

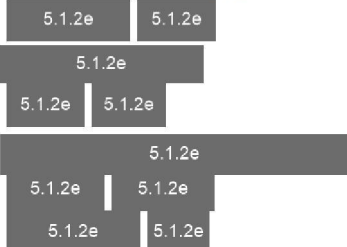
# SARS-Cov-2 re-infection cases (The Netherlands 2020)

5.1.2e 5.1.2e 5.1.2e, RIVM  
WHO/LTWG, october 9, 2020

- measles example of breakthrough/re-infection
- antibody response after primary SAR-Cov-2 infection
- description of re-infection cases
- antibody response after re-infection
- discussion

# Acknowledgements

## Medical Microbiology Labs

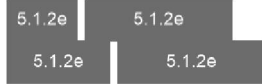


## Municipal Health Services



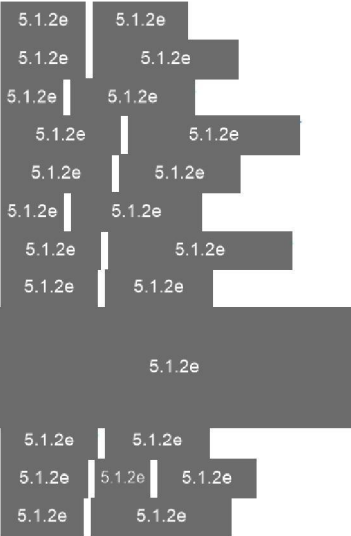
..and several others

## RIVM LCI



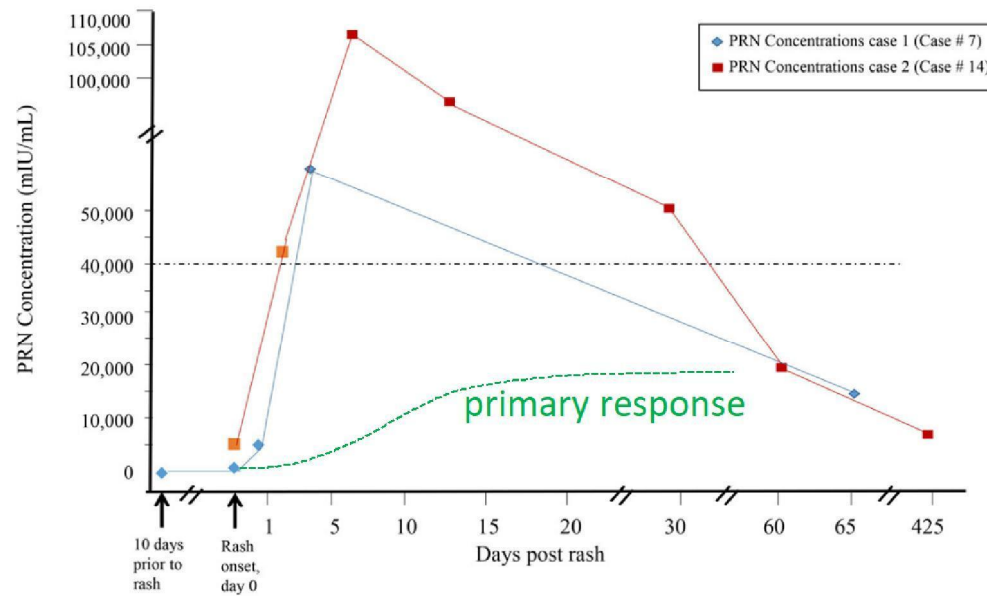
..and several others

## RIVM Virology and Immunology depts.



Example: measles 'breakthrough' infection (re-infection)

