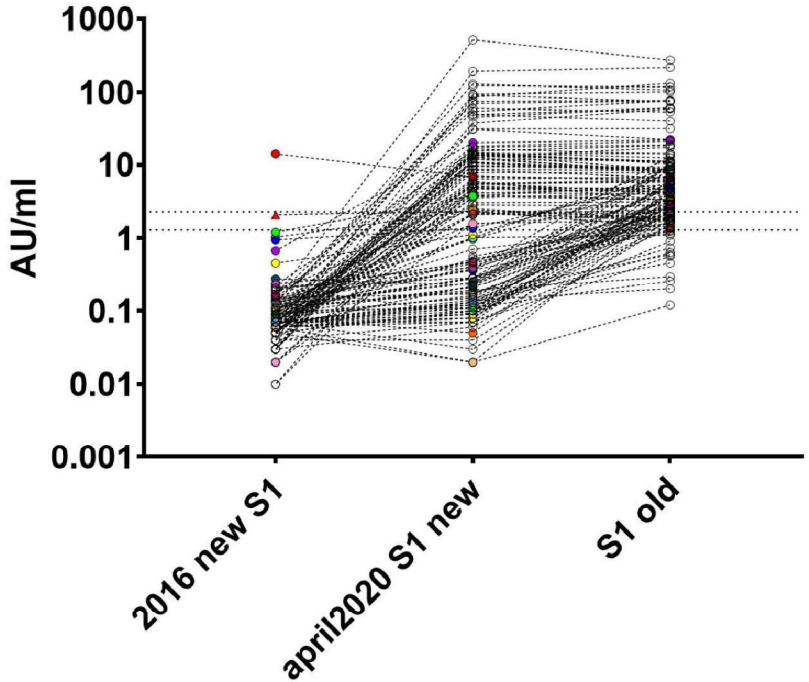
COV-19 Spike S1
V1 cut-off >2.0



COV-19 Spike S1
V2 cutoff >1.0 (N=73)



**COV-19 Spike S1
V1 vs V2**



16 juli validatie panel
Conjugatie 7

Conjugatie goed gekeurd (zie tabblad conjugaties)

21-7-2020

Bepaling IgA blok 3 + 4 FFX (pluk 2)

S1 = 1/200

Potency S1 0.2/0.5/0.75 S1, N, RBD resp.

Samples 1/800!

27 + 28 juli

Blok 4 tm6 IgA + IgG

S1 1/400

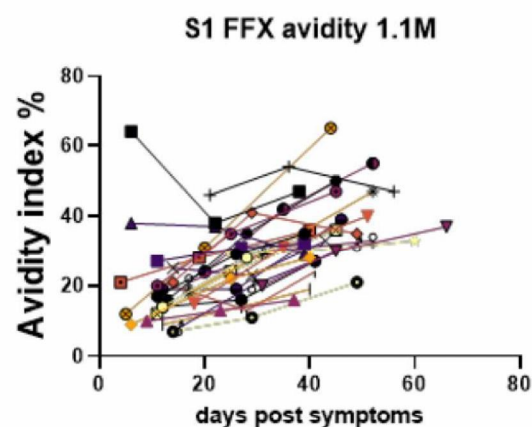
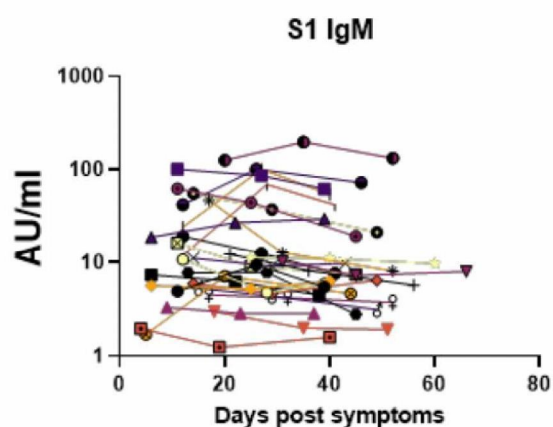
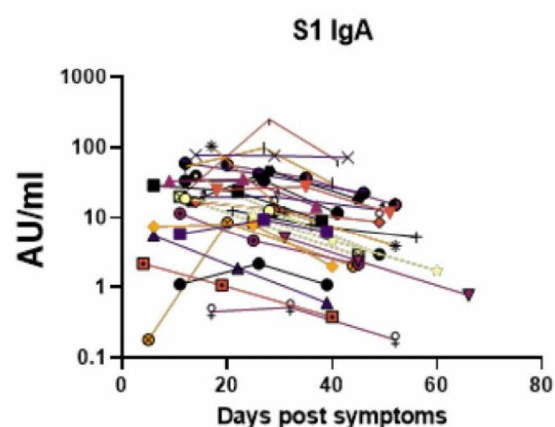
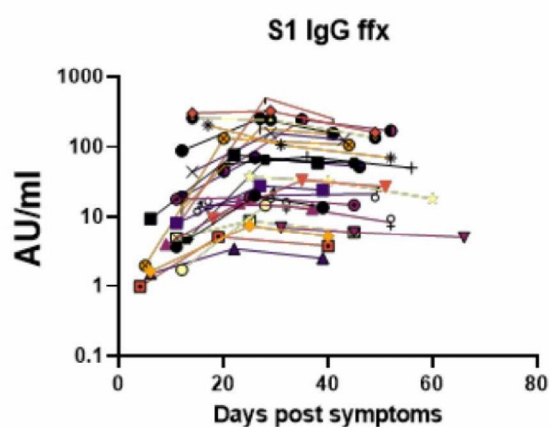
Samples 1/800

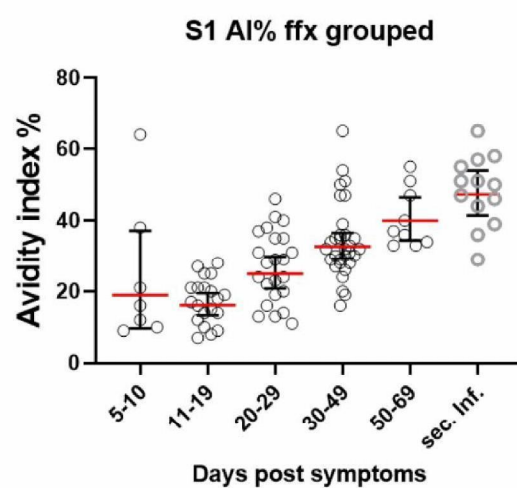
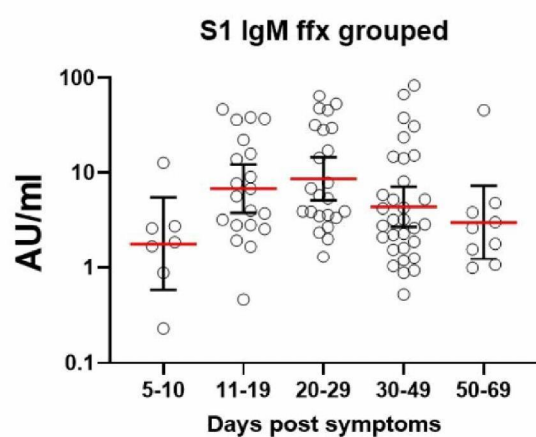
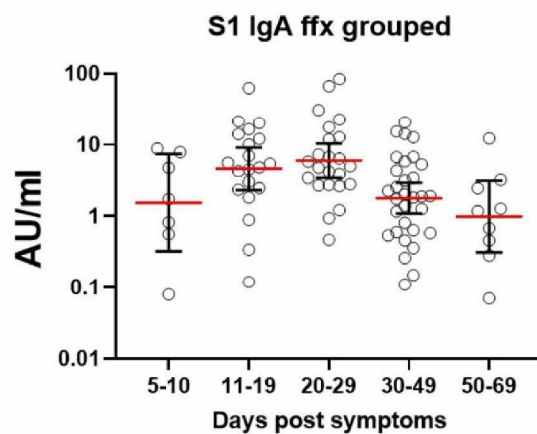
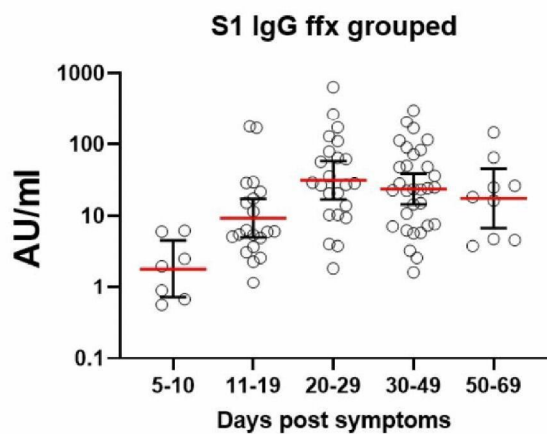
8-13 augustus, Meten IDS 5.1.2e panel

13 samples herinfectie.

IgG< IgA en IGM tevens

Aviditeti met 1.1 M NH4 SCN





nCOV19 - Mia Protocol

vrijdag 20 maart 2020

11:15

Buffers and Biologicals:

Buffers

- EDC (Sigma, Cat# E1769)
- Sulfo-NHS (Sigma, Cat# 56485)
- Activation buffer: 2-(N-morpholino)ethanesulfonic acid (MES) buffer (50 mM, pH 5.5) of 10x MES buffer pH 5.5
- Coupling buffer; 1X Phosphate Buffered Saline (PBS) pH 7.4 (PBS)
- Washing buffer; 1X Phosphate Buffered Saline + 0.05% Tween 20 (PBST)
- Storage buffer; 1X Phosphate Buffered Saline + 0.05% Tween 20 + 1.0 % BSA + 0.03% Sodium Azide
- Goat Anti human IgG-PE F(ab')₂(Jackson ImmunoResearch Laboratories ,0.1mg/ml)
- goat F(ab')₂ anti-human IgA-PE (Southern Biotech) (0.1mg/ml)
- Mouse anti HisTaq -RPE (0.1mg/ml) (abcam; AB....)

