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COVID-19 serology

October 28, 2020

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IIV COVID-19 meetings

- IMM-IMS-KIM joined meeting
  - Birds-eye view
- Subsequent meetings
  - More details / in-dept
  - Discussion
    - Aims
    - content
  - Connection to other COVID-19 projects

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Acknowledgements

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

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Serology for SARS-CoV-2

- Identification of infection (in addition to PCR)
- Serosurveillance at population level
- Cost-effective
- Highly quantitative

- Develop a multiplex bead-based immunoassay to
  - Study seroprevalence
  - Investigate host immune responses
- Approach
  - Nucleoprotein, Spike subunits S1 and RBD

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Program today

- Seroprevalence in the Pienter-Corona study
- Extension of the assay: IgM, IgA, avidity
- Longevity of antibody responses
- Antibodies in relation to symptoms
- Spike S1 versus Trimeric Spike and D614G

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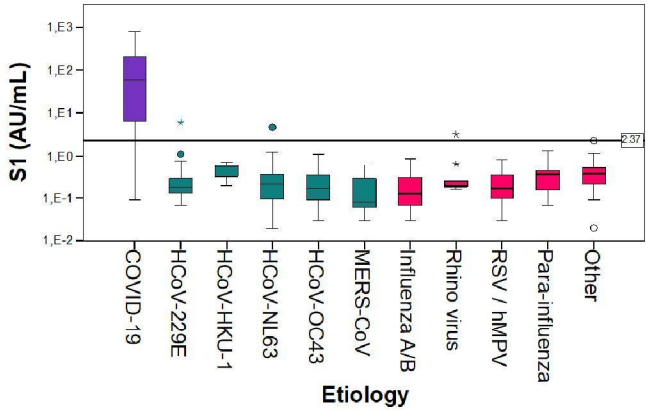
Ability to discriminate samples with different etiology



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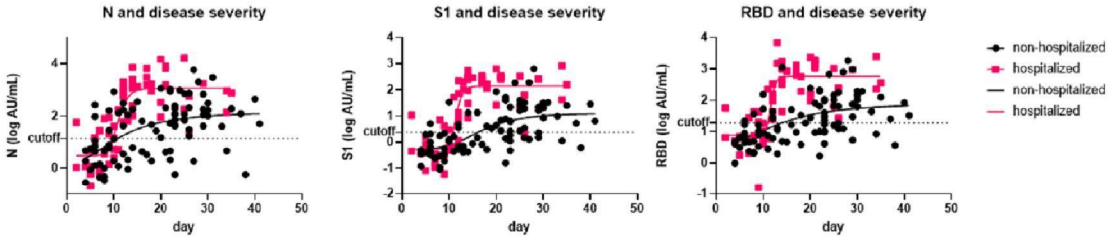
Severity of symptoms



N	Hospitalized	
	No	Yes
Slope	2.95	8.43
R square	0.284	0.705
P-value	<0,0001	

S1	Hospitalized	
	No	Yes
Slope	3.64	51.1
R square	0.293	0.753
P-value	<0,0001	

RBD	Hospitalized	
	No	Yes
Slope	2.36	17.7
R square	0.192	0.707
P-value	<0,0001	

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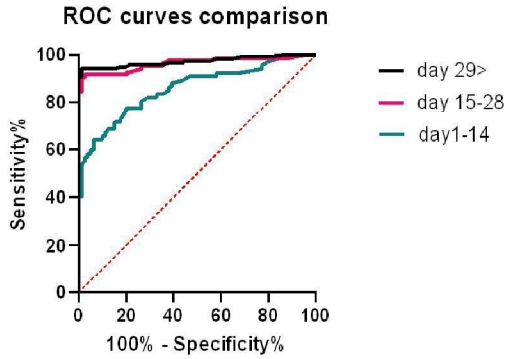
Sample selection ~ Assay performance

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



	Cutoff	Sensitivity%	Specificity%	Likelihood ratio	Area	SE	z	P
Day 1-14	> 0.5614	53,73	98,73	42,45	0,858	0,0318	reference	
Day 15-28	> 0.6618	90,48	98,73	71,48	0,964	0,0151	3,023	**0,0025**
Day >29	> 0.6350	94,21	98,73	74,43	0,973	0,0114	3,404	**0,0007**

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The PiCo study

- Pienter 3 – 2016/2017
  - Municipality-based
- PiCo1 – April – N ~ 3400
  - Within P3
- PiCo2 – June – N ~ 7400
  - PiCo1 + additional random sample
- PiCo3 – October – N > 6500

- Extensive questionnaire

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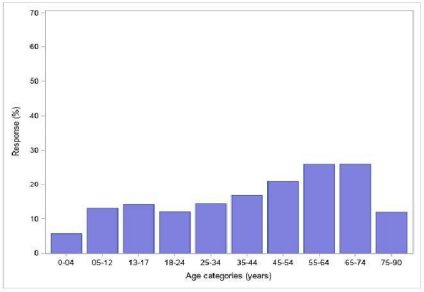
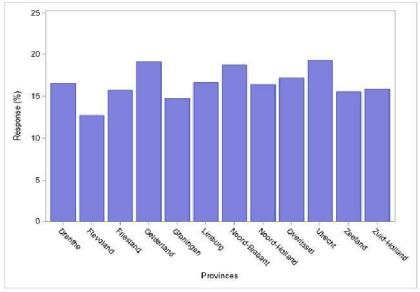
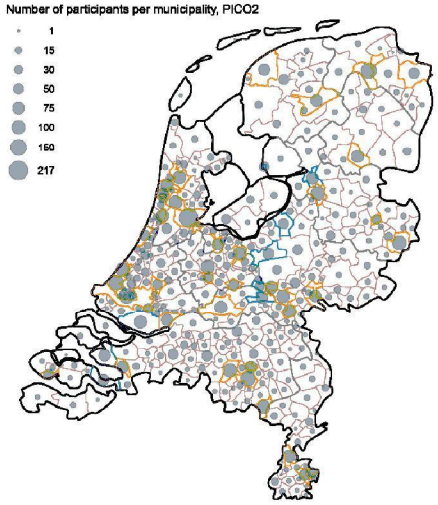
PICO2 – June, 2020

