

COVID-19 research @ IIV

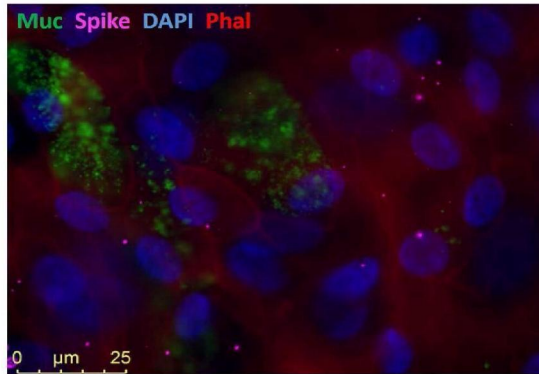
Joined IMS IMM KIM meeting October 12, 2020



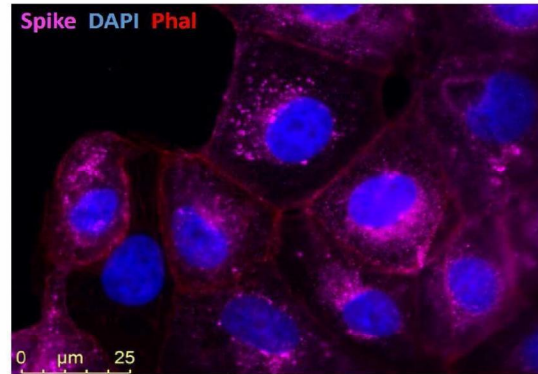
program

- Epithelial cells
- Innate immunity
- T cells
- *In vivo*, ferret infection model
- Serology
- Reinfections and serology
- B cells

Cell models: Cero versus primary nasal epithelium



96h 100x overlay all phalloidin bottom

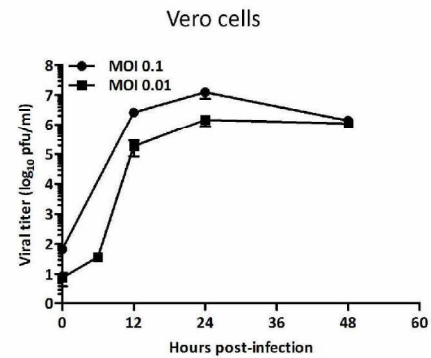
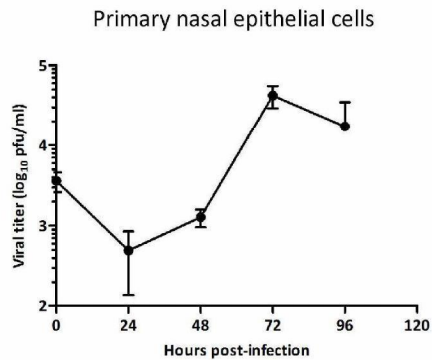


VERO SARS staining 2 100X low overlay

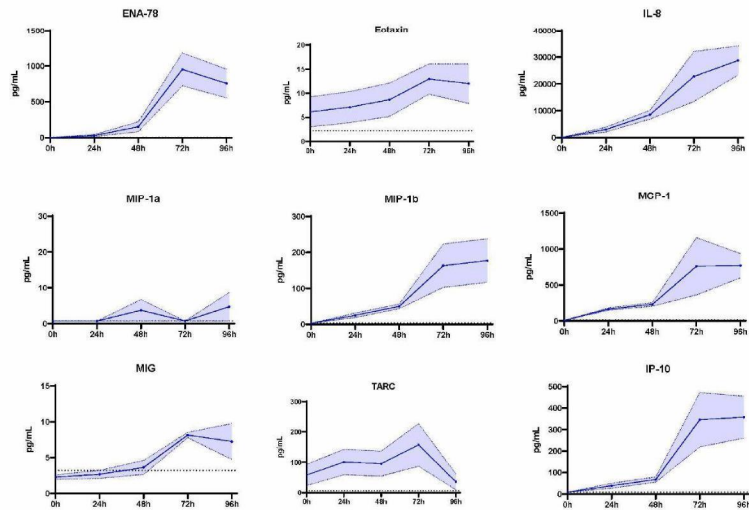


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Ministry of Health, Welfare and Sport

Viral loads



Chemokine production following infection



PMN

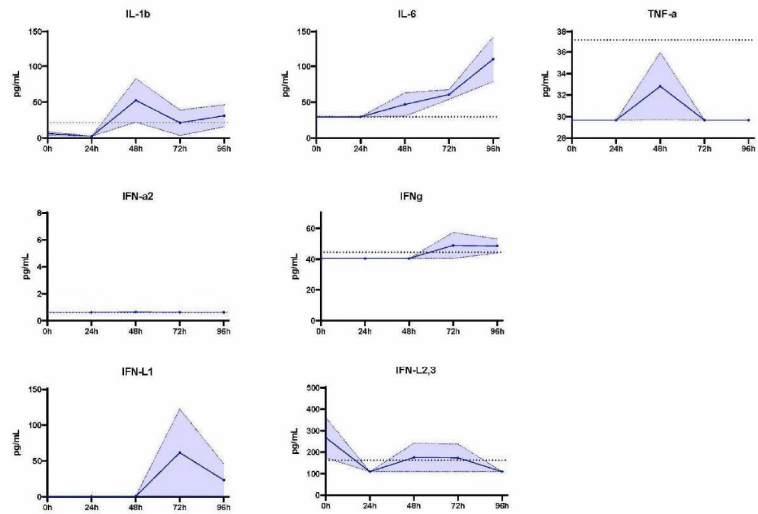
Mono/macro

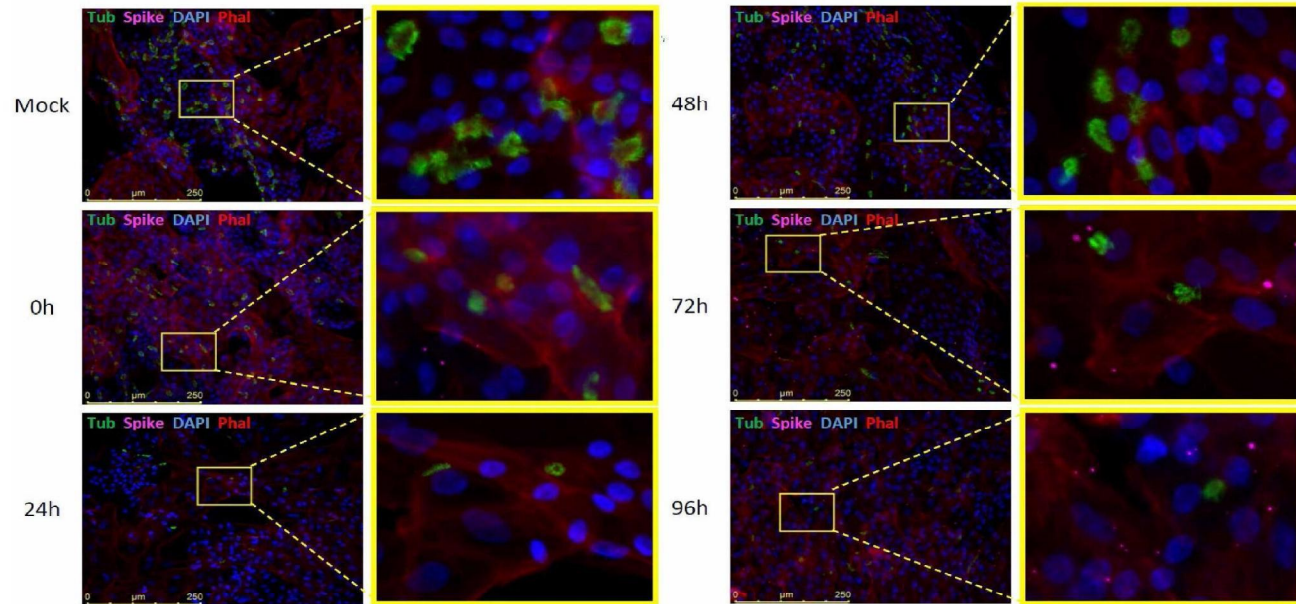
NK / T cell



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Antiviral cytokines produced following infection







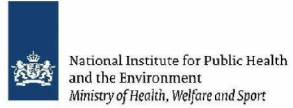
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Acknowledgements