Activation and coupling of carboxylated microspheres

EDC should be stored at -20 °C in a desiccated container and allowed to equilibrate to room temperature before use.

EDC is extremely sensitive to moisture and should not be used if damp or clumped

All reagents should be at room temperature before use.

Note: EDC/NHS rapidly hydrolyzes in aqueous solutions and should be prepared fresh just prior to conjugation

Wanneer MES pH 5.5 nog niet binnen is activeren in PBS, dit lijkt namelijk wieing uittemaken omdat de EDC de pH verlaagd

First activation was done using PBS 7.4

Second conjugation was done in MES , no observational difference

- **800 µl** beads (COOH Microspheres, Luminex Corp) per region are collected in 1.5 ml coupling tubes (**starlab**)
- Spin (= 5 min, 13000rpm) on minispin and wash beads 1x with 1ml 50 mM MES pH 5.5.
- Spin down beads remove supernatant and add 400 µl of 50mM MES pH 5.5, vortex 30 sec and add 50 µl NHS (50mg/ml in AD), mix and subsequently and 50 µl EDC (50mg/ml in AD) and vortex 10 seconds
- Incubate 30 min at Room temp on a rotator.
- Spin down beads remove activation buffer and wash beads twice with 1ml PBST.
- Remove PBST and add protein in 1ml PBS
- Incubate 2 to 4h at RT with mixing/rotating.
- Spin and remove supernatant and wash once with 1 ml PBST
- Spin and remove supernatant and store in 1 ml Storage buffer. Store at 4C in the dark.

MIA (concept 1, update 26-03-2020)

-Standard protocol(like RSV)

Note, The histag doenst appear to be reachable, since the anti 6x HisTag doesn't give any signal for the proteins

Dilute samples in **100% surmodics**, 1/200 and 1/4000 (1/2000)

Dilute beads in **100% surmodics**

30 maart; 2% FCS aan surmodics toegevoegd, lijkt geen significant effect te hebben.

Voorlopig aan zo houden

25-03-20**Serum pool: 5x pos serum (total ca 40ul)**

NL63 = 663*600/10e5	5 AU/ml
HKU1 = 1000*500/10e5	6 AU/ml
OC43 = 9400 * 600/ 10e5	60 AU/ml
S1 = 14500*1800 / 10e5	260 AU/ml
RBD = 90000*1800/10e5	1620 AU/ml
N= 75000*1800 /10e5	1360 AU/ml

S1(200x)	0.025 <u>aU/ml</u>
	0.03 <u>aU/ml</u>
	0.3 <u>aU/ml</u>
	1.3 <u>aU/ml</u>
	8.1 <u>aU/ml</u>
	6.8 <u>aU/ml</u>

9-4-2020

Iga ref potency

(mix serum IDS n=5)

OC43 = 300 *600/ 10e5	2 AU/ml
S1 =	30 AU/ml
RBD =	260 AU/ml
N =	110 AU/ml

C1 approx 50 ug protein to 500ul beads

C2 approx 50 ug protein to 700ul beads

No significant difference.

10-4-2020**C2 N conjugation #40588V083 lot LC14MC309**

C2: N; 100 ug to 1400 ul beads in 1 ml PBS

C2; OC43;40 ug to 500 ul beads in 500 ul PBS

Validatie 12 april: ok

Ijken ref

23 april

	oc43 (29)	S1 (73)	N (75)	RBD (23)
Pienter C biopl	45.6	180.8	2958.0	
pienter C r	48.8	176.2	4000.0	
Ref inijk MB	46.1	183.6	3337.7	770.1
Ref Inijk RS	49.4	191.2	3358.7	775.0
ref inijk MK	47.8	165.6	2633.4	694.9
gmc all 27april	45.9	180.9	3032.8	746.6
gesteld 27 april(final)	46	182	3050	750
S1; 1/200	0.23	0.91	15.25	3.75
1e ref terug berekend				
potency gesteld	60	260	1360.00	1650

MB	62.1	239.9	1275.2	1504.6
RS	60.6	276.7	1378.2	1647.6
Mk	58.2	278.7	1471.7	1885.9

24-4-2020

Conjugation C3/C4

Bij N koppelen in PBS op 0.025% tween20

27 april validatie nieuwe koppeling

N lijkt nu meer beads te vinden.

EMC x-react blok 1 400x + cov19 + RIVM panel (FFX etc) 400 + 10.000x
opFM3d

Final

	oc43 (29)	S1 (73)	N (75)	RBD (23)	S1+S2
Pienter C biopl	45.6	180.8	2958.0		
pienter C r	48.8	176.2	4000.0		
Ref inijk MB	46.1	183.6	3337.7	770.1	
Ref Inijk RS	49.4	191.2	3358.7	775.0	
ref inijk MK	47.8	165.6	2633.4	694.9	
gmc all 27april	45.9	180.9	3032.8	746.6	
gesteld 27 april(final)	46	182	3050	750	750
S1; 1/200	0.23	0.91	15.25	3.75	3.75
1e ref terug berekend					
potency gesteld	60	260	1360.00	1650	
MB	62.1	239.9	1275.2	1504.6	
RS	60.6	276.7	1378.2	1647.6	
Mk	58.2	278.7	1471.7	1885.9	

4mei

S1+ S2 SINO

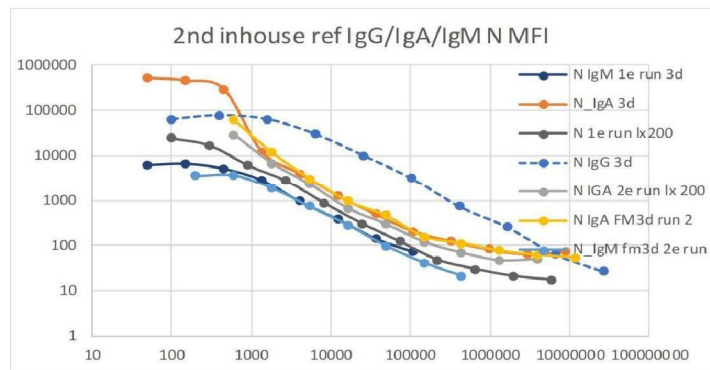
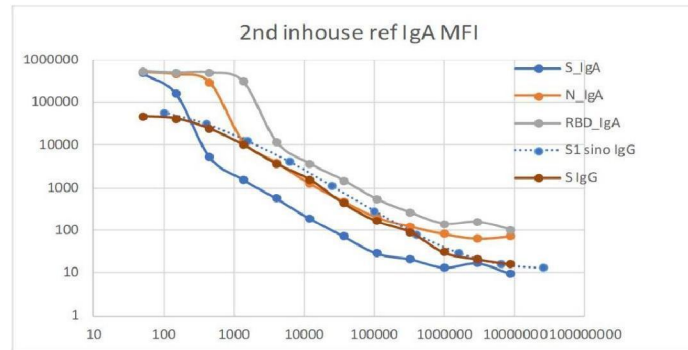
Ref gesteld op 750 (gelijk aan RBD) (updatet 30 juni2020)

2nd inhouse ref CoV19	oc43 (29)	S1 (73)	N (75)	RBD Hek (23)	S1+S2 (64)
IgG	46	182	3050	200	750
IgM		40	100	60	
IgA		40	100	150	
S1; 1/200 IgG	0.23	0.91	15.25	1.0	3.75
S1; 1/400 IgG	X	0.456	7.63	0.5	1.88
S1; 1/200 IgM		0.2	0.5	0.3	ND
S1; 1/200 IgA		0.2	0.5	0.75	ND
UI beads per plate	13	14	18	14	20
Control: Mr X 27000x	58	65	2500	360/93	184
Control: hCOV Low; 400x	48	1.3	5.4	10.6/2.7	15.0

- RBD eerst baculo nu ook uit HEK (IgG/IgA/IgM waarden 750/500/60 au/ml)
- Nieuwe batch (kopelingen vanaf C5) zijn zuiverder en RBD is nu ook uit HEK en nu vergelijkbaar met S1

- ZIE GRAFIEK.. IN lage verdunning zeer hoge MFI's
IgM
Ref gesteld op 30/60 en 90 AU/ml voor S1/N en RBD

Iga is sky high in de oxford samples en wordt blijkbaar niet geremd en worden Mfi's boven de 500.000
Gevonden in een 400x verdunning
Dit is nog niet eerder gezeien

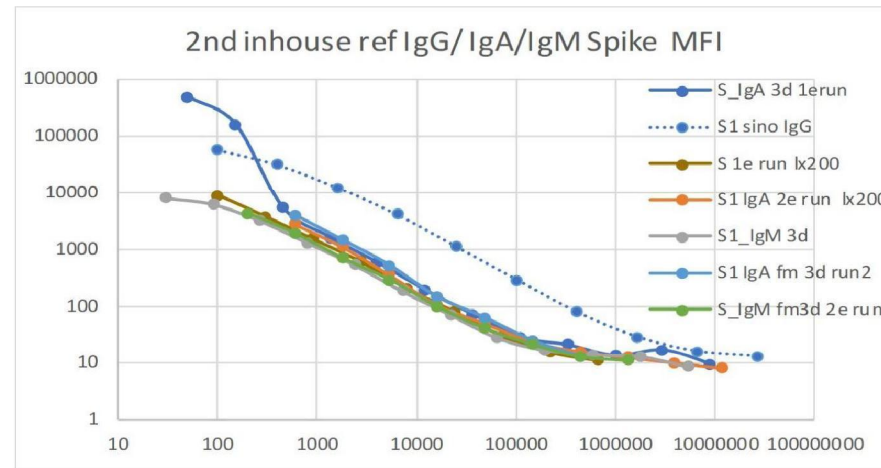


MR X IgM

S1 (73)	N (75)	RBD (23)
23.6	166.2	34.7

MrX IgA

22.5	29.0	99.72
23.8	33.7	107.3
18.19	34.5	97.5
21.59	36.6	106.4
	43.94	109.52
	51.51	128.44



IgA voor N lastig te bepalen.