- **Longitudinal measures:**
  - PICO2-NS sample: n=> 2,300
  - PICO2-LVC sample: approx. 500

- An additional sample to enhance the geographical spread (sampling across the NL and all ages)
  PICO2-PLUS: extra n=> 4,600







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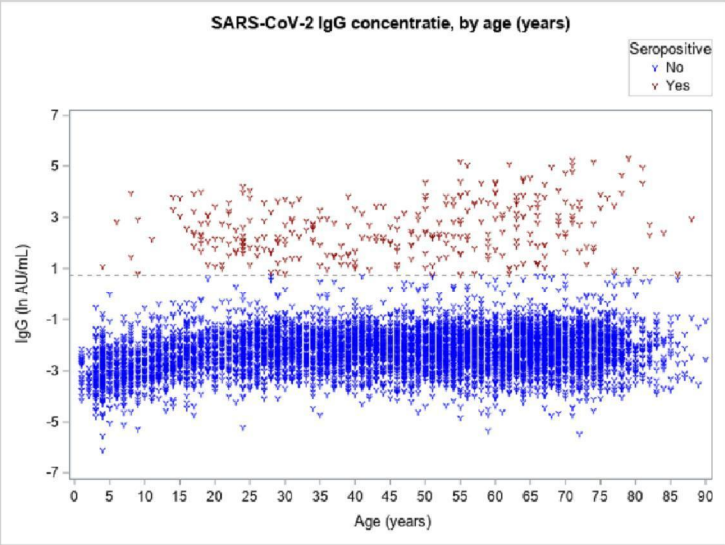
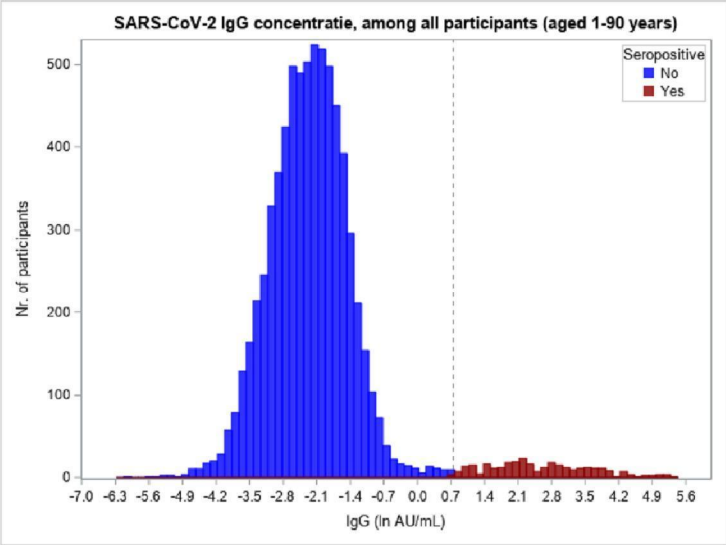
SARS-CoV-2 IgG concentration (S1, In AU/mL), PICO2





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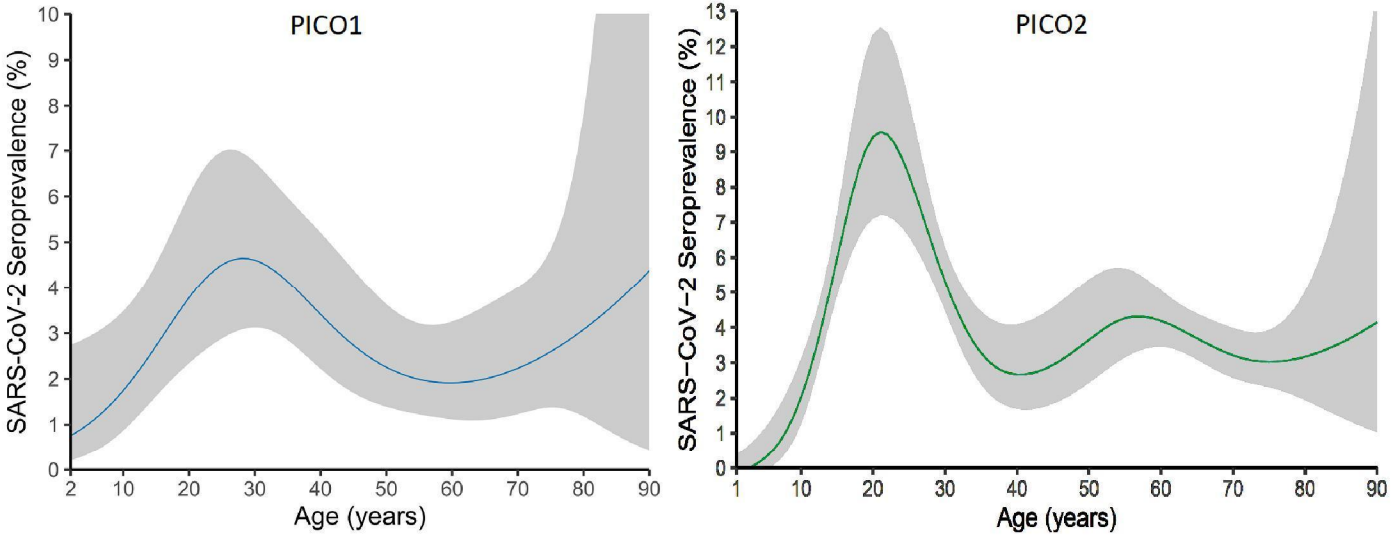
April versus June 2020



