Community based sero-epidemiological study of COVID-19 to provide data in real time on age-stratified infection attack rates, disease severity and population-immunity, for guiding intervention policy (Ref. No.: COVID190126)

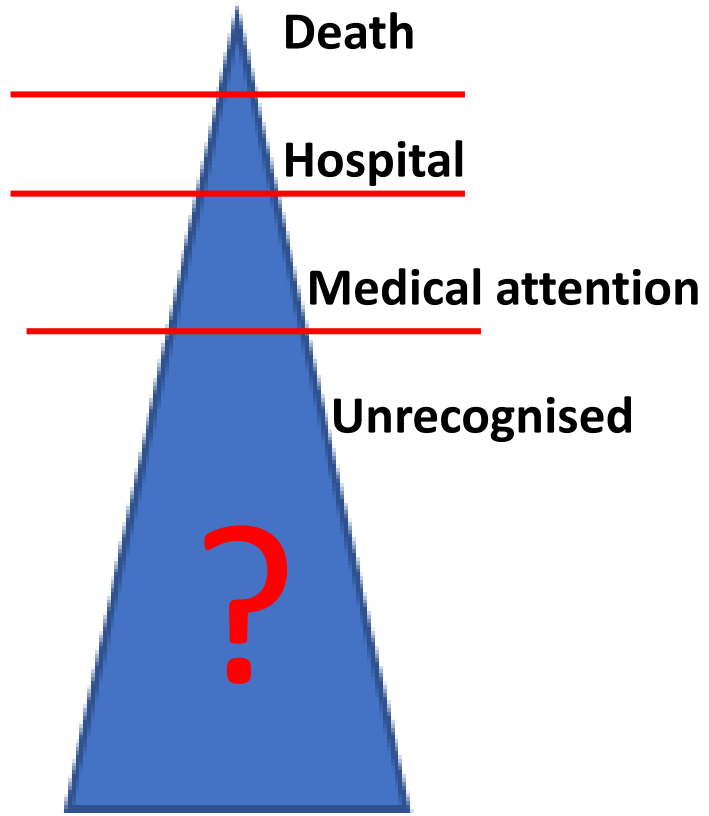
M Peiris[1], BJ Cowling[1], JT Wu[1], DKM Ip[1], MY Ni[1], RSL Au Yeung[1], KSM Leung[1], EHY Lau[1], DSC Hui[2], OTY Tsang[3], WH Chan[4], MYW Kwan[5], SS Chiu[6] & GM Leung[1]

[1]School of Public Health, The University of Hong Kong; [2]Department of Medicine and Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong; [3]Infectious Diseases Centre, Princess Margaret Hospital, [4]Department of Paediatrics, Queen Elizabeth Hospital; [5]Department of Paediatric and Adolescent Medicine, Princess Margaret Hospital; [6]Department of Paediatric and Adolescent Medicine, The University of Hong Kong.

Research Fund Secretariat
Food and Health Bureau
Hong Kong Special Administrative Region

- **Introduction:** During an epidemic, diagnosed cases represent a tip-of-the-iceberg of infection taking place in the community. Sero-epidemiological studies provide an effective means to assess true infection attack rates in a community to inform control strategies.
- **Overall Aims:** to define infection attack rates in the Hong Kong population, development and duration of population immunity to SARS-CoV-2 through population based serial cross sectional and longitudinal sero-epidemiology studies.

Epidemic iceberg



Specific objectives / research questions

- **Define age-stratified infection attack rate in the community over time? What proportion of infections are being captured by our surveillance / case detection / confirmation?**
- What is duration of immunity (detectable antibody and T cell immunity after natural infection)
  - ➢effects the reliability of sero-epidemiology studies
  - ➢Development of population immunity → relevant to vaccine policy
- Validate testing strategy for large scale sero-epidemiology studies (N ELISA, S ELISA, S RBD ELISA, neutralizing antibody, other antibodies)?

Methods: Study cohorts

Five study cohorts chosen to provide different levels of exposure risk.

- **Cohort A)** Age-stratified community-based longitudinal cohort (Jul 2020-Oct 2021)(PI: Ben Cowling);
- **Cohort B)** Age-stratified serial cross-sectional sampling of blood donors (Apr 2020-Sept 2021) (PI: J Wu & K Leung);
- **Cohort C)** Serial cross sectional sampling of individuals working in specific occupations with increased social contacts with presumed increased risk of infection (June 2020-Oct 2021) (PI: M Ni & R Au);
- **Cohort D)** RT-PCR negative persons discharged from quarantine centres (June 2020-Aug 2021) (PI: DKM Ip)
- **Cohort E)** longitudinal follow up of a cohort of RT-PCR confirmed COVID-19 infections (Feb 2020-Aug 2021) (PIs: David Hui, Owen Tsang, Mike Kwan, Susan Chu; Wai-hung Chan).

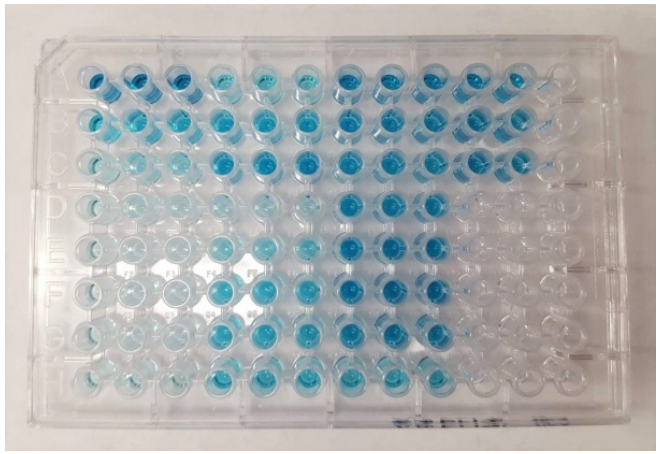
Methods: Study cohorts and numbers recruited

Five study cohorts chosen to provide different levels of exposure risk.

- **Cohort A)** Age-stratified community-based longitudinal cohort (n=4736; unvaccinated n=1788)(Jul 2020-Oct 2021)(PI: Ben Cowling);
- **Cohort B)** Age-stratified serial cross-sectional sampling of blood donors (n=13,968; unvaccinated n=13,043)(Apr 2020-Sept 2021) (PI: J Wu);
- **Cohort C)** Serial cross sectional sampling of individuals working in specific occupations with increased social contacts with presumed increased risk of infection (n=2336; unvaccinated n=1301) (June 2020-Oct 2021) (PI: M Ni & R Au);
- **Cohort D)** RT-PCR negative persons discharged from quarantine centres (n=4,044; unvaccinated n=3203) (June 2020-Aug 2021) (PI: DKM Ip)
- **Cohort E)** longitudinal follow up of a cohort of RT-PCR confirmed COVID-19 infections (n=2282) (Feb 2020-Aug 2021) (PIs: David Hui, Owen Tsang, Mike Kwan, Susan Chu; Wai-hung Chan).