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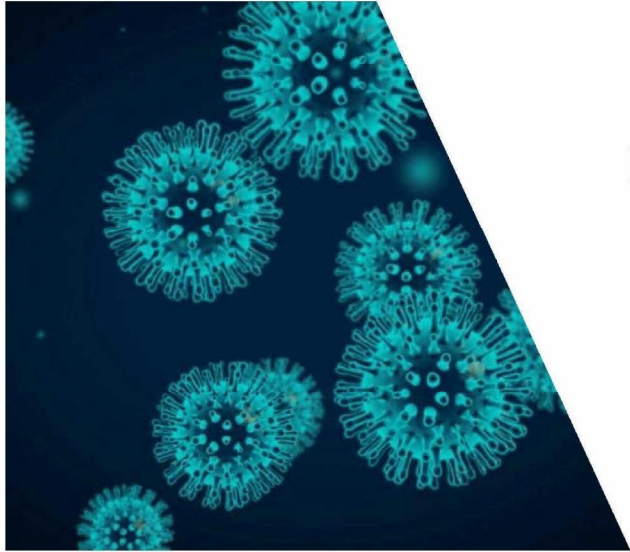
Radboud UMC

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-
-



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Innate immune responses in COVID-19: projects and First Few results

(10)(2e)

October 12th 2020

IIV-IMS joint meeting



Programma
COVID-19 Programma

UMCU:

RIVM:

VUmc:

(10)(2e)

“Understanding the two faces of the COVID-19 immune response to predict clinical course and define strategies for early and late phase intervention”

(non) Symptomatic → COVID-19 patients hospitalized/ ICU

Aim: To understand the dynamics and interaction of immune events and link it to the course of the disease



Programma
COVID-19 Programma

UMCU:

RIVM:

VUmc:

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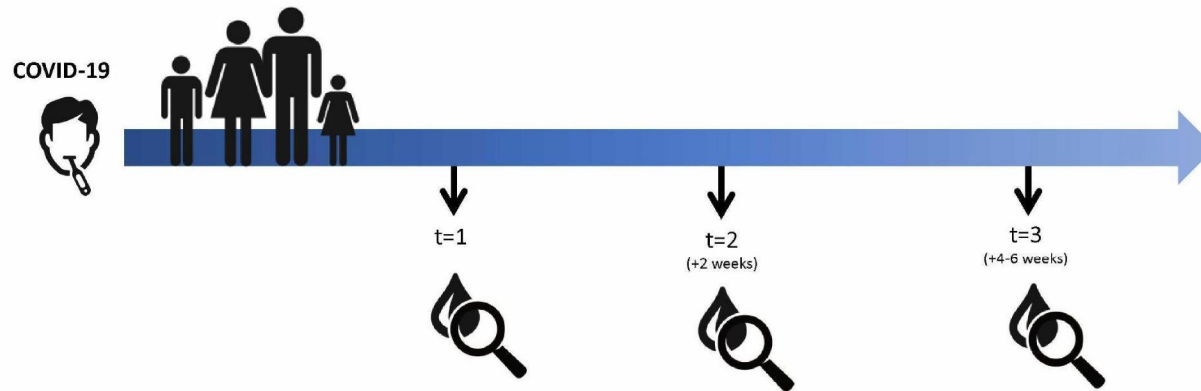
“Understanding the two faces of the COVID-19 immune response to predict clinical course and define strategies for early and late phase intervention”

- Characterization of innate and adaptive immune responses across the course COVID-19
- Identification of dysregulated networks of the circulating proteome over time (markers associated with inflammation and immune regulation) O-link technology
- Multilayer data integration, selection and validation of relevant biomarkers, and development of a comprehensive prognostic model for clinical use.



FFX-RIVM

Families with at least 1 confirmed SARS-CoV-2 infected member





FFX-Research questions

- Why do some SARS-CoV2 PCR+ individuals (non)symptomatic or hospitalized?
- Why do men get more severe symptoms?
- Can we predict severity of disease and/or successful outcome? (biomarkers)

Cellular immune responses

Innate Immune Responses

- Is there a dysregulation of the innate immune response during the course of the disease?
- Can the kinetics of PRR activation and cytokine production be used as a biomarker of disease progression and/or successful outcome?



Cellular analysis

basic phenotyping

- PPR stimulation of PBMC

deep phenotyping

- ✓ - Cytokine/chemokine secretion
(type-I IFNs, IFN γ , IL6, IL8, IL10, TNF α , IL12p70, GMCSF, IP-10, IL1 β)



innate responses

- Cellular response by flow cytometry
(CD14/CD16, CD80, CD86, CD11b, CD11c, CD163, CCR2, CX3CR1)

T cell responses

- *RNA-seq PBMC (deconvolution analysis)



Stimulation of PBMC

- ✓○ Medium control
- ✓○ Heat-inactive SARS-CoV-2
- ✓○ TLR7/8 (R848)
- ✓○ TLR2 (HKLM)
- ✓○ TLR4 (LPS)
 - TLR4 + h.i. SARS-CoV-2
 - TLR7/8 + h.i. SARS-CoV-2
 - TLR2 + h.i. SARS-CoV-2