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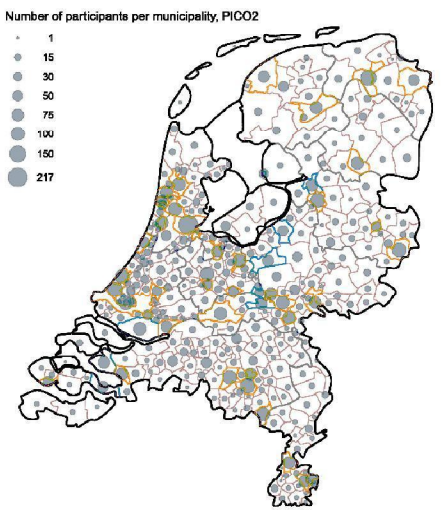
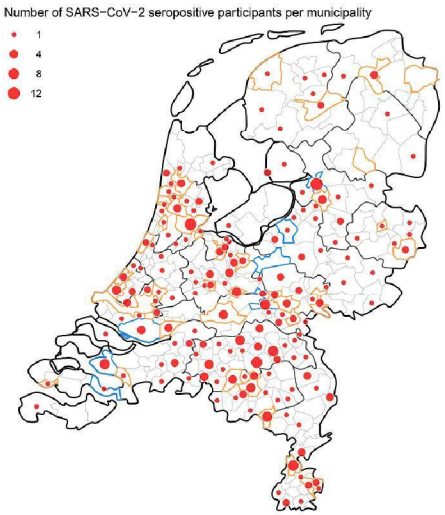
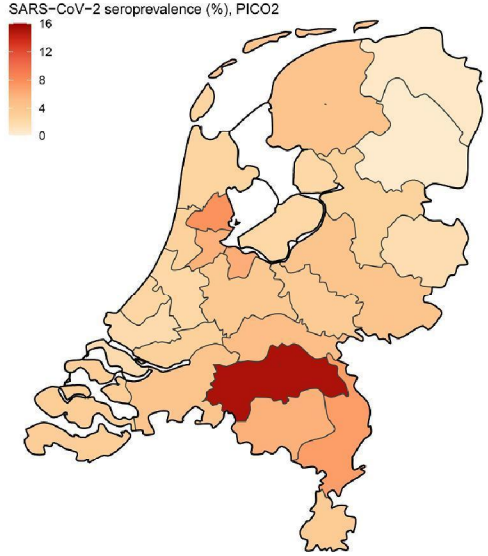
Seroprevalence – NL, by geo spread







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Ongoing analyses (PiCo2, PiCo3)

- Risk factors
  - Contact patterns
- Symptoms
- Age-specific risk factors?
- Ig concentrations
  - Time
  - symptoms

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CNN health Food Fitness Wellness Parenting Vital Signs

British study shows evidence of waning immunity to Covid-19

By Jen Christensen, CNN
Updated 0359 GMT (1159 HKT) October 27, 2020

CNN health Food Fitness Wellness Parenting Vital Signs Edition

New reports show coronavirus immunity can last for months

By Maggie Fox, CNN
Updated 1155 GMT (1955 HKT) October 14, 2020

The New York Times

The Coronavirus Outbreak › | LIVE Latest Updates | Maps and Cases | Vaccine Tracker | Spike in Hospitalizations | Answers to Your Questions

Why You Shouldn't Worry About Studies Showing Waning Coronavirus Antibodies

Experts say it's normal for levels of antibodies to drop after clearing an infection, and that they represent just one arm of the immune response against a virus.

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Coronavirus immunity: Can you catch it twice?

By James Gallagher
Health and science correspondent

24 August

Coronavirus pandemic

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Persbericht

Corona-antistoffen bij donors landelijk gedaald

15 juli 2020 Deel dit bericht

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What causes the confusing flow of news

- A lot at stake
- Researchers with different backgrounds involved
- Sampling strategy
  - Representative / corrected?
  - Independent samples / Longitudinal?
- Assay
  - Performance
  - Antigen
  - Which Ig
- Interpretation and communication..

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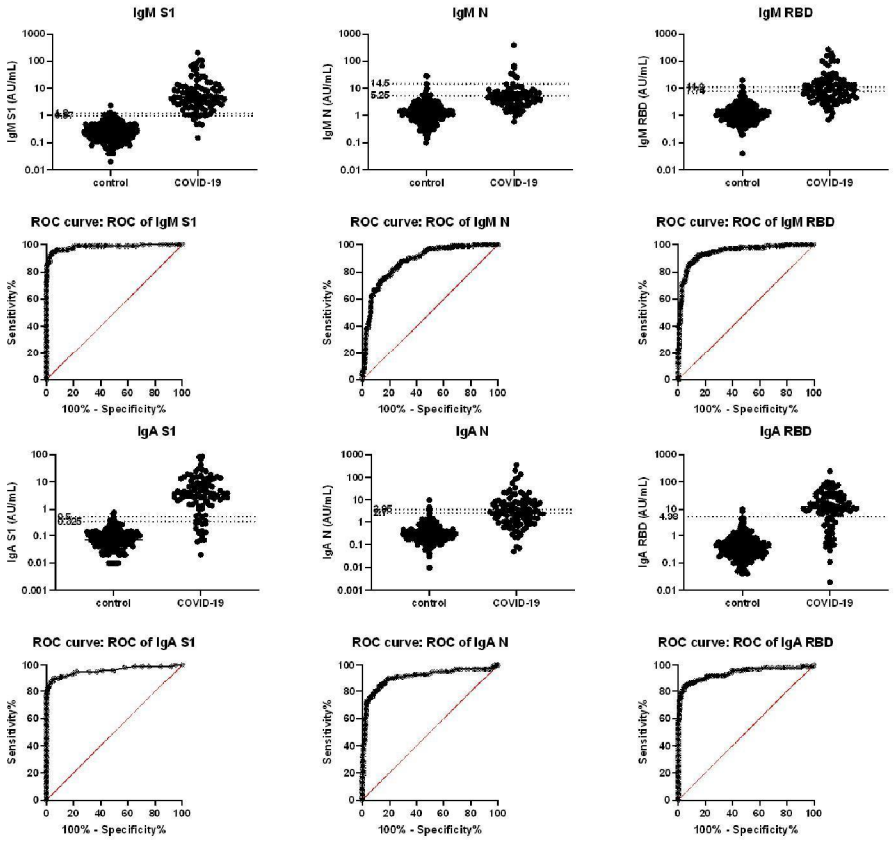
What we do