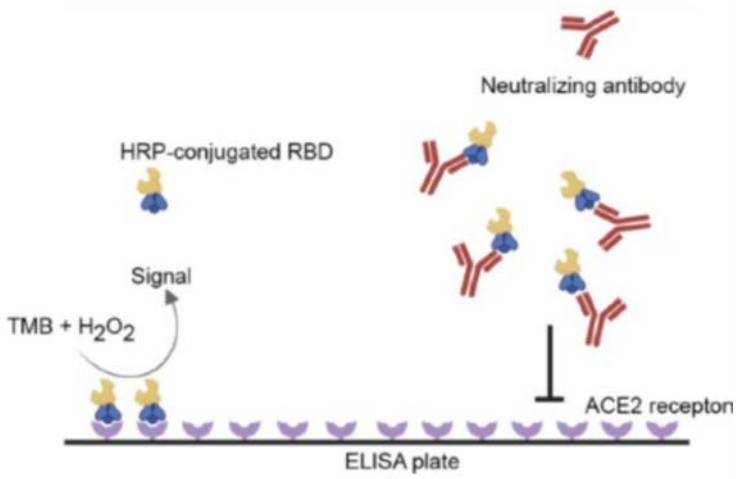
Methods

Spike RBD ELISA



- High throughput
- Inexpensive

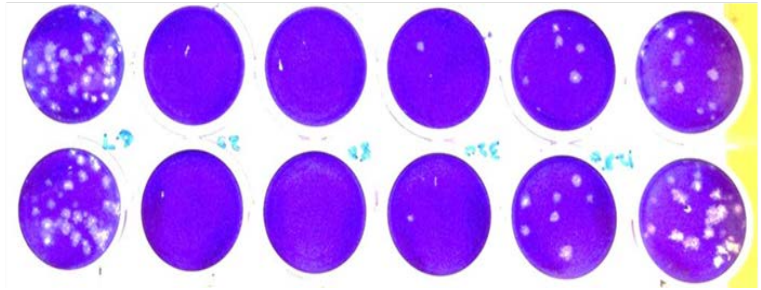
Surrogate virus neutralization test (sVNT)



- Can be done in BSL-2 containment laboratory
- Simple and rapid
- More costly than ELISA

Plaque reduction neutralization test

(the "gold standard" test for specificity and immunity)



No serum | 1:10 | 1:20 | 1:40 | 1:80 | 1:160

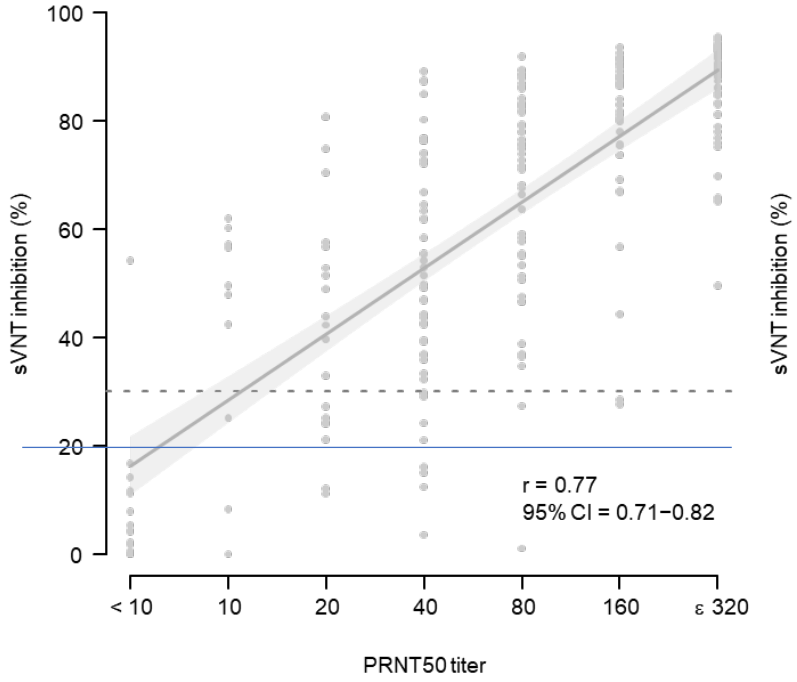
50% plaque neutralization: PRNT50
90% plaque neutralization: PRNT90

- Requires work in BSL-3 containment laboratory
- Highly labour and expertise intensive
- 5 days to complete assay

Perera et al Eurosurveillance 2020

SARS-CoV-2 antibody detectable by ELISA, sVNT and neutralization tests ≥ 90 days after RT-PCR confirmed infection (329 sera; 124 patients)

		Days after onset of illness			
Test		**90-150**	**151-200**	**201-386**	**Total**
PRNT50	Tested	50	39	26	115
	% Pos	98%	100%	100%	99.1%
PRNT90	Tested	50	39	26	115
	% Pos	92%	90%	92%	91%
RBD ELISA	Tested	52	41	27	120
	% Pos	100%	98%	93%	98%
sVNT >20%	Tested	41	39	26	106
	% Pos	100%	95%	96%	97%
sVNT >30%	Tested	41	39	26	106
	% Pos	100%	85%	73%	90%



196 negative controls: 100% negative

Sera 151-389 days post infection, 97.5% positive by spike RBD ELISA; 100% by PRNT50, 94.9% positive by sVNT (cut off 20%) and 73% by sVNT (cut off 30%).