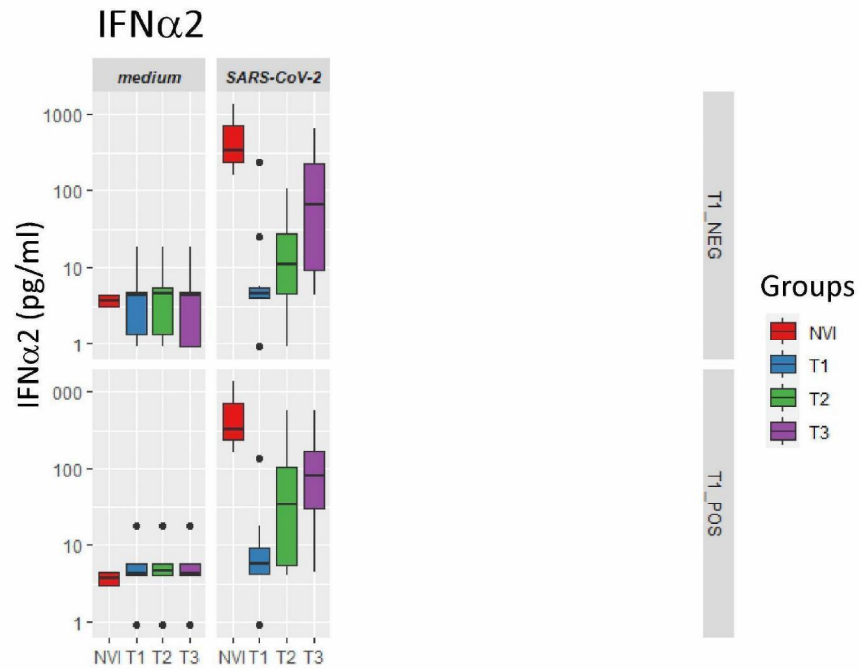


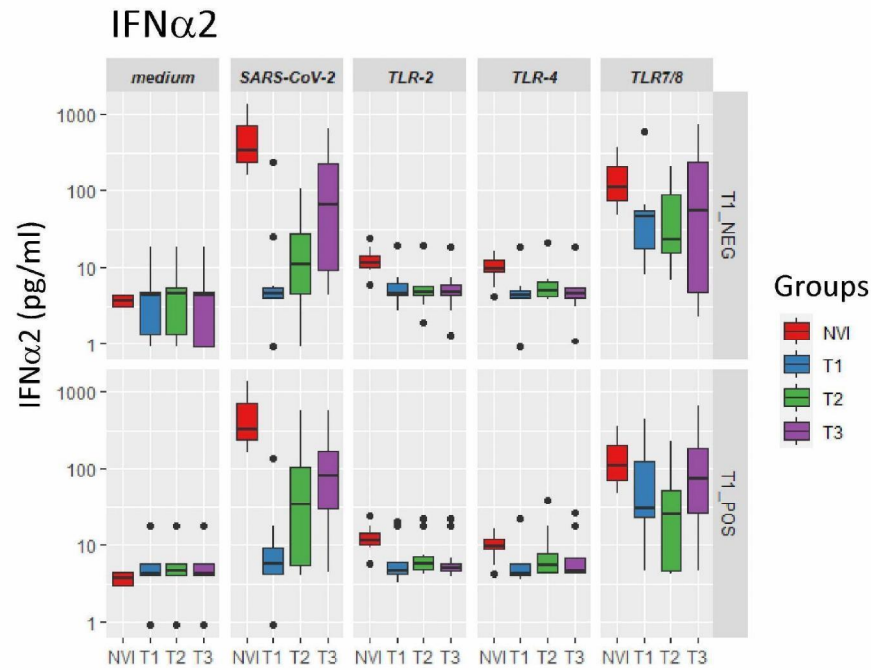
Results today: FFX selection-Innate

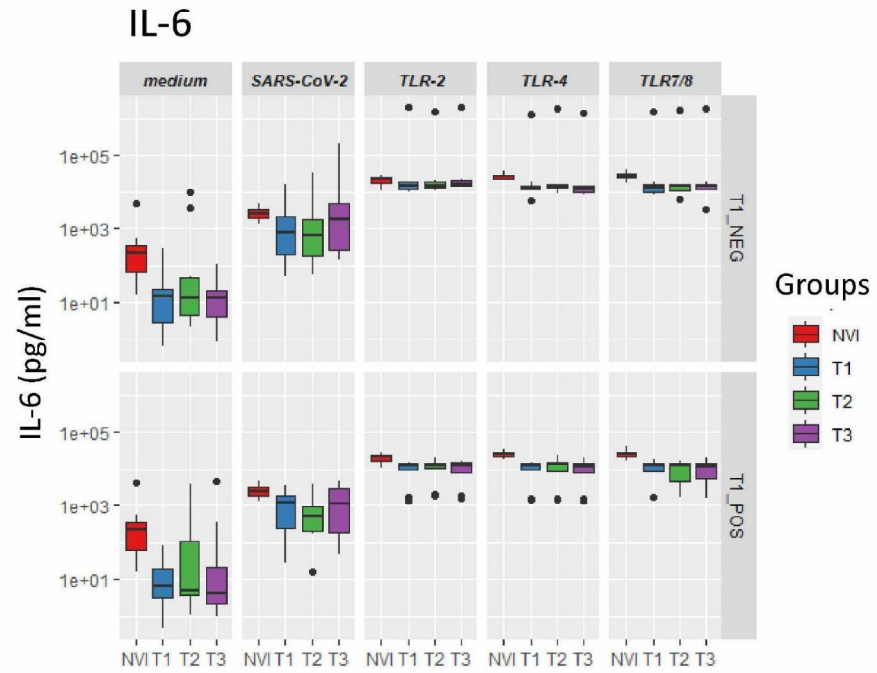
- Adults (M/F)
- SARS-CoV-2 PCR+
- Symptoms
- Few days after onset of symptoms (T1= 3-7d)

Groups

- | | |
|--|------|
| - Healthy Controls (NVI-255/2009-2010) | n=6 |
| - T1 Ab-Negative | n= 9 |
| - T1 Ab-Positive | n= 8 |









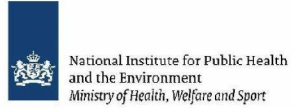
FXX- To-do

Groups

- SARS-CoV-2 PCR+ Adults/No-Symptoms
- SARS-CoV-2 PCR+ Children with/without Symptoms
- Healthy controls Children
- SARS-CoV-2 PCR-

Interpretation

Link to Symptoms; Ab, Cell subset activation,
Cytokines in sera (ZonMw); children vs Adults; Male vs Female



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COVID-19 T cell immunity

IIV - IMM/IMS/KIM MEETING 12 OCT 2020



SARS-CoV-2 immune response

Antibody response

- Neutralizing
- Help in clearance of virus and infected cells

Innate

- Cytokine storm in severe cases
- Shaping adaptive response

Adaptive response

- B cells
 - T cells
- 

T cell immunity – Research questions

Is T cell immunity induced?

What type of response?

Longevity?

Cross-reactivity with other (corona) viruses



FFX cohort

FFX – setting up T cell assays

Stimulations:

- Peptide pools
- Heat-inactivated virus
- Recombinant proteins

Read-out

- ELIspot
- Flow cytometry

24h stimulation
48h stimulation
24h pre-stimulation
Additional co-stimulation
NK cell depletion
Peptide pools
Titration heat-inact. virus
Recombinant spike protein
Other cytokine secretion
Intracellular cytokine staining

FFX – setting up T cell assays

Stimulations:

- Peptide pools
- Heat-inactivated virus
- Recombinant proteins

Read-out

- ELISpot
- Flow cytometry

- ✓ 24h stimulation
- ✗ 48h stimulation
- ✗ 24h pre-stimulation
- ✗ Additional co-stimulation
- ✗ NK cell depletion
- ✓ Peptide pools
- ✓ Titration heat-inact. virus
- ✗ Recombinant spike protein
- ✗ Other cytokine secretion
- ✗ Intracellular cytokine staining

Current conditions

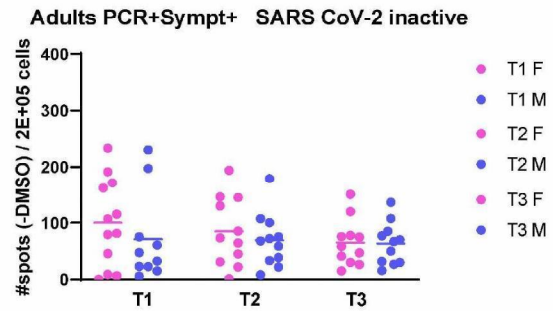
ELIspot

- Peptide pool SARS-CoV-2 spike
- Peptide pool SARS-CoV-2 nucleocapsid
- Heat-inactivated SARS-CoV-2
- Peptide pool OC-43 spike

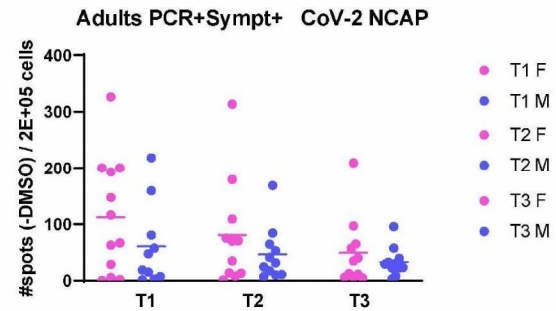
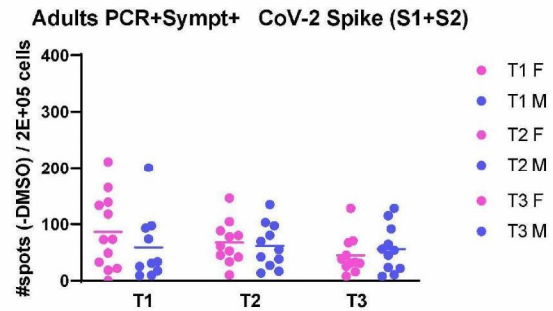
Flow cytometry



First results

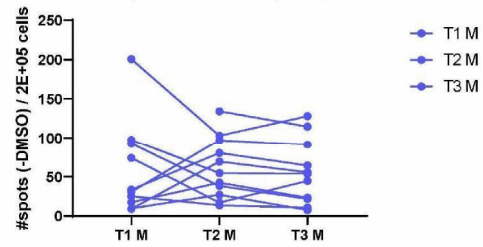


First results

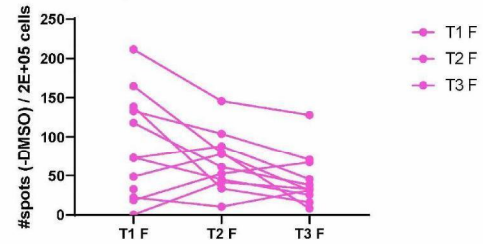


Kinetics

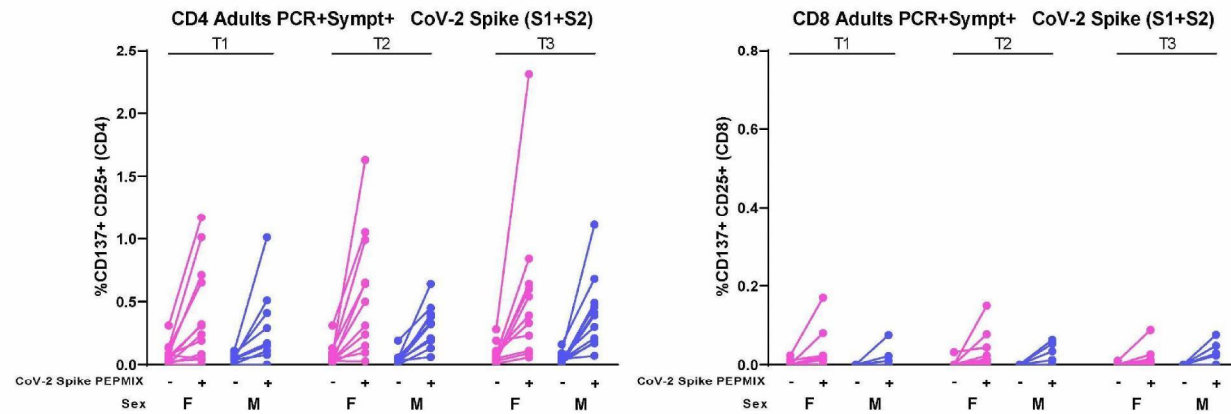
Adults PCR+Sympt+ CoV-2 Spike (S1+S2) Male