- PiCo
- Include N and Spike
- Measure IgM, IgA, IgG separately
- Longitudinal

- Disseminate

IgM, IgA

- Less separation
  - N
  - RBD
- IgG superior for classification of samples



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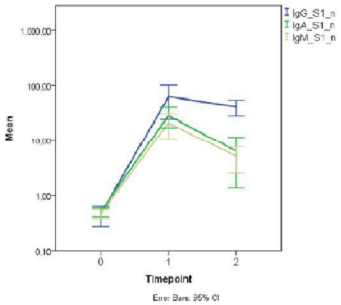
Ig kinetics vary between isotype and specificity (PiCo)

A



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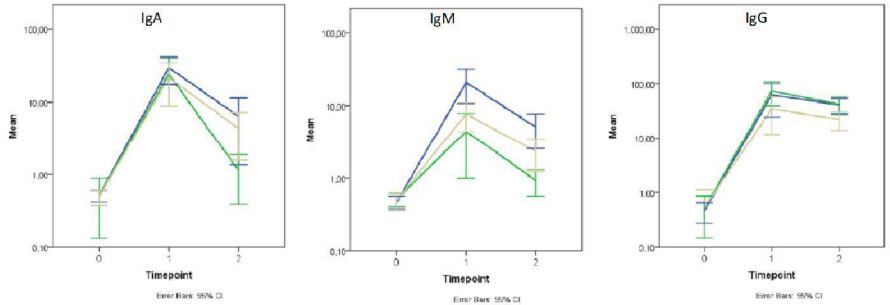

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FFX versus PiCo

- Fundamentally different study design
- Similar data collected
  - Questionnaire
  - Humoral data
- Could datasets be merged?
  - More samples
  - Better distribution of (early) time points
  - If 2 independent studies show the same result…

  - Restriction: no epi conclusions

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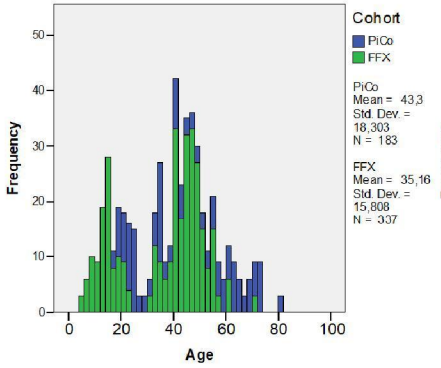

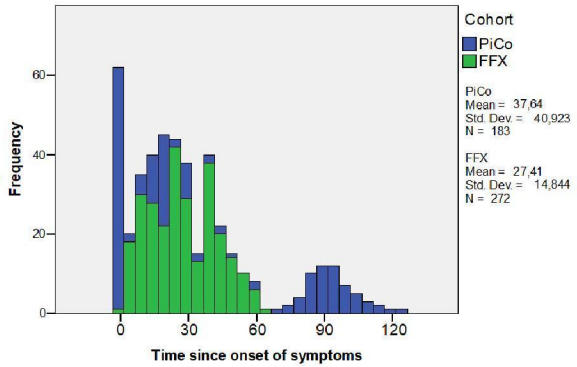

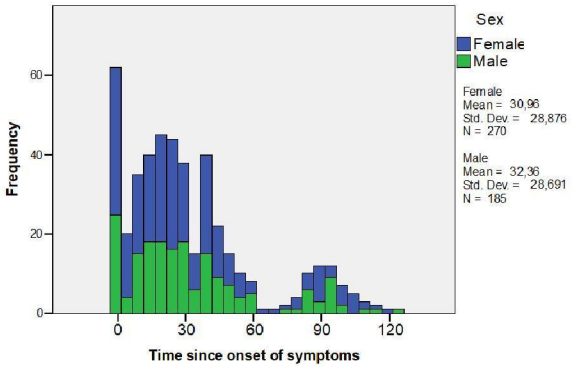

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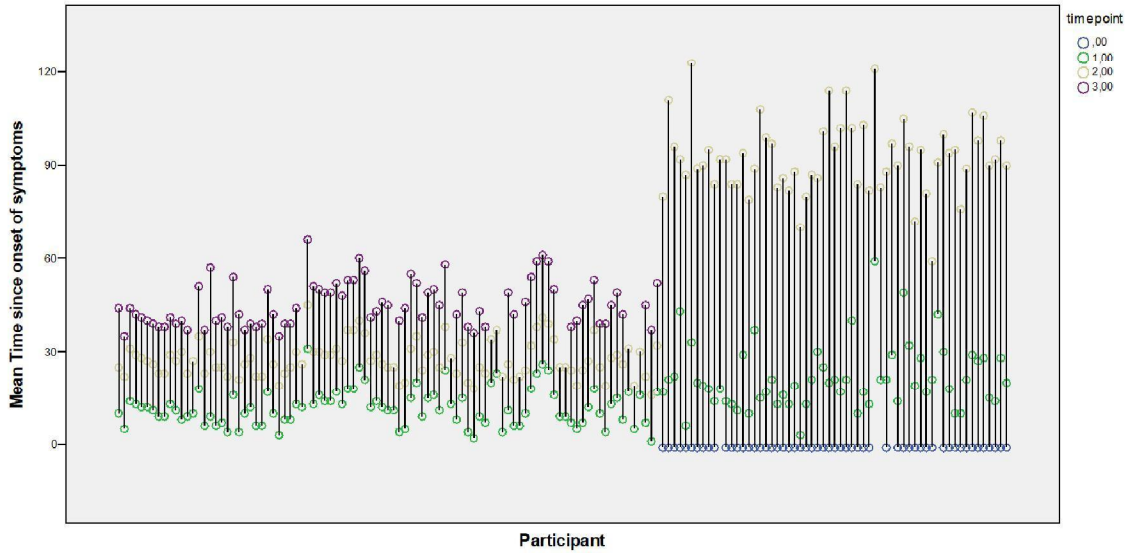
Longitudinal samples FFX & PiCo (P3, Pico1,2)