Lau et al EClinMed 2021

Validated strategy for serological testing for sero-epidemiology study

Screen sera using spike RBD ELISA

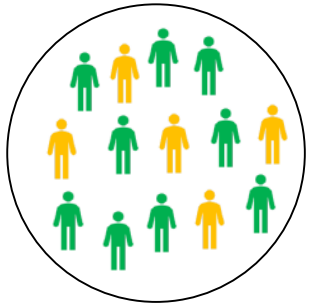
Pre-confirm using surrogate neutralization test (sVNT)

(rapid, no need for BSL-3 lab)

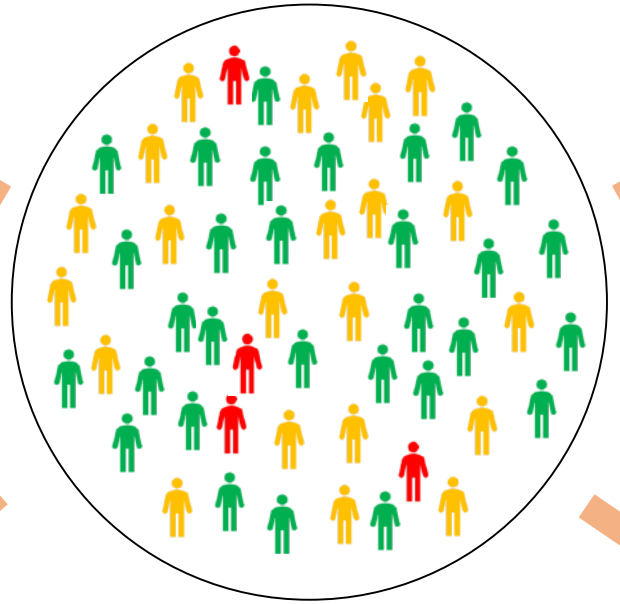
Final confirmation with plaque reduction neutralization test (PRNT)

(Requires biosafety level 3 containment)

Cohort 1: Community (age-stratified)

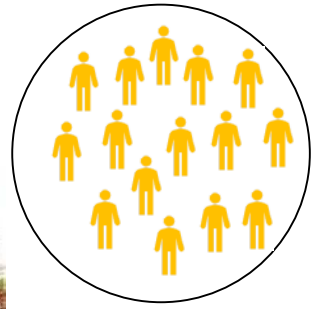


Hong Kong Population

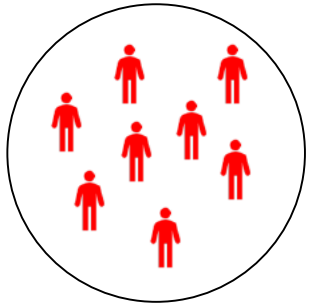


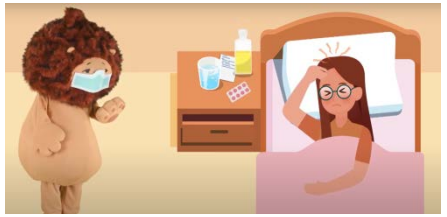
Cohort 2: Healthy blood donors (relatively healthier)

Cohort 3: High-risk occupations with high number of contacts (increased risk)



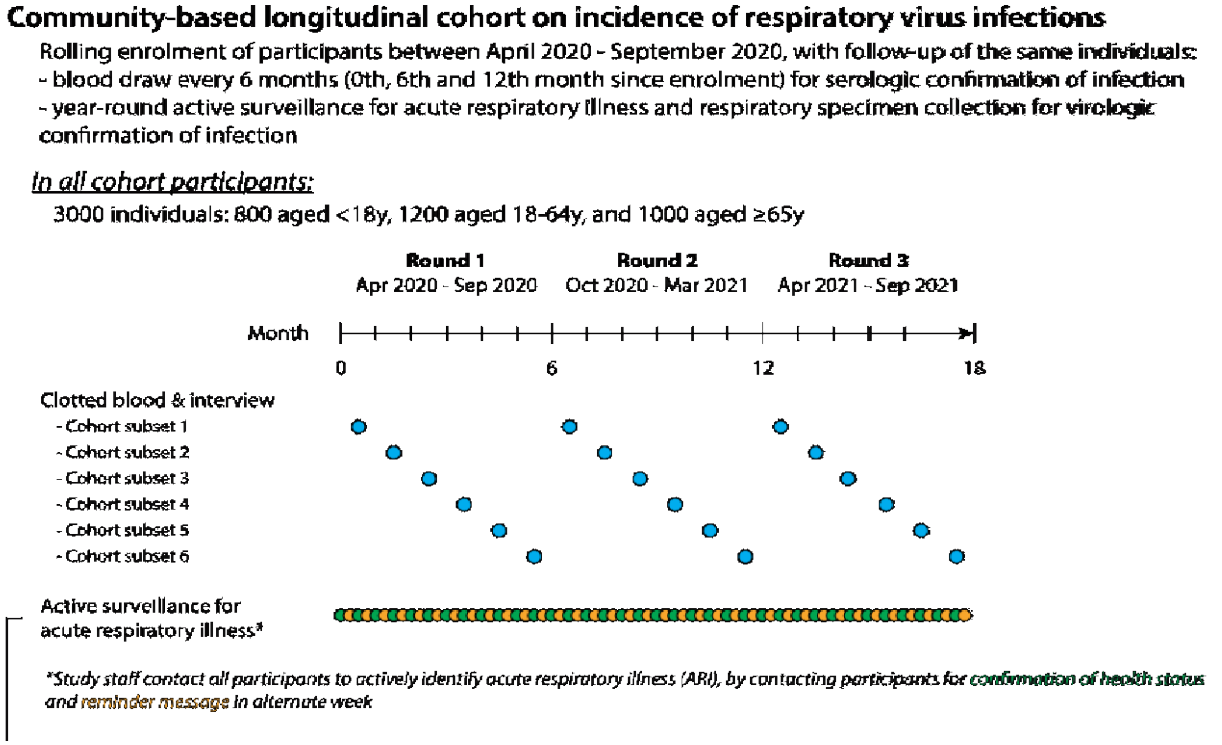
Cohort 4: Close contacts of confirmed cases from quarantine camps (highest risk)





Cohort A: “EPI-HK” community cohort study

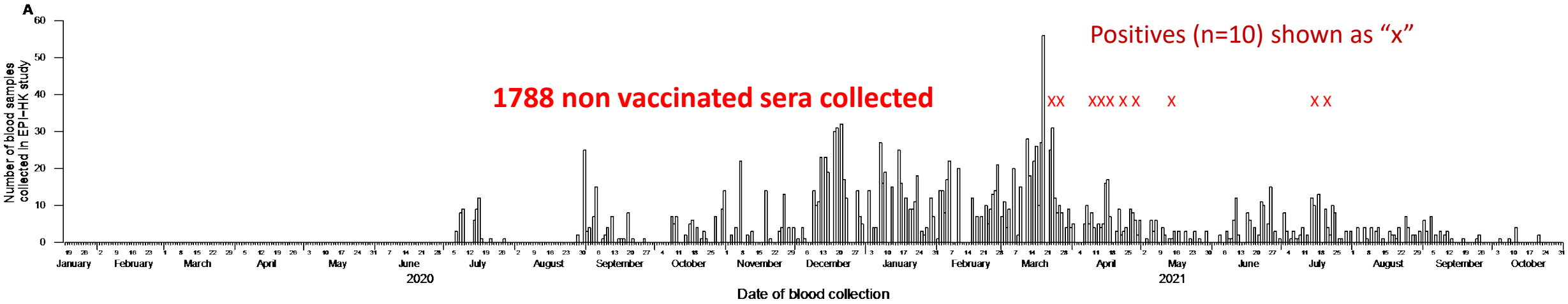
- **Longitudinal observational cohort study of all ages. Household composition recorded but participation from all household members is not required.**
- **Started in July 2020**
- **Participant enrolment through multiple mass promotion efforts:**
  - **mass mailing (community centres, residential estates, schools, general practitioners, churches, and companies)**
  - **advertisements in newspapers**
  - **advertisements in social media**
  - **mass emailing**
  - **COVID-19 community vaccination centres (pre-vaccination samples relevant for identifying prior natural infections)**
- **Blood collection every 6 months in all participants**
- **Continuous enrolment to replace dropouts**



