EVOLUZIONE COVID 2019
LA CLINICA TRA VARIANTI E RIABILITAZIONE

MARTEDÌ 25 MAGGIO 2021 - ORE 20.30

Responsabile scientifico: Giovanni Leoni

PROGRAMMA

ORE 20.30 - APERTURA DEI LAVORI
Giovanni Leoni, *Presidente OMCeO Venezia e Vicepresidente FNOMCeO*

ORE 20.40 - L'EVOLUZIONE DELLA PANDEMIA NEL MONDO E IN EUROPA - DATI E IMMAGINI CORRELATE
Guido Sattin, *Direttore Sanitario ORAS*
Ospedale Riabilitativo di Alta Specializzazione, Motta di Livenza, Treviso

ORE 21.00 - DIAGNOSTICA DI LABORATORIO NEL COVID-19
Mario Plebani, *Cattedra di Biochimica Clinica e Biologia Molecolare, Università di Padova*
Direttore Dipartimento Servizi di Diagnostica Integrata, Azienda Ospedale Università di Padova

ORE 21.40 - ESPERIENZA DEL COVID HOSPITAL DI JESOLO
Lucio Brollo, *Direttore UOC Medicina Generale e Cardiologia Riabilitativa, PO Jesolo*
Responsabile Malattie Infettive Covid-19 PO Jesolo

ORE 22.20 - EFFETTI DEL COVID: RIABILITAZIONE RESPIRATORIA E NEUROLOGICA
Guido Sattin

ORE 22.40 - DISCUSSIONE CON I RELATORI

ORE 23.00 - CHIUSURA DEI LAVORI

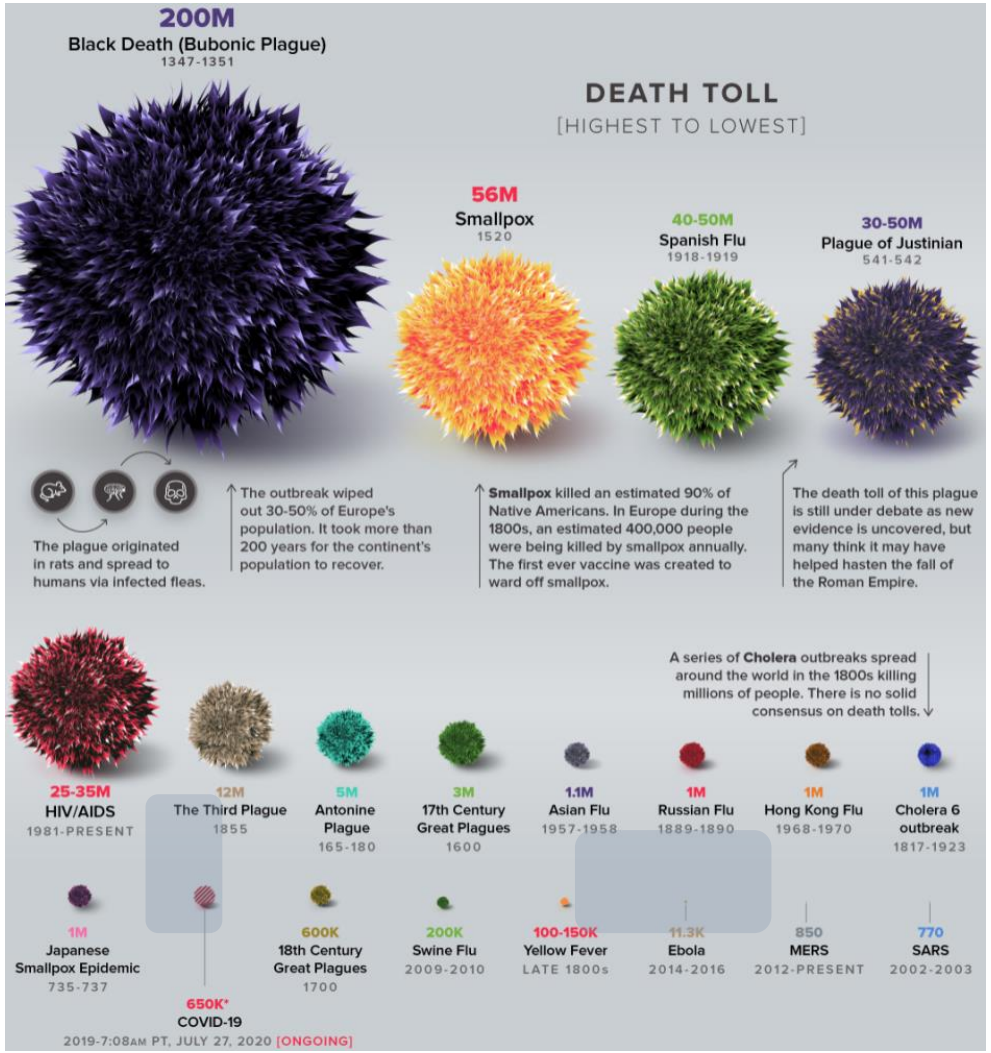
ISCRIZIONE OBBLIGATORIA ON LINE A QUESTO LINK:
https://zoom.us/webinar/register/WN_WRNirquXS_-sIueQKnoqtQ

Coronavirus disease 2019 (COVID-19)

Coronavirus disease 2019 (COVID-19),
The first official case diagnosed in Wuhan (China) on November 17, 2019,
The third coronaviruses outbreak occurring during the past 20 years

- Severe acute respiratory syndrome (**SARS**) in 2002-2003

- Middle-East respiratory syndrome (**MERS**) in 2012



https://www.visualcapitalist.com/history-of-pandemics-deadliest/

MEDICINA DI LABORATORIO e PANDEMIA

- La pandemia da SARS-CoV-2, nella sua drammatica manifestazione, oltre alle migliaia di decessi, pazienti con malattia severa e lunghe degenze in reparti ospedalieri e in isolamento domiciliare, ha portato finalmente alla luce ***il valore e la centralità della medicina di laboratorio***.
- Più che decine di pubblicazioni scientifiche, relazioni a congressi e documenti di Società Scientifiche e Organismi professionali, il "COVID" ha illustrato a tutti i cittadini e pazienti quale sia il ***valore dell'analisi di laboratorio***.

Plebani M. Biochimica Clinica 2020

MEDICINA DI LABORATORIO e PANDEMIA

- ***La pandemia da Sars-Cov-2, molto più di numerose pubblicazioni scientifiche, ha fatto capire a tutti quale sia il valore dell'analisi di laboratorio.*** Il messaggio dell'importanza della diagnostica è arrivato forte e chiaro quando, nel corso della prima fase, alcuni lavori scientifici hanno dimostrato che anche gli asintomatici possono essere contagiosi. Il caso della nave da crociera Diamond Princess è stato, sotto quest'aspetto, quasi un modello di studio che si è avvalso della diagnostica molecolare per scovare i positivi.
- «La medicina di laboratorio – spiega Mario Plebani, docente di Biochimica clinica e Biologia molecolare e direttore del Dipartimento di Servizi di diagnostica integrata presso l'Azienda Ospedaliera Università di Padova – è fondamentale per poter avere una diagnosi, sia per confermare un'ipotesi clinica basata sull'osservazione dei sintomi, sia quando il paziente è asintomatico».

La Repubblica 17.11.2020

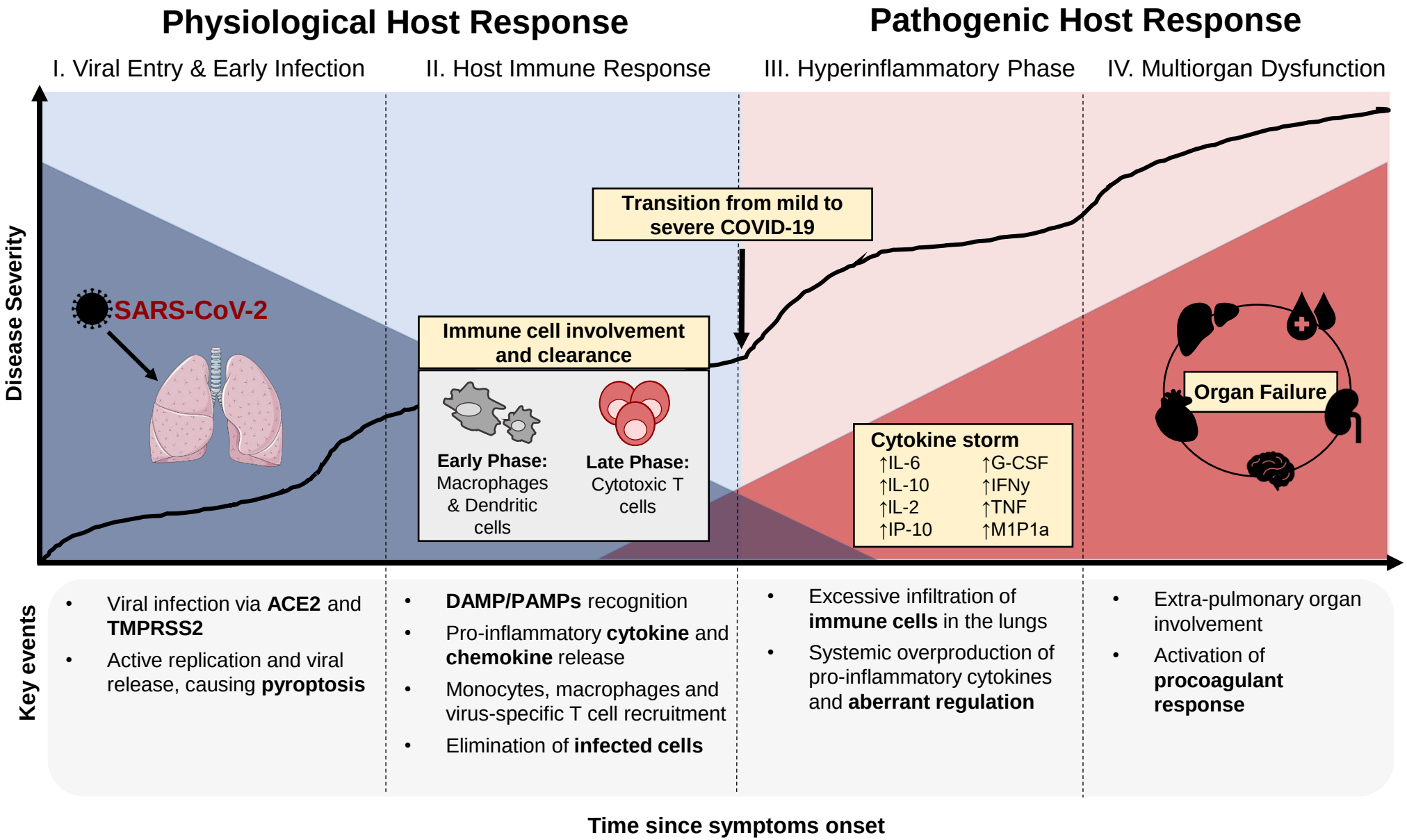

Physiological Host Response
Pathogenic Host Response
I. Viral Entry & Early Infection
II. Host Immune Response
III. Hyperinflammatory Phase
IV. Multiorgan Dysfunction
Disease Severity
SARS-CoV-2
Transition from mild to severe COVID-19
Immune cell involvement and clearance
Early Phase: Macrophages & Dendritic cells
Late Phase: Cytotoxic T cells
Cytokine storm
↑IL-6
↑IL-10
↑IL-2
↑IP-10
↑G-CSF
↑IFNy
↑TNF
↑M1P1a
Organ Failure
Key events
• Viral infection via ACE2 and TMPRSS2
• Active replication and viral release, causing pyroptosis
• DAMP/PAMPs recognition
• Pro-inflammatory cytokine and chemokine release
• Monocytes, macrophages and virus-specific T cell recruitment
• Elimination of infected cells
• Excessive infiltration of immune cells in the lungs
• Systemic overproduction of pro-inflammatory cytokines and aberrant regulation
• Extra-pulmonary organ involvement
• Activation of procoagulant response
Time since symptoms onset

Figure 1

Giuseppe Lippi and Mario Plebani

The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks.

There are at least ***three major areas*** where in vitro diagnostics can provide essential contributions to diagnostic reasoning and managed care of patients with suspected or confirmed SARS-CoV-2 infection.

These include:

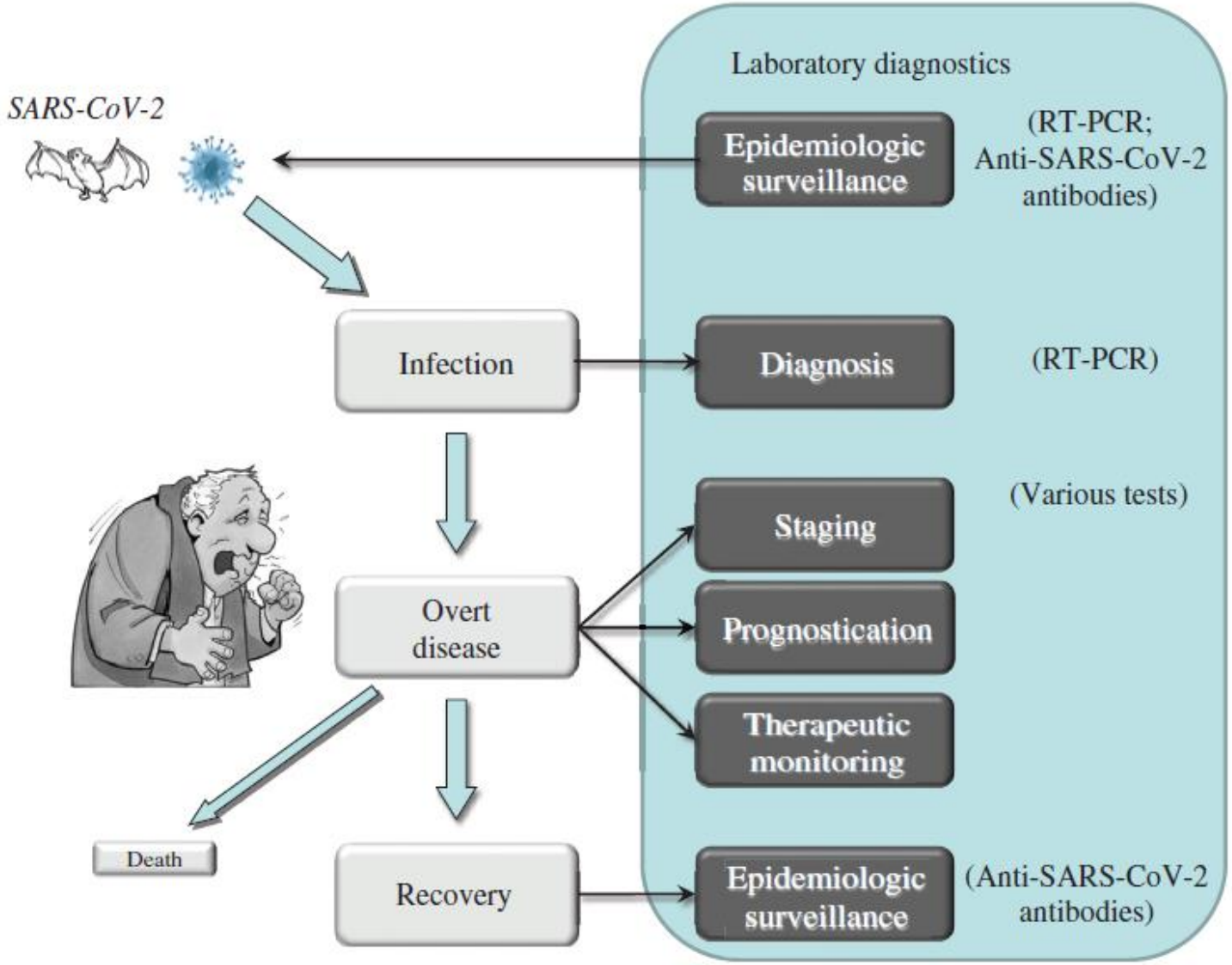
etiological diagnosis,

patient monitoring,

epidemiologic surveillance

Lippi G, Plebani M. Clin Chem Lab Med. 2020 Mar 19. doi: 10.1515/cclm-2020-0240. [Epub ahead of print] PubMed PMID: 32191623.

Figure 2: The essential role of laboratory diagnostics in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. RT-PCR, reverse transcription-polymerase chain reaction.