Protocol voor IgA start S1 1/400 (3staps verdunning tm s12)

Samples 1 op 2000

Conjugaat 1/500

IgM S1 = 1/200 samples 1/1000

In lagere verdunning vaak aspecifieke effecten bij patienten met hoge antistof concentraties,

Laag positieven zijn nog te meten in een 1/1000 en 1/2000x v

Conjugaat 1/1000

Update 30juni2020

IgA en IgM samples 1 op 400 meten Ref S1 = 1/200

Conjugaat 1/500

Wanneer MES ph 5.5 nog niet binnen is activeren in PBS, dit lijkt namelijk wieing uittemaken omdat de EDC de ph verlaagd

First activation was done using PBS 7.4

Second conjugation was done in MES , no observational difference

Note, The histag **doenst** appear to be reachable, since the anti 6x H1Stag doesn't give any signal for the proteins

9 juni2020 - Nieuwe Conjugatie S, N en RBD-HEK

10 juni validatie met geplukte pos samples uit PICO ronde 1

Blok 1 80 samples: 400 en 8000 x

Oude vs nieuwe bead conjugatie (c4 vs C5)

Ref 1/400 S1, staps

Controle 1; mr x; 16000x

Controle 2; low; 400x

Controle 3; NIBSC code 20/130 400x en 8000x

Conjugations - Assay Conditions

woensdag 25 maart 2020
13:30

HCoV-OC43 - Beadregio -29
HCoV-NL63- beadregio -37
HCoV-HKU1 -beadregio 64

Ca 7-10ugr (ca 1mg/ml)
7-10 ug to 120 ul activated beads in 200 ul PBS pH7.4

Sars-Cov-2 Spike RBD- histag t0 bead #23
Sars-CoV-2 Nucleocapsid to bead #75 dialysed
Sars-CoV-2 Nucleocapsid to bead #75 non- dialysed
Sars-CoV-2 Spike protein S1 subunit to bead #73

Concentration diluted to 0.2mg/ml InPBS

50-60ug protein per 500ul activated beads.

S1 is PBS

Nucelocapside

A) dialysed to PBS pH7.4

B) 20mM TRIS, 500mM NaCl, 10% glycerol pH 8.0, 5% trehalose, 5% mannitol, 0.01% tw80) Diluted in 500 ul PBS (0.2mg/ml) (20ug to 200ul beads #75)

RBD; 20 mMPB, 300mM NaCl Ph7.0, 10% glycerol, 5% mannitol 5% trehalose, 0.01% tween, diluted to 0.2mg/ml in PBS

Samples were diluted 100% surmodics

Beads diluted in 50% surmodics/ 50 % assay buffer (1xPBS 1% bsa 0.05%tw20) of 100% surmodics (no clear difference between the two but not tested with panels

30 maart 2020

2% FCS added to surmodics as assaybuffer and bead ix buffer

Sapril Conjugation 2

S1 100ug (50ul) + 450 ul PBS -> 250ul + 500 ul PBS + 700 ul activated beads (2x) #73

OC43 50 ul (1mg/ml) in 700 ul PBS to 700 ul Activated beads (29).

S1 Uvu- koppersveld ca 15ug aan 200ul Beads (#40)

2nd ref: pooled; 10x S.Stoof samples + mr X (ca 50%) + 2x ffx samples (laag pos) eerste serie

S1 van utrecht zit in tris, Echter te weinig om te dialyseren, daarom dieet gekoppeld aan 200 ul geactiveerde beads (in 250ul PBS)

In een klelne vergelijking met IVIG leek Surmodics minder achtergrond te geven tov 1xPBS, 1% BSA en 0.05% tw20.

Nog uitesten 100% surmodics vs Surmodics+BSA of FCS.
Met 2% FCS maakt niet veel verschil vooralsnoch toegevoegd

24-27 april

Nieuwe conjugatie

100 ug S en N aan 1.5ml geactiveerde beads in MES

100 ug OC43 aan 1.5 ml beads

Ca 30-25 ug RBD aan 500 ul beads

N koppelen in PBS met beetje tween lijkt een verbetering.

Tevens wassen in pBS tween zorgt ervoor dat je een beter/compactere en vaster pellet krijgt bij wassen.

*(minder beads verlies)

N gekoppeld in PBS + 0.025% tween 20, dit lijkt beter (geen effect op max mfi, wel minder klontering), volgende keer 0.05%

2-5-2020

Conjugation

S1 en RBD van UvU tevens S1+S@ van sino.

Alle 3 de eiwitten bevatten tris, daarom omgebufferd met een 10 Kda Amicon buis

Alle eiwitten van Sino 100 ug aan 1.5 ml beads

Bij >20mM TRIS ombufferen!

OC43 100 ug aan 1200 ul beads.

Alle eiwitten ca 100 ug verdund naar ca 0.2 mg in 500ul PBS.

Na afdraaien ca 100 ul opgenomen in 1 ml pb = 0.1 mg/ml

En 500 ul gekoppeld aan 550 ul (ca 6 milj beads) aan geactiveerde beads

Overige 500 ul ingevroren -80C (vak 2, rij 4 la 2)

UvU - RBD bead # 37 (sars-s1B-2st 0.9mg/ml)

UvU- S1 bead #40 C2 (NcovS1)

Sino S1+S2 # 64 (50 ug aan 550 ul beads)

9 juni 2020

Koppelen

1. RBD- Hek (c1) ref; 40592-V08H, LOT# LC14AP1403, 100ug (in 1ml pBS) aan 1.5ML beads #23
2. S1_ PBS ref 40591-V08H, LOT MM14Y0701-C: 100ug (in 1 mlPBS) aan 1.5mml beads #73
3. N - Baculo ref 405888-V083 LC 14AP2101 100ug (in 1 ml PBS) aan 1.5ml beads #75

19 juni 2020 nieuwe koppeling (C6)

Koppelen

1. RBD- Hek (c2) ref; 40592-V08H, LOT# LC14AP1403, 100ug (in 1ml pBS) aan 1.6ML beads #23
2. S1_ PBS ref 40591-V08H, LOT MM14Y0701-C: 100ug (in 1 mlPBS) aan 1.6mml beads #73
3. N - Baculo ref 405888-V083 LC 14AP2101 100ug (in 1 ml PBS) aan 1.6ml beads #75