4736 sera collected:
1788 non-vaccinated sera analysed

Estimating the community incidence of infection

Longitudinal observational cohort study in individuals of all ages. Blood collection every 6 months in all participants. PI BJ Cowling

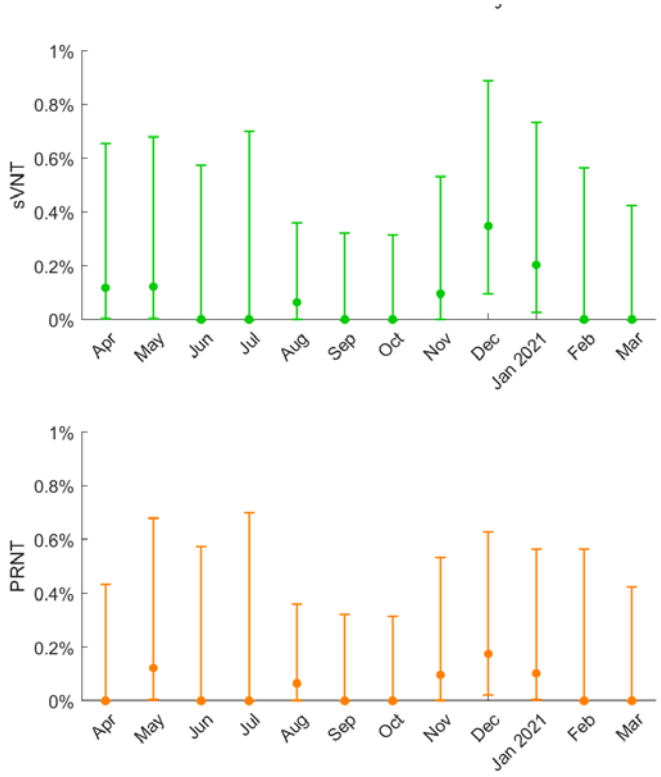


- **Using a statistical model allowing for change in risk of SARS-CoV-2 infection over time, estimate that cumulatively there have been 60,000 (95% CI: 12,000 – 140,000) unrecognized SARS-CoV-2 infections in Hong Kong. That corresponds to a cumulative incidence of 0.9%.**
- **If adding the 12300 confirmed cases, the cumulative incidence of SARS-CoV-2 infection in the community would be approximately 1%.**
- **Suggests case ascertainment level of approx. 17%**

Seroprevalence among blood donors aged 16 to 65 before COVID vaccination

- We detected 6 positives by PRNT among 10,534 blood samples collected from unvaccinated blood donors on or before 28 Feb 2021 (prior to vaccination roll-out)
- Using a Bayesian inference model, estimate case ascertainment rate of local cases was 23.1% (17.4-32.0) by PRNT. Consistent with estimate of 17% from Cohort A and 23% (17-47%) from the "Octopus model"
- Applying this case ascertainment rate to all cases as of 18 Nov 2021 (12,396 cases), we estimate the total number of infections in Hong Kong to be 54,000-62,000, or slightly less than 1% of Hong Kong population.

Blood donors	No.	sVNT positive	sVNT % positive	PRNT positive	PRNT % positive
2020					
April	848	1	0.12% (0.00-0.66)	0	0.00% (0.00-0.43)
May	819	1	0.12% (0.00-0.68)	1	0.12% (0.00-0.68)
June	640	0	0.00% (0.00-0.57)	0	0.00% (0.00-0.57)
July	526	0	0.00% (0.00-0.70)	0	0.00% (0.00-0.70)
August	1544	1	0.06% (0.00-0.36)	1	0.06% (0.00-0.36)
September	1149	0	0.00% (0.00-0.32)	0	0.00% (0.00-0.32)
October	1178	0	0.00% (0.00-0.31)	0	0.00% (0.00-0.31)
November	1044	1	0.10% (0.00-0.53)	1	0.10% (0.00-0.53)
December	1150	4	0.35% (0.00-0.89)	2	0.17% (0.00-0.63)
2021					
January	984	2	0.20% (0.02-0.73)	1	0.10% (0.00-0.56)
Feburary	652	0	0.00% (0.00-0.56)	0	0.00% (0.00-0.56)



Cohort C: High occupational risk (excluding those vaccinated)

Occupations	Initial recruitment N (%)	2nd visit N (%)	3rd visit N (%)
Bus Driver	139 (16%)	64 (17%)	14 (22%)
Construction site workers	19 (2%)	2 (0.5%)	0 (0%)
Courier services (delivery or courier)	65 (8%)	2 (0.5%)	0 (0%)
Cross-border Truck Drivers	4 (0.5%)	2 (0.5%)	0 (0%)
Foreign domestic helpers	9 (1%)	0 (0%)	0 (0%)
Frontline Staff of MTR	240 (28%)	133 (35%)	34 (53%)
Mini-bus Driver	31 (4%)	15 (4%)	0 (0%)
Taxi Driver	260 (30%)	130 (34%)	12 (19%)
Work in Supermarkets	45 (5%)	15 (4%)	1 (1%)
Work in Wet Markets	44 (5%)	18 (5%)	3 (5%)
Total	856 (100%)	381(100%)	64 (100%)