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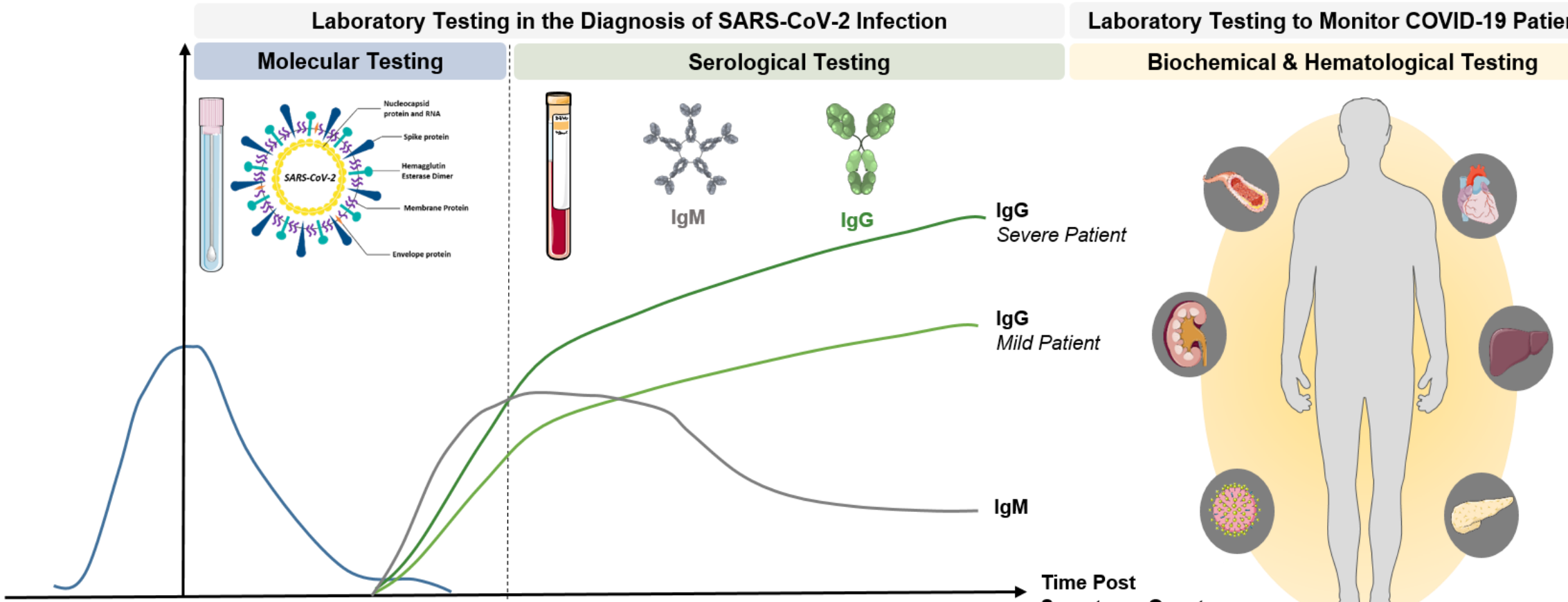
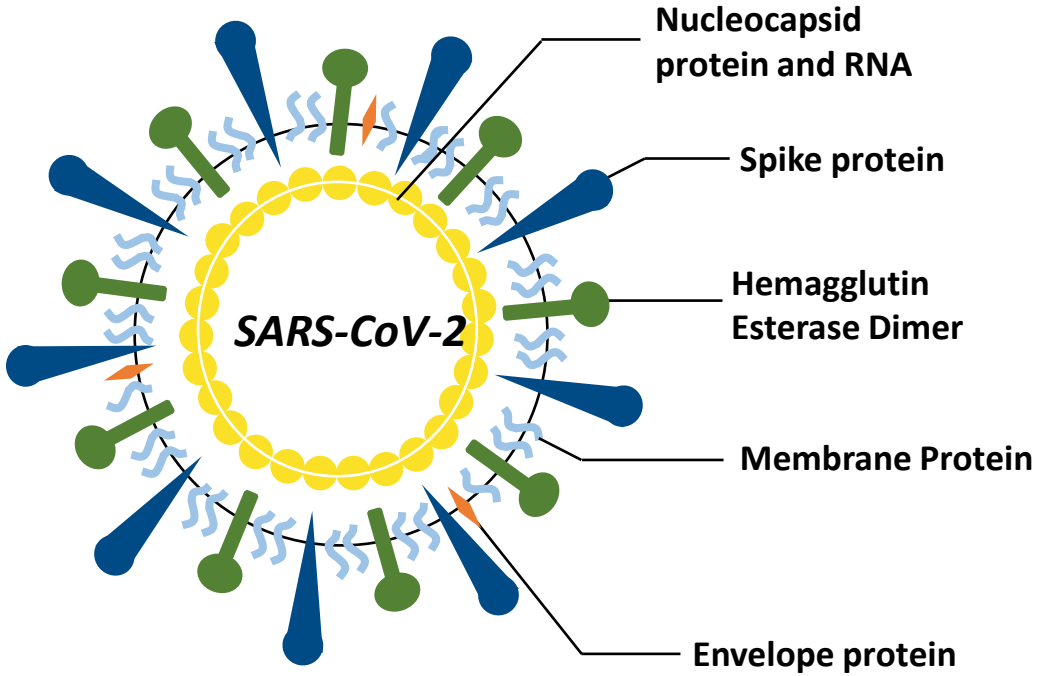
Clinical Laboratory Testing during the COVID-19 Pandemic



Specimen Type:	Nasopharyngeal	Blood (serum, plasma, whole blood, finger prick)
Assay Principle:	NAAT	LFA, CLIA, or ELISA
General Use:	Identification of current SARS-CoV-2 infection	Identification of past SARS-CoV-2 infection

Lippi G, Plebani M., CCLM 2020

SARS-CoV-2: *Overview of Viral Characteristics*



SARS-CoV-2 consists of four main structural glycoproteins:

- ***spike (S),***
- ***membrane (M)***
- ***envelope (E)***
- ***nucleocapsid (N)***

The M, E, and N proteins are critical for viral particle assembly and release, whereas the S protein is responsible for viral binding and entry into host cells

Molecular testing uses RT-PCR to identify viral SARS-CoV-2 RNA in a variety of specimens. Available assays target different viral RNA sequences

TESTS FOR COVID-19 FALL INTO TWO BROAD GROUPS

TESTS THAT DETECT THE PRESENCE OF SARS-CoV-2 **VIRUS**

Sample type: respiratory samples such as nasopharingeal swabs, oropharyngeal swabs and saliva

Methods: molecular assays (rRT-PCR, DigPCR) and rapid antigen tests (both lab-based and POCT)

TESTS THAT DETECT THE PRESENCE OF **ANTIBODIES** TO SARS-CoV-2

Sample type: whole blood and/or serum plasma

Methods: lab-based and POCT, ELISA/CLIA and immunochromatographic

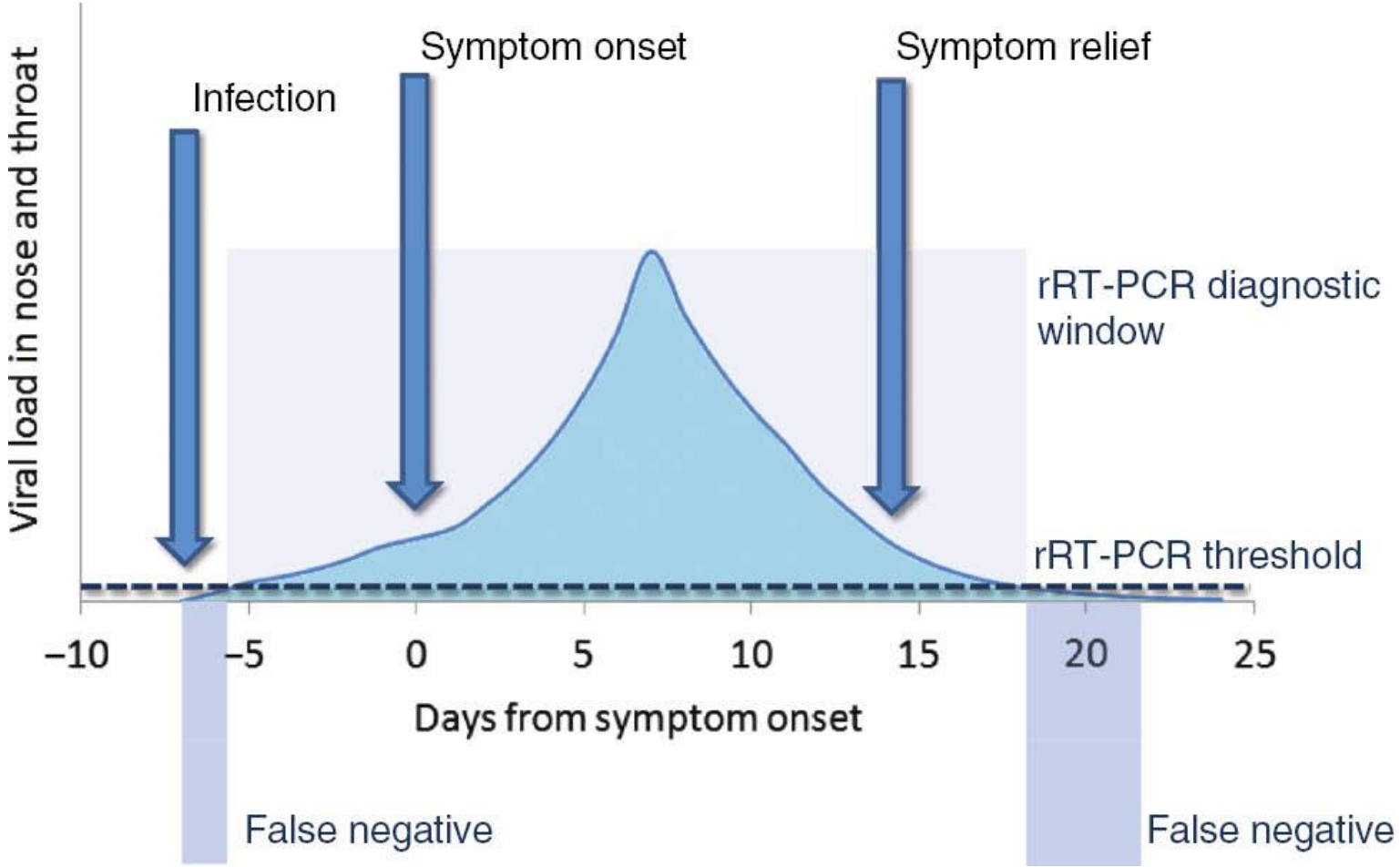


Figure 1: Correspondence between development of viral load during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, clinical course and positivity of (real time) reverse transcription polymerase chain reaction (rRT-PCR) assays.

Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19)

Lippi G, Simundic AM, Plebani M. Clin Chem Lab Med. 2020 Mar 16. [Epub ahead of print] PMID: 32172228.

THE GOLD STANDARD (RT-PCR)

The current ***gold standard*** for the etiological diagnosis of SARS-CoV-2 infection is (real-time) reverse transcription polymerase chain reaction (**rRT-PCR**) on respiratory tract specimens.

The diagnostic accuracy of this technique shall be considered a foremost prerequisite but, as for all laboratory tests there are some ***pre-analytical*** and ***analytical vulnerabilities***.

Lippi G, Plebani M. Clin Chem Lab Med. 2020

DIAGNOSTIC ACCURACY OF LABORATORY TESTS

SARS-CoV-2 (MOLECULAR TESTING)

Pooled ***sensitivity***: 87,8%	16 studies =3818	assay: RT-PCR
Pooled ***specificity***: 98,3%	n= 108	assay: RT-PCR
98.7%	n= 154	assay: RT-LAMP

Jarrim D et al. BMJ BMJ Evid Based Med. 2020

Table 1: Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19) using (real time) reverse transcription polymerase chain reaction (rRT-PCR).

Preanalytical
General
– Lack of identification/misidentification
– Inadequate procedures for specimen (e.g. swab) collection, handling, transport and storage
– Collection of inappropriate or inadequate material for quality or volume
– Presence of interfering substances
– Manual (pipetting) errors
Specific
– Sample contamination
– Testing in patients receiving antiretroviral therapy
Analytical
– Testing carried out outside of the diagnostic window
– Active viral recombination
– Use of non-adequately validated assays
– Lack of harmonization of primers and probes
– Instrument malfunctioning
– Insufficient or inadequate material
– Non-specific PCR annealing
– Misinterpretation of expression profiles

The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks

Lippi G, Plebani M. Clin Chem Lab Med. 2020 Mar 19. doi: 10.1515/cclm-2020-0240. [Epub ahead of print] PMID: 32191623.

Table 3: Practical indications to minimize the risk of diagnostic errors in identifying severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Combine results of SARS-CoV-2 RT-PCR infection with – Clinical and epidemiologic evidence (probability of exposure, signs, symptoms, negative diagnostic tests especially for other respiratory illnesses) – Chest computed tomography (CT; most frequently appear with ground-glass opacities, consolidation with or without vascular enlargement, air bronchogram signs, interlobular septal thickening)
Recollect and test upper respiratory specimens in patients with negative RT-PCR test results and high suspicion or probability of SARS-CoV-2 infection
Provide clear instructions on how nasopharyngeal and oropharyngeal swabs shall be correctly collected, managed and stored
Thorough compliance with assay procedures, including quality assurance
Validate extensively RT-PCR assay before clinical usage
Further refinement of molecular target(s)

rRT-PCR, (real time) reverse transcription polymerase chain reaction.

Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19)

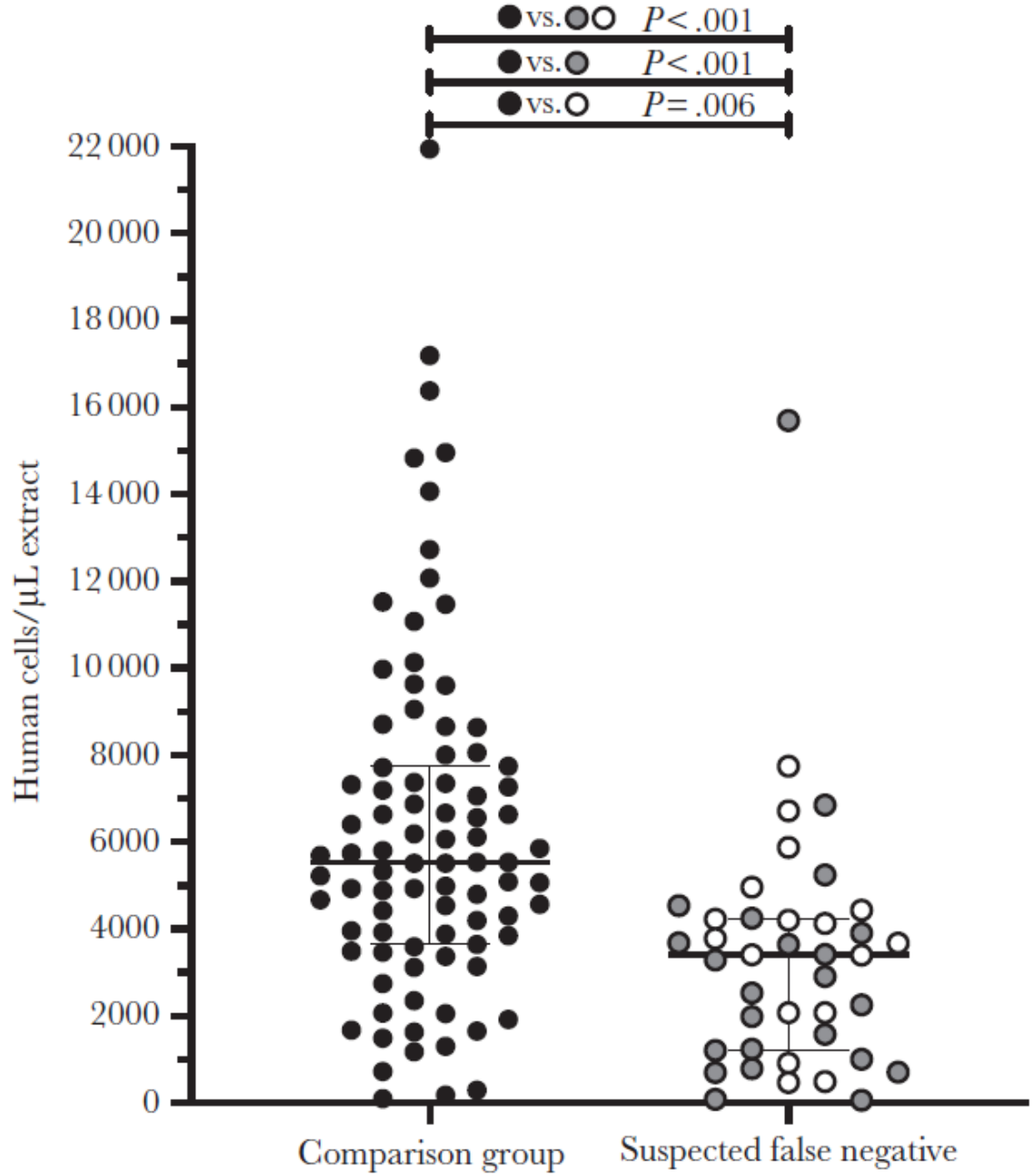
Lippi G, Simundic AM, Plebani M. Clin Chem Lab Med. 2020 Mar 16. [Epub ahead of print] PMID: 32172228.