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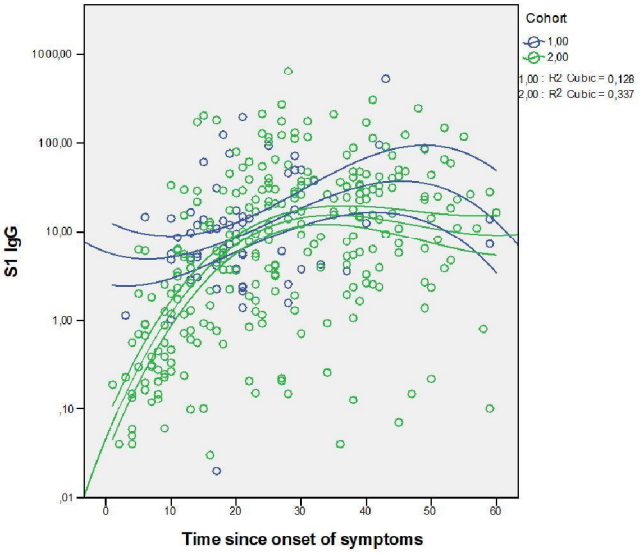

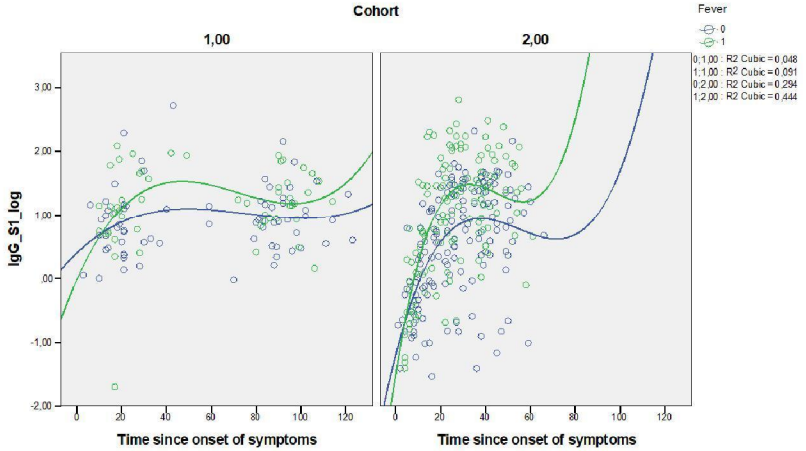

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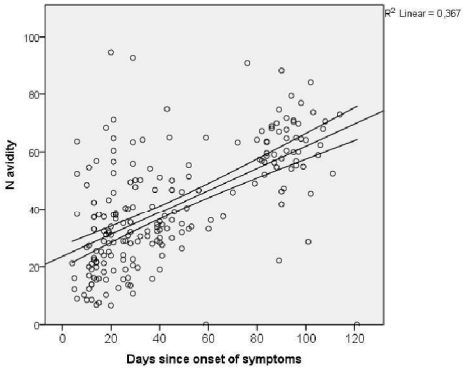
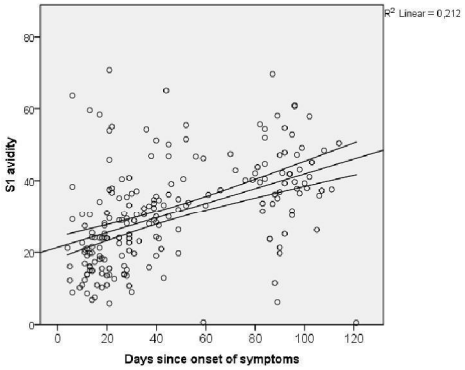
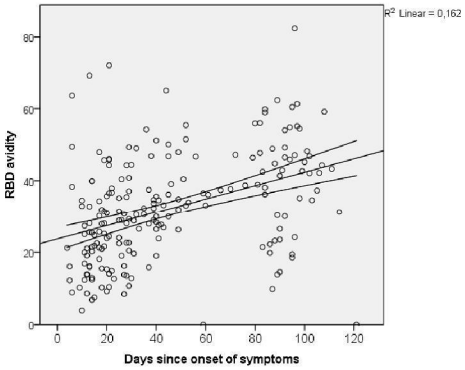
IgG avidity







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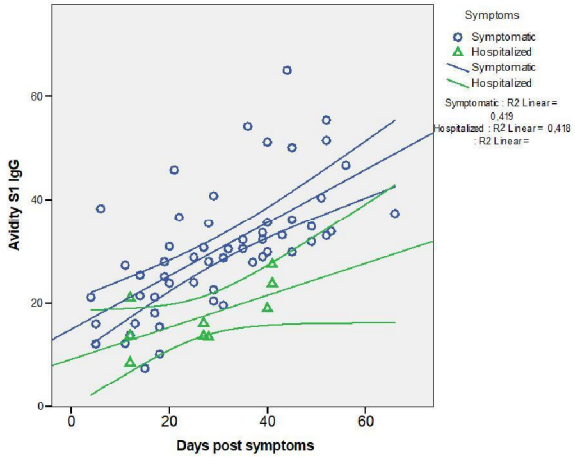
IgG avidity & hospitalization (FFX)