Sero-positives

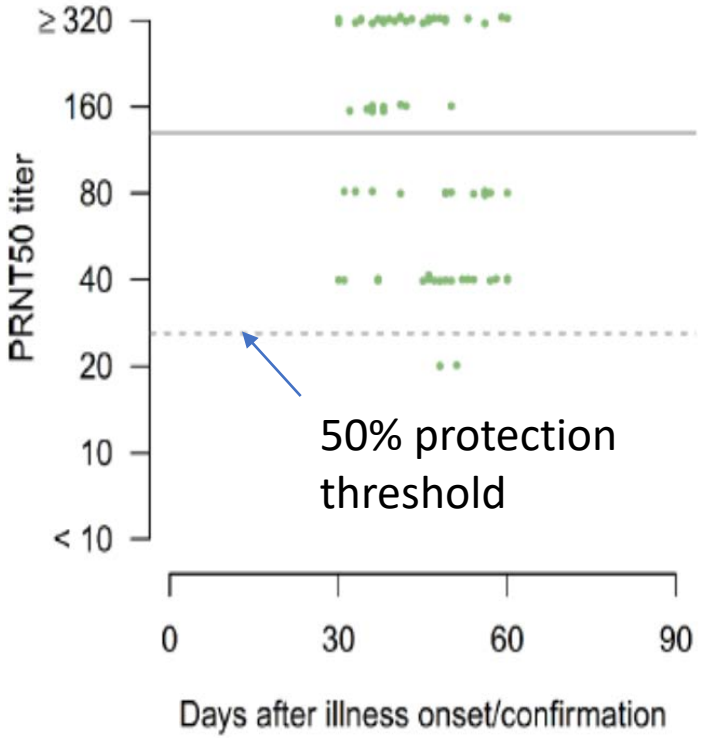
Date of Blood Drawn	Gender	Age	Occupation
23 Apr 2021	Female	64	Bus Driver
25 May 2021	Female	64	Frontline staff of MTR

Positive rate for

Bus drivers 0.7% (95% CI 0.04-3.9)

MTR workers 0.4% (95% CI 0.02-2.3)

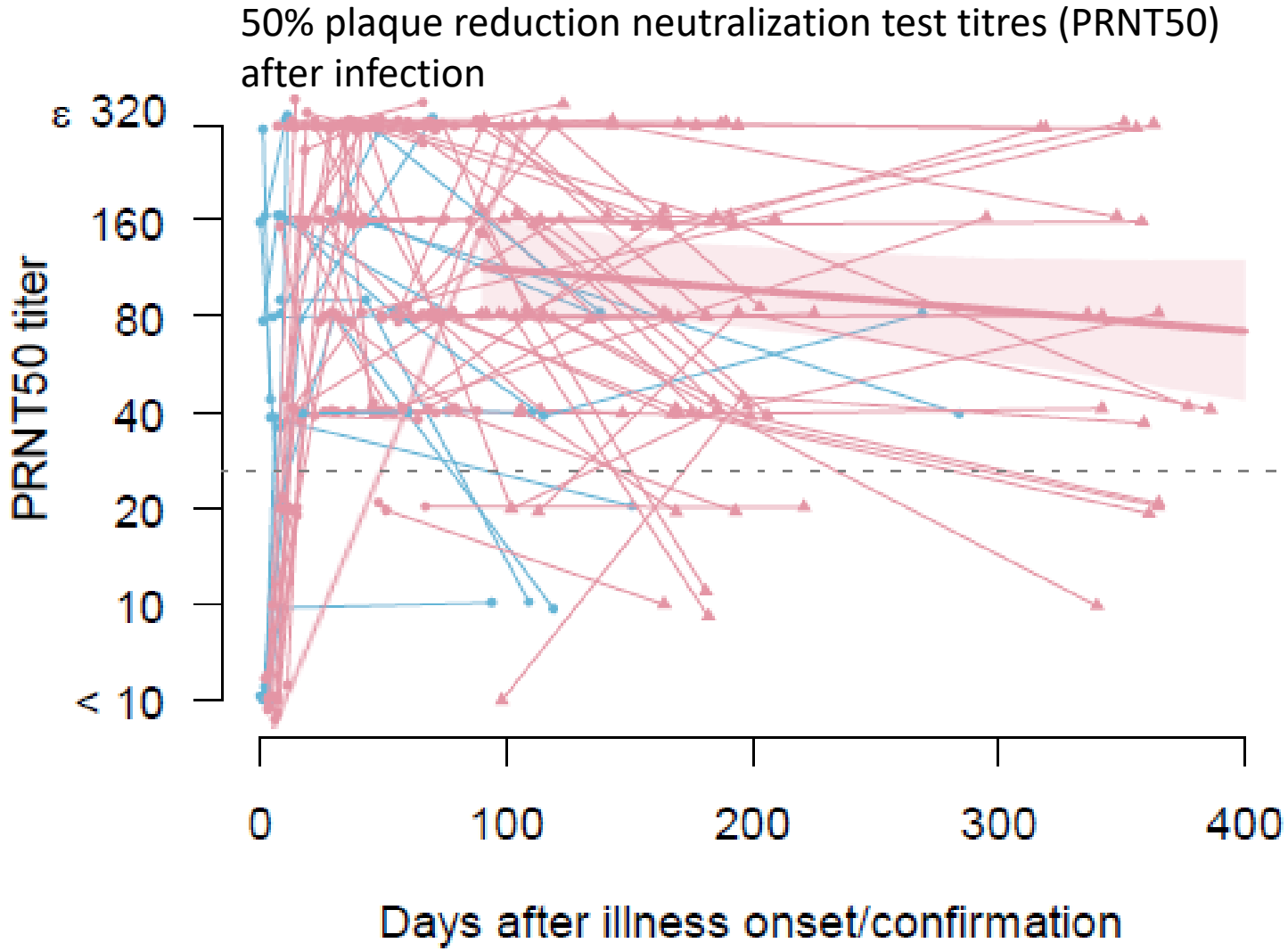
Duration of protection from re-infection in symptomatic COVID-19 infections



- Recent studies have estimated that the correlate of 50% protection from re-infection was 20% of the convalescent neutralizing antibody titre (Khoury et al 2021).
- From the PRNT50 antibody titres in symptomatic COVID-19 cases between 30 to 60 days after illness onset in this study, we estimated the geometric mean antibody titers (GMT) and then, estimated 20% of the GMT, which represents the 50% correlate of protection.
- The threshold for 50% protection from re-infection for PRNT50 was 1:25.9 (95% CI 1:24.7-1:27.6).