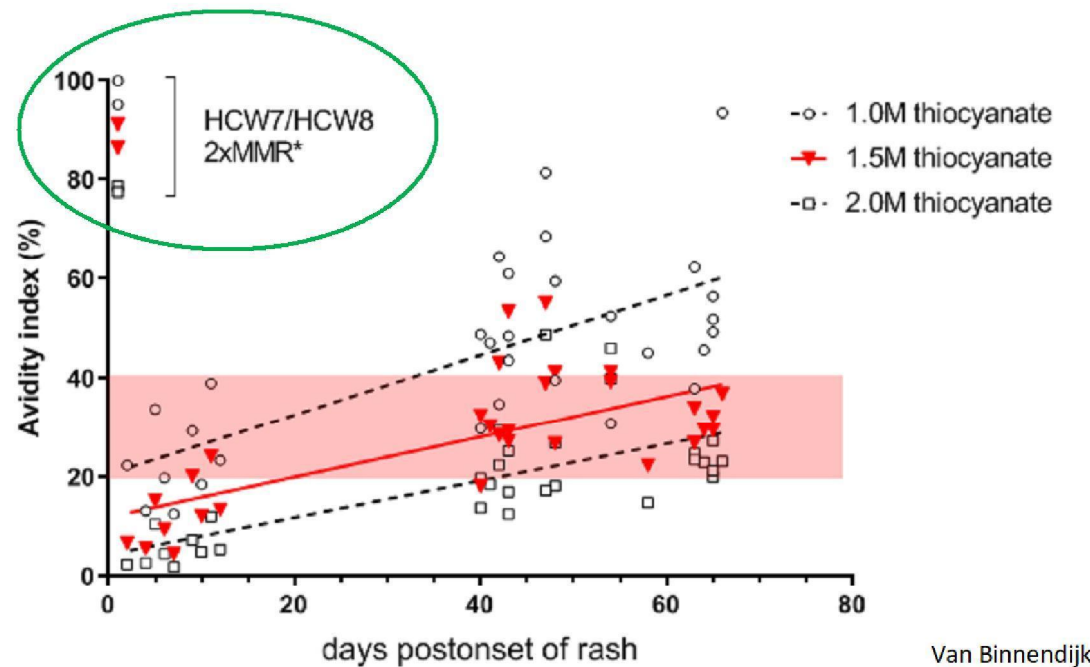
1st characteristic; secondary immune response > faster & higher



Sowers et al. 2016

## 2<sup>nd</sup> characteristic > avidity

- binding strength between antibodies and antigen
- chaotropic reagents like ureum/thiocyanate usefull disrupt hydrophobic ab-ag interaction
- Avidity expressed as ratio (avidity index or AI): binding in presence or absence of the reagent
- AI = ab concentration independent
- Primary (unvaccinated) measles cases versus breakthrough infection
- High avidity ab's discriminate secondary from primary response



Van Binnendijk, unpublished data  
(except for HCW7/8, Hahné et al. 2016)

## The measles framework of breakthrough/ re-infection cases

- rapid antibody response, high antibody titers
- good correlation between IgG and virus neutralization (VNT)
- (mild) disease symptoms, lower viral load, low transmission risk

Case ID	Birth Year Age (years)	Measles vaccination status	Days between rash and serum sample	IgM	PRN (mIU/ml)	MIA(IgG) (mIU/ml)	Avidity index (EuroImmune)	CT value	Measles severity	Infected others	Hospitalized
HCW 1	1987	2 doses	<b>1</b>	Neg	<b>3,670</b>	<b>1,670</b>	High (87%)	37.5	Mild	No	No
	26 years		<b>&gt; 7 days</b>	-	<b>66,020</b>	<b>35,740</b>					
HCW 2	1982	2 doses	<b>1</b>	Neg	<b>7,970</b>	<b>3,560</b>	High (88%)	26.6	-	No	No
	31 years		-	-	-	-					
HCW 3	1985	1 dose	<b>0</b>	Pos	<b>1,080</b>	<b>ND</b>	Low/Interm. (36%)	23.3	Severe	No	No
	29 years		<b>&gt; 7 days</b>	-	<b>7,750</b>	<b>4,360</b>					
HCW 4	1970	0 doses	<b>-1</b>	Neg	<b>50/neg</b>	<b>40/neg</b>	NA	17.2	Severe	Yes	Yes
	43 years		<b>&gt; 7 days</b>	-	<b>19,110</b>	<b>13,330</b>					
HCW 5	1988	2 doses	-	-	-	-	-	29.4	-	No	No
	25 years		-	-	-	-					
HCW 6	1987	2 doses	<b>0</b>	Neg	<b>6,940</b>	<b>4,350</b>	High (89%)	28.1	Mild-moderate	No	No
	26 years		<b>&gt; 7 days</b>	-	<b>200,640</b>	<b>120,480</b>					
HCW 7	1987	2 doses	<b>0</b>	Neg	<b>5,970</b>	<b>3,620</b>	High (84%)	26.5	Mild	No	No
	26 years		<b>&gt; 7 days</b>	-	<b>107,150</b>	<b>87,640</b>					
HCW 8	1990	2 doses	<b>2</b>	Neg	<b>46,100</b>	<b>13,730</b>	High (95%)	33.7	-	No	No
	23 years		-	-	-	-					