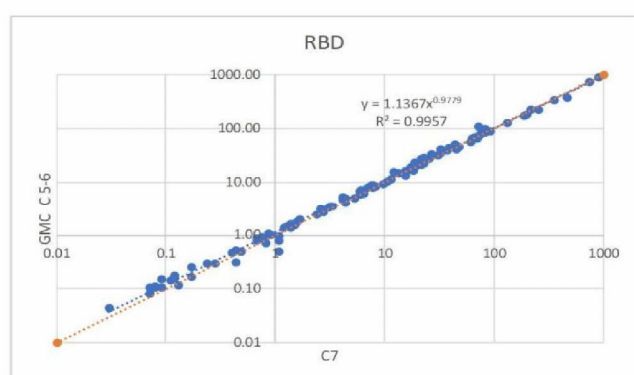
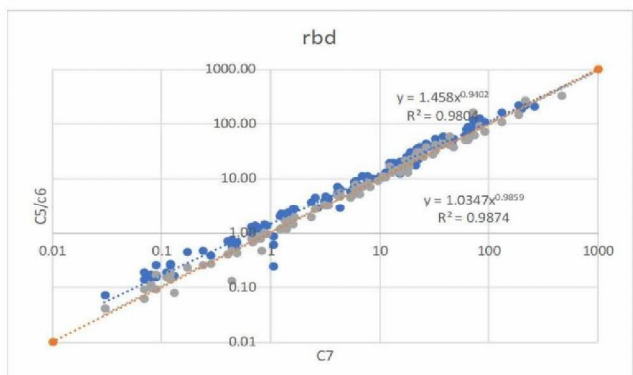
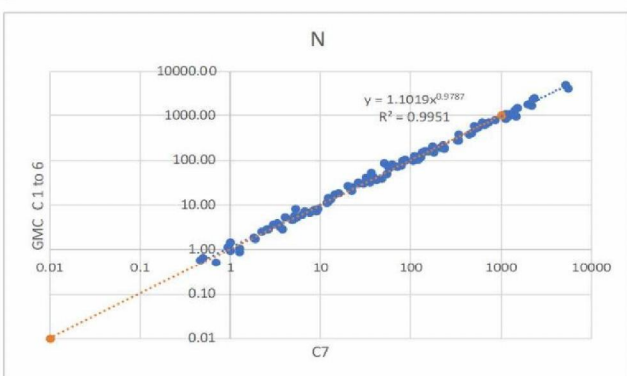
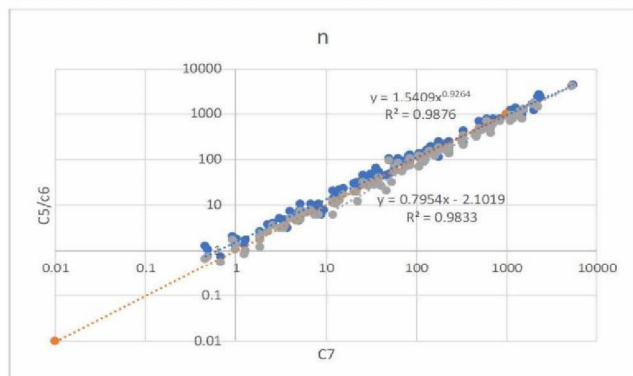
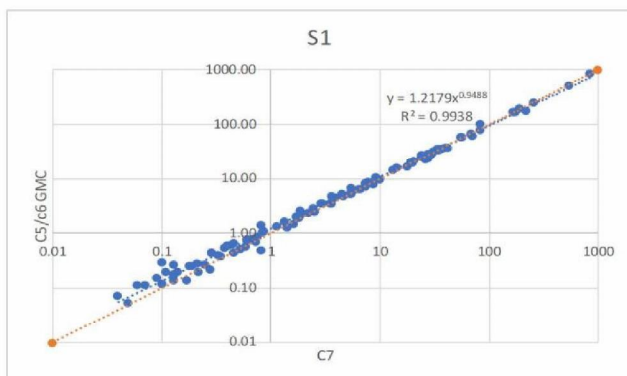
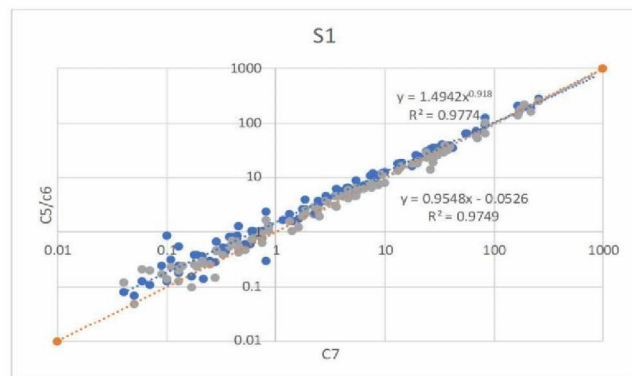
22 juni
Meten validatie panel
(FFX + BI26 P3)

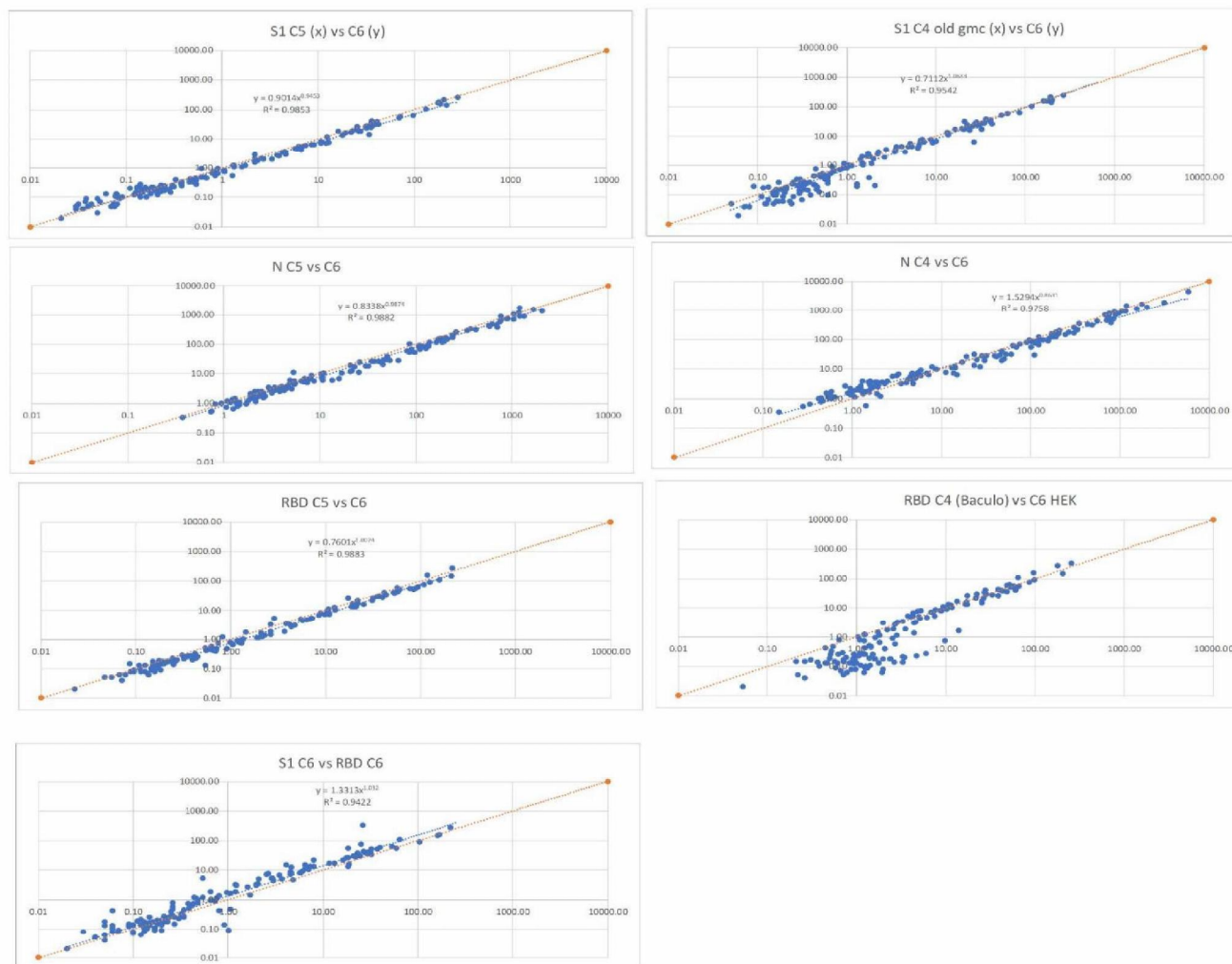
Ref 2nd inhouse ref 1/400
FFx samples 800x/1000x
P3 samples 400x
Controls Low = 600x
Mr X = 15,000x
FM3d

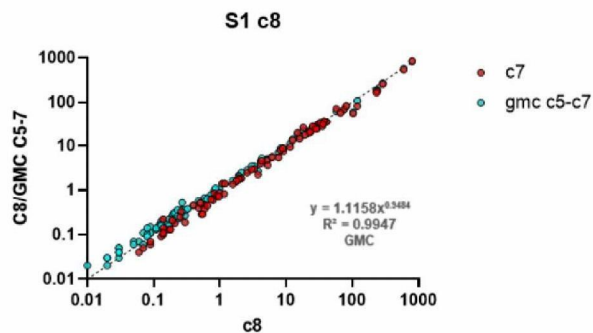
Validatie resultaten samengevoegd in folder
R:\IIV\Afdeling - IMS\9. Assay info\Coronavirus MIA\Beads conjugation validation

15 juli 2020

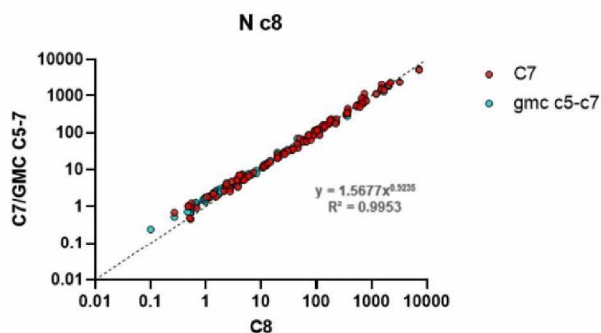
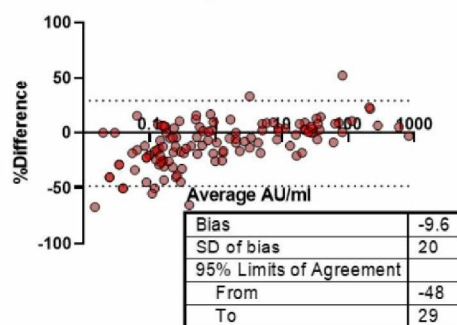
Koppeling C7 100ugr S, N en RBD aan 1600 ul beads
Volgens protocol, geen bijzonderheden.