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Preliminary statistical results

	IgG	IgA	Avidity IgG
Fever	++	+	
Headache			+/-?
Taste/smell loss			+/-
Runny nose		-	-
Hospital	++	++	-
Time	-	--	++

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Preliminary statistical results IgG (week2-10)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	,713	,110		6,451	,000
	Fever	,356	,107	,223	3,334	,001
	Fatigue	,301	,140	,143	2,147	,033
	Headache	-,004	,134	-,002	-,032	,974
	Joint pain	-,044	,252	-,011	-,176	,861
	Myalgia	,046	,102	,028	,448	,655
	Anosmia/Ageusia	-,131	,106	-,080	-1,235	,218
	Coughing	,277	,103	,163	2,685	,008
	Sore throat	,085	,105	,052	,813	,417
	Runny nose	-,196	,099	-,120	-1,980	,049
	Shortness of breath	,107	,107	,065	,999	,319
	Pain breathing	,092	,177	,035	,523	,602
	Diarrhea	-,284	,121	-,154	-2,339	,020
	Nausea	-,151	,110	-,093	-1,365	,174
	GP visit	,250	,143	,112	1,745	,082
	Hospitalized	1,103	,195	,341	5,656	,000

a. Dependent Variable: IgG_S1_log

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	,671	,091		7,411	,000
	Fever	,416	,096	,260	4,341	,000
	Fatigue	,296	,121	,141	2,444	,015
	Coughing	,298	,098	,176	3,035	,003
	Runny nose	-,182	,093	-,112	-1,960	,051
	Diarrhea	-,272	,111	-,148	-2,449	,015
	Hospitalized	1,041	,188	,321	5,545	,000

a. Dependent Variable: IgG_S1_log

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Preliminary statistical results IgA (week2-10)

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-,217	,102		-2,126	,035
	Fever	,404	,098	,276	4,103	,000
	Fatigue	,285	,129	,148	2,209	,028
	Coughing	,281	,095	,181	2,959	,003
	Runny nose	,040	,091	,027	,436	,663
	Diarrhea	-,261	,112	-,155	-2,334	,020
	Hospitalized	,784	,180	,264	4,360	,000
	Headache	-,042	,123	-,022	-,344	,731
	Joint pain	,034	,232	,010	,148	,882
	Myalgia	,032	,094	,021	,340	,734
	Anosmia/Ageusia	-,037	,098	-,025	-,375	,708
	Sore throat	-,047	,097	-,032	-,492	,623
	Shortness of breath	,127	,099	,084	1,285	,200
	Pain breathing	,031	,163	,013	,193	,847
	Nausea	-,050	,102	-,034	-,488	,626
	GP visit	,135	,132	,066	1,024	,307

a. Dependent Variable: lgA_S1_log

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-,221	,079		-2,809	,005
	Fever	,443	,087	,302	5,086	,000
	Fatigue	,296	,110	,154	2,698	,007
	Coughing	,309	,089	,199	3,481	,001
	Diarrhea	-,245	,101	-,145	-2,415	,016
	Hospitalized	,777	,171	,262	4,536	,000

a. Dependent Variable: lgA_S1_log

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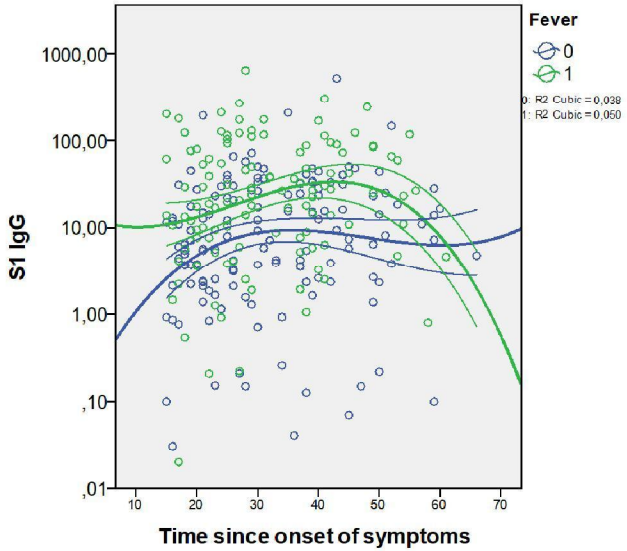

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Preliminary results PiCo3

Table 2: Numbers and % of participants with antibodies to SARS-CoV-2 Spike S1 in the PiCo study rounds

		S1_IgG	S1_IgA	S1_IgM	Day Median (95% CI)
PiCo 1	N tot	59	59	59	19
	N pos	59	49	51	(17-21)
	%	**100**	**83,1**	**86,4**	
PiCo 2	N tot	59	59	59	92
	N pos	58	31	33	(90-97)
	%	**98,3**	**52,5**	**55,9**	
PiCo 3	N tot	50	50	50	197
	N pos	47	26	22	(196-203)
	%	**94**	**52**	**44**	

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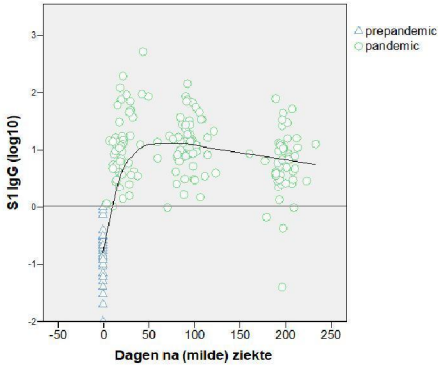
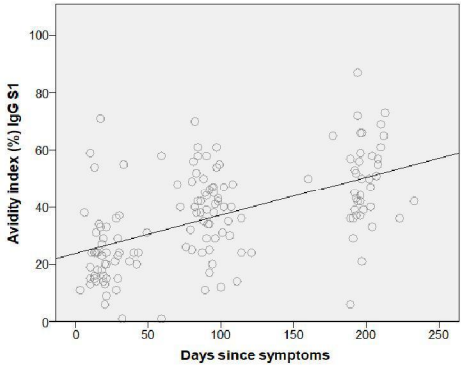
IgG