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

### cases

- 30-35 possible re-infection cases under investigation
- clinical symptoms, non-hospitalized
- laboratory-confirmed for the second time (PCR+)
- additional serum collected on 2<sup>nd</sup> visit

### selection

- first and second 'episode' confirmed by PCR
- availability of serum (n=18)

### basics (n=18):

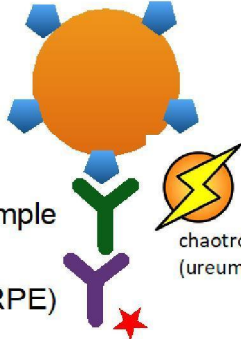
- median age: 51 years (range 18-85 years)
- male/female: 8/10
- median days between first and second episode: 85 days (57-133)

SARS-Cov-2 serology carried out by the multiplex beads assay (MIA)



microsphere bead 1

S1 [HPLC, gen-2]  
Sino Biological



IgG(IgM, IgG) in sample

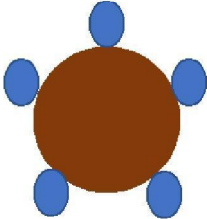


chaotropic reagent  
(ureum/isothyanaat)

detection (anti-IgG RPE)



bead 2  
N (Sino)



bead 3  
RBD (Sino)

Bead 4, 5, 6, ...  
OC43, etc.

## IgG quantitative response against SarS-Cov-2 S1 after primary infection

*The Journal of Infectious Diseases*

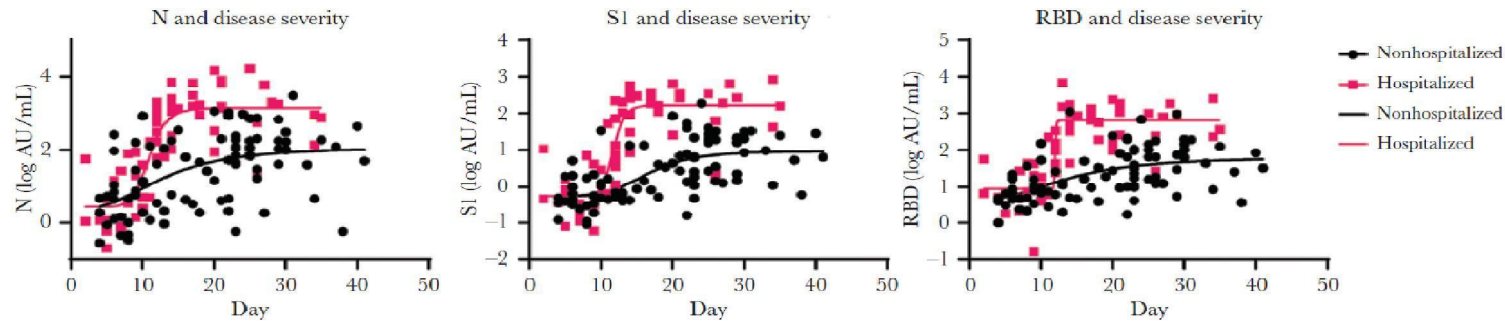
MAJOR ARTICLE



## SARS-CoV-2–Specific Antibody Detection for Seroepidemiology: A Multiplex Analysis Approach Accounting for Accurate Seroprevalence