Lau et al EClinMed 2021

Estimated duration of protection from re-infection for 50% of those with prior SARS-CoV-2 infection



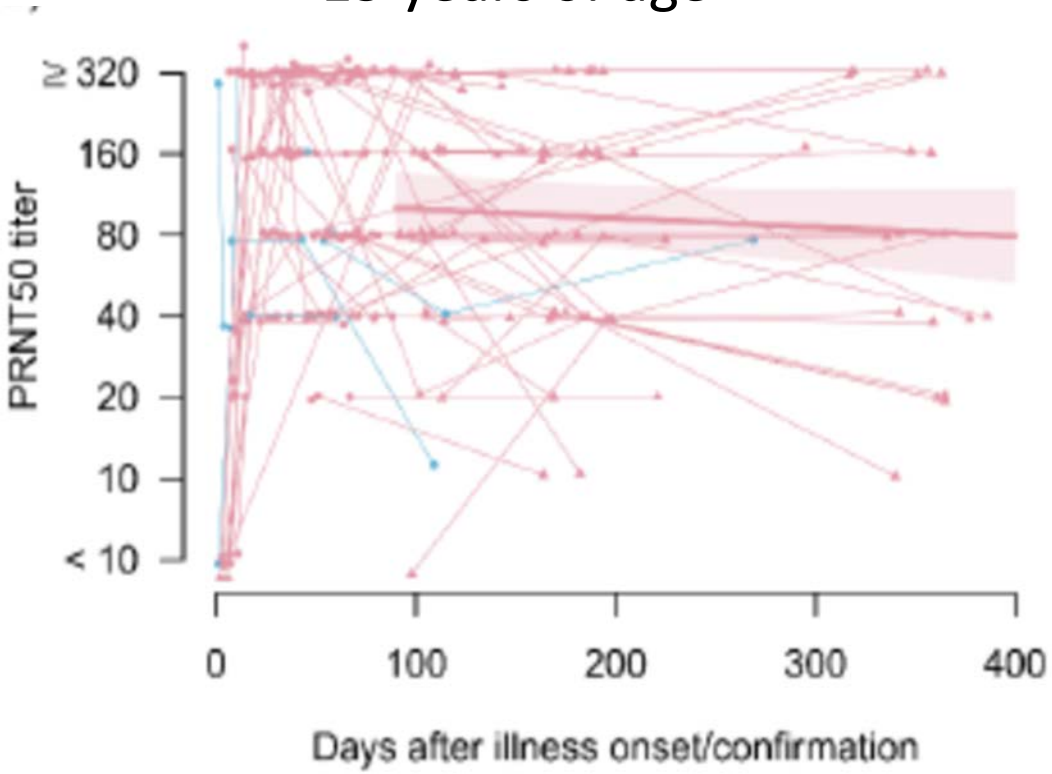
Estimated that PRNT50 will drop to the threshold of protection (1:25) at 990 days (95% CI lower bound 441, decline not statistically significant) days after symptom onset in symptomatic patients.

Lau et al EClinMed 2021

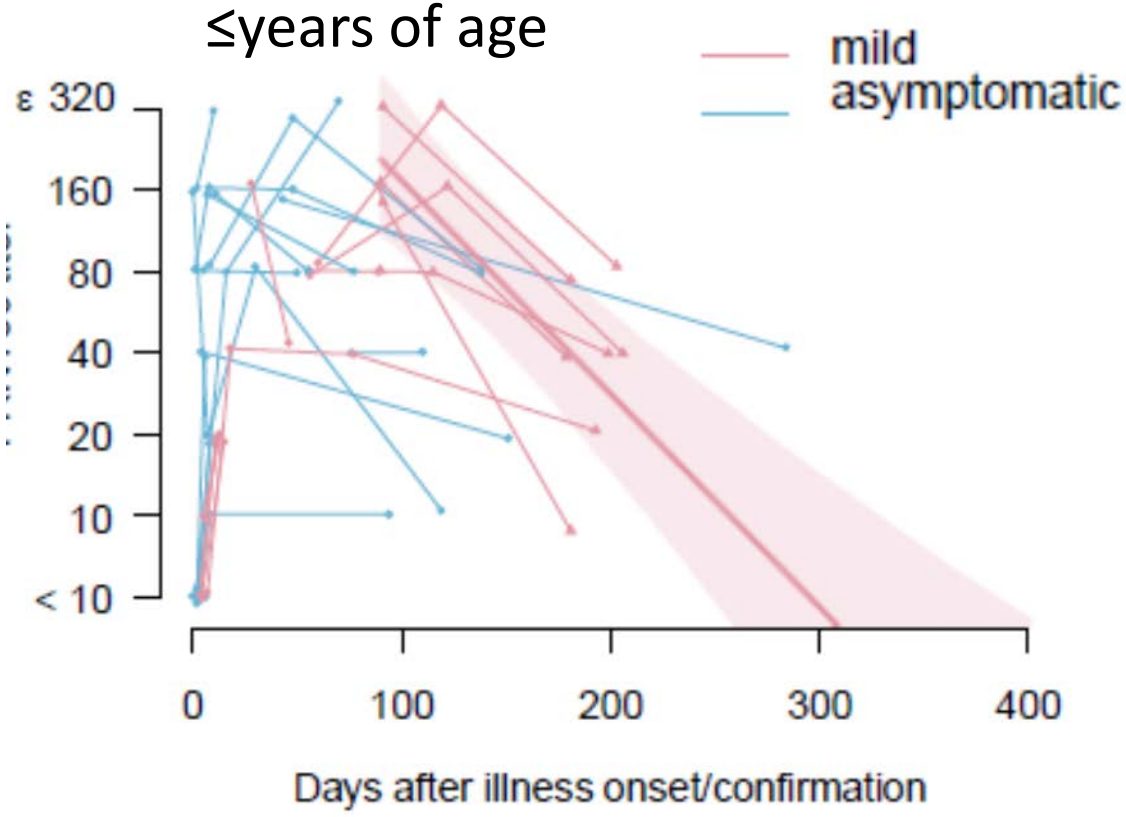
Comparison of duration of 50% plaque reduction neutralization test (PRNT50) antibody responses after natural infection

Compared to adults, children had significantly faster waning of antibody titers for PRNT50 ($p < 0.001$) and PRNT90 ($p = 0.004$) (data not shown). Difference in peak (between 30 to 60 days) PRNT titers did not differ significantly between children and adults with mild disease.