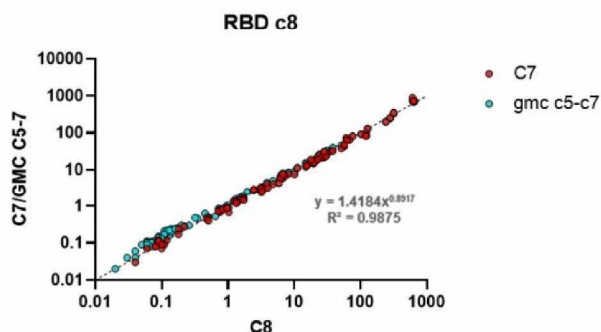
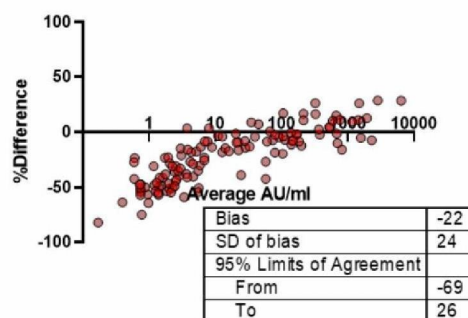




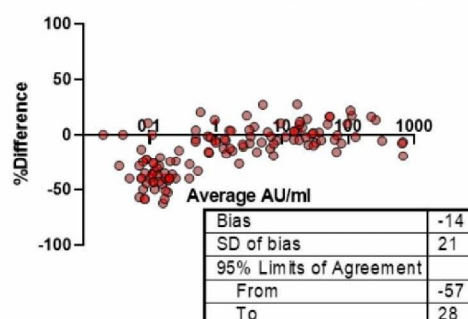
%Difference C8 and gmc c5-c7 : Bland-Altman S1



%Difference C8 and gmc c5-c7 : Bland-Altman N



%Difference C8 and gmc c5-c7 : Bland-Altman RBD



18 augustus

Koppeling C8 100ugr S, N en RBD aan 1600 ul beads

Volgens protocol, geen bijzonderheden.

Koppelen

1. RBD- Hek (c1) ref; 40592-V08H, LOT# LC14AP1403, 100ug (in 1ml pBS) aan 1.5ML beads #23
2. S1 _ PBS ref 40591-V08H, LOT MM14Y0701-C: 100ug (in 1 mlPBS) aan 1.5mml beads #73
3. N - Baculo ref 405888-V083 LC 14AP2101 100ug (in 1 ml PBS) aan 1.5ml beads #75

18 september

Koppeling C9

100ug aan 1.6 ml beads

Volgens protocol

RBD laatste vial gebruikt

Van S1 laatste in PBS vial (nog 2 gevriesdroogde vials)

Van N nog 1 Vial

Koppelen

1. RBD- Hek (c1) ref; 40592-V08H, LOT# LC14AP1403, 100ug (in 1ml pBS) aan 1.6ML beads #23
2. S1 _ PBS ref 40591-V08H, LOT MM14Y0701-C: 100ug (in 1 mlPBS) aan 1.6ml beads #73
3. N - Baculo ref 405888-V083 LC 14AP2101 100ug (in 1 ml PBS) aan 1.6ml beads #75

29 october 2020
Koppeling N (c10)
MF14AP3008
500ug @ 0.76mg/ml In 20 mM tris PH8.0

= 132 ug bij 1600 ul beads
Rest gealiquote in 100ug porties (ca 134 ul)

10 november 2020

Opnieuw koppelen N

Ca 60 ug aan 800 ul beads #75
Activation in 50mM MES ph 5.5
Conjugation in 20mM TRIS, 0.5M NaCl, 0.1% tween20 Ph 8.1
Wash idem conjugation buffer
Storage; 20mM TRIS, 0.5M NaCl, 0.1% tween20 Ph 8.1 + 1% BSA + 0.03% Sodium Azide

Crossreaction (depletion specificity)

vrijdag 20 maart 2020

11:13

Overview MIA dilutions and ref/CNTR potencies

woensdag 2 september 2020

10:06

2nd inhouse ref CoV19	oc43 (29)	S1 (73)	N (75)	RBD Hek (23)	S1+S2 (64)
IgG	46	182	3050	200	750
IgM		40	100	60	
IgA		40	100	150	
S1; 1/200 IgG	0.23	0.91	15.25	1.0	3.75
S1; 1/400 IgG	X	0.456	7.63	0.5	1.88
S1: 1/200 IgM		0.2	0.5	0.3	ND
S1: 1/200 IgA		0.2	0.5	0.75	ND
UI beads per plate	13	13	17	13	20
Control: Mr X 27000x	58	65	2500	360/93	184
Control: hCOV Low; 400x	48	1.3	5.4	10.6/2.7	15.0

controles

low	IgA S	N	RBD		MrX	IgA S	N	RBD		low	IgM S	N	RBD		MrX	IgM S	N	RBD
	1.23	1.25	5.08			22.5	29	99.72			2.55	4.4	7.46			23.6	166.2	34.7
	1.35	1.42	5.89			23.8	33.7	107.3			2.81	4.17	6.33			24.44	143.46	32.68
	1.24	1.42	5.98			18.19	34.5	97.5			2.59	3.97	5.99			25.16	146.81	34.25
	1.49	1.35	5.7			21.59	36.6	106.4			2.1	5	5.03			23.94	178.87	33.98
	1.42	1.49	6.61				43.94	109.52			2.12	4.05	5.01			21.31	155.85	34.72
						13.26	29.25	79.3								23.25	136.66	31.64
						12.63	26.94	80.42										
						12.73	25.18	72.75										
						16.95	38.15	97										
						14.49	36.35	104.59										
gmc	1.34	1.38	5.83			16.86	32.91	94.57			2.42	4.30	5.90			23.59	153.99	33.64

IgG Mr X: S/N/RBD; 72/2787/93

Low: S/N?RBD; 1.4/7.0/2.9

NIBCS 30/120;

Conjugate

IgG	1/400
-----	-------

IgA/IgM 1/500

Samples IgG 1/200 and 1/8000

IgA/IgM	1/400
Pos low (piCO)	1/800
Pos high (hosp. /reinfect.)	1/2000

potency AU/ml	182	3050	750	200			8000x												
	S1	N	RBD baculo	RBD HEK		S1	N	RBD baculo	RBD HEK		S1	N	RBD baculo	RBD HEK		S1	N	RBD baculo	RBD HEK
NIBSC 20/130	40.57	4278.89	205.27	54.74	NIBSC 20/130						NIBSC 20/130	40.57	4278.89	205.27	54.74				
20/128	0.05	2.83		0.01	20/128						20/128	0.05	2.83		0.01				
20/120	98.59	219.10		99.92	20/120	100.86	180.64		91.81		20/120	99.72	198.94		95.78				
20/122	28.82	113.63		39.48	20/122	22.41	112.58		46.29		20/122	25.41	113.10		42.75				
20/124	3.55	160.21		5.25	20/124	3.75	181.74		4.46		20/124	3.65	170.64		4.84				
20/126	1.01	16.12		10.93	20/126	4.41	74.99		8.20		20/126	2.11	20.07		9.47				
20/130	43.22	*2120.51		51.46	20/130	42.11	4270.30		58.46		20/130	42.66	4270.30		54.85				

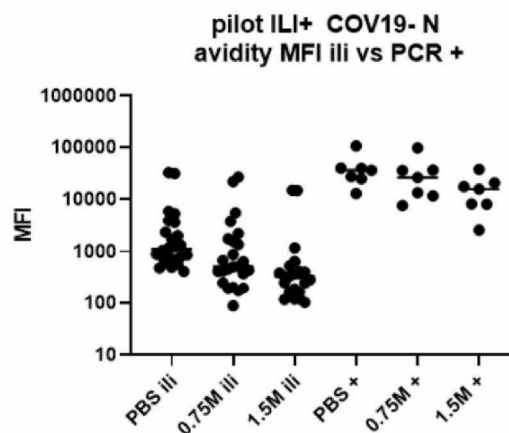
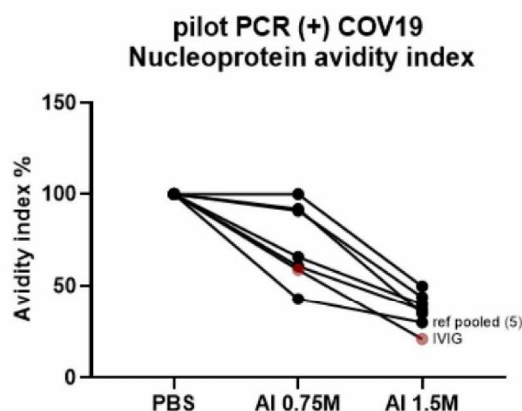
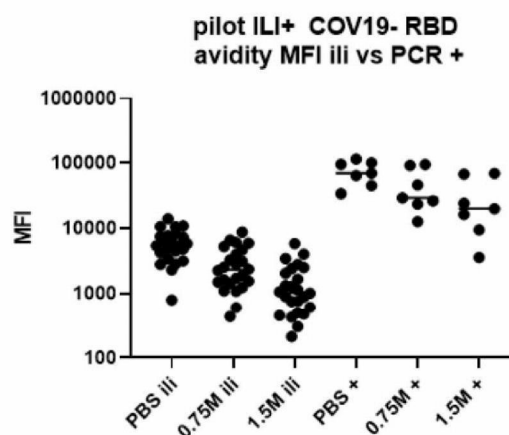
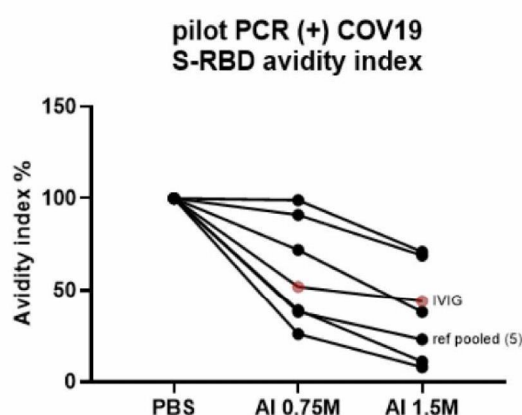
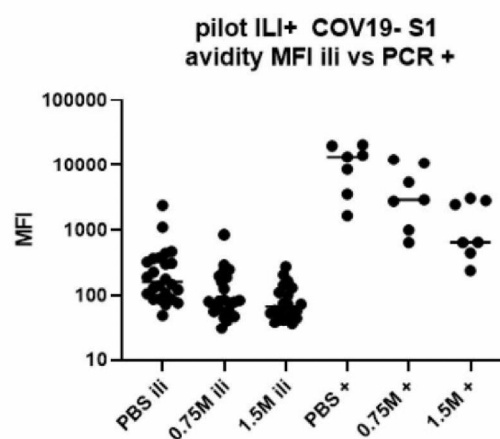
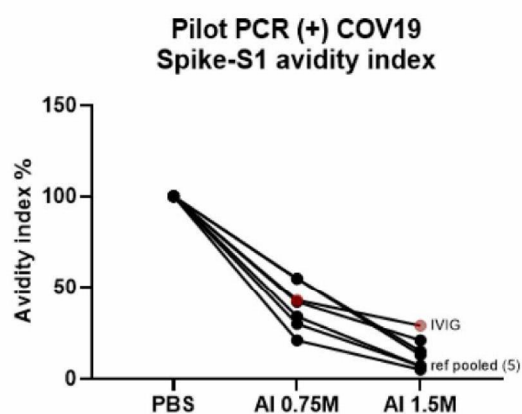
NIBSC 20/130