Adults PCR+Sympt+ CoV-2 Spike (S1+S2) Female

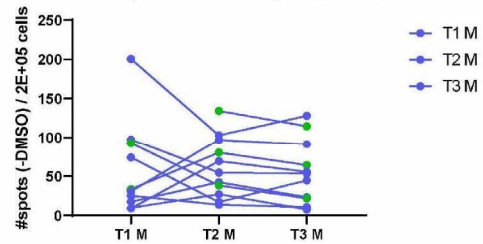


Activated T cells

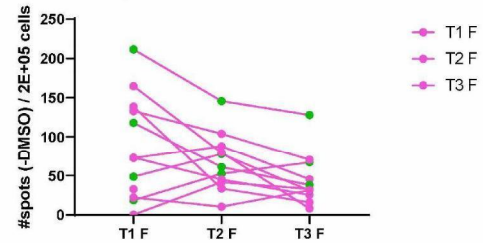


Kinetics

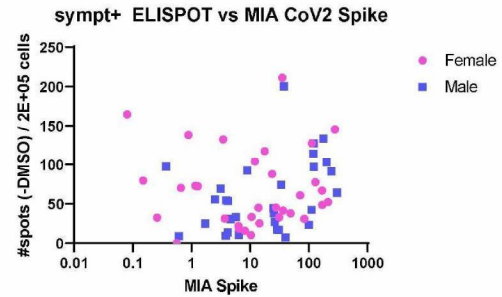
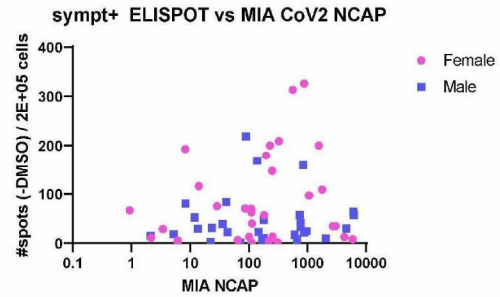
Adults PCR+Sympt+ CoV-2 Spike (S1+S2) Male



Adults PCR+Sympt+ CoV-2 Spike (S1+S2) Female



Association with antibody-levels



Next steps

T cell responses

- asymptomatic adults
- T cells responses in children

Analyses

- Flow cytometry – CD4 or CD8 response
 - Other cytokines produced?
 - Link to onset of disease; symptoms; severity (also with UMCU)
 - Link to antibody/B cell responses and deep phenotyping
- 

Future

Long-term follow up

In depth characterizations

Cross-reactivity



Acknowledgements

IIV FFX team

(10)(2e)

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FFX innate

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FFX deep phenotyping

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FFX T cell

(10)(2e)

IDS

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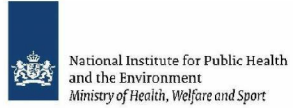
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COVID-19 samples

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BSL-3 team

(10)(2e)



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SARS-CoV-2 infection in young and adult ferrets

Is severity of disease dependent on age
and route of infection

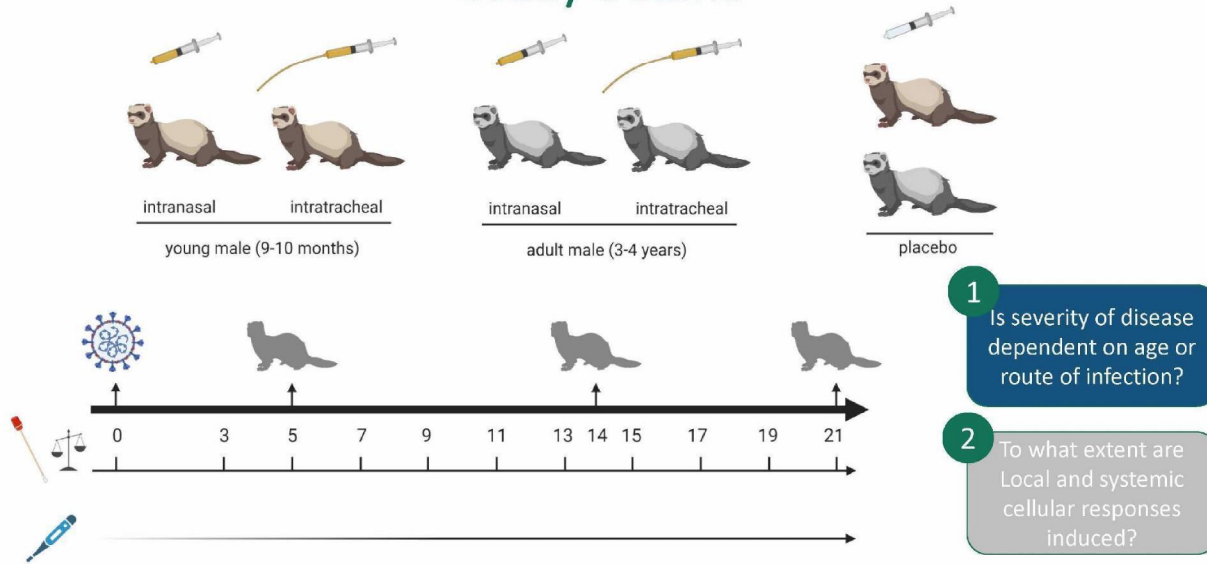
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Centre for Immunology of Infectious diseases and Vaccines
Centre for Infectious Disease Control
National Institute of Public Health and the Environment (RIVM)
The Netherlands



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Ministry of Health, Welfare and Sport

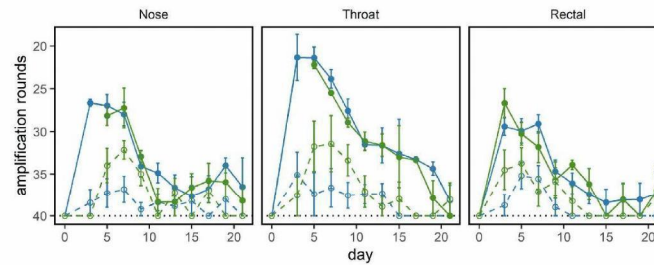
Study outline





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Viral RNA and replication in swabs

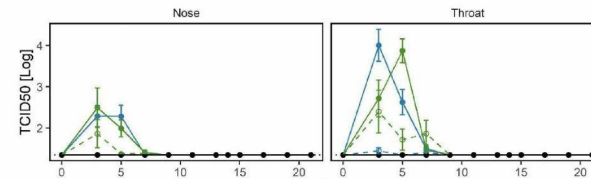


Detection of Viral RNA 5 d.p.i. in:

Respiratory system

Brain

Gut

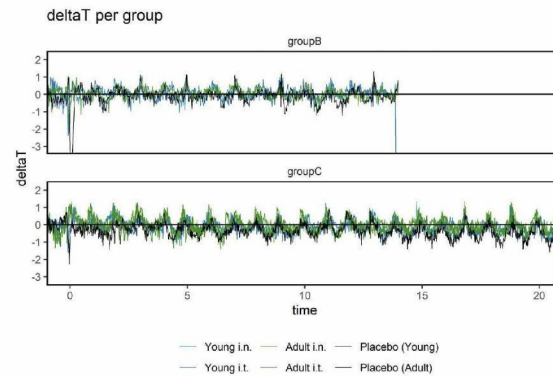




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Clinical Signs

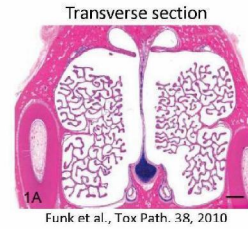
- No weight loss
- No Fever
- No change in breathing or activity
- Diarrhea (intranasally infected group)





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Pathology of the Nasal Turbinates

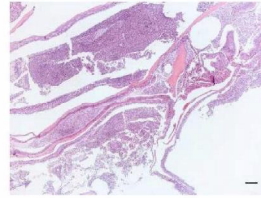
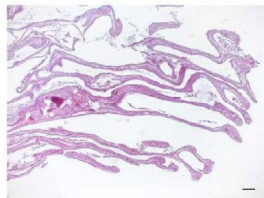
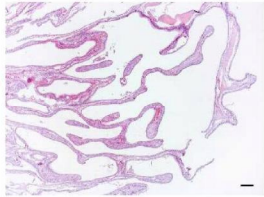