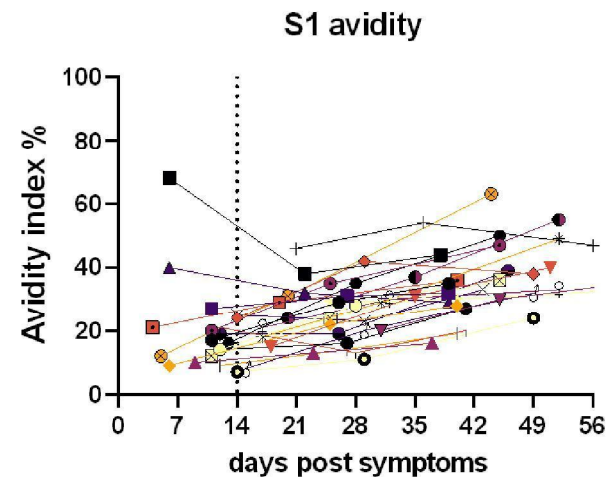
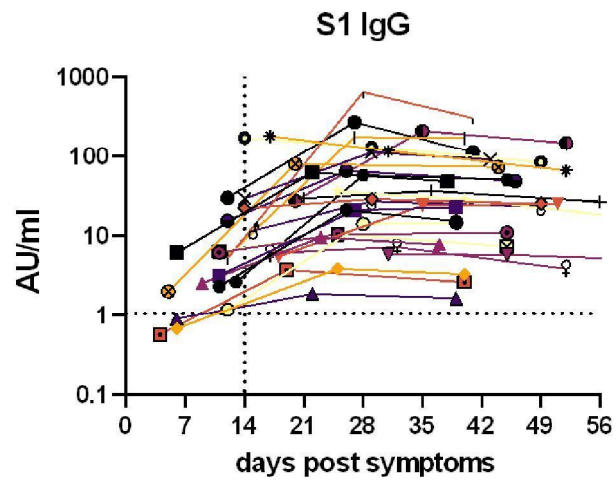
Gerco den Hartog,<sup>1,2</sup> Rutger M. Schepp,<sup>1</sup> Marjan Kuijper,<sup>1</sup> Corine GeurtsvanKessel,<sup>2</sup> Josine van Beek,<sup>1</sup> Nynke Rots,<sup>1</sup> Marion P. G. Koopmans,<sup>2</sup> Fiona R. M. van der Kluis,<sup>1</sup> and Robert S. van Binnendijk<sup>1</sup>

<sup>1</sup>Centre for Immunology of Infectious Diseases and Vaccines, National Institute for Public Health and the Environment, Bilthoven, the Netherlands, and <sup>2</sup>Department of Viroscience, Erasmus Medical Center, Rotterdam, the Netherlands



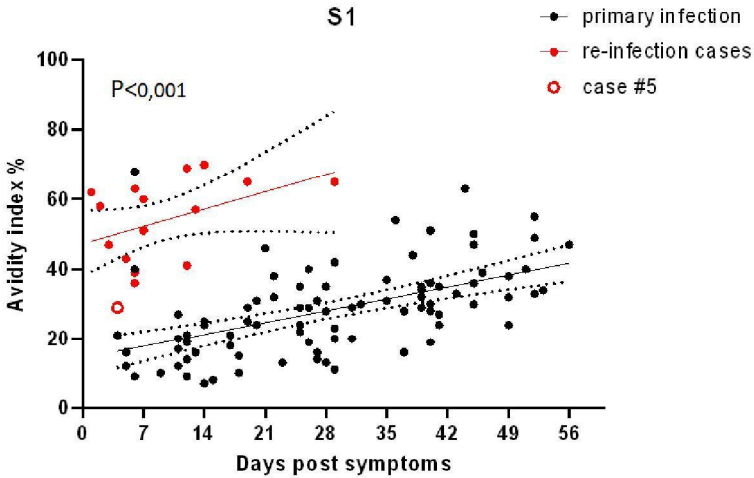
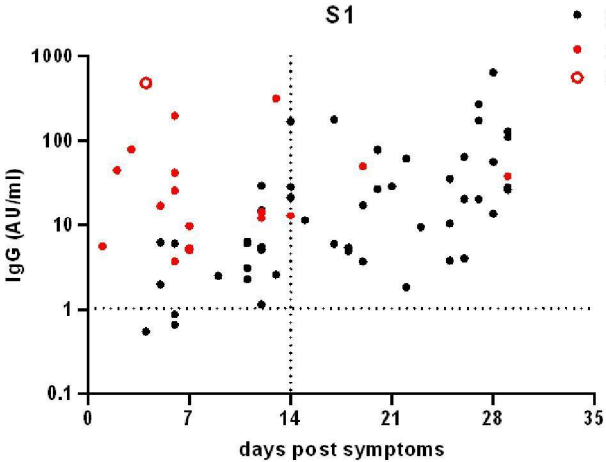


IgG quantitative response to S1 in relation to avidity maturation  
(improved mk-2 MIA: specificity/sensitivity 99.7-99.9%/ > 95%)