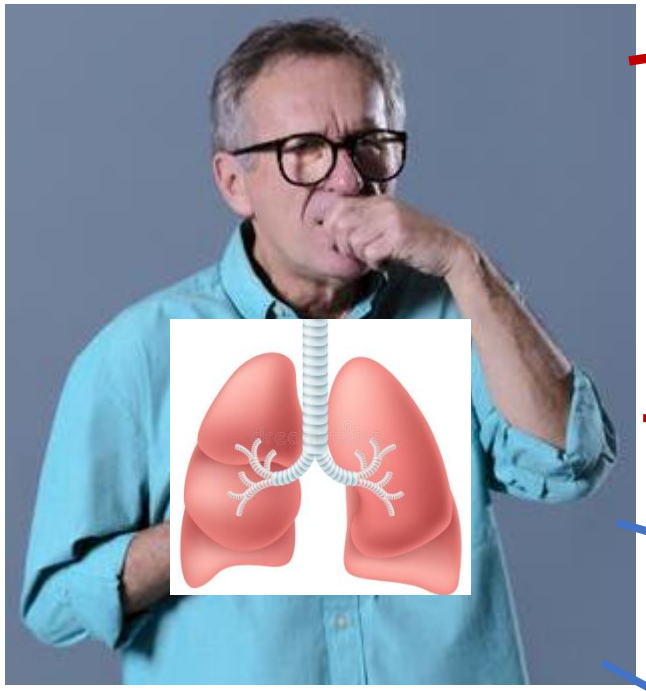
The Journal of Infectious Diseases

BRIEF REPORT

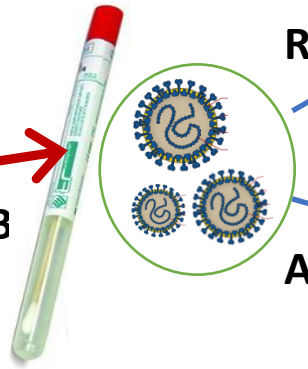
Suboptimal Biological Sampling as a Probable Cause of False-Negative COVID-19 Diagnostic Test Results

Natalie N. Kinloch,[1,2] Gordon Ritchie,[3,4] Chanson J. Brumme,[2,5] Winnie Dong,[2] Weiyan Dong,[2] Tanya Lawson,[3] R. Brad Jones,[6] Julio S. G. Montaner,[2,5] Victor Leung,[3,4] Marc G. Romney,[3,4] Aleksandra Stefanovic,[3,4] Nancy Matic,[3,4] Christopher F. Lowe,[3,4,a] and Zabrina L. Brumme[1,2,a]





SWAB



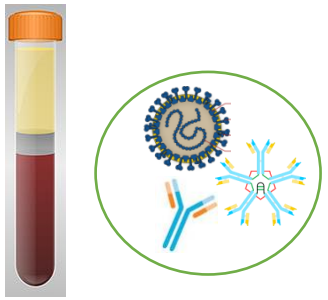
RNA
- RT-qPCR
- RT-LAMP
- dd PCR
- CRISPR

ANTIGEN
- Lateral Flow (ICT)
- Chemiluminescence (CLIA)
- Fluorescent (FIA)

SALIVA
- RT-PCR
- CLIA

EXHALED BREATH
- RT-PCR
- Gas-chromatography-ion mobility spec
- Multiplexed nano material-based sensor

BLOOD



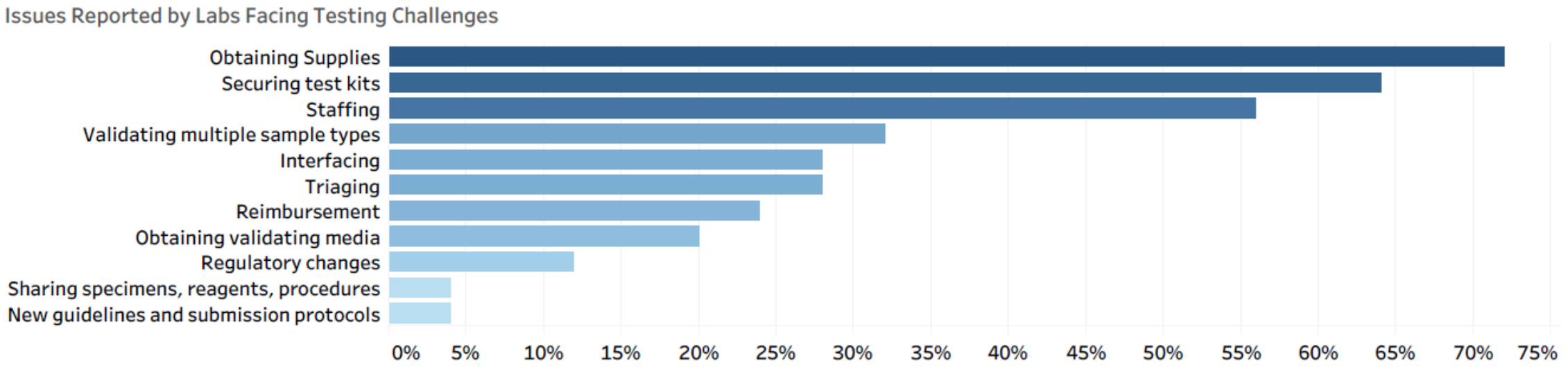
- Lateral Flow (??)
- ELISA
- Chemiluminescence immunoassays

LABORATORY MEDICINE

AACC: COVID-19 Survey Results

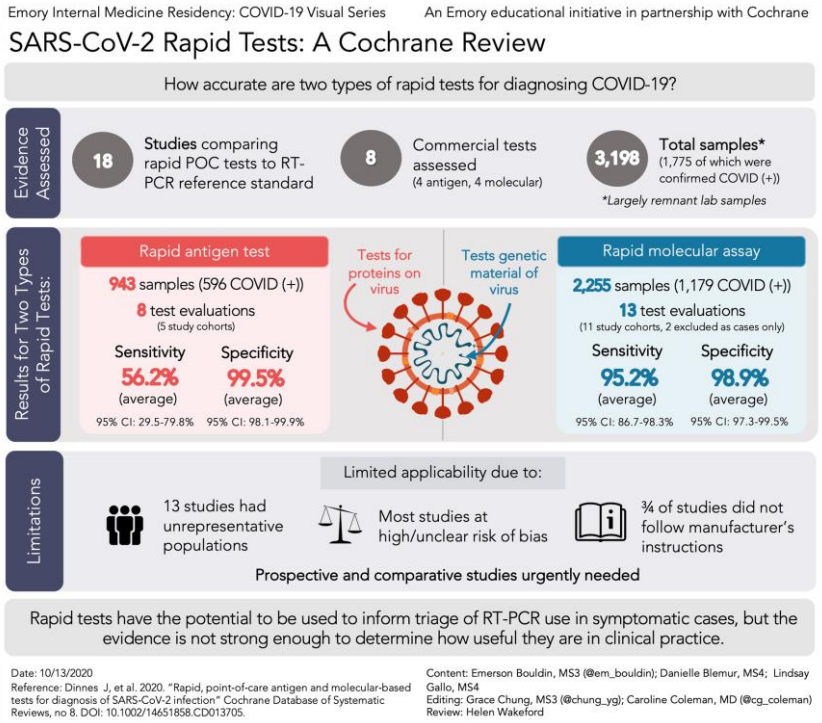
- **57% of respondent labs report being unable to obtain supplies necessary to run COVID-19 tests in the week before they were surveyed**
- **21% of labs offering COVID-19 testing expect to be unable to process all requested COVID-19 tests within the week after they were surveyed**

83% of respondent labs report facing challenges in testing or increasing their testing capacity for COVID-19.



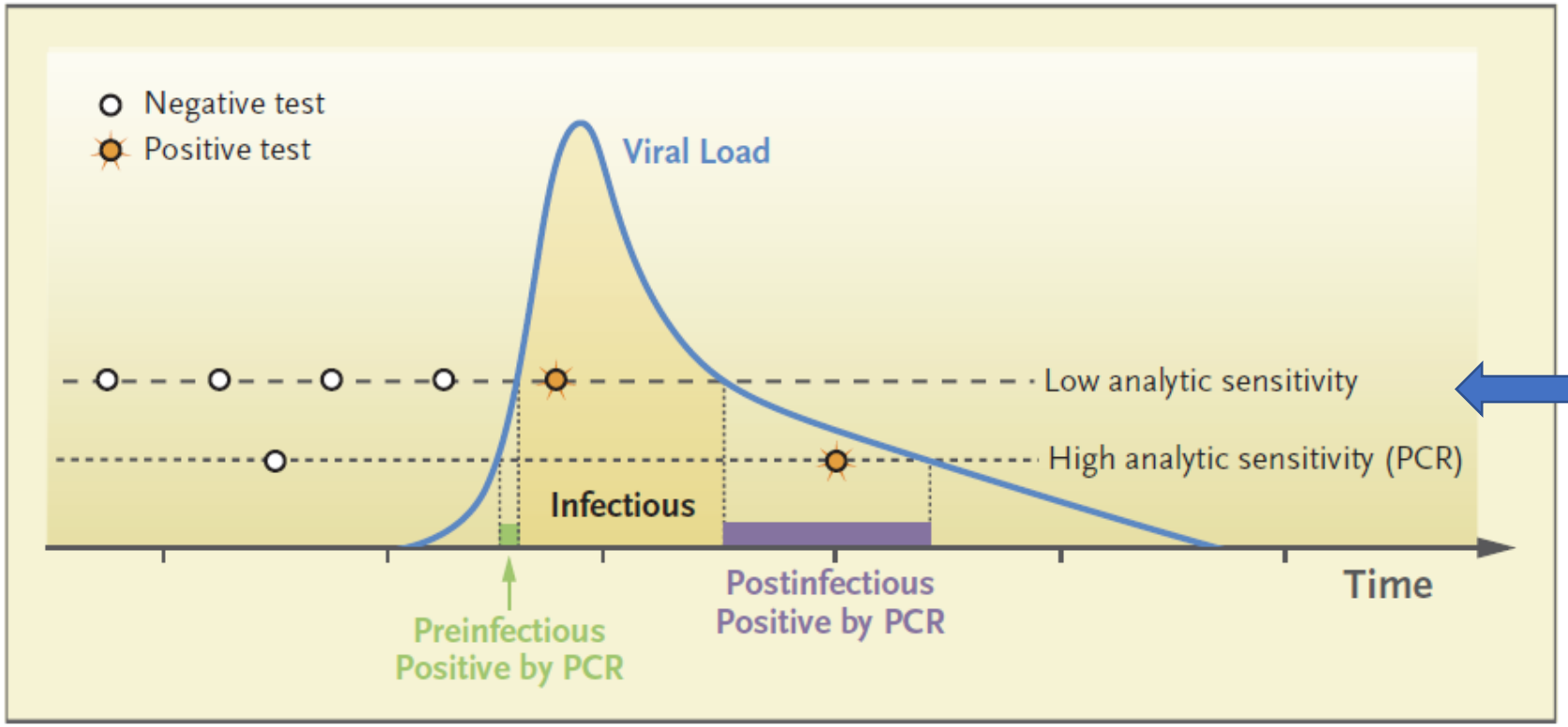
ANTIGEN TESTING for SARS-CoV-2

- «RAPID» Antigen testing (lateral flow tests)
- Laboratory-based Antigen testing
- Antigen testing on salivary samples



Rethinking Covid-19 Test Sensitivity — A Strategy for Containment

Michael J. Mina, M.D., Ph.D., Roy Parker, Ph.D., and Daniel B. Larremore, Ph.D.



High-Frequency Testing with Low Analytic Sensitivity versus Low-Frequency Testing with High Analytic Sensitivity.

N Engl J Med 2020

Rapid Antigen

Cochrane Library

Cochrane Database of Systematic Reviews

Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection (Review)

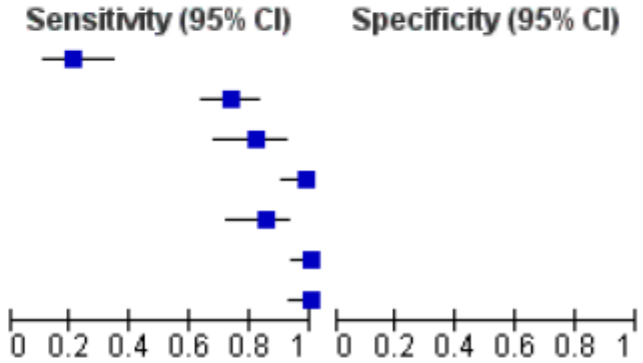
Antigen tests

Sensitivity varied considerably across studies (from 0% to 94%): the average sensitivity was 56.2% (95% CI 29.5 to 79.8%) and average specificity was 99.5% (95% CI 98.1% to 99.9%; based on 8 evaluations in 5 studies on 943 samples). Data for individual antigen tests were limited with no more than two studies for any test.

Figure 5. Forest plot of studies evaluating antigen tests according to viral load: high (≤ 25 Ct) versus low viral load (≤ 30 Ct in Diao 2020). Studies grouped by test

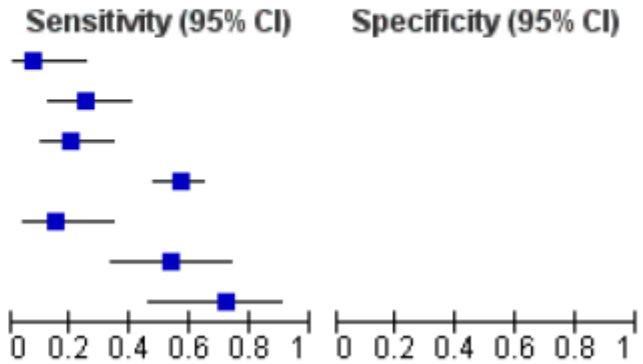
Antigen tests - high viral load

Study	TP	FP	FN	TN	Test	Sensitivity (95% CI)	Specificity (95% CI)
Weitzel 2020 [C]	11	0	41	0	Beijing Savant - FIA	0.21 [0.11, 0.35]	Not estimable
Mertens 2020	65	0	23	0	Coris BioConcept - CGIA	0.74 [0.63, 0.83]	Not estimable
Lambert-Niclot 2020	37	0	8	0	Coris BioConcept - CGIA	0.82 [0.68, 0.92]	Not estimable
Diao 2020	55	0	1	0	In-house - FIA	0.98 [0.90, 1.00]	Not estimable
Weitzel 2020 [A]	45	0	8	0	RapiGEN Inc - CGIA	0.85 [0.72, 0.93]	Not estimable
Weitzel 2020 [D]	54	0	0	0	Shenzhen Bioeasy - FIA	1.00 [0.93, 1.00]	Not estimable
Porte 2020	52	0	0	0	Shenzhen Bioeasy - FIA	1.00 [0.93, 1.00]	Not estimable