Normal

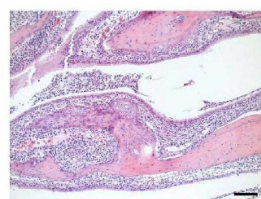
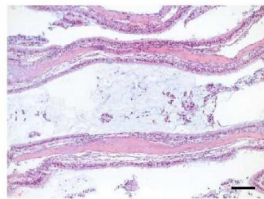
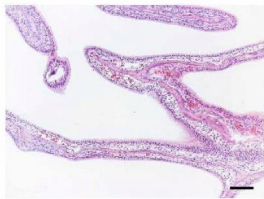
Moderate

Severe

2.5x



10x





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Pathology of the lung

Normal



Perfused lungs

Minimal

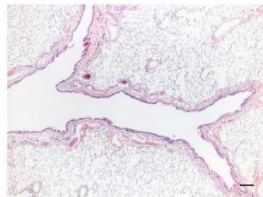


Moderate



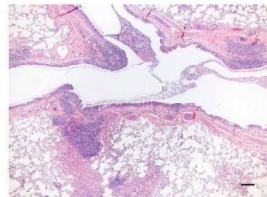
Follicular hyperplasia:

Normal

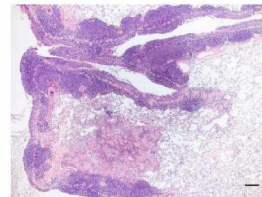


2.5x

Moderate



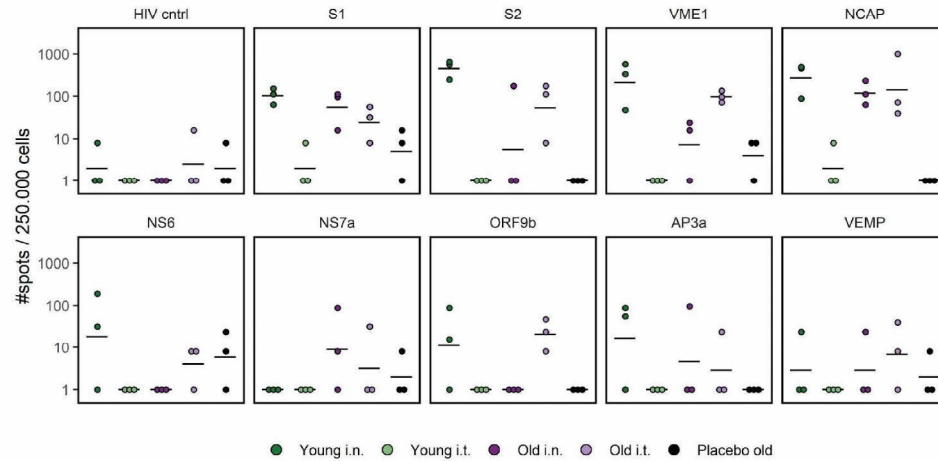
Severe





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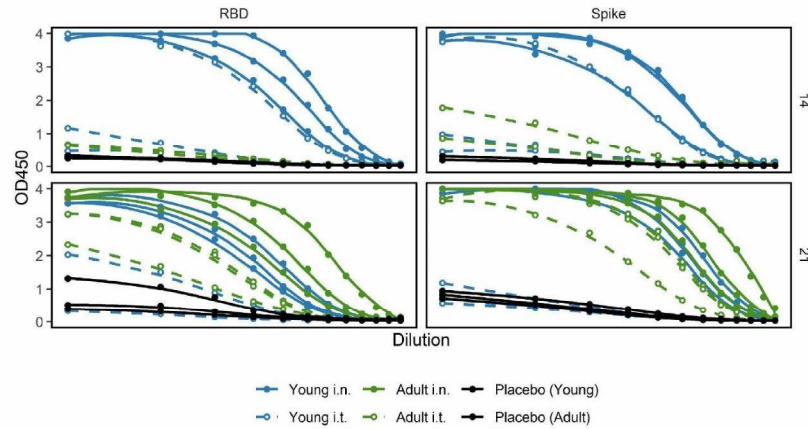
Responses against pep. pools – Lung (21 days)

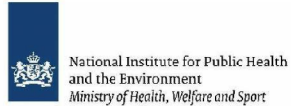




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Antibody response Against Spike and RBD (ELISA)





Conclusions and goals

- The young ferret intranasal SARS-CoV-2 infection model is a model that represents a persistent common cold (without fever) and similar virus dissemination as in humans.
- T-cel (local and systemic) and Antibody response responses can be clearly detected.
- Nor intratracheal infection, nor age are a factor for increase of disease severity in ferrets.

Future plans:

Compare immunity (humoral and cellular) induced by different vaccine concepts and the relation to protection against infection.



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Acknowledgements

Department of immune mechanisms (IMM), RIVM

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Centre for Infectious Disease Research, Diagnostics and Laboratory
Surveillance (IDS), RIVM

- (10)(2e) (10)(2e) (10)(2e) (10)(2e)

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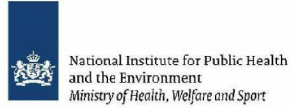
Microscope consultancy

- (10)(2e)

Animal Research Centre (ARC), Poonawalla Science Park

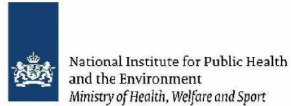
- (10)(2e)

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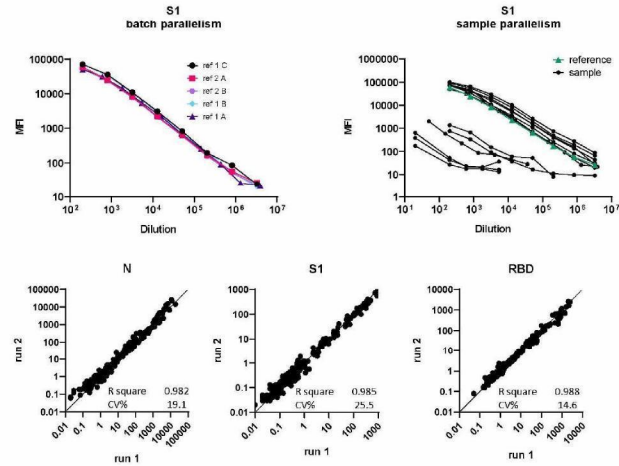


Serology for SARS-CoV-2

- Identification of infection (in addition to PCR)
- Serosurveillance at population level
- Immunity
 - Protection?
- Develop a multiplex bead-based immunoassay to
 - Study seroprevalence
 - Investigate host immune responses
- Approach
 - Nucleoprotein, Spike subunits S1 and RBD

Assay development

- Parallelism between refs and samples

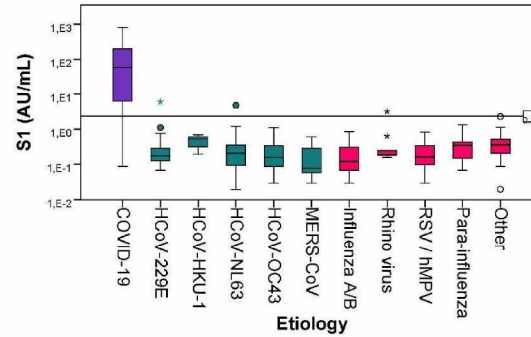
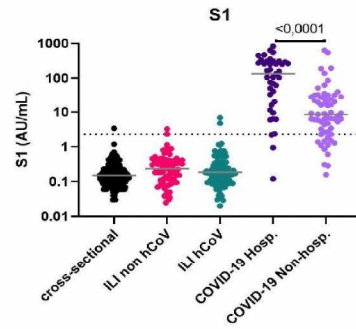


- Reproducibility



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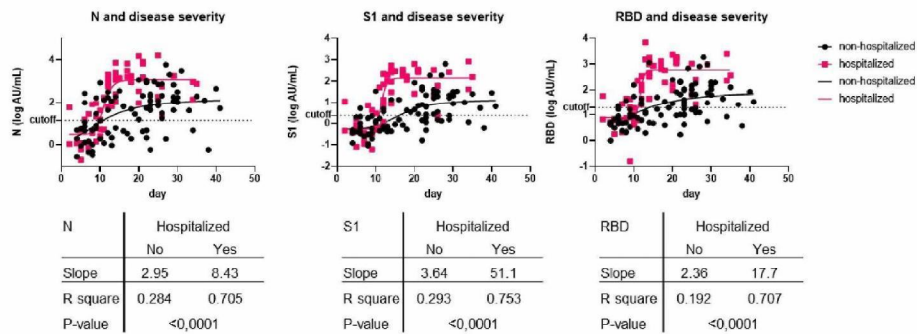
Ability to discriminate samples with different etiology