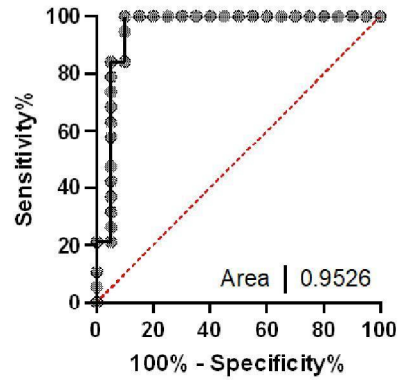
IgG concentrations of re-infection cases (n=18)

and avidity



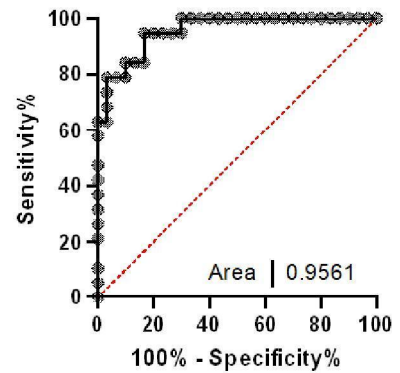
### Optimal avidity 'cut-off' to define re-infection

ROC of Avidity Index S1 d1-14

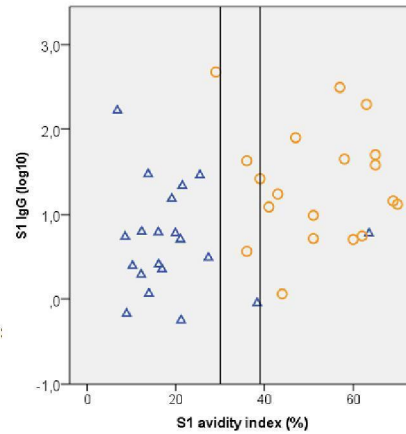


Cut-off	Sensitivity%	95% CI	Specificity%	95% CI
> 26.38	100,0	83,18% to 100,0%	85,00	63,96% to 94,76%
> 28.17	100,0	83,18% to 100,0%	90,00	69,90% to 98,22%
> 32.50	94,74	75,36% to 99,73%	90,00	69,90% to 98,22%
> 37.17	84,21	62,43% to 94,48%	90,00	69,90% to 98,22%
> 38.67	84,21	62,43% to 94,48%	95,00	76,39% to 99,74%