COV19-S1	43.0	6.7	13.5	20.2	49.8	56.5	63.3	15.6
		-6.7	-13.5	-20.2	36.3	29.6	22.8	
COV19-N	4581.5	557.1	1114.2	1671.4	5138.7	5695.8	6252.9	12.2
		-557.1	-1114.2	-1671.4	4024.4	3467.3	2910.2	
COV19-RBD	60.1	8.3	16.6	25.0	68.5	76.8	85.1	13.8
		-8.3	-16.6	-25.0	51.8	43.5	35.2	

Avidity

dinsdag 7 april 2020

10:11



5 pos samples , primaire SARS-cov2 infection. PCR + (IDS)

3 augustus 2020

Selectie ffx gepaarde samples

Verdunning 1/1000

Concentratie NH₄SCN

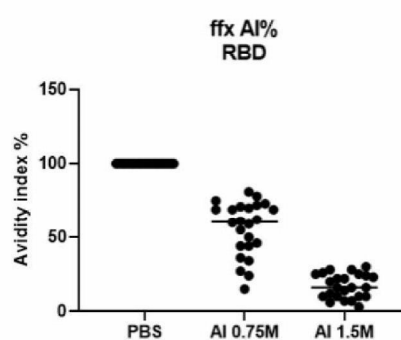
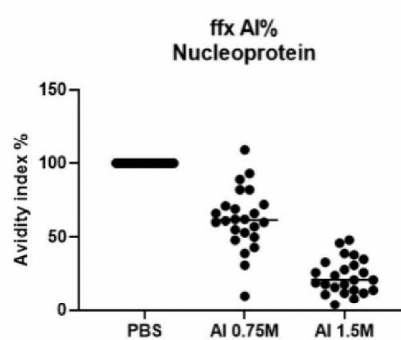
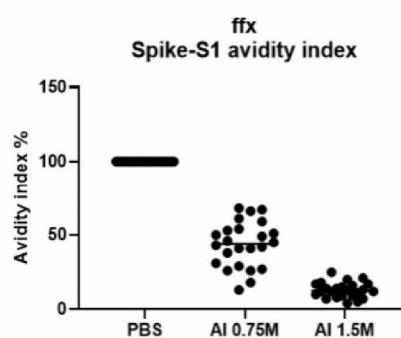
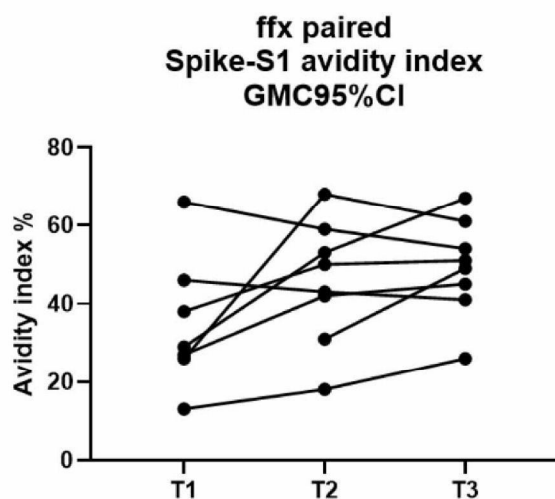
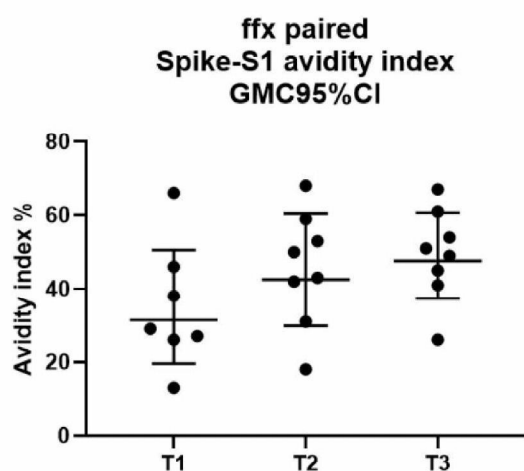
0.75 en 1.5M 10 min KT

Metten op 3D

4 augustus.

Vervolg ca 28x3 gepaarde samples

0.75m NH₄ScN



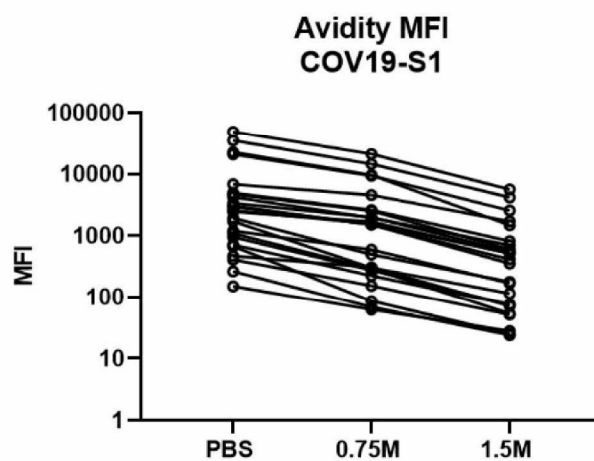
5 en 10 augustus

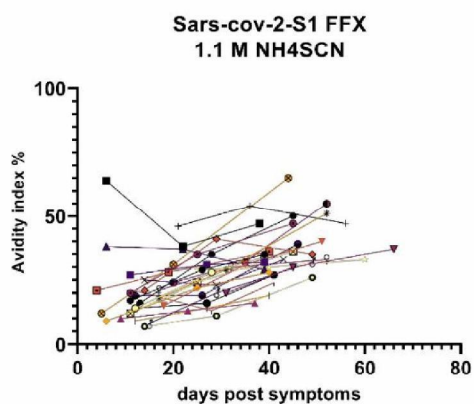
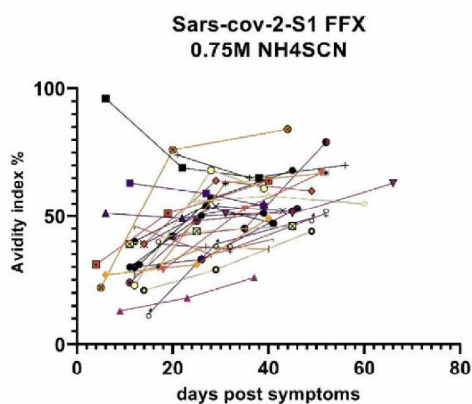
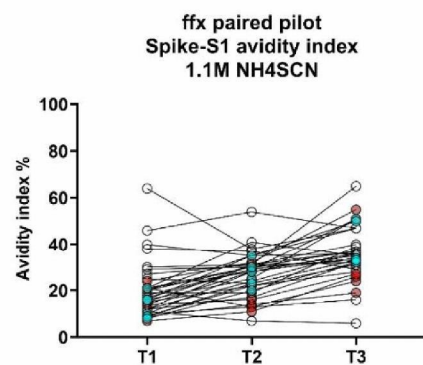
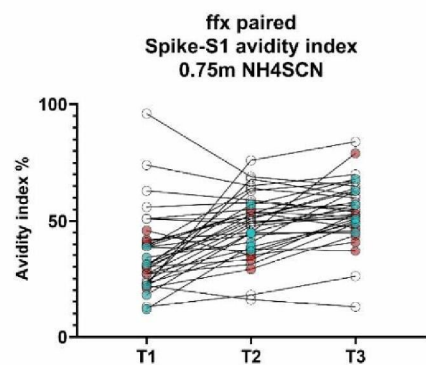
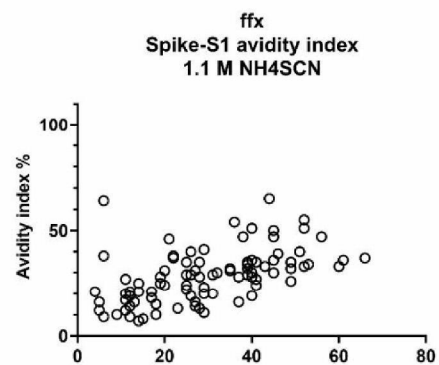
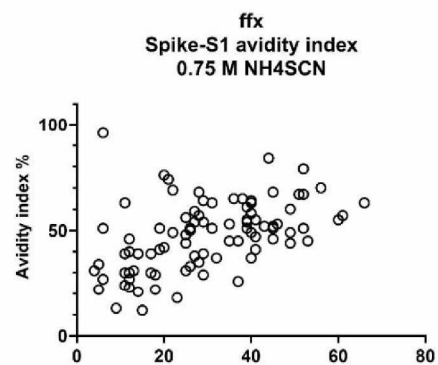
FFX samples meten met 1.1M ScN