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Severity of symptoms

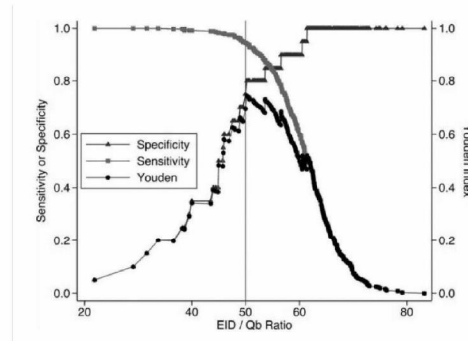




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Translate assay to serosurveillance

- Clinical background, time since infection
- Assay sensitivity and specificity
- True seroprevalence

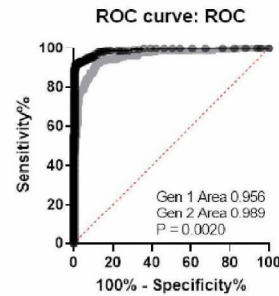
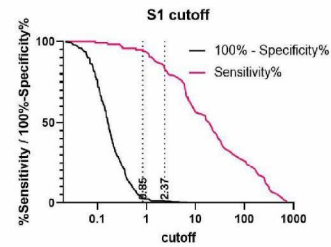
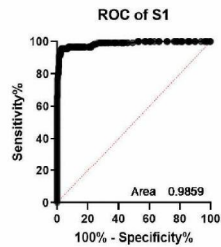




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ROC analyses to assess seroconversion

- Establish cutoff
- Determine assay characteristics
- Assay MarkII



Likelihood ratio 342



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Assay performance and true prevalence determine accuracy of prevalence estimates

Table S1. Prevalence estimated by the test relative to true prevalence and different levels of specificity and sensitivity

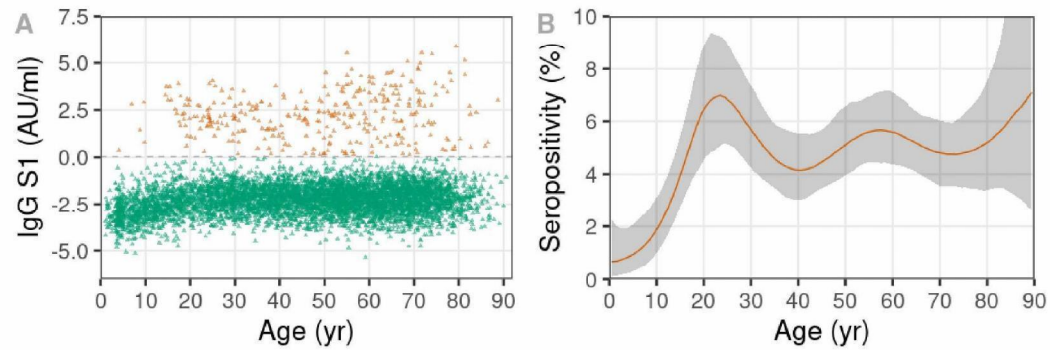
Specificity	Sensitivity	true prevalence							relative error
		1	3	5	10	20	50	90	
100	100	1,00	3,00	5,0	10,0	20,0	50,0	90,0	+/- 5%
	98	0,98	2,94	4,9	9,8	19,6	49,0	88,2	+/- 10%
	95	0,95	2,85	4,8	9,5	19,0	47,5	85,5	+/- 20%
	90	0,90	2,70	4,5	9,0	18,0	45,0	81,0	
	85	0,85	2,55	4,3	8,5	17,0	42,5	76,5	
	80	0,80	2,40	4,0	8,0	16,0	40,0	72,0	
99	100	1,99	3,97	6,0	10,9	20,8	50,5	90,1	
	98	1,97	3,91	5,9	10,7	20,4	49,5	88,3	
	95	1,94	3,82	5,7	10,4	19,8	48,0	85,6	
	90	1,89	3,67	5,5	9,9	18,8	45,5	81,1	
	85	1,84	3,52	5,2	9,4	17,8	43,0	76,6	
	80	1,79	3,37	5,0	8,9	16,8	40,5	72,1	
98	100	2,98	4,94	6,9	11,8	21,6	51,0	90,2	
	98	2,96	4,88	6,8	11,6	21,2	50,0	88,4	
	95	2,93	4,79	6,7	11,3	20,6	48,5	85,7	
	90	2,88	4,64	6,4	10,8	19,6	46,0	81,2	
	85	2,83	4,49	6,2	10,3	18,6	43,5	76,7	
	80	2,78	4,34	5,9	9,8	17,6	41,0	72,2	



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PiCo2 Prevalence by age

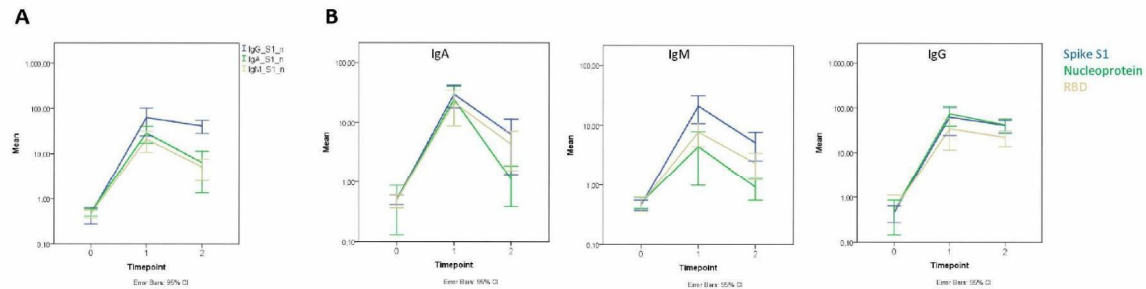
- N~7400
- Self-collected finger-prick blood





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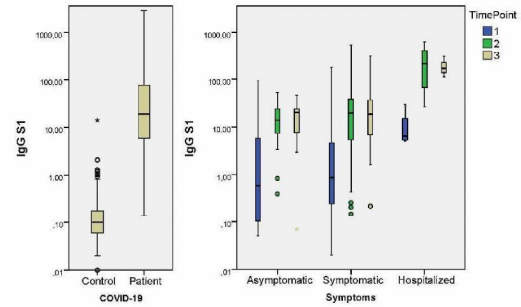
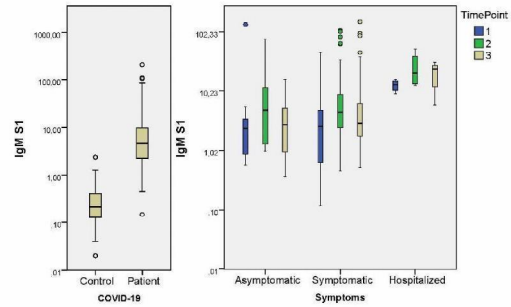
Ig kinetics vary between isotype and specificity (PiCo)





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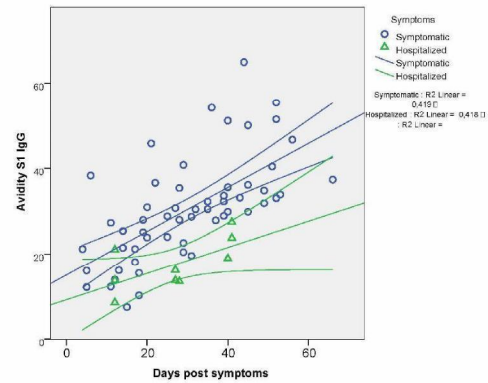
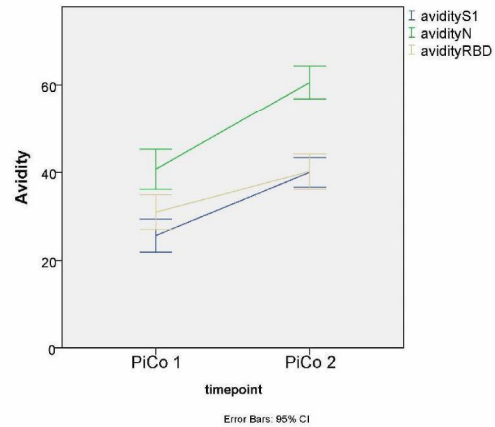
Kinetics (FFX)

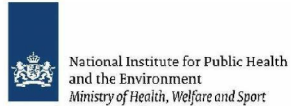




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IgG avidity





Conclusions

- A reproducible, quantitative multiplex assay
- Characterized and evaluated for accurate seroprevalence studies
- Used to assess seroprevalence (few μL), longitudinal (PiCo)
- Able to precisely study kinetics (PiCo, FFX)
 - IgG, IgA, IgM, Avidity
- Decay of IgA, persistence of IgG, specificity dependent
- Increasing IgG avidity



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Acknowledgements

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- Study participants
- Volunteers
- IIV sidekicks
- PiCo study team
- FFX study team

SARS-Cov-2 re-infection cases (The Netherlands 2020)

101261 1101261 (10)(2a)

Compilation of serological data shared with IDS, and WHO/lab working group

- antibody response after primary SAR-Cov-2 infection
- description of re-infection cases
- antibody response after re-infection
- discussion

Acknowledgements

Medical Microbiology Labs

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Municipal Health Services

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..and several others

RIVM LCI

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..and several others

RIVM Virology & Immunology collaboration.