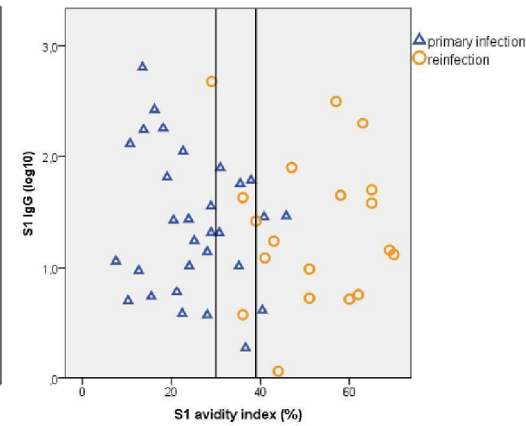
ROC of Avidity Index S1 d15-30



day 1-14

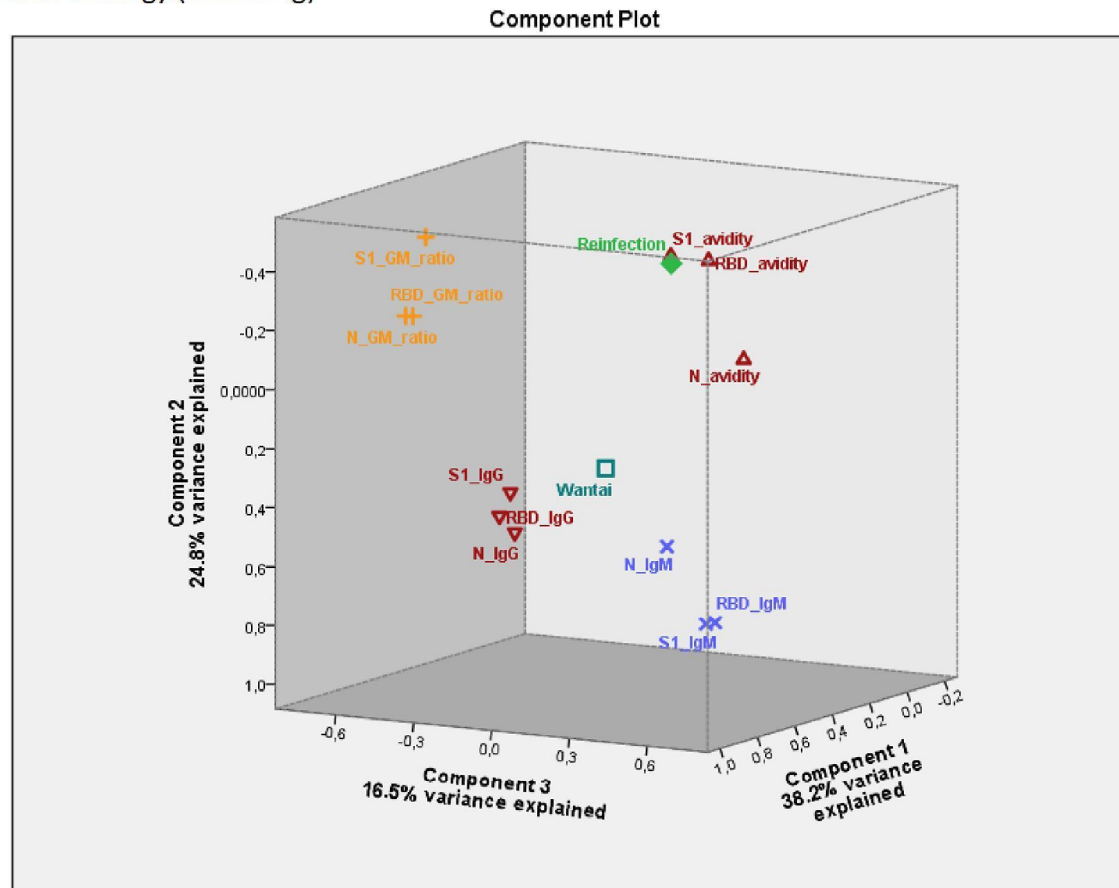


day 15-30



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 (not shown): 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, but only in combination with IgG (eg ratio)
- One exceptional case at day 4 with very high IgG (and VNT) antibodies, but of low avidity

Not presented here:

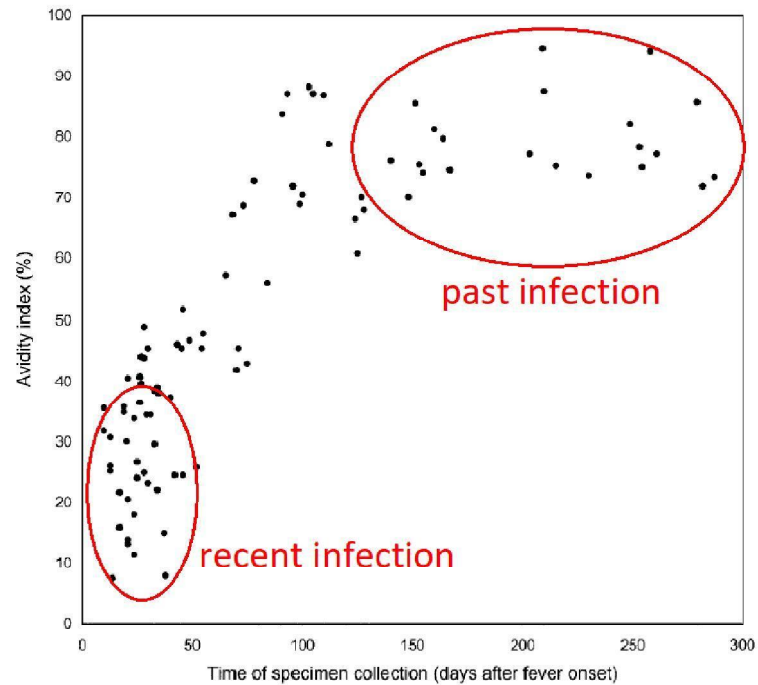
PCR and sequence data ( 5.1.2e 5.1.2e )