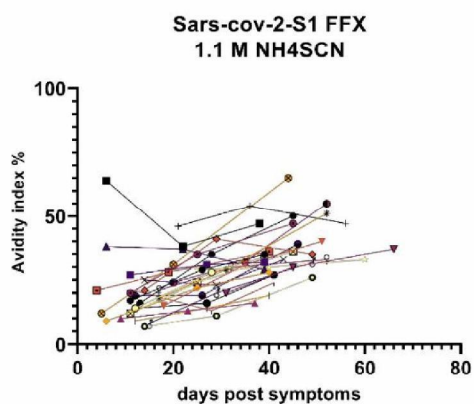
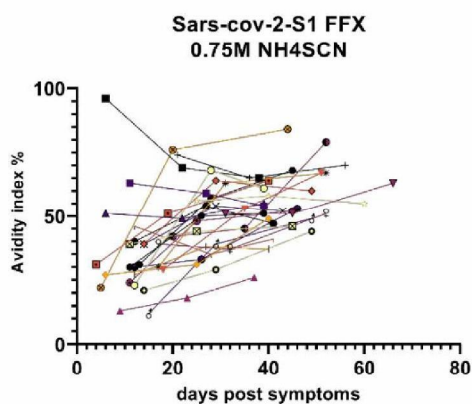
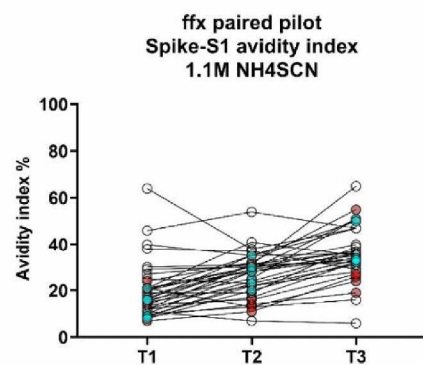
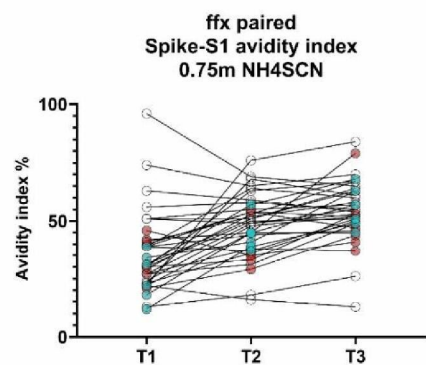
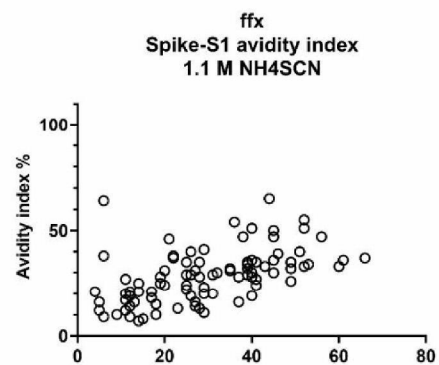
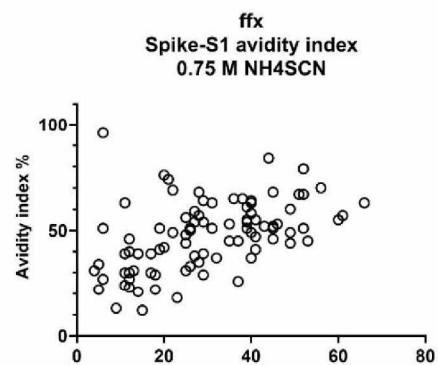
Tevens 13 samples Cov19 herinfectie COV19IgG en

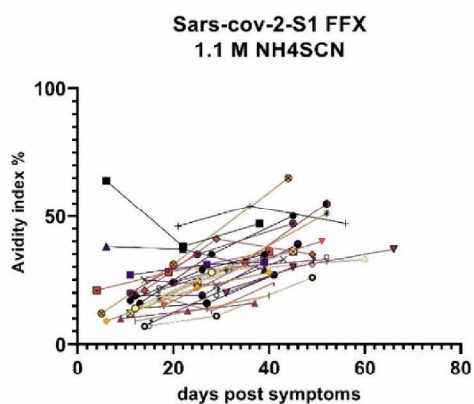
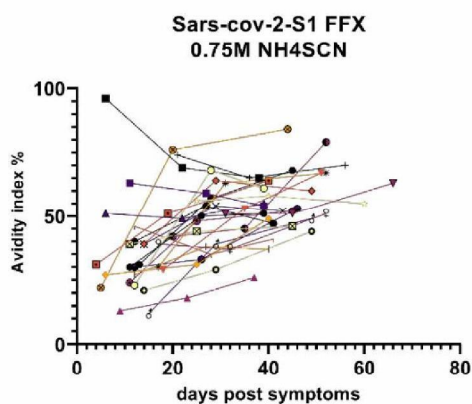
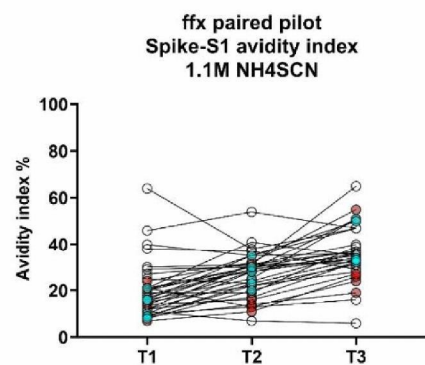
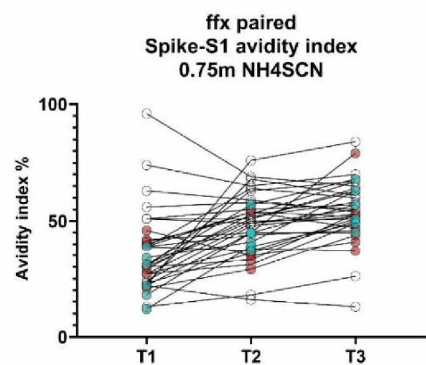
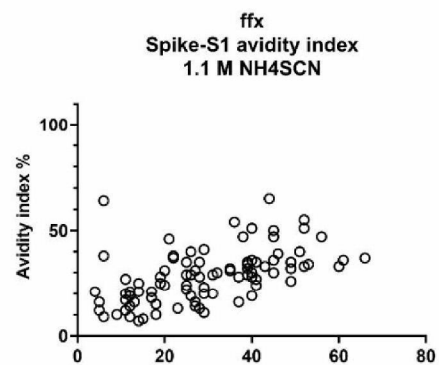
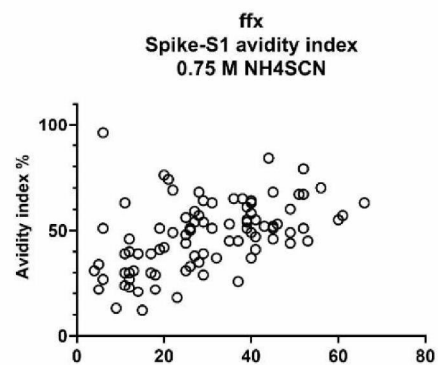
Aviditeit meten