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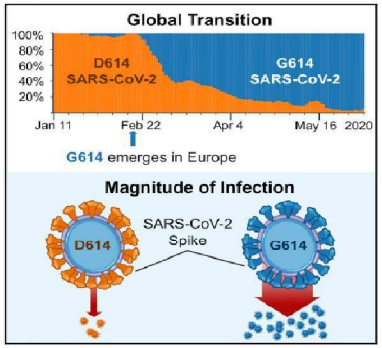
Are we still assessing abs to the right target?

Cell Article

Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus

Graphical Abstract



Authors
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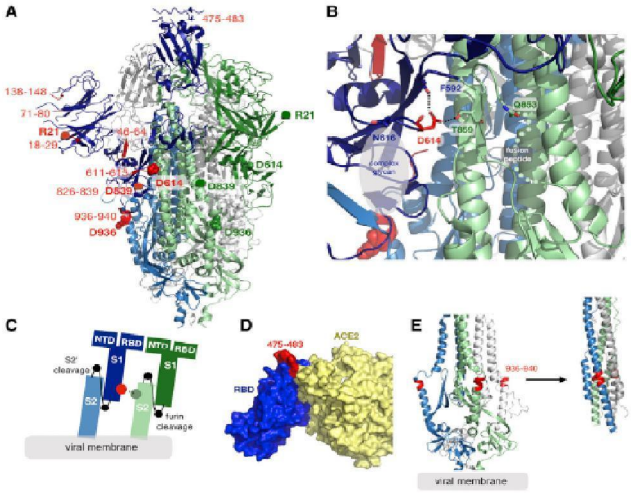
Correspondence
btk@lanl.gov

In Brief
Korber et al. present evidence that there are now more SARS-CoV-2 viruses circulating in the human population globally that have the G614 form of the Spike protein versus the D614 form that was originally identified from the first human cases in Wuhan, China. Follow-up studies show that patients infected with G614 shed more viral nucleic acid compared with those with D614, and G614-bearing viruses show significantly higher infectious titers *in vitro* than their D614 counterparts.



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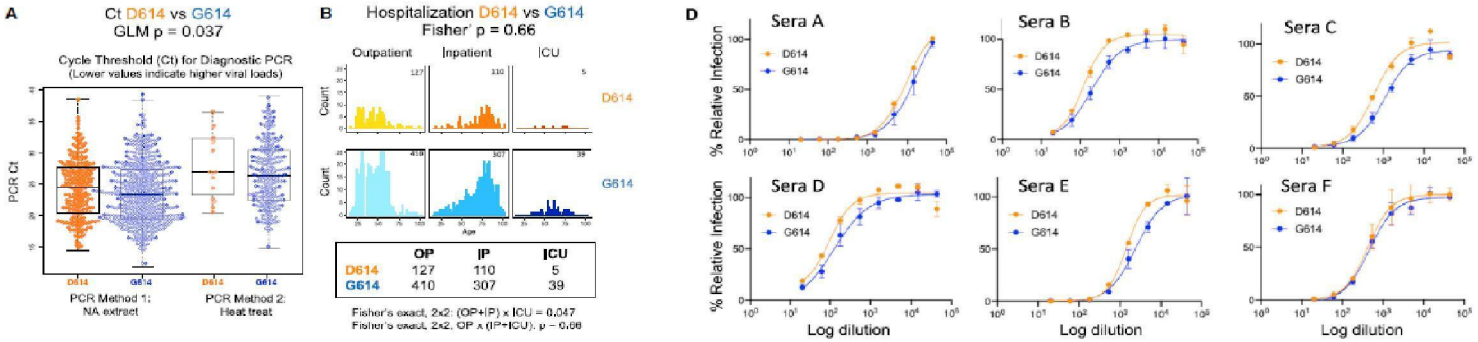
Implications of the D614G mutation



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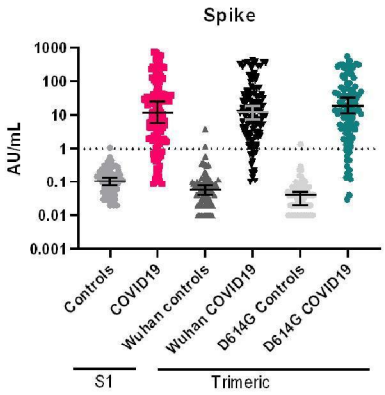
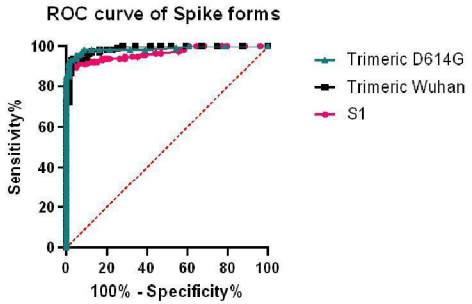
S1 v trimeric Spike RIVM MIA