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First notification of reinfection in Hong Kong

33-yr male with good past health

First episode:

- 23 Mar: fever, throat ache, cough for 3 days
- 26 Mar: PCR pos
- 29 Mar: hospital, symptoms subsided, PCR pos
- 02 Apr: PCR pos, IgG neg
- 14 Apr: discharged 3x PCR neg

Second episode:

- 15 Aug: return from Spain, routine screening at airport, asymptomatic, 4.5 mo after first
- 17 Aug: PCR pos, IgG neg
- 19 Aug: PCR pos, IgG neg
- 21 Aug: PCR pos, IgG pos

Determined by WGS: 24 nt difference

- Phylogeny: GISAID Clade V vs G, Nextstrain Clade 19A vs 20A, Pangolin B.2 vs B.1.79

Additional confirmed reinfections in other countries:

- US, Belgium, Ecuador, India, Netherlands, South Africa

<https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa1275/5897019>



Caveats when assessing reinfection

Prolonged duration of virus shedding can appear as reinfection

- Virus replication continues but viral load decreases below limit of detection of the NAAT:

false negative

- *This may be interpreted as reinfection as in this case a positive test result can occur after one or multiple (false) negative PCR tests*
- *Viral persistence is not uncommon and can last up to 100 days*

Potential coinfection with different strains

Recrudescence/re-activation

- Unlikely/not expected for SARS-CoV-2



Laboratory Technical Working Group

1. Definition
2. Case confirmation (clinical, epidemiological, laboratory)
 - Molecular
 - Sequencing (clonal spread)
 - Serology
3. Clinical relevance (symptoms)
4. Epidemiologic relevance (quarantine measures)
5. Surveillance relevance
6. Publication
7. Research questions



Questions to be answered

Is there a minimum elapsed time between two consecutive SARS-CoV-2 infections to be considered?

Which laboratory methods need to be performed?

Which clinical specimens need to be collected from first and subsequent episode?

Is genome sequencing needed, which interval and by what technique(s)?

Does immunity status play a role and how should this be determined?

- T-cell, Ab-mediated, avidity, VNT

What would be the clinical criteria?

SARS-Cov-2 re-infection cases

cases

- 30-35 assumed re-infection cases under investigation
- mostly healthcare workers, PCR+ diagnosis in march/april
- clinical symptoms, non-hospitalized
- **second disease episode, varying from 12-133 days after first episode**
- laboratory-confirmed again (PCR+)
- additional serum collected (specific GGD visits)
- extensive molecular characterization, including sequencing

criteria

- first and second 'episode' confirmed by PCR
- in between 'healthy/virus-free' period of ~2 months
- availability of serum (n=18)

basics of the 18 selected cases:

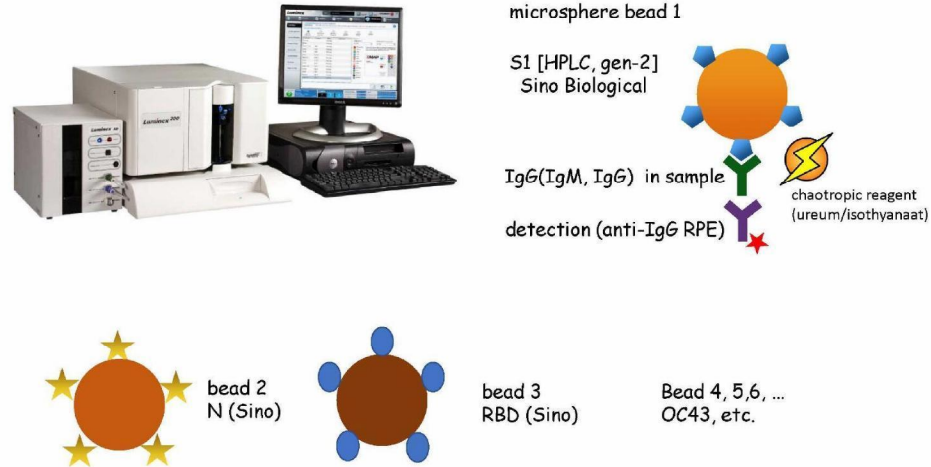
- median age: 51 years (range 18-85 years)
- male/female: 8/10
- median days between first and second episode: 85 days (57-133)

Serological profiling

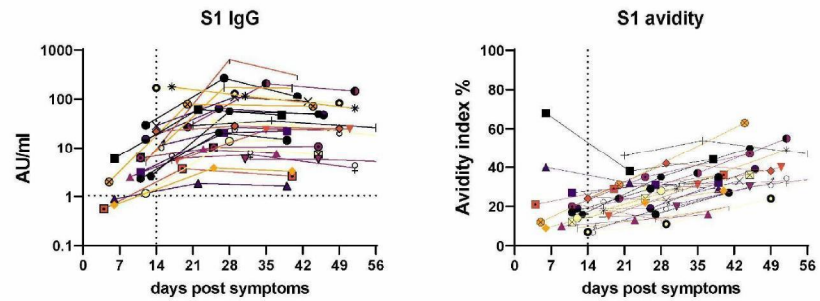
- IgG response after primary COVID-19 infection (MIA data)
- Serological profile after re-infection
 - MIA data (S1, N, RBD)
 - Virus neutralization (VNT) & Wantai data
 - IgG avidity (MIA)
- Correlation(s) between data, statistics
- Added value of serology in SARS-Cov-2 in diagnostic & population screening
- Serology and (molecular) virology as instruments in defining infectiousness

SARS-Cov-2 serology: multiplex bead assay (MIA)

Including avidity (ab-ag binding strength)

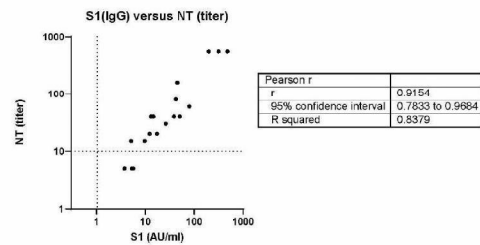
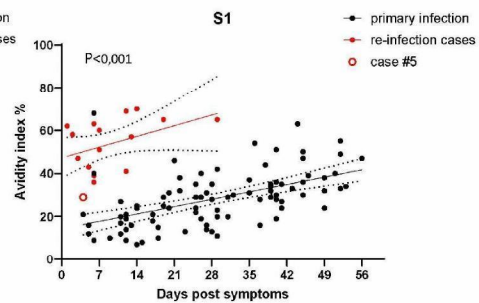
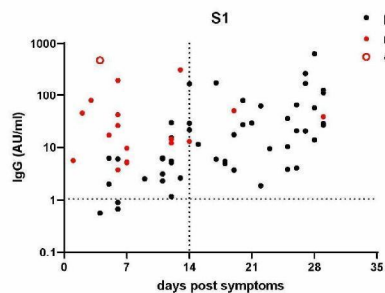


IgG quantitative response to S1 in relation to avidity maturation
(improved mk-2 MIA: specificity/sensitivity 99.7-99.9% / > 95%)



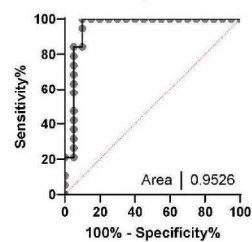
IgG concentrations of re-infection cases (n=18)

... and avidity



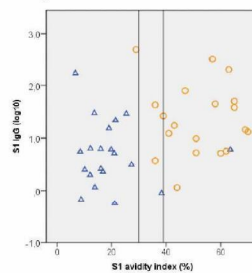
Optimal avidity 'cut-off' to define re-infection