> 15 years of age



≤years of age

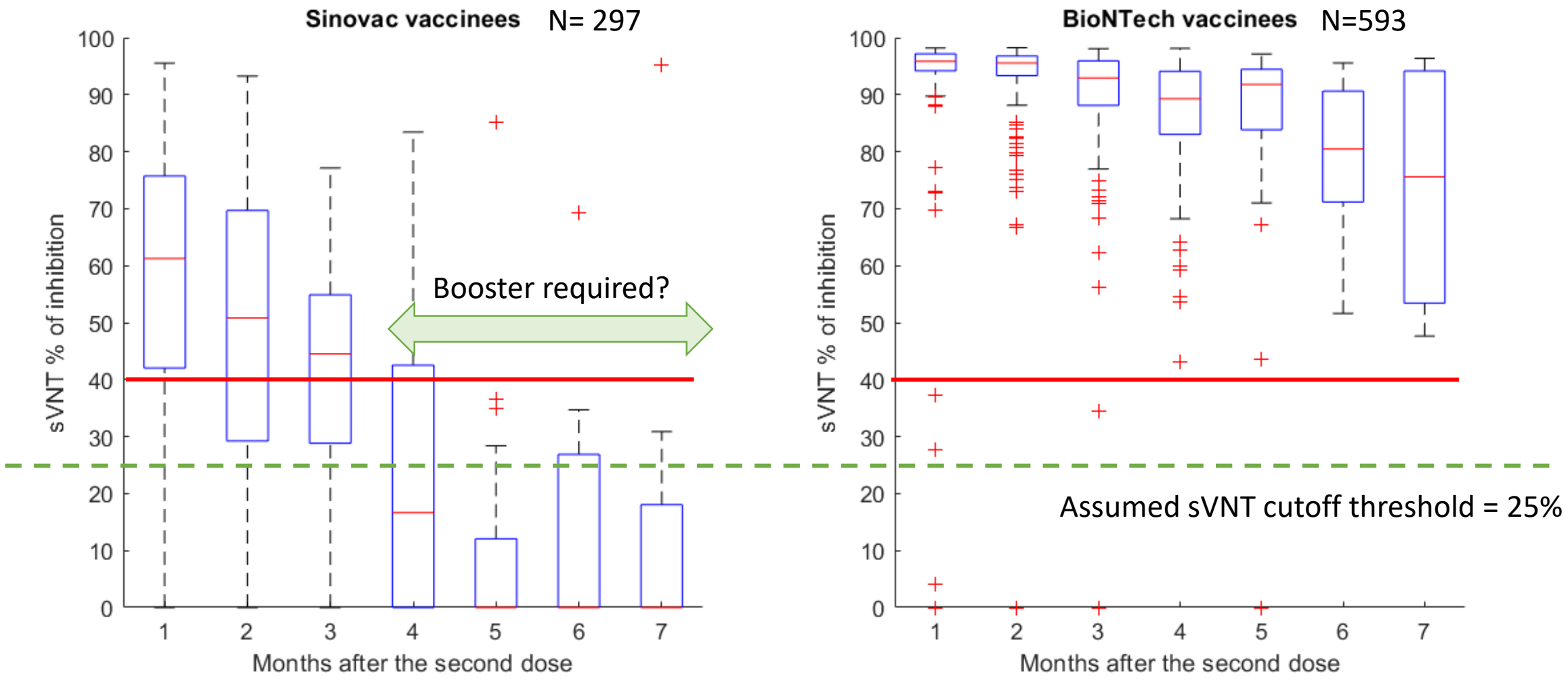


Lau et al EClinMed 2021

The waning immunity among blood donors over time in blood donor cohort

Median age: 48.7 (IQR: 41.8-55.2)

Median age: 39.9 (IQR: 30.3-49.3)



nature COMMUNICATIONS

2021

ARTICLE

https://doi.org/10.1038/s41467-020-20247-4 OPEN

Neutralizing antibody titres in SARS-CoV-2 infections

Eric H. Y. Lau[1], Owen T. Y. Tsang[2], David S. C. Hui[3], Mike Y. W. Kwan[4], Wai-hung Chan[5], Susan S. Chiu[6], Ronald L. W. Ko[1], Kin H. Chan[1], Samuel M. S. Cheng[1], Ranawaka A. P. M. Perera[1], Benjamin J. Cowling[1], Leo L. M. Poon[1] & Malik Peiris[1,7]✉

EClinicalMedicine 2021

Long-term persistence of SARS-CoV-2 neutralizing antibody responses after infection and estimates of the duration of protection

*Eric HY Lau,[a] David SC Hui,[b] Owen TY Tsang,[c] Wai-Hung Chan,[d] Mike YW Kwan,[e] Susan S Chiu,[f] Samuel MS Cheng,[a] Ronald LW Ko,[a] John KC Li,[a] Sara Chaothai,[a] Chi H Tsang,[a] Leo LM Poon,[a,g] and Malik Peiris,[a,g]**

IMMUNOASSAYS

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

2021

Evaluation of a SARS-CoV-2 Surrogate Virus Neutralization Test for Detection of Antibody in Human, Canine, Cat, and Hamster Sera

Ranawaka A. P. M. Perera,[a] Ronald Ko,[a] Owen T. Y. Tsang,[b] David S. C. Hui,[c] Mike Y. M. Kwan,[d] Christopher J. Brackman,[e] Esther M. W. To,[e] Hui-ling Yen,[a] Kathy Leung,[a,f] Samuel M. S. Cheng,[a] Kin Ho Chan,[a] Karl C. K. Chan,[a] Ka-Chi Li,[a] Linda Saif,[g] Vanessa R. Barrs,[h] Joseph T. Wu,[a,f] Thomas H. C. Sit,[e] Leo L. M. Poon,[a,i] Malik Peiris[a,i]