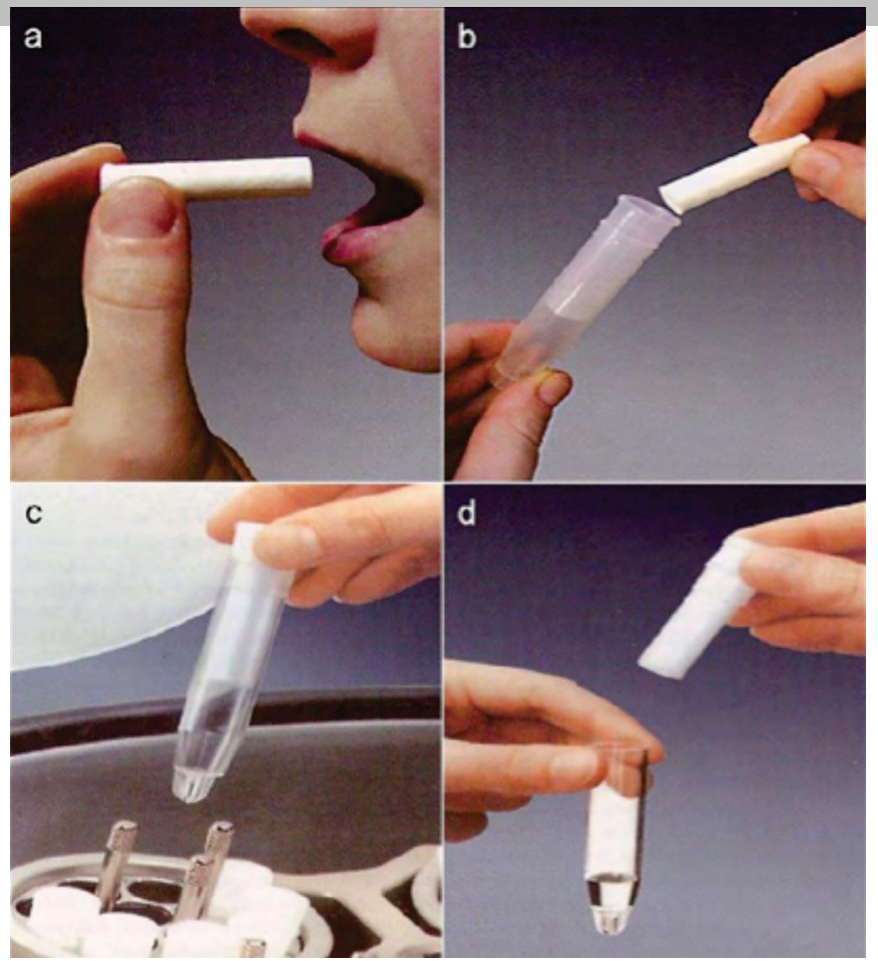


Antigen tests - low viral load

Study	TP	FP	FN	TN	Test	Sensitivity (95% CI)	Specificity (95% CI)
Weitzel 2020 [C]	2	0	24	0	Beijing Savant - FIA	0.08 [0.01, 0.25]	Not estimable
Mertens 2020	11	0	33	0	Coris BioConcept - CGIA	0.25 [0.13, 0.40]	Not estimable
Lambert-Niclot 2020	10	0	39	0	Coris BioConcept - CGIA	0.20 [0.10, 0.34]	Not estimable
Diao 2020	86	0	66	0	In-house - FIA	0.57 [0.48, 0.65]	Not estimable
Weitzel 2020 [A]	4	0	22	0	RapiGEN Inc - CGIA	0.15 [0.04, 0.35]	Not estimable
Weitzel 2020 [D]	14	0	12	0	Shenzhen Bioeasy - FIA	0.54 [0.33, 0.73]	Not estimable
Porte 2020	13	0	5	0	Shenzhen Bioeasy - FIA	0.72 [0.47, 0.90]	Not estimable



LA SALIVA COME CAMPIONE «ALTERNATIVO»



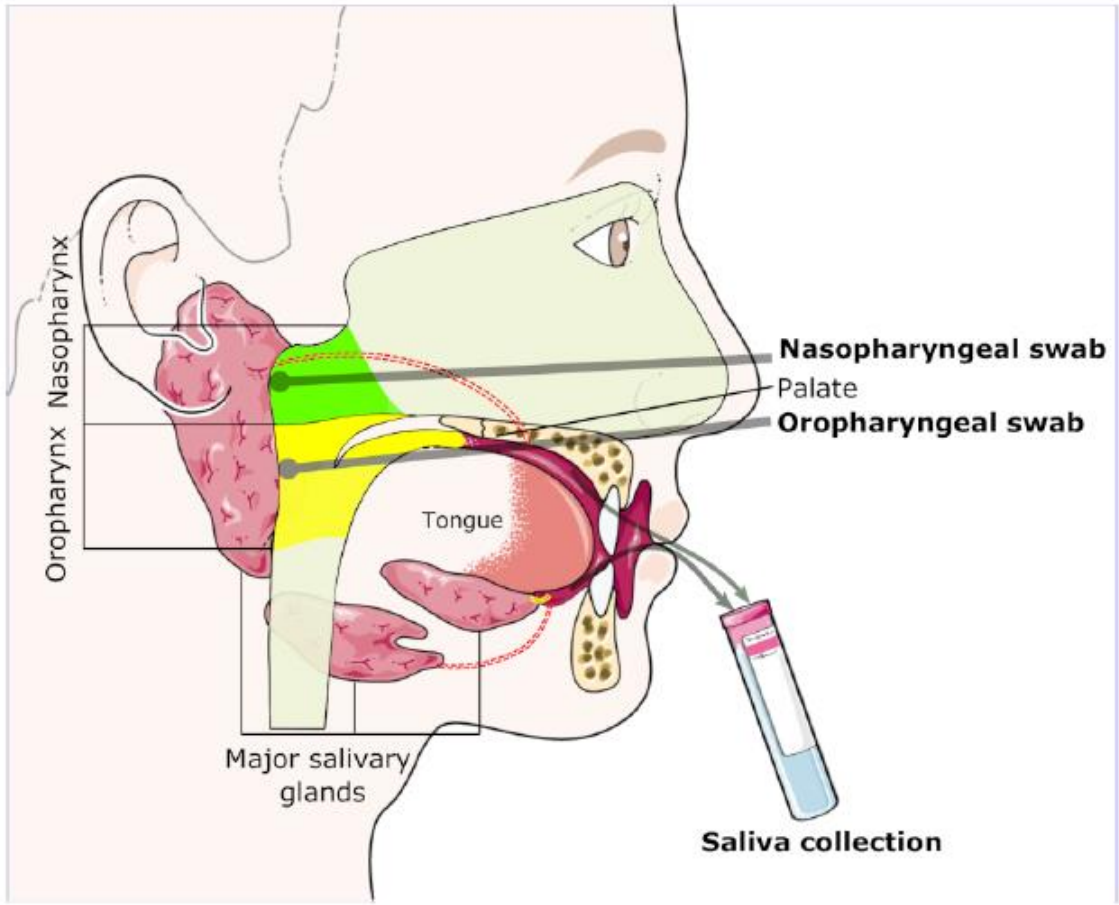


Figure 2 Schematic illustration demonstrating major salivary glands (parotid, submandibular and sublingual) and their respective ducts, oropharynx and nasopharynx, and approximate anatomic locations for collection of oropharyngeal and nasopharyngeal swabs.

Sapkota D et al. J Clin Pathol 2020

Table 1 Advantages and disadvantages of saliva sampling

Advantages	Disadvantages
Non-invasive approach for disease diagnosis and monitoring of general health.	Not always reliable for measurement of certain markers.
Painless (no patient discomfort and anxiety for sampling).	Contents of saliva can be influenced by the method of collection, degree of stimulation of salivary flow, interindividual variation and oral hygiene status.
Easy collection and applicable in remote areas.	Serum markers can reach whole saliva in an unpredictable way.
Relatively cheap technology.	Medications may affect salivary gland function and consequently the quantity and composition of saliva.
Cost-effective applicability for screening large populations.	Possibility for degradation of salivary proteins due to presence of proteolytic enzymes.
Suitable for children, anxious/disabled/elderly patients.	
Possible multisampling.	
Safer collection for health professionals than other biological samples such as nasopharyngeal swabs and blood.	
Cheap to store and ship.	
Easy to handle.	
No need for expensive equipment/instruments (swabs, suction tubes or special collection devices) for collection. Only needs a sterile container.	

Sapkota D et al. J Clin Pathol 2020

Annals of Internal Medicine

REVIEW

The Sensitivity and Costs of Testing for SARS-CoV-2 Infection With Saliva Versus Nasopharyngeal Swabs

A Systematic Review and Meta-analysis

Mayara Lisboa Bastos, MD; Sara Perlman-Arrow; Dick Menzies, MD; and Jonathon R. Campbell, PhD

Conclusion: Saliva sampling seems to be a similarly sensitive and less costly alternative that could replace nasopharyngeal swabs for collection of clinical samples for SARS-CoV-2 testing.

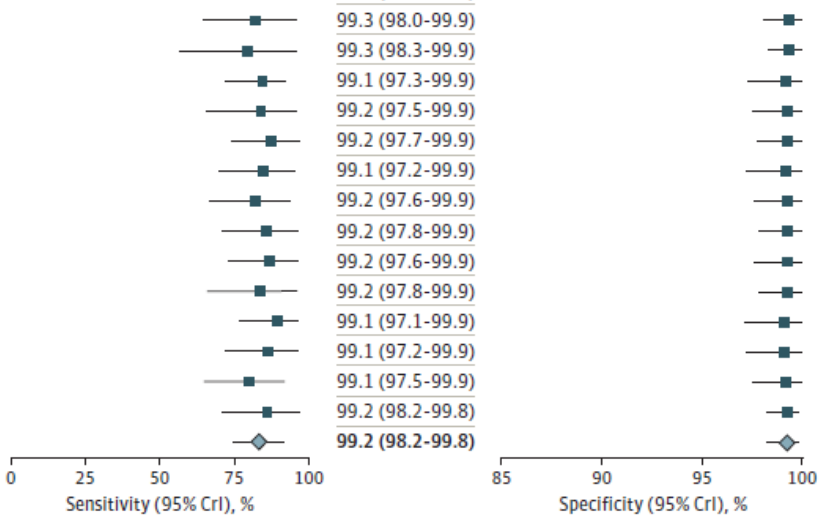
JAMA Internal Medicine | Original Investigation

Comparison of Saliva and Nasopharyngeal Swab Nucleic Acid Amplification Testing for Detection of SARS-CoV-2
A Systematic Review and Meta-analysis

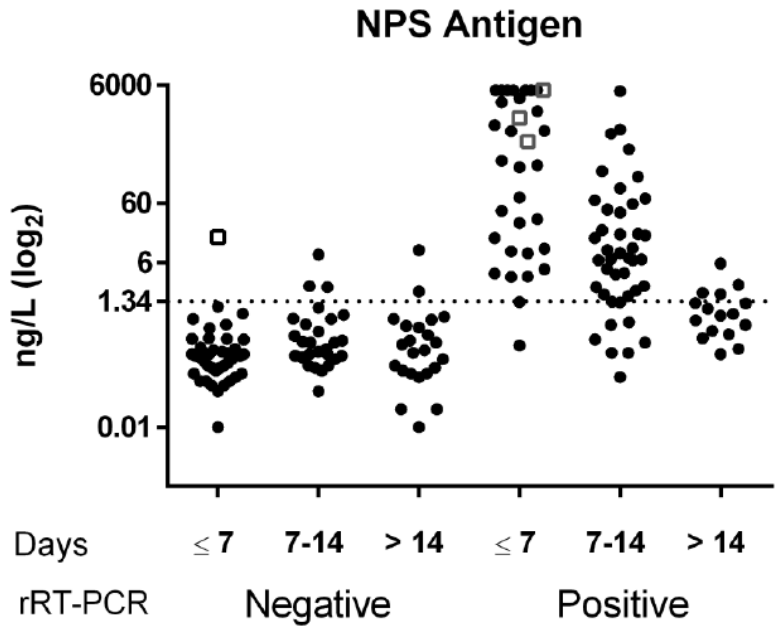
Guillaume Butler-Laporte, MD; Alexander Lawandi, MD, MSc; Ian Schiller, MSc; Mandy C. Yao, MSc; Nandini Dendukuri, PhD; Emily G. McDonald, MD, MSc; Todd C. Lee, MD, MPH

Figure 3. Primary Meta-analysis Results for the Detection of Severe Acute Respiratory Syndrome Coronavirus 2 in Saliva Samples

Source	Test finding, No. TP	FP	FN	TN	Sensitivity (95% CrI), %	Specificity (95% CrI), %
Akgun et al[14]	30	5	25	38	60.6 (46.3-77.0)	98.8 (95.4-99.9)
Becker et al[15]	11	2	10	62	65.4 (44.3-89.8)	99.0 (96.5-99.9)
Byrne et al[16]	12	0	2	96	82.2 (64.8-95.5)	99.3 (98.0-99.9)
Caulley et al[22]	34	14	22	1869	79.5 (56.8-95.8)	99.3 (98.3-99.9)
Cheuk et al[25]	104	37	18	70	84.5 (72.4-92.0)	99.1 (97.3-99.9)
Iwasaki et al[26]	8	1	1	66	83.8 (65.8-96.1)	99.2 (97.5-99.9)
Hanson et al[23]	75	6	5	268	87.3 (74.1-96.5)	99.2 (97.7-99.9)
Kojima et al[17]	20	6	3	16	84.6 (69.9-95.0)	99.1 (97.2-99.9)
Landry et al[27]	28	2	5	89	81.8 (66.8-93.9)	99.2 (97.6-99.9)
McCormick-Baw et al[29]	47	1	2	105	86.0 (71.1-96.0)	99.2 (97.8-99.9)
Miller et al[21]	33	1	1	56	86.7 (73.1-96.6)	99.2 (97.6-99.9)
Pasomsub et al[3]	16	2	3	179	83.3 (66.1-96.0)	99.2 (97.8-99.9)
Teo et al[18]	139	70	11	116	89.4 (77.1-96.3)	99.1 (97.1-99.9)
Vogels et al[19]	32	3	2	30	86.4 (72.1-96.1)	99.1 (97.2-99.9)
Williams et al[28]	33	1	6	49	79.9 (65.0-91.6)	99.1 (97.5-99.9)
Yokata et al[20]	42	6	4	1872	85.9 (71.0-96.6)	99.2 (98.2-99.8)
Pooled	**664**	**157**	**120**	**4981**	**83.2 (74.7-91.4)**	**99.2 (98.2-99.8)**



CONCLUSIONS AND RELEVANCE These results suggest that saliva NAAT diagnostic accuracy is similar to that of nasopharyngeal swab NAAT, especially in the ambulatory setting. These findings support larger-scale research on the use of saliva NAAT as an alternative to nasopharyngeal swabs.



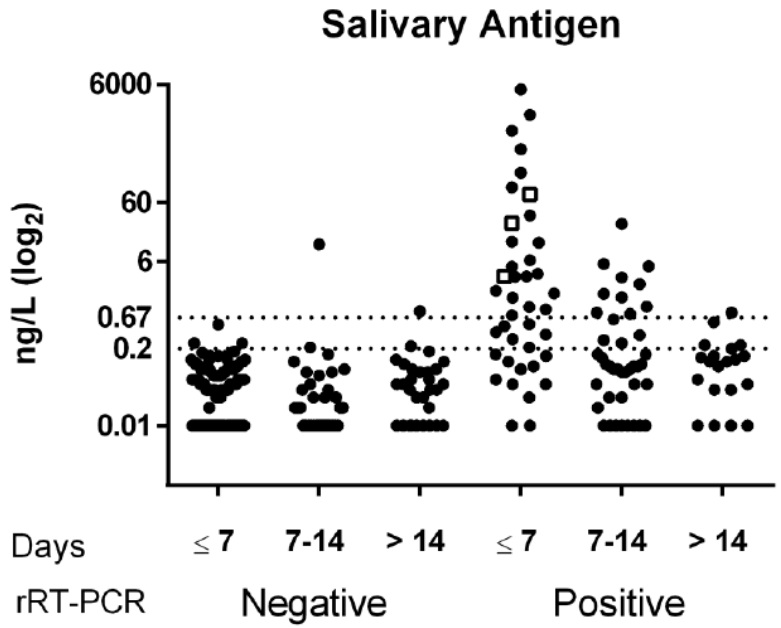


Table 5. Area under the ROC curve (AUC) with 95% confidence intervals (95% CI) of SARS-CoV-2 antigen measured in NPS and saliva by means of CLEIA. Patients were considered overall and after they have been subdivided on the basis of the time lapse between onset of symptoms and enrollment. Patients were classified as positive or negative on the basis of rRT-PCR on NPS.