# Additional slides

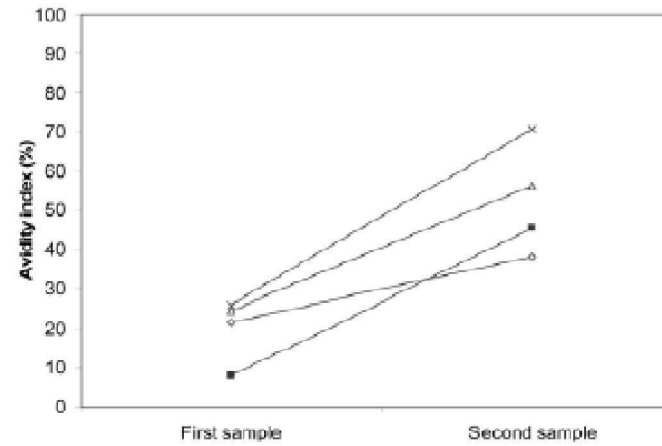
# Antibody Avidity Maturation during Severe Acute Respiratory Syndrome–Associated Coronavirus Infection

The Journal of Infectious Diseases 2005;192:166–9

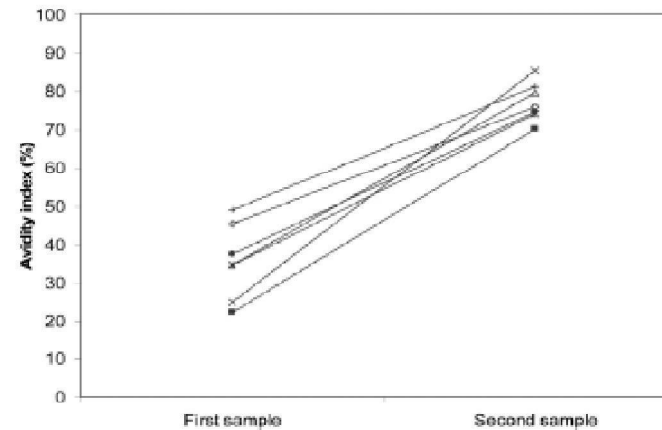
Paul K. S. Chan,<sup>1,2,3</sup> Pak-Leong Lim,<sup>4</sup> Esther Y. M. Liu,<sup>2</sup> Jo L. K. Cheung,<sup>2</sup> Danny T. M. Leung,<sup>4</sup> and Joseph J. Y. Sung<sup>1</sup>



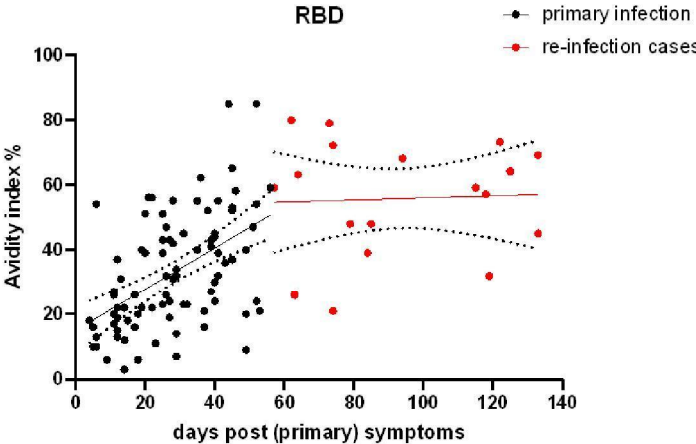
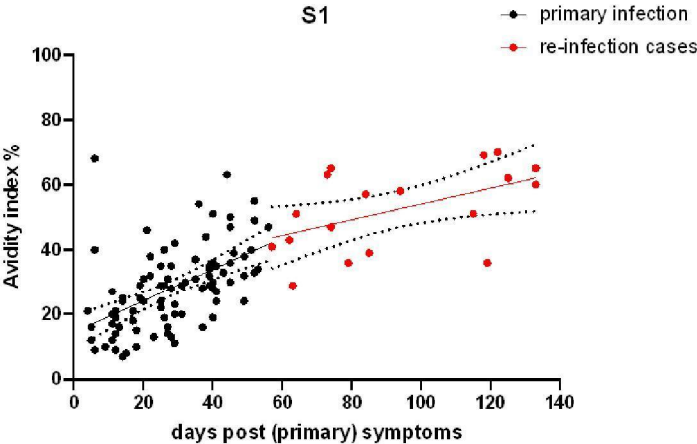
A. Paired samples collected  $\leq 50$  days apart



C. Paired samples collected 101-150 days apart



IgG avidity index primary and re-infect cases  
time/disease relationship according to "first episode"





## Correlation IgG (MIA) with VNT - re-infection cases only