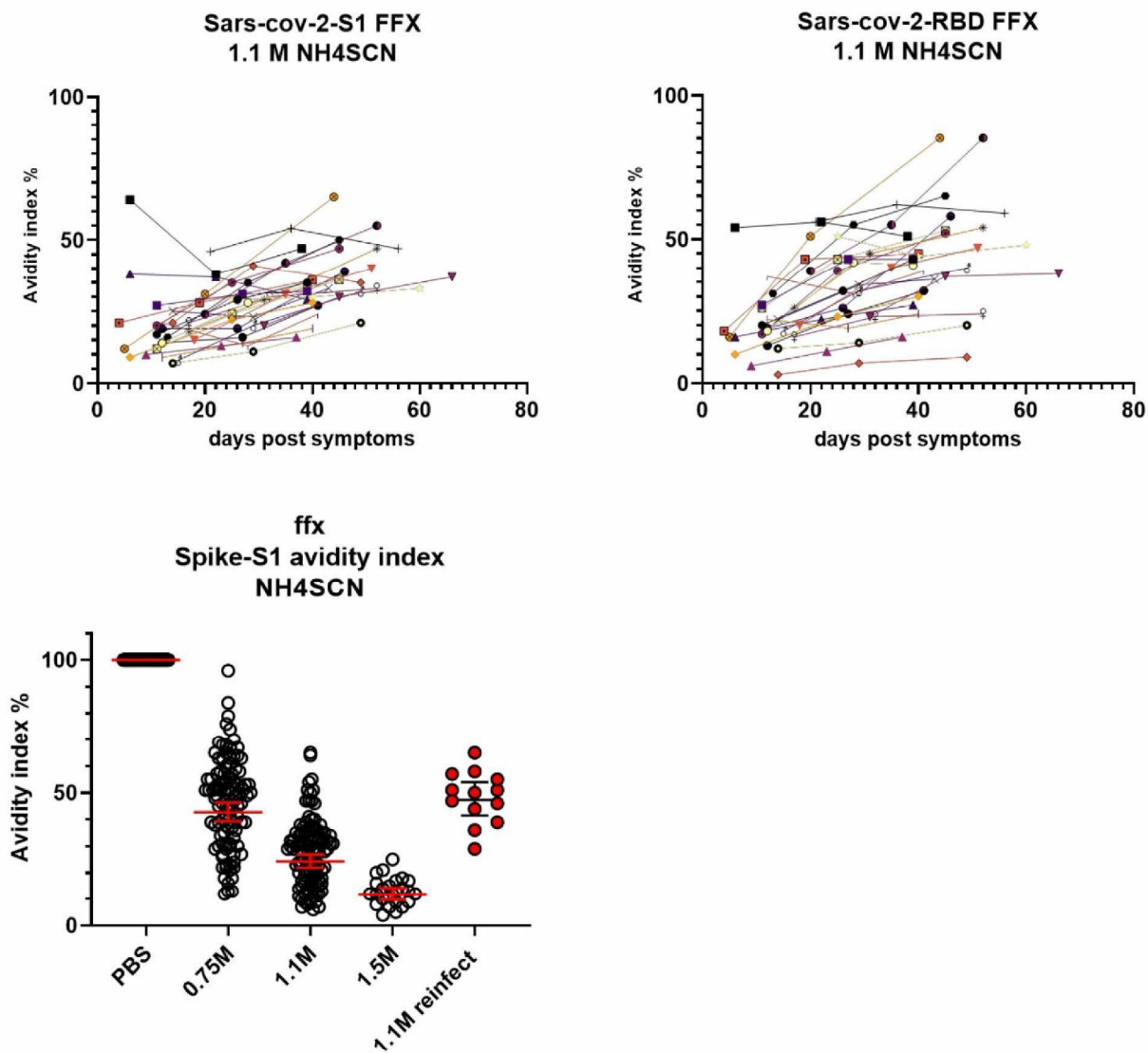
(verdunning 400 en 8000)











13 augustus Redos RBD hoge samples

ExcellGene COV proteins

woensdag 9 september 2020

10:40

Ontvangen

2x1 ml Trimeric Spike Wuhan (lot 20345PPT) @ 1mg/ml
2x1 ml Trimeric Spike D614G (lot20345PMT) @ 1 mg/ml
2x1ml RBD (Lot 203021RBD) @ 1mg/ml

OPGESLAGEN -70C

S113 (-06 IFIX)

Vriezer 4745 (31082086)

Vak 2 (van boven)

Rek 4 (van links)

Rij 2 (van boven)

Doosje incl UvU eiwitten

24 sept 2020

Conjugeren 25 ul (=25ug) aan 200 ul beads

S1 #33

RBD #58

Ampullen ca 1 ml a 1 mg/ml uitgevuld in 5x 200ul

En weer ingevroren -70C

6 okt

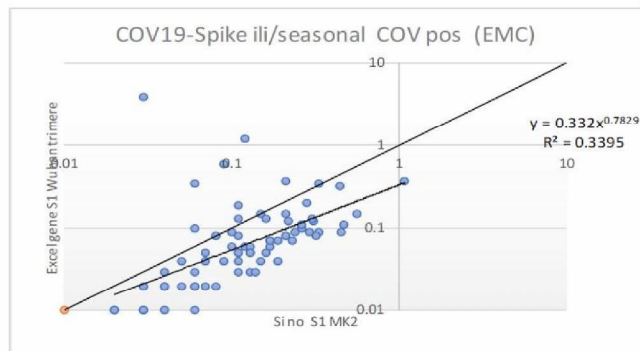
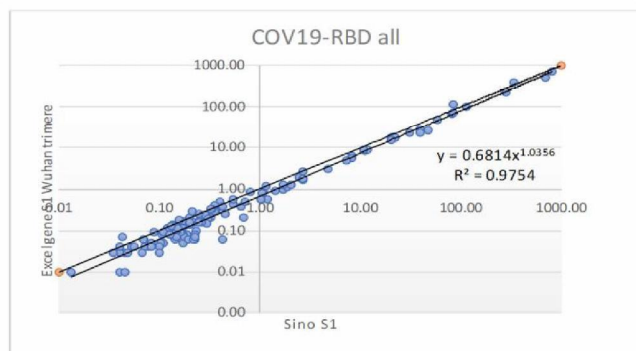
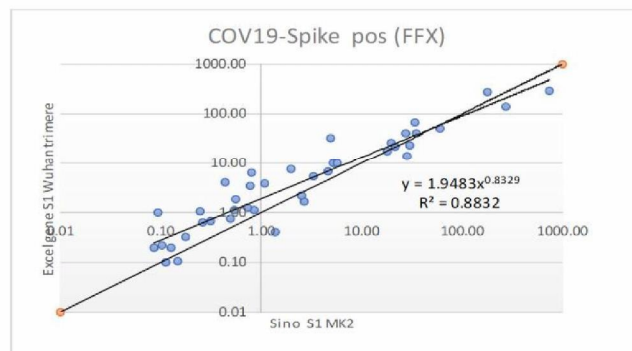
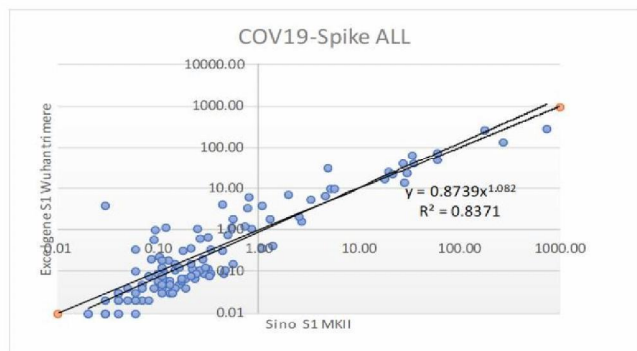
Testen 40 x ffx samples (blok 1) 400 + 8000x

Ids panel x reactive 15 samples

1x blankoi

64 x ILI samples (EMC)

R:\IIV\Afdeling - IMS\9. Assay info\Coronavirus MIA\Beads conjugation validation\Excellgene



12oct 2020

Conjugation D614 To bead #45

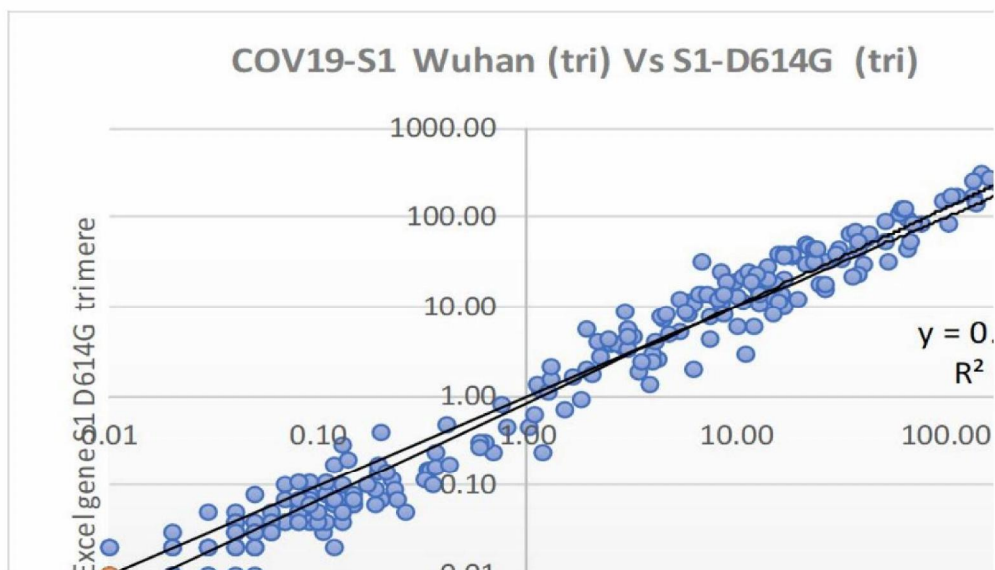
15ul (15ug) to 250ul beads.

Rest gealiquote

13 oct testen uitgebreid panel

5.1.2h

Filename; validation C1 excellgene



Opslag COV19 eiwitten

donderdag 8 oktober 2020

14:15

Ontvangen

2x1 ml Trimeric Spike Wuhan (lot 20345PPT) @ 1mg/ml
2x1 ml Trimeric Spike D614G (lot20345PMT) @ 1 mg/ml
2x1ml RBD (Lot 203021RBD) @ 1mg/ml

In PBS 7.4

Aliquoted ca 100-120ug

OPGESLAGEN -70C

S113 (-06 IFIX)

Vriezer 4745 (31082086)

Vak 2 (van boven)

Rek 4 (van links)

Rij 2 (van boven)

Tevens

Ca 400ug S1+s2 Sino

RBD + S1 + N Uvu eiwiiten

SINO bulk

S1-His tag 1 mg conc 2.4 ug/ul in PBS 7.5

40591-V08H

MM14y0702-C

RBD-His-tag

40592-V08H

MA14AP1602

500 ug @ 1.34 mg/ml

In PBS ph 7.4

Nucleocapsid His-tag

Baculo

40588-v08b

MF14AP3008

500ug @ 0.76mg/ml In 20 mM tris PH8.0