	Positive (N.)	Negative (N.)	NPS antigen CLEIA AUC (95% CI)	Positive (N.)	Negative (N.)	Saliva antigen CLEIA AUC (95% CI)
Overall	75	81	0.939 (0.903-0.977)	80	141	0.805 (0.740-0.870)
≤ 7 days	32	42	0.985 (0.965-1.00)	39	94	0.879 (0.801-0.957)
7-14 days	37	25	0.897 (0.819-0.976)	34	30	0.784 (0.668-0.899)
> 14 days	6	14	0.809 (0.607-1.00)	7	17	0.697 (0.428-0.967)

medRxiv preprint doi: https://doi.org/10.1101/2020.12.24.20248825;

CLEIA SALIVARY TESTING FOR SARS-COV-2 ANTIGEN

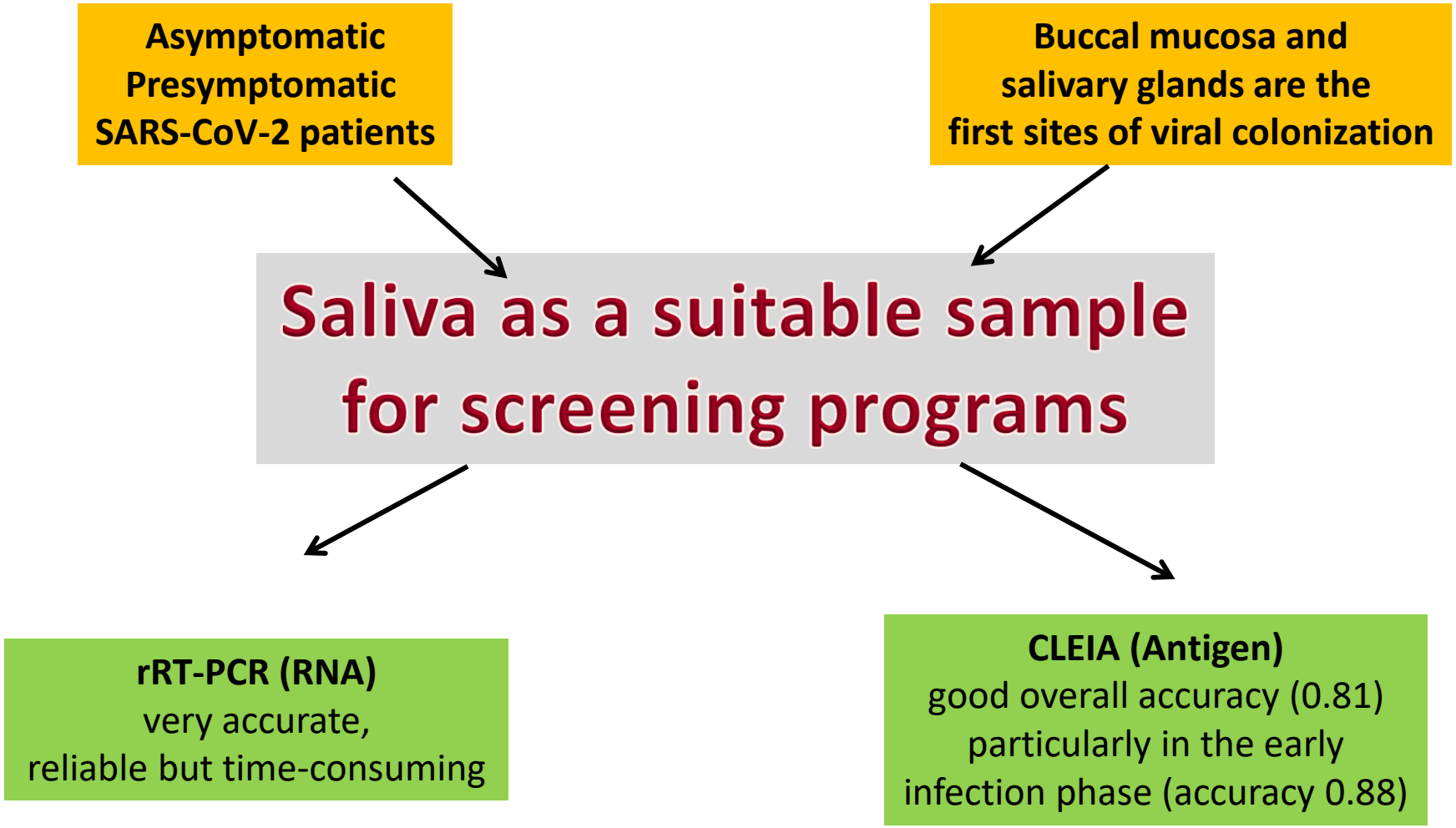
> **0.67 ng/L** → **POSITIVE**

< **0.20 ng/L** → **NEGATIVE**

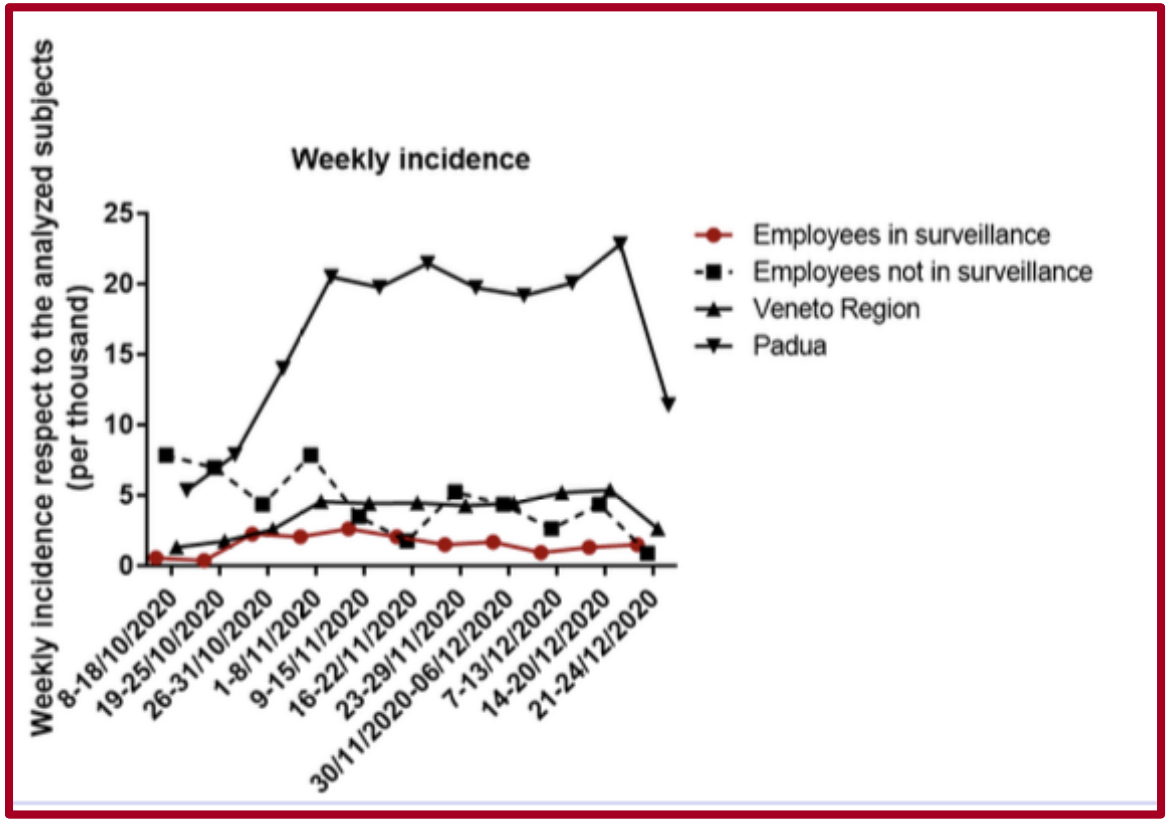
From 0.20 to 0.67 ng/L → GREY ZONE

SAMPLES TO BE RE-TESTED BY rRT-PCR

(REFLEX TESTING)

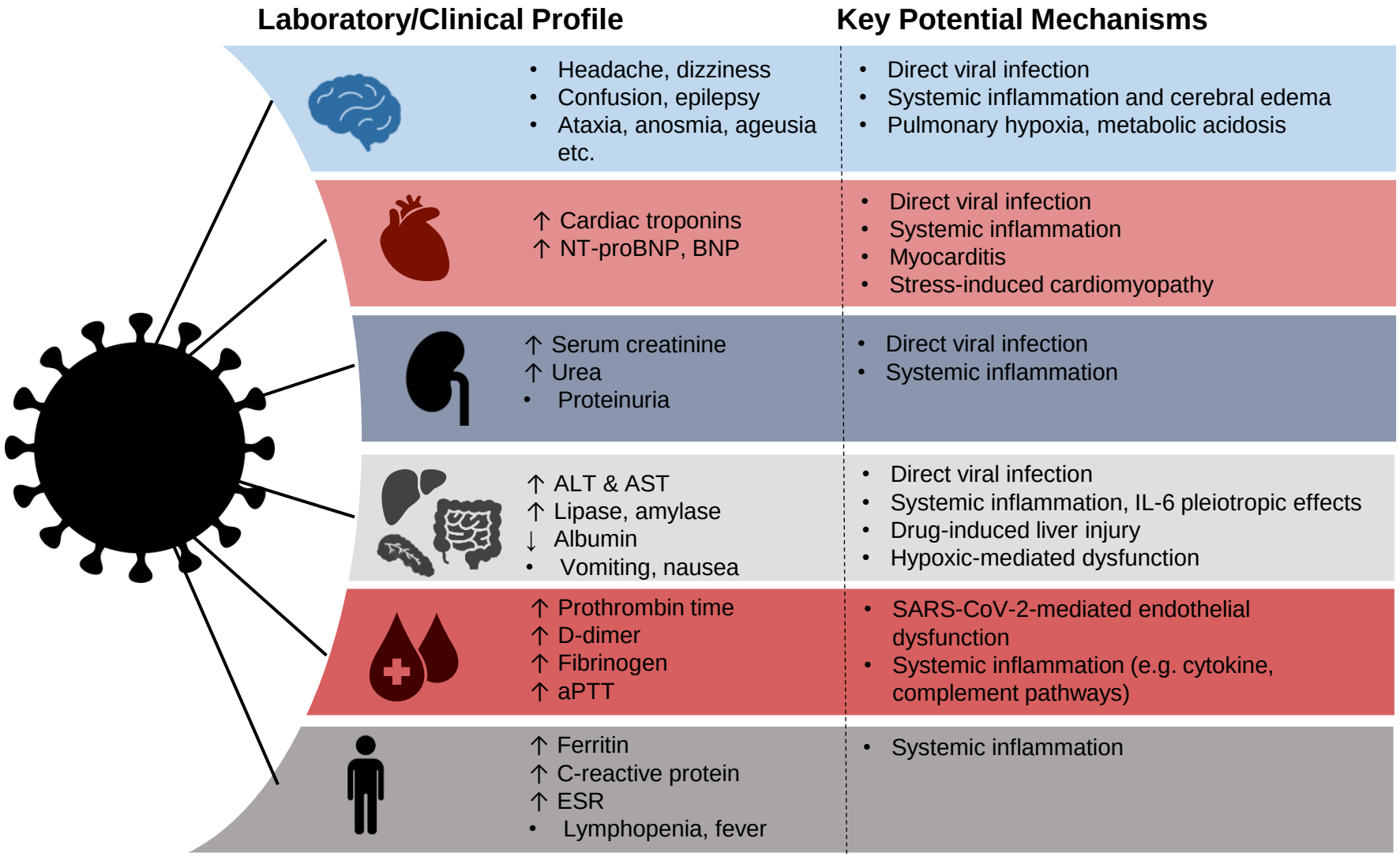

Asymptomatic
Presymptomatic
SARS-CoV-2 patients
Buccal mucosa and
salivary glands are the
first sites of viral colonization
Saliva as a suitable sample
for screening programs
rRT-PCR (RNA)
very accurate,
reliable but time-consuming
CLEIA (Antigen)
good overall accuracy (0.81)
particularly in the early
infection phase (accuracy 0.88)

SALIVA-BASED MOLECULAR TESTING FOR ACTIVE CONTROL OF SARS-COV-2 INFECTION



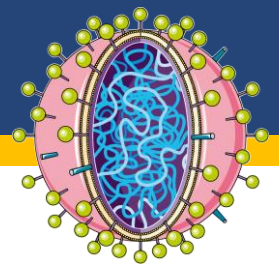
5579 employees
a total of 19850 salivary samples

COVID-19 Clinical Presentation and Pathophysiological Mechanisms



Key potential mechanisms link back to inflammation!

COVID-19: *Monitoring Markers of Inflammation*



Clinical Manifestations/Complications:

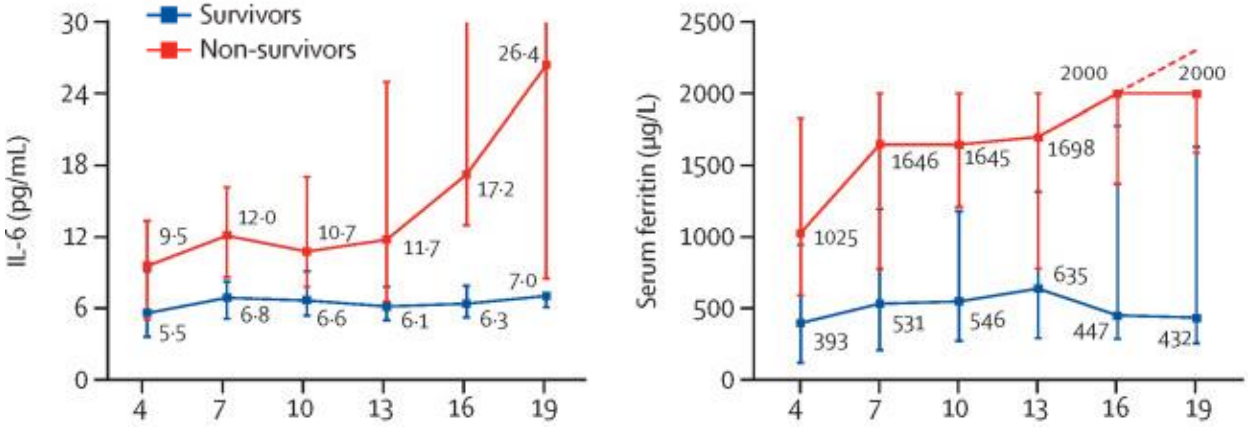
- Cytokine storm (hyperinflammatory reaction)
- Progression to multisystem organ failure and death

Key Prognostic Laboratory Indicators:

- ↑ CRP, ferritin, IL-6, ESR
- ↓ Lymphocyte count

Potential Pathophysiological Mechanisms:

- Maladaptive cytokine release as a result of a combined Th1 and Th2 cell response
- T-cell redistribution via pulmonary recruitment, exhaustion, as well as depletion through TNF-α-mediated apoptosis or even direct cytopathic injury
- Direct viral infection of immune cells such as monocytes and macrophages
- Antibody-dependent enhancement (ADE)



Temporal changes in IL-6 and ferritin from illness onset in patients hospitalized with COVID-19.

(Zhou , et al. Lancet. 2020 Mar 28;395(10229):1054-1062)

Tay MZ et al. Nature Reviews Immunology. 2020 Apr 28:1-2.

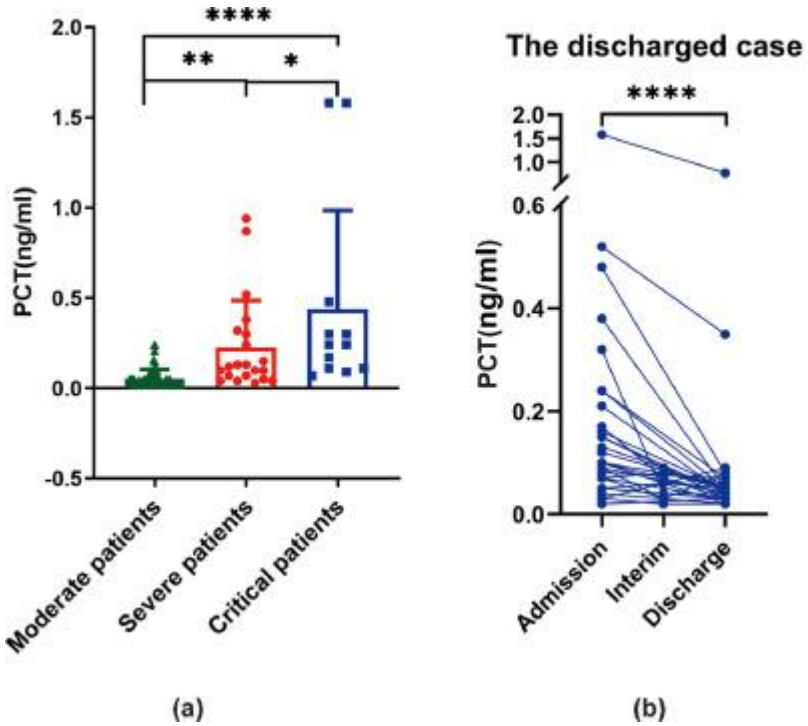
COVID-19 Patient Monitoring: PCT

- Several studies reported that elevated PCT levels are positively associated with the severity of COVID-19 (**5 fold risk** of complications)

- PCT levels appear to be **disease severity-dependent** and may be associated with bacterial co-infection:
  - **co-infection rate**: ~50% in critical patients
  - **elevated PCT rate**: ~80% in critical patients

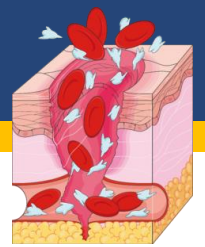
Potential mechanisms include: *bacterial co-infection, extrathyroid tissues synthesis mediated by increased concentrations of TNFα and IL-6*

PCT levels in COVID-19 patients. (A) patients with differing severity. (B) Serial PCT values for COVID-19 patients who were discharged



International journal of antimicrobial agents, *56*(2), 106051.