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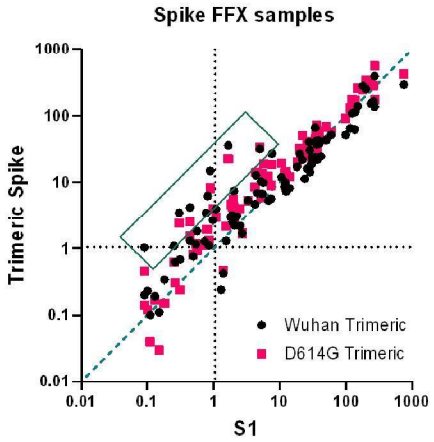
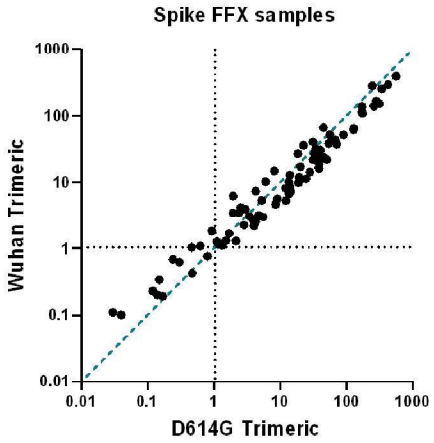
FFX





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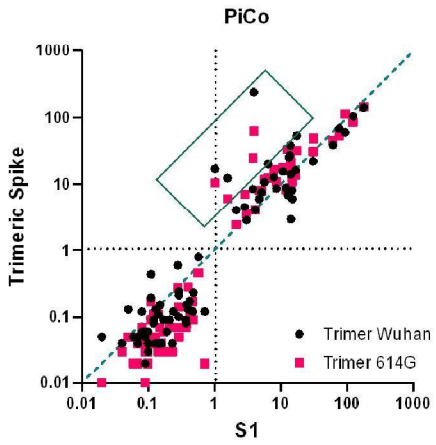
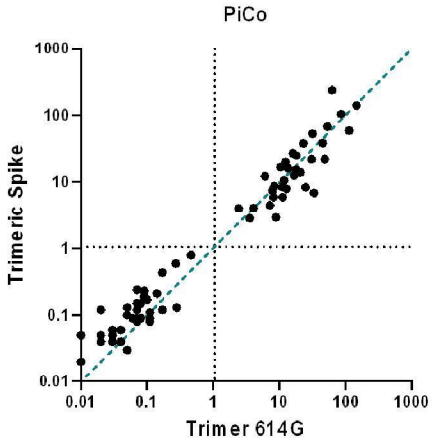
PiCo





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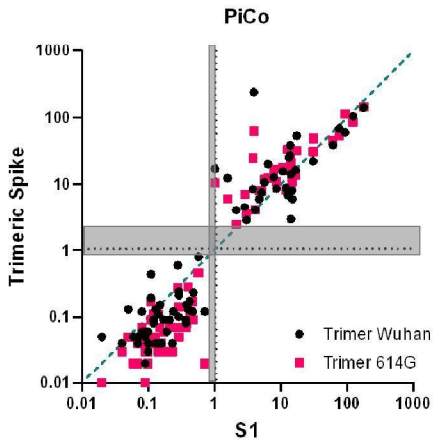
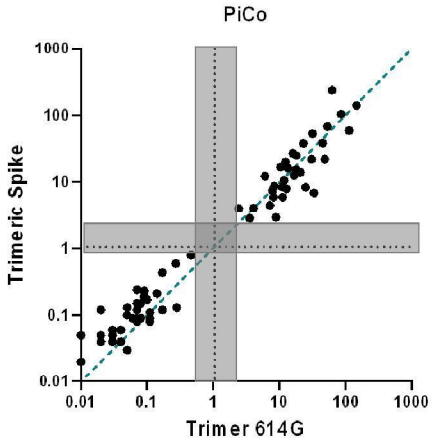
Superior separation by trimeric spike?





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Conclusions

- A reproducible, quantitative multiplex assay
- Characterized and evaluated for accurate seroprevalence studies

- Used to asses seroprevalence (few µL), longitudinal (PiCo)
- Able to precisely study kinetics (PiCo, FFX)
  - IgG, IgA, IgM, Avidity
- Decay of IgA, persistance of IgG, specificity dependent
- Increasing IgG avidity
- Concentrations associate with symptoms
- Trimeric Spike may enhance assay performance
  - no evidence for altered binding due to D614G

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Ongoing projects

- Gene expression profiling of SARS-CoV-2 infected innate cells: a role for epigenetic reprogramming
- SARS-CoV-2-specific T cell responses in COVID-19: characterization, cross-reactivity and longevity
- Immunity induced by different SARS-CoV-2 vaccine concepts and correlation with protection in the ferret model
- SARS-CoV-2 epithelial infection model and antibody Fc-functionality
- Serology
  - In-dept and functional serology
  - Big data analyses
  - Support for PiCo

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Connect to..

- Connection seroprevalence and immunity study
  - Ig functionality
- B cell cloning
- T cells
- Innate
- Mucosal response (FFX saliva)

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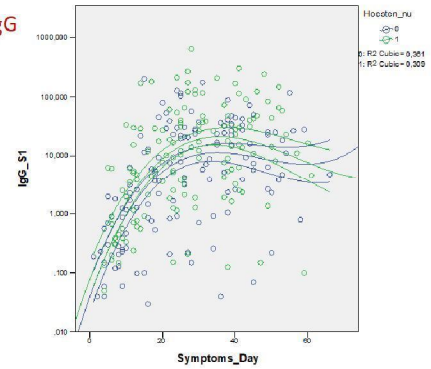
FFX symptoms nows or past 14 days

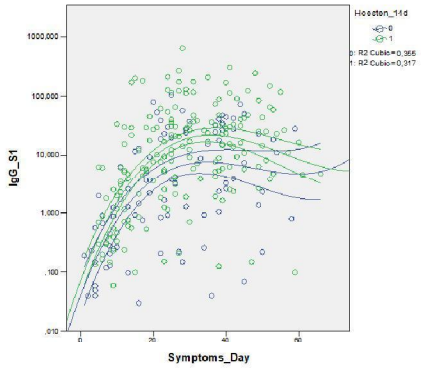
Coughing

IgG

Now



14 days



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Kinetics (FFX)