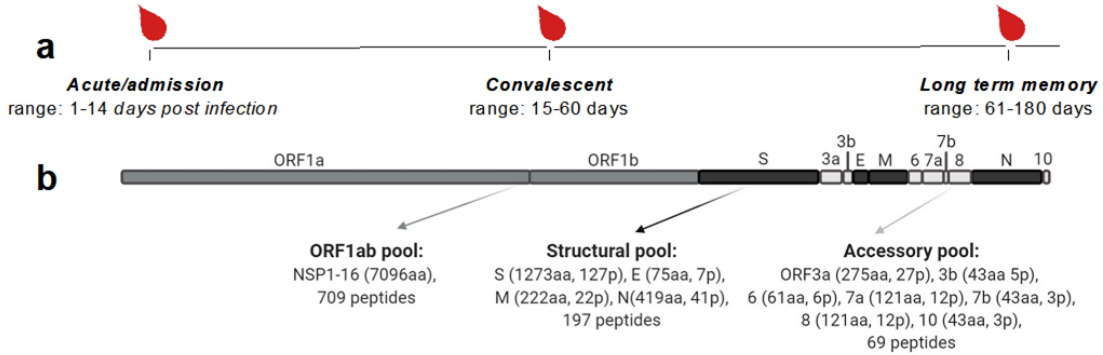
nature COMMUNICATIONS

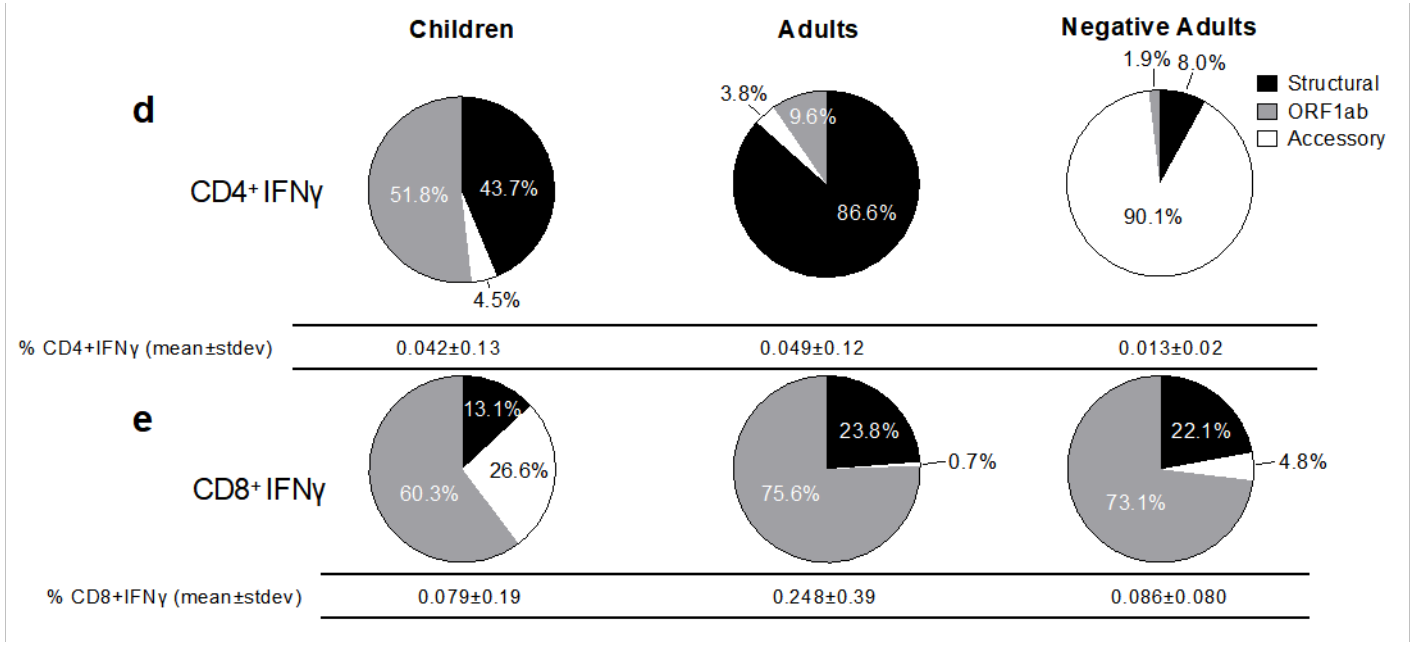
ARTICLE

https://doi.org/10.1038/s41467-021-24938-4 OPEN

SARS-CoV-2 specific T cell responses are lower in children and increase with age and time after infection

Carolyn A. Cohen[1], Athena P. Y. Li[1], Asmaa Hachim[1], David S. C. Hui[2], Mike Y. W. Kwan[3], Owen T. Y. Tsang[4], Susan S. Chiu[5], Wai Hung Chan[6], Yat Sun Yau[6], Niloufar Kavian[1], Fionn N. L. Ma[1], Eric H. Y. Lau[7], Samuel M. S. Cheng[8], Leo L. M. Poon[1,8], Malik Peiris[1,8] & Sophie A. Valkenburg[1]✉





- Children have reduced T cell immunity after SARS-CoV-2 infection compared to infected adults
- Biased towards non-structural proteins
- Reduced proportion of effector memory T cells for recall -> may impact their long-term protection

Summary of outcomes

- Validate testing strategy and define duration of protective antibody responses which is now being used in the different vaccine studies being carried out by Profs Hui, Lau and Cowling.
- Infection attack rate in the Hong Kong population is ~1% and 17%-23% of all infections are being detected and confirmed.
- Occupational groups such as taxi drivers, supermarket employees were not at higher risk than the general population? Perhaps because of PPE and precautional practices?
- Protective antibody responses following natural infection are relatively long lived.
- Antibody responses in children appear to be shorter lasting.
- Natural infection is associated with robust T cell responses but these are weaker in children and more directed to the ORF1 non-structural proteins.

Acknowledgments

- Cohort A: Ben Cowling, Nancy Leung, Eunice Shiu, Irene Wong
- Cohort B: Joe Wu, Kathy Leung, D Liu, Shirley Kwok, Miky Wong; and from BTS: Dr CK Lee, Dr Jennifer Leung, Ms Wing-Ching Ling, Ms Rita Lo
- Cohort C: M Ni, Ryan Au, A Lee, A Kwok, V Ip, S Ding, CY Chan, K Hon, Y Cheng, T Leung, V Lai, Q Li, K Yau, A Chan, M Kan, M Kan, V Fung, A Mok
- Cohort D: Dennis Ip, Teresa So and Ada Lin, May Ked Tham, Cecilia Fan, Joan Yu from CHP
- Cohort E: David Hui, Owen Tsang, Wai hung Chan, Mike Kwan, Susan Chiu, Eric Lau
- Planning and advice: Gabriel Leung
- Serology testing: SMS Cheng, R Ko, HK Chan, JKC Li, S Chaothai, CH Tsang, K Kwan, K Chan, Y Leung, L Luk, Z Chai
- T cell testing: Sophie Valkenburg, Carolyn Cohn

Research Fund Secretariat
Food and Health Bureau
Hong Kong Special Administrative Region

Month-by-month data on unvaccinated individuals

Month	Samples from unvaccinated individuals (or pre-first-dose)	PRNT positive samples	Crude % positive	Age-standardized % with 95% CI
Jul 2020	50	0	0%	0% (0%, 0%)
Aug 2020	27	0	0%	0% (0%, 0%)
Sep 2020	56	0	0%	0% (0%, 0%)
Oct 2020	71	0	0%	0% (0%, 0%)
Nov 2020	76	0	0%	0% (0%, 0%)
Dec 2020	254	0	0%	0% (0%, 0%)
Jan 2021	227	0	0%	0% (0%, 0%)
Feb 2021	194	0	0%	0% (0%, 0%)
Mar 2021	366	2	0.54%	0.55% (0%, 1.08%)
Apr 2021	143	5	3.31%	3.50% (0.29%, 5.11%)
May 2021	41	1	2.44%	2.44% (0%, 13.13%)
Jun 2021	92	0	0%	0% (0%, 0%)
Jul 2021	101	2	1.83%	1.98% (0%, 3.41%)
Aug 2021	51	0	0%	0% (0%, 0%)
Sep 2021	31	0	0%	0% (0%, 0%)