COVID-19: *Monitoring Hematology & Coagulation*



Clinical Manifestations/Complications:

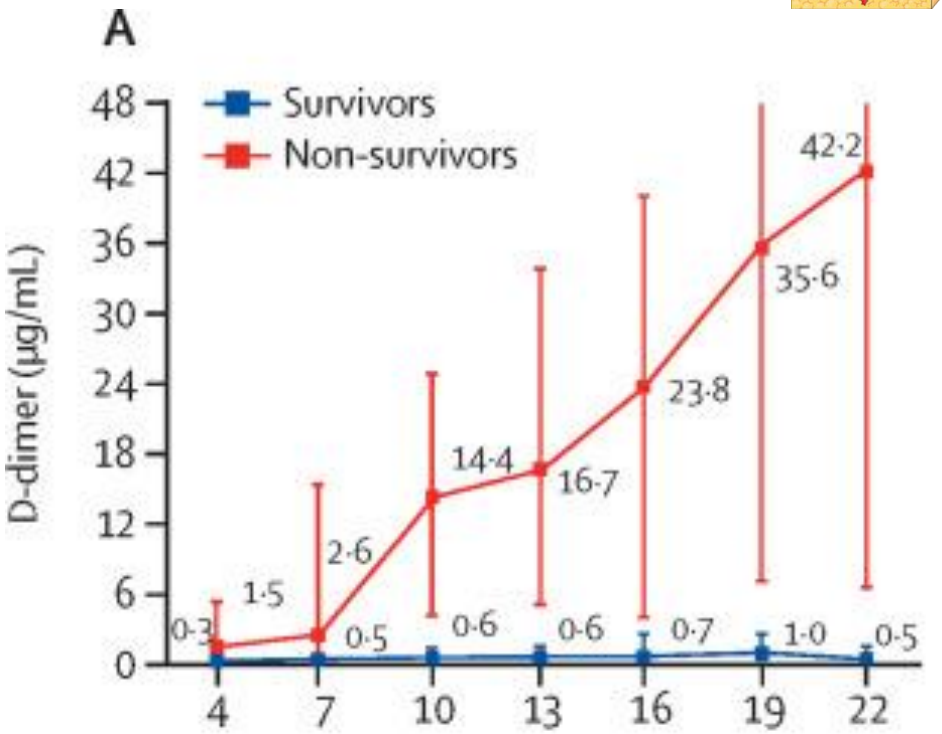
- Venous thromboembolism (VTE)
- Disseminated intravascular coagulation (DIC)

Key Prognostic Laboratory Indicators:

- ↑ D-dimer & fibrinogen
- ↓ Platelet count

Potential Pathophysiological Mechanisms:

- Likely tightly linked to inflammation and cytokine release – *immuno-thrombosis*
  - Complement-mediated pulmonary tissue damage and microvascular injury
  - Procoagulant response as a result of cytokine release in the vascular endothelium, including increased vascular permeability and damage as a result of immune-cell infiltration
  - Presence of neutrophil extracellular traps (NETs) and activation of intrinsic coagulation

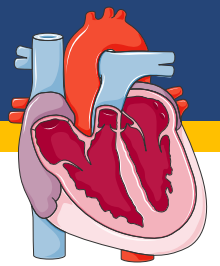


Temporal changes in D-dimer concentrations from illness onset in patients hospitalised with COVID-19

(Zhou , et al. Lancet. 2020 Mar 28;395(10229):1054-1062)

Connors JM, et al. Blood. 2020 Jun 4;135(23):2033-2040.

COVID-19: *Cardiovascular Complications*



Clinical Manifestations/Complications:

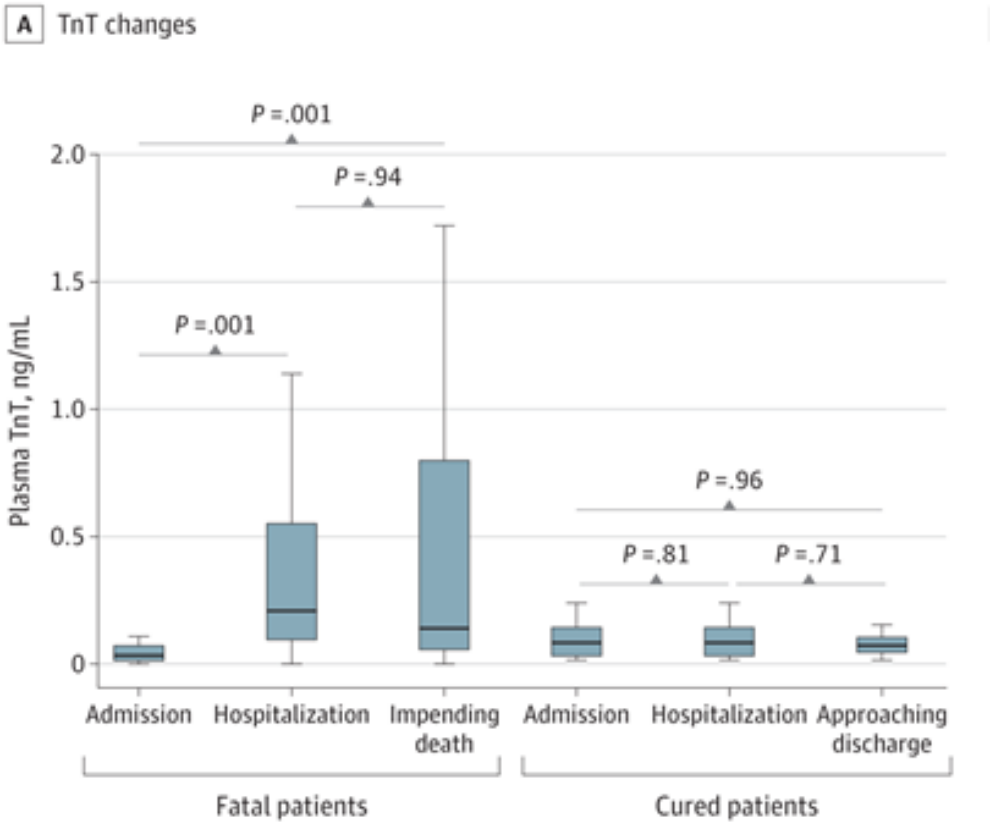
- Acute coronary syndrome
- Arrhythmias
- Heart Failure

Key Prognostic Laboratory Indicators:

- ↑ cardiac troponin (marker of cardiac injury)
- ↑ brain natriuretic peptides (marker of cardiac injury)

Potential Pathophysiological Mechanisms:

- Endothelial cell reprogramming and dysfunction as a result of maladaptive cytokine release
- Myocarditis and stress-related cardiomyopathy due to respiratory failure and hypoxemia
- Direct viral infection of cardiomyocytes

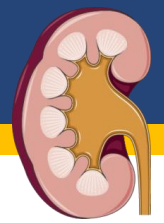


COVID-19 Stratification by cTnT values

(Guo T, et al. JAMA Cardiol. 2020 Jul 1;5(7):811-818)

Guo T, et al. JAMA Cardiol. 2020 Jul 1;5(7):811-818. doi: 10.1001/jamacardio.2020.1017.

COVID-19: *Renal Manifestations & Complications*



Clinical Manifestations/Complications:

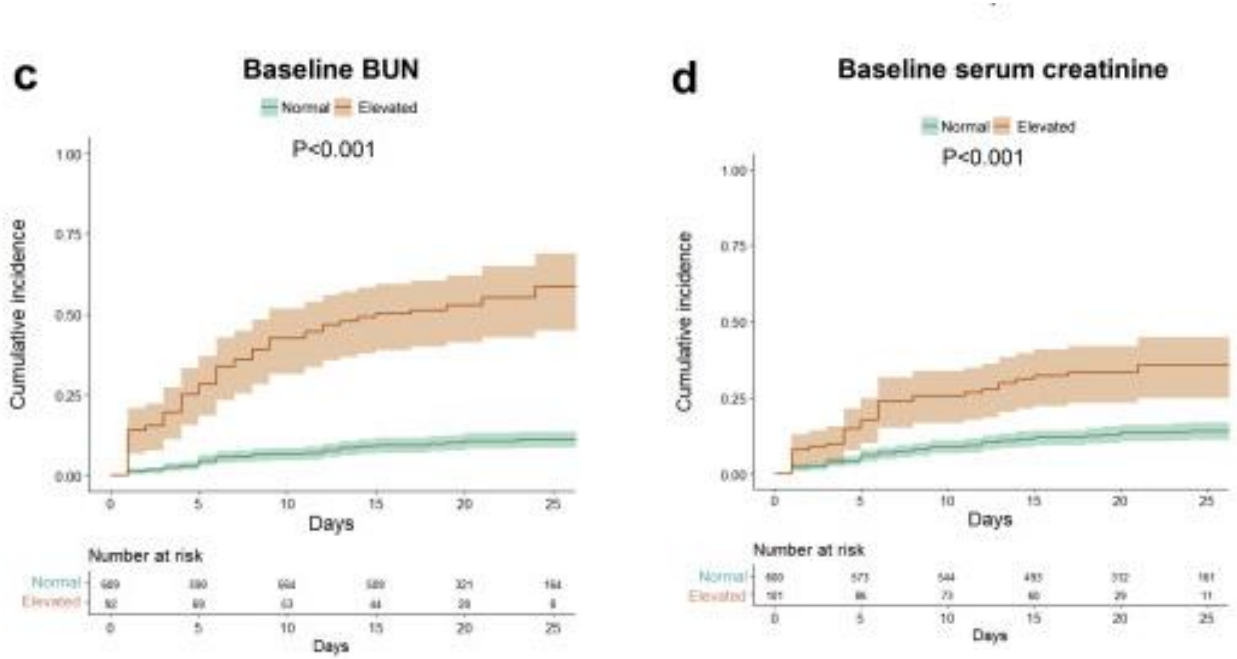
- Acute Kidney Injury
- Renal Failure

Key Prognostic Laboratory Indicators:

- ↑ serum creatinine and urea
- ↑ proteinuria

Potential Pathophysiological Mechanisms:

- Direct SARS-CoV-2 infection of the renal epithelium resultant in mitochondrial dysfunction, acute tubular necrosis, and protein leakage
- Uncontrolled cytokine release, thrombosis, and ischemia



Cumulative incidence for in-hospital death of patients with COVID-19 subgrouped by kidney disease indicators
(Cheng Y, et al. Kidney Int. 2020 May;97(5):829-838.)

Ronco C, et al. Lancet Respir Med. 2020 Jul;8(7):738-742.

POTENTIAL UTILITY OF SARS-COV-2 ANTIBODY TESTING

EVIDENCE SUPPORTING THE APPLICATION

- *Seroprevalence studies (general population and high-risk subgroups)*
- *Contact tracing*
- *Identify donors of convalescent plasma therapy*
- *Identify prior infection (late diagnosis)*
- *Assess vaccine response in clinical trials and monitoring*

EVIDENCE DOES NOT SUPPORT THE APPLICATION

- *Diagnose acute infection*
- *Provide disease prognosis*
- *Screen units of blood for SARS-CoV-2*

SARS-CoV-2 RNA versus ANTIBODY KINETICS

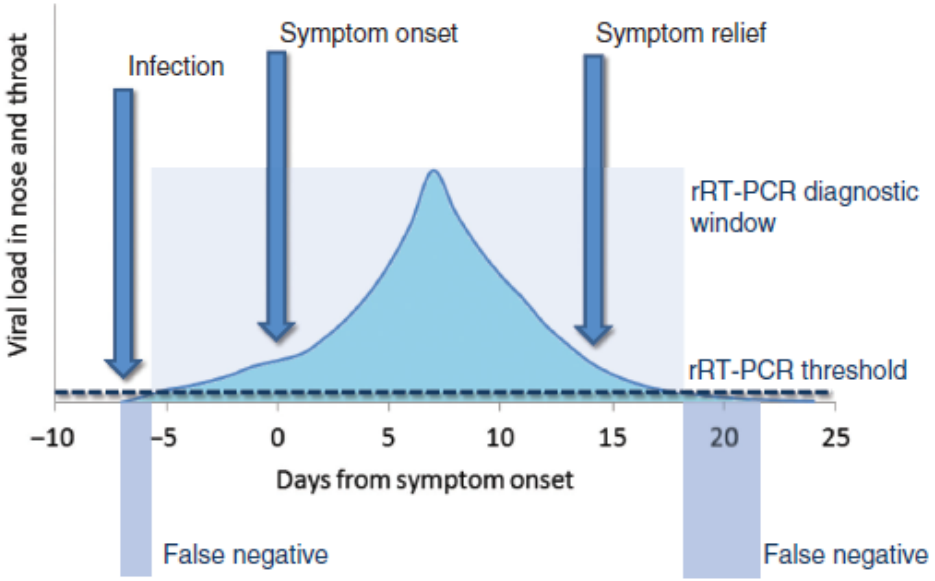
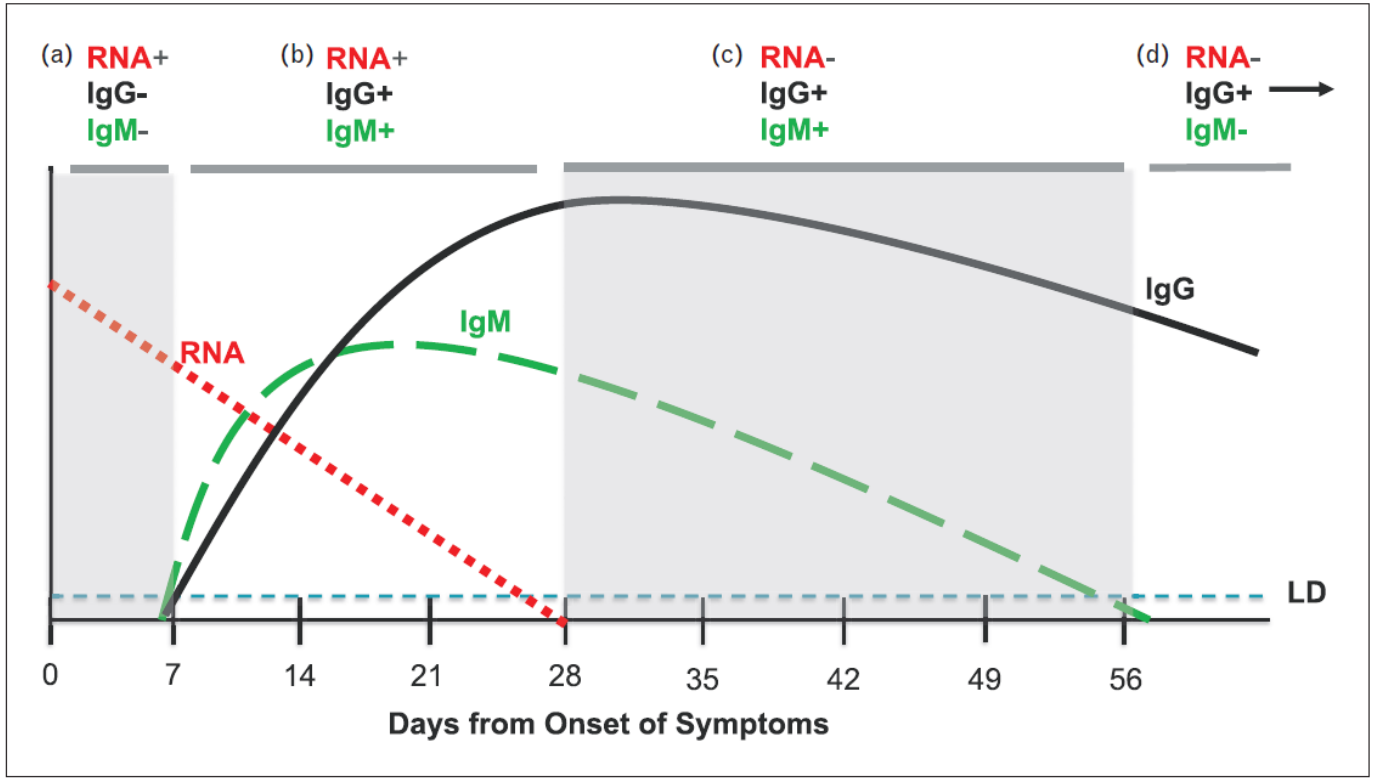


Figure 1: Correspondence between development of viral load during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, clinical course and positivity of (real time) reverse transcription polymerase chain reaction (rRT-PCR) assays.