ROC of Avidity Index S1 d1-14

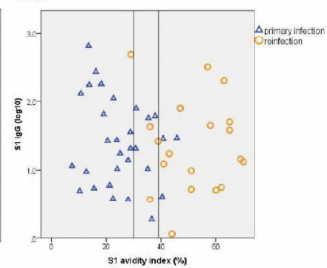


Cut-off	Sensitivity%	95% CI	Specificity%	95% CI
> 26.38	100.0	83.18% to 100.0%	85.00	63.96% to 94.76%
> 28.17	100.0	83.18% to 100.0%	90.00	69.90% to 98.22%
> 32.50	94.74	75.36% to 99.73%	90.00	69.90% to 98.22%
> 37.17	84.21	62.43% to 94.48%	90.00	69.90% to 98.22%
> 38.67	84.21	62.43% to 94.48%	95.00	76.38% to 99.74%

day 1-14

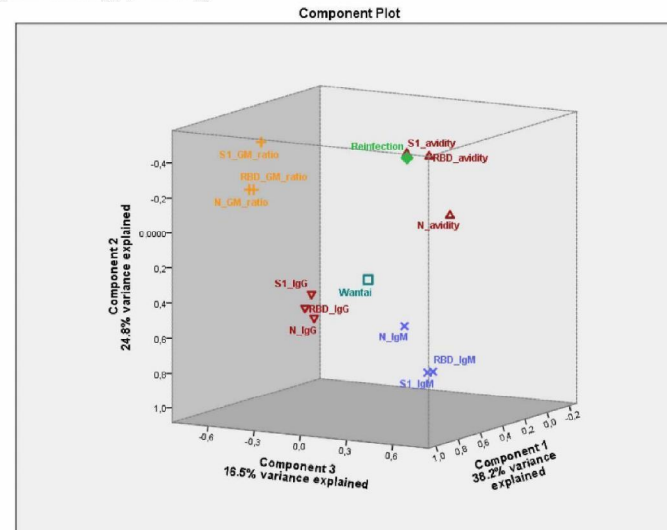


day 15-30



Principle component analysis; multivariate method/visualization of different types of data

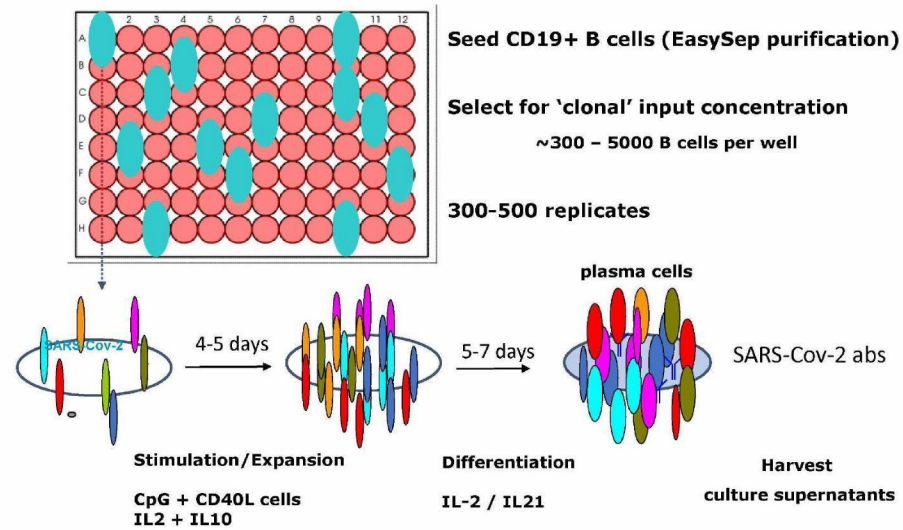
1. IgG against 3 SARS-Cov-2 proteins (S1, N and RBD)
2. avidity (against S1, N and RBD)
3. SARS-Cov-2 specific IgM and quantitative IgG/IgM ratio
4. SARS-Cov-2 diagnostic serology (Wantai Ig)



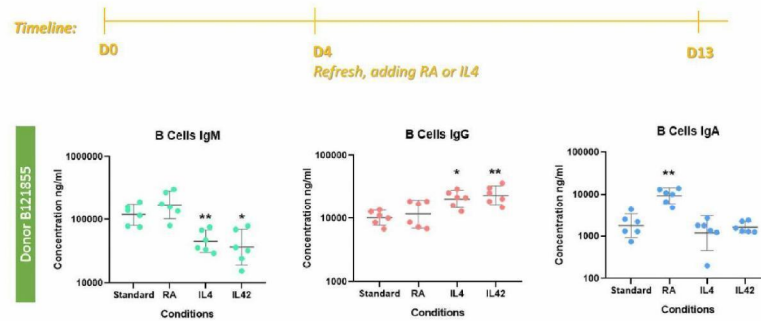
Summary, discussion

- Majority of re-infection cases show high avidity (IgG) antibodies
- IgG avidity supports best discrimination between recent and past Cov-2 infection
- Quantitative IgG and VNT data: high titers support secondary immune response as a result of re-infection
 - > Discrimination dependent on timing of sampling (< 7 days)
- Other serological data (ie IgM) could be usefull, preferably in combination with IgG (eg ratio)
- One exceptional case at day 4 with very high IgG (and VNT) antibodies, but of low avidity
- Implications for infectiousness
 - Viral loads: PCR Ct-values (nasopharyngeal swabs), recovery of infectious virus on cell lines
 - Secondary (booster) immune response instrumental for rapid viral clearance > protection

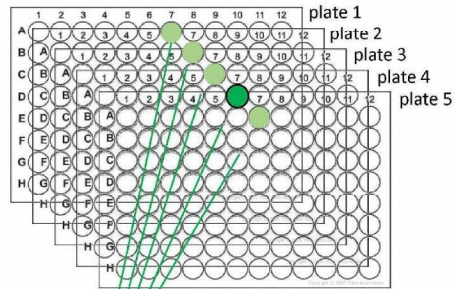
Memory B cell responses in COVID-19
analysis of specificity and function of memory B cells at the 'clonal' level



Improved plasmacell formation (ab production) in the presence of RA (retinoic acid) and IL4



- ❖ IL-4 suppresses IgM production
- ❖ IL-4 → memory B cells → IgG production
- ❖ RA → memory B cells → IgA production

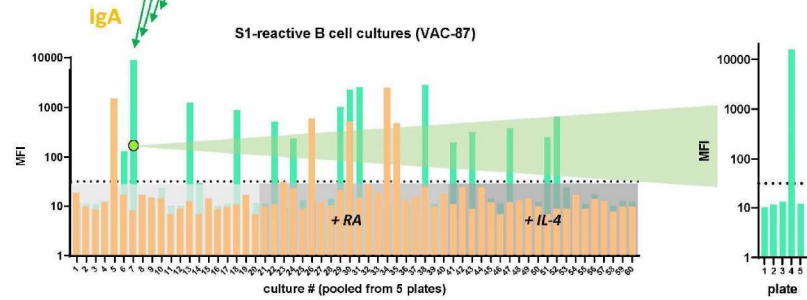


VAC-87, seropositive for SARS-Cov-2
1000 CD19+ B cells per well (60 cultures/plate)

- 5 plates (300 individual cultures)

2-step expansion procedure
 - cpg/CD40L + IL2/IL10 (proliferation)
 - IL2+IL21 [RA/IL4] (plasmacells)

- harvest individual supernatants (day 11)
- Pool sups per position from each plate for 1st testing
- test by (COVID-19) multiplex MIA
- 2nd testing of positive pools for B cell clonality

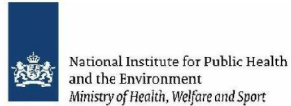


B cell memory roadmap COVID-19

- Repertoire analysis
 - clonal specificities (N, S1, S1S2 mono/trimer, RBD, ..)
 - development/ longevity
 - isotype contribution (eg mucosal, IgA)
- Maturation, function (avidity, VNT), duration
- cross-reactivity with eg human coronaviruses (array/luminex)
- vaccine response: diff. vaccine/wildtype virus response
- Relate mem-B development to Th
- Recall immune response to re-infection, CoP

Acknowledgements:

(10)(2e), (10)(2e), (10)(2e), (10)(2e)
(10)(2e) (students)
(10)(2e)



Ongoing projects

- Gene expression profiling of SARS-CoV-2 infected innate cells: a role for epigenetic reprogramming
 - (10)(2e) 1 technician
- SARS-CoV-2-specific T cell responses in COVID-19: characterization, cross-reactivity and longevity
 - (10)(2e) 1002e 1002e; 1 Post doc
- Immunity induced by different SARS-CoV-2 vaccine concepts and correlation with protection in the ferret model
 - (10)(2e) 1002e (10)(2e); 1 Post-doc + 1 technician
- SARS-CoV-2 epithelial infection model and antibody Fc-functionality
 - (10)(2e) 1002e (10)(2e); 1 technician
- At IMS, (10)(2e) technician, 2 post-docs, technician
 - In-dept and functional serology
 - Big data analyses
 - Support for PiCo