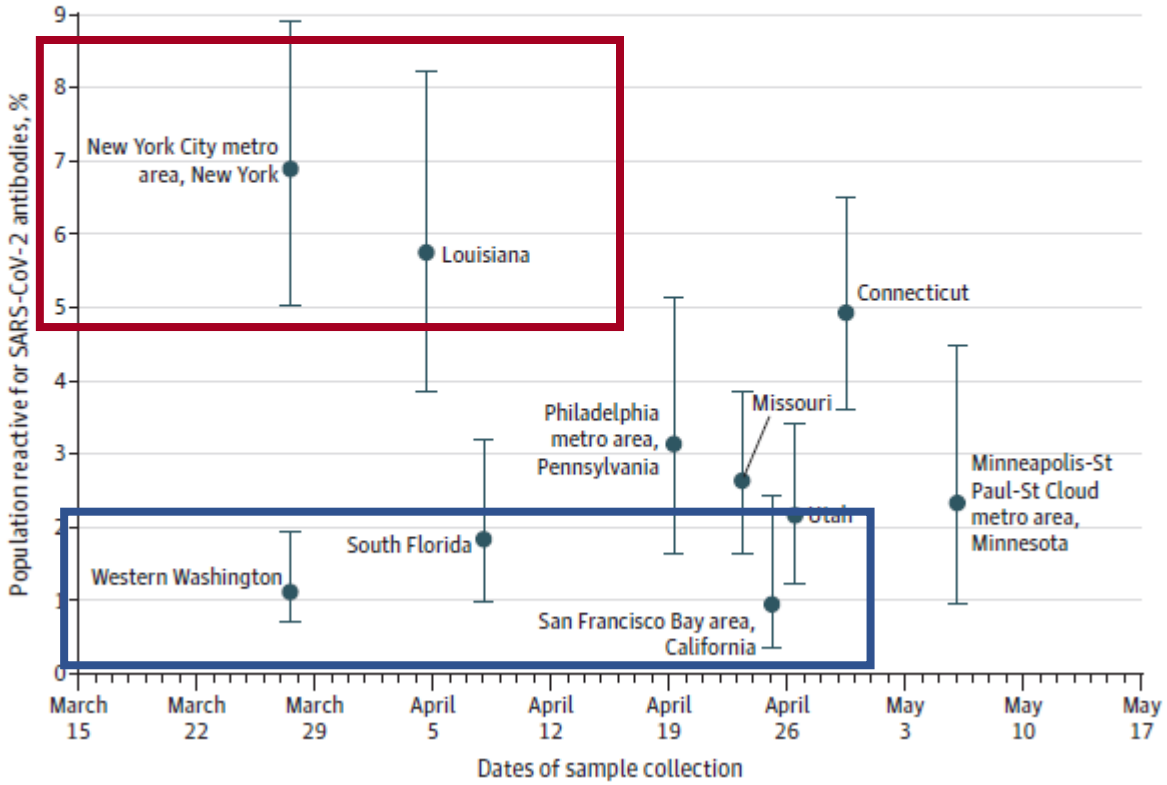


JAMA Internal Medicine | Original Investigation

Seroprevalence of Antibodies to SARS-CoV-2 in 10 Sites in the United States, March 23-May 12, 2020

Figure 1. Estimates of Seroprevalence to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antibodies and Timeline of Specimen Collection

A Estimates of seroprevalence



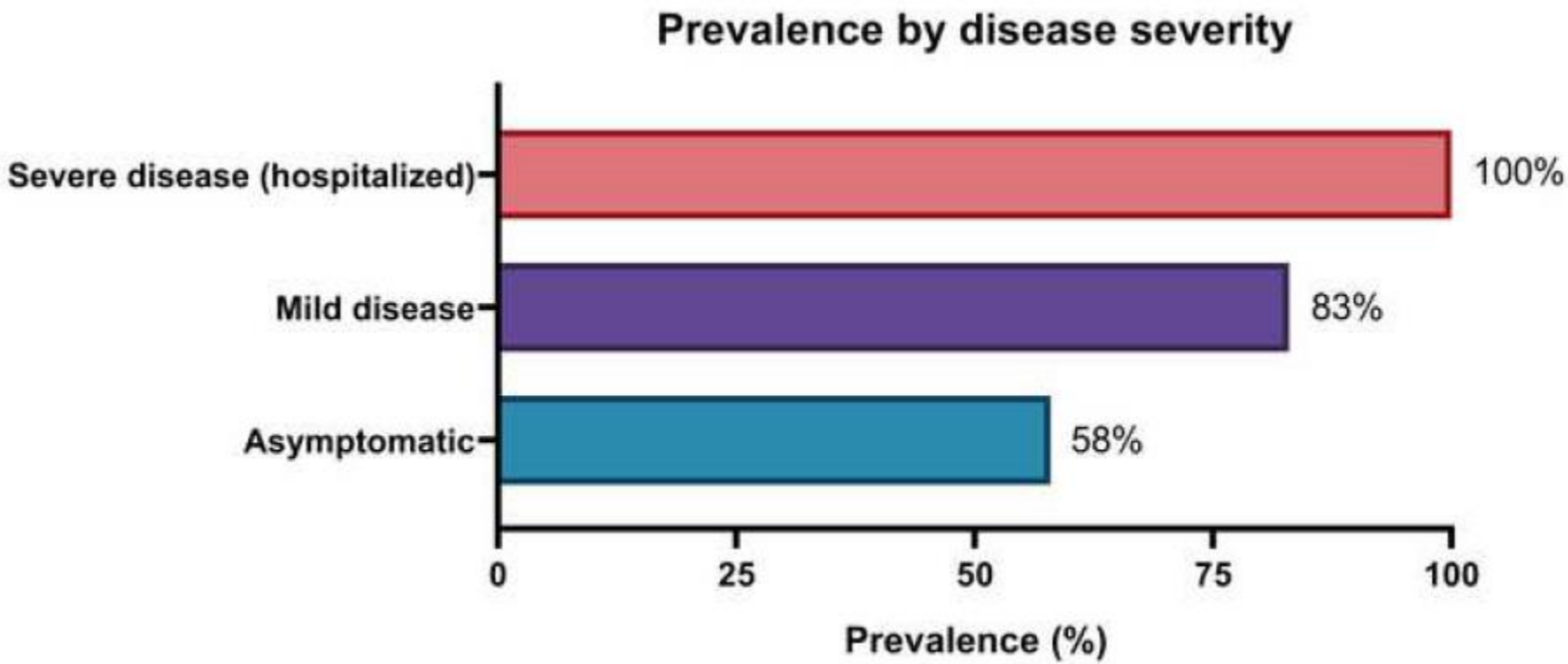
Mario Plebani*, Andrea Padoan, Ugo Fedeli, Elena Schievano, Elena Vecchiato, Giuseppe Lippi, Giuliana Lo Cascio, Stefano Porru and Giorgio Palù

SARS-CoV-2 serosurvey in health care workers of the Veneto Region

Age classes (yrs)	Total number of tests	Percentage (%) of positive tests	Percentage 95% CI
< 30 yrs	1512	4.1%	3.2-5.2%
30-39 yrs	1826	3.5%	2.7%-4.4%
40-49 yrs	1962	4.4%	3.6%-5.4%
50-59 yrs	2389	6.0%	5.1%-7.1%
> 60 yrs	596	3.7%	2.3-5.5%

Table 2: Total number and percentages of positive tests with 95% confidence intervals (CI), subdivided by the different health care figures

Healthcare figures	Total number of tests	Percentage (%) of positive tests	Percentage 95% CI
Physicians	2337	3.6%	2.8%-4.4%
Nurses	3230	4.7%	4.0-5.5%
Healthcare assistants	1040	6.0%	4.6%-7.6%
Others	1678	4.8%	3.8%5.9%


Prevalence by disease severity
Severe disease (hospitalized)
Mild disease
Asymptomatic
100%
83%
58%
0
25
50
75
100
Prevalence (%)

SARS-CoV-2 Antibody Testing: YES for……..

Evaluating the risk of reinfection

	Positive cohort (n=8278)*			Negative cohort (n=17 383)†		
	n	Incidence of reinfections		n	Incidence of new infections	
		Cumulative (cases per 1000 participants)	Density (reinfections per 100 000 days)		Cumulative (cases per 1000 participants)	Density (new infections per 100 000 days)
Probable	2	0·2	0·1	..	..	..
COVID-19 symptoms‡	50	6·0	2·4	1126	64·8	37·9
Other symptoms§	28	3·4	1·4	243	14·0	8·2
Asymptomatic	76	9·2	3·7	293	16·9	9·9
All events	155	18·7	7·6	1704	98·0	57·3

*Person-time at risk was 2 047 113 days. †Person-time at risk was 2 971 436 days. ‡COVID-19 symptoms included any of cough, fever, anosmia, or dysgeusia. §Other symptoms include any of sore throat, runny nose, headache, muscle aches, fatigue, diarrhoea, vomiting, or itchy red patches.

Table 3: **Frequency of new infections and reinfections by cohort, characterised by case definitions and symptoms 14 days before and after date of positive PCR test**

Positive cohort (antibody positive, or previous positive PCR or antibody test) had *99.8% lower risk of new infection* than did participants in the negative cohort, adjusted IRR (aIRR) 0,002 (95% CI 0.00-0.01)

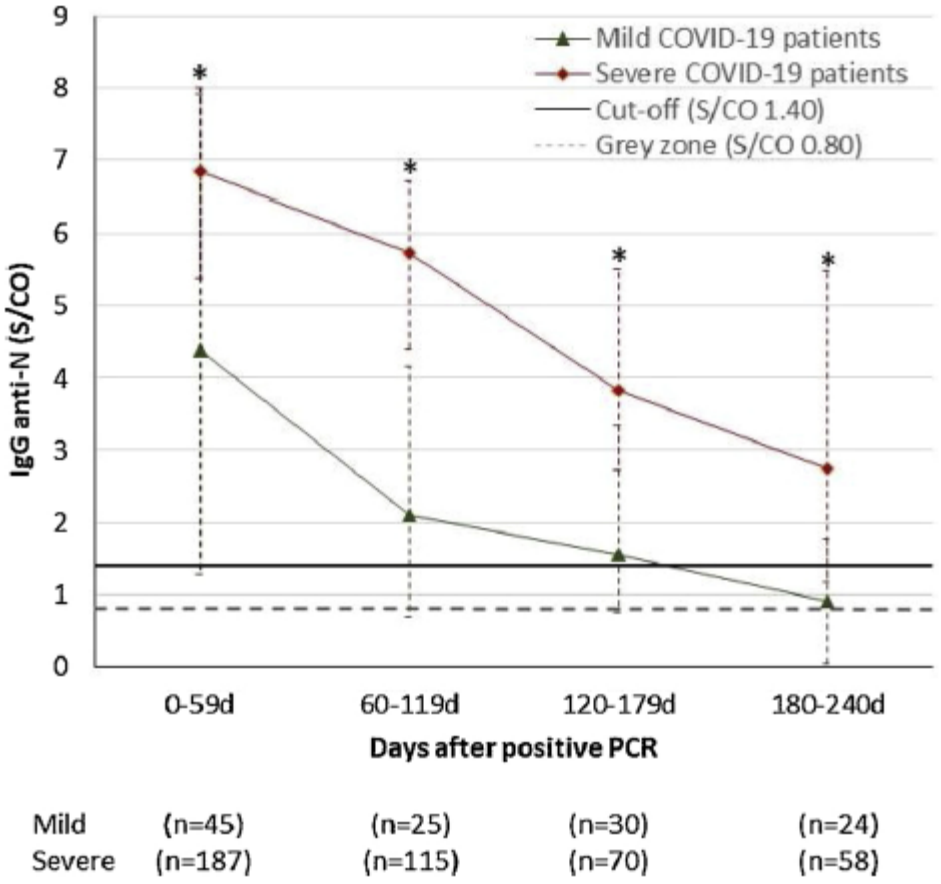
	n	IRR (95% CI)	p value	aIRR (95% CI)	p value
Probable	2	0·002 (0·00–0·01)	<0·0001	0·002 (0·00–0·01)	<0·0001
COVID-19 symptoms	50	0·079 (0·06–0·11)	<0·0001	0·074 (0·06–0·10)	<0·0001
Other symptoms	28	0·219 (0·15–0·33)	<0·0001	0·215 (0·14–0·32)	<0·0001
Asymptomatic	76	0·503 (0·39–0·65)	<0·0001	0·484 (0·37–0·63)	<0·0001
All events	155	0·169 (0·14–0·20)	<0·0001	0·159 (0·13–0·19)	<0·0001

IRR unadjusted model was adjusted for period and site. IRR adjusted model included fixed effects (adjusted for week group, age group, gender, ethnicity, staff role, index of multiple deprivation, region); time-varying effects (adjusted for vaccination and B.1.1.7 variant prevalence); and random effect (adjusted for site). SIREN=The SARS-CoV-2 Immunity and Reinfection Evaluation study. IRR=incidence rate ratio. aIRR=adjusted incidence rate ratio. *Both probable cases had COVID-19 symptoms and one reinfection case did not provide details on symptoms so the results for this participant are unknown.

Table 4: **Univariable and multivariable analysis of risk of infection by cohort during SIREN follow-up, using a range of reinfection case definitions, between June 18 and Jan 11, 2021***

DECAY or not DECAY: THIS IS THE QUESTION

Journal of Clinical Virology 136 (2021) 104765



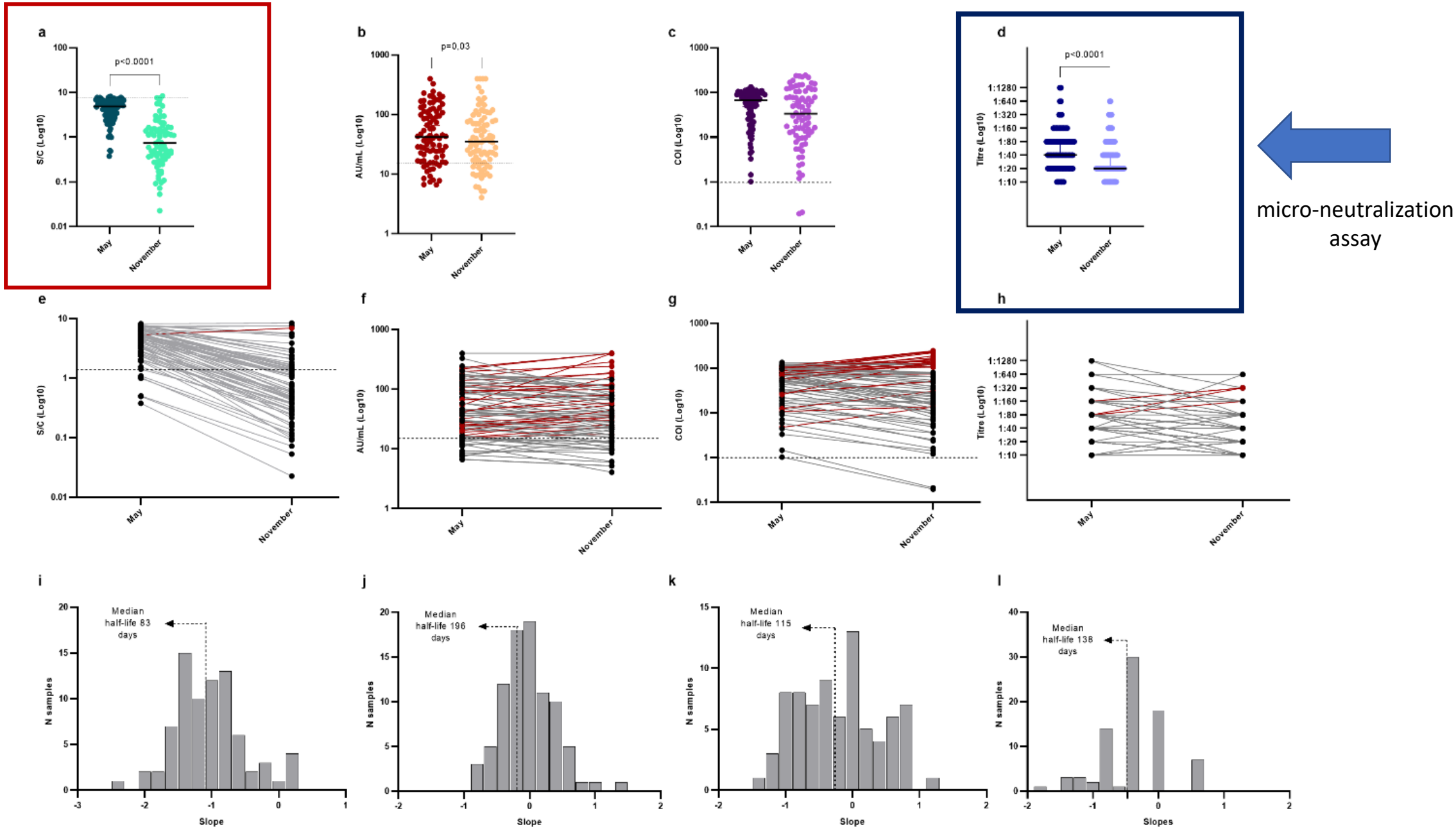
IgG anti-N assay

PERSISTENCE OF SARS-CoV-2 ANTIBODY RESPONSES

- SARS-CoV-2 ***Spike IgG*** titers were relatively stable from 20-240 days PSO (half-life= ***103 days***)
- SARS-CoV-2 ***RBD IgG*** titers gave an estimated half-life of ***83 days***
- SARS-CoV-2 ***Nucleocapsid IgG*** gave an estimated half-life of ***68 days***
- PSV ***neutralization*** titers gave an estimated half-life of ***90 days***

Dan J. et al Science 2021

Evidence: The stability of the antibody response over time may also depend on the target antigen



Submitted for publication

HETEROGENEITY OF THE HUMORAL IMMUNE RESPONSE IN COVID-19

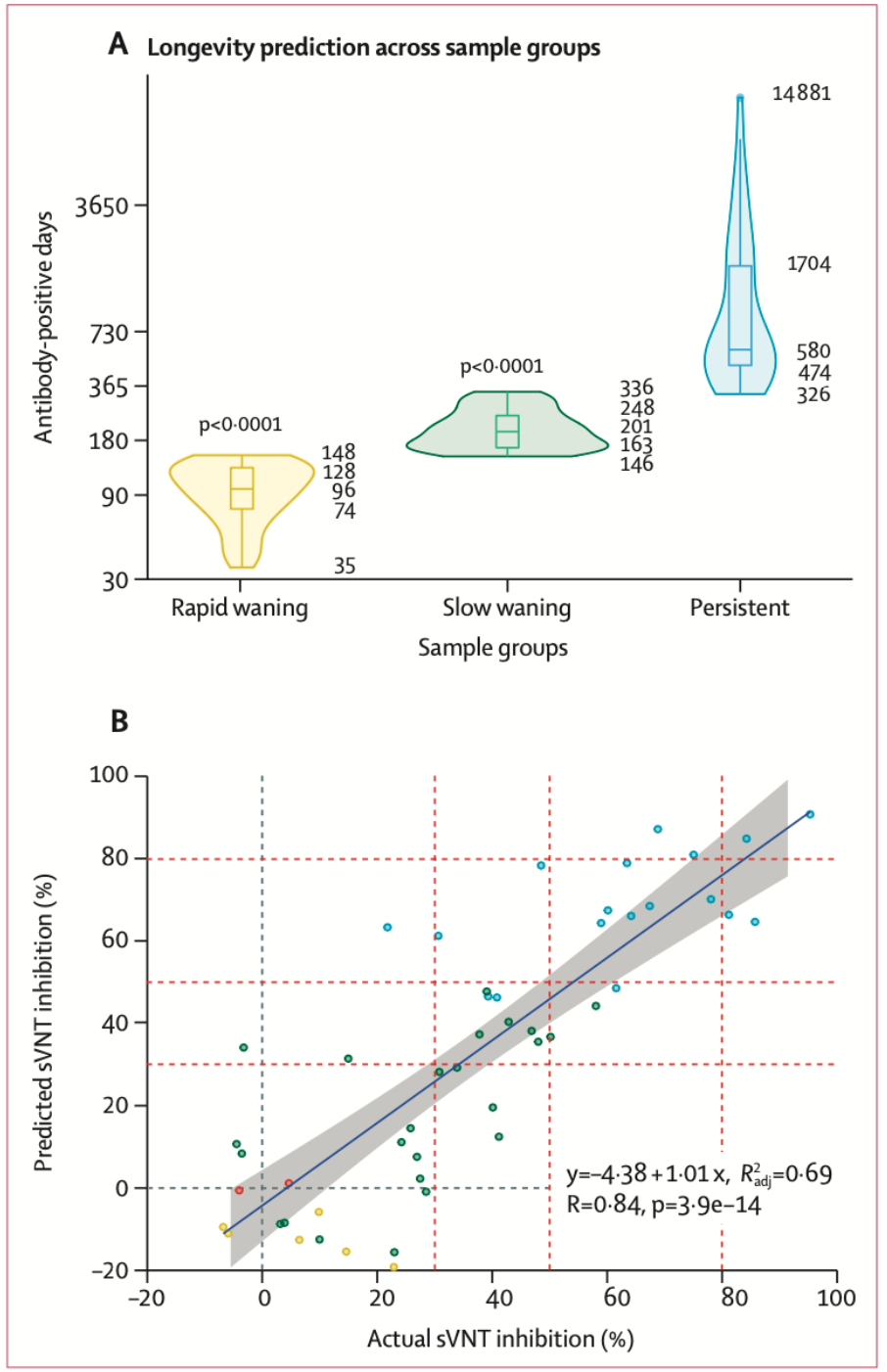
The median neutralizing antibody positive days for the ***rapid waining***, ***slow waining***, and ***persistent*** groups were:

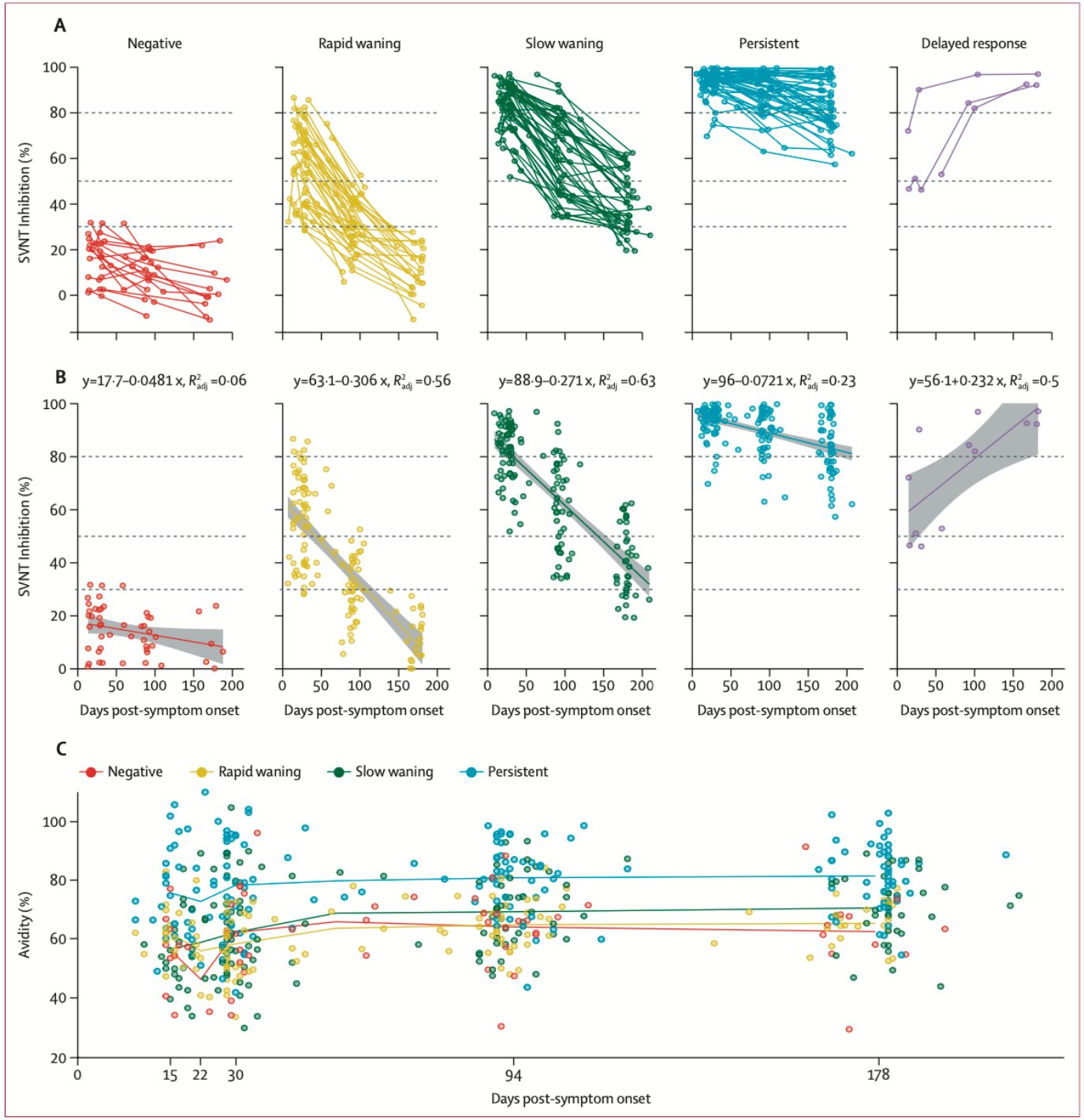
96 days,

201 days, and

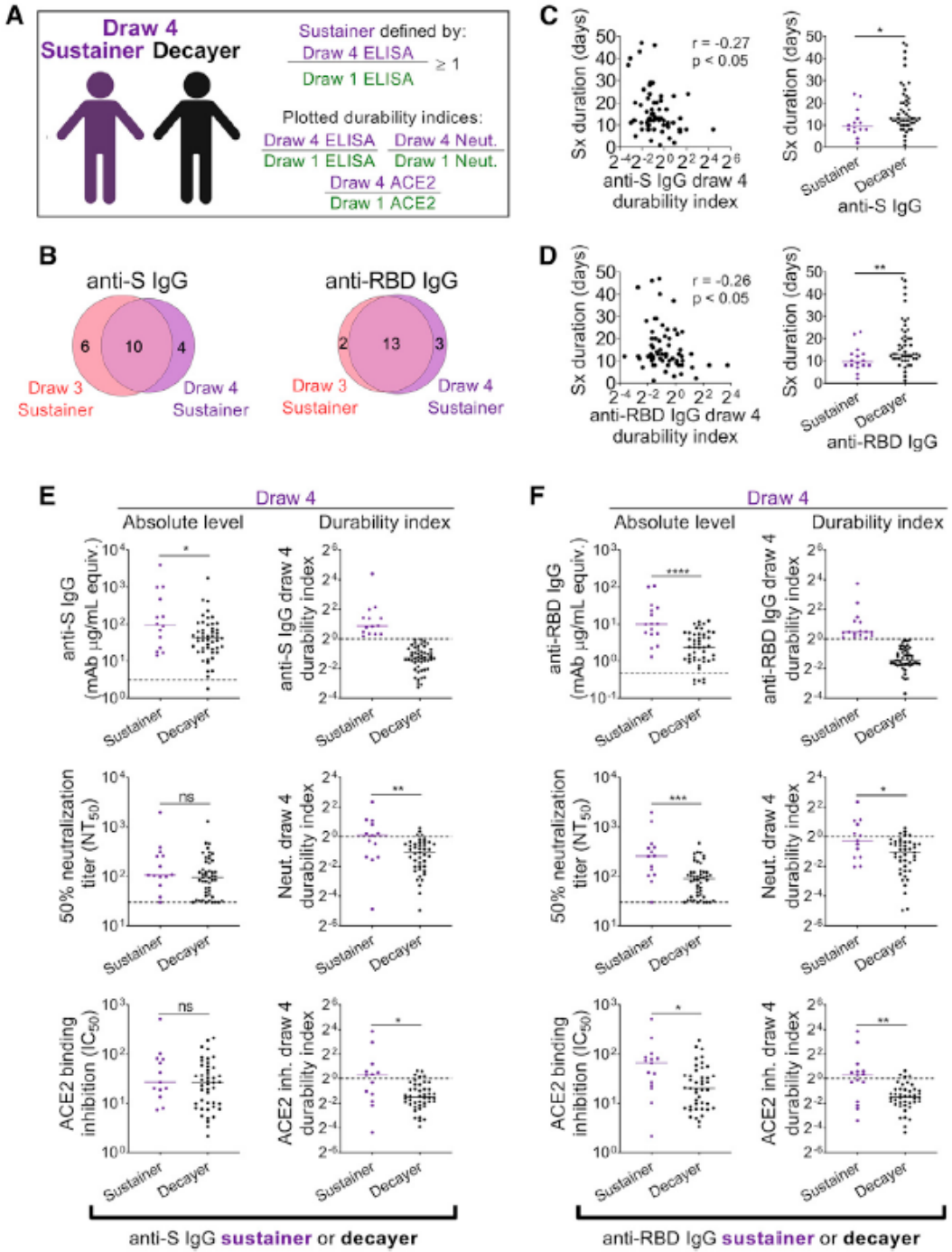
580 days

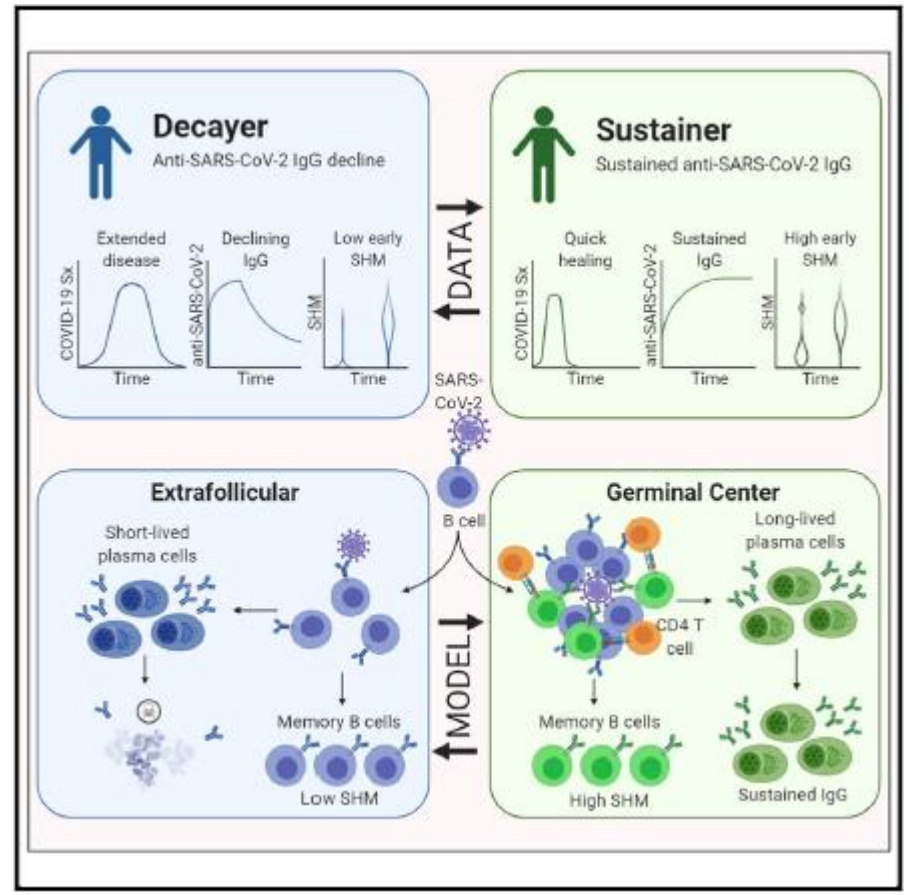
Chia WN et al Lancet Microbe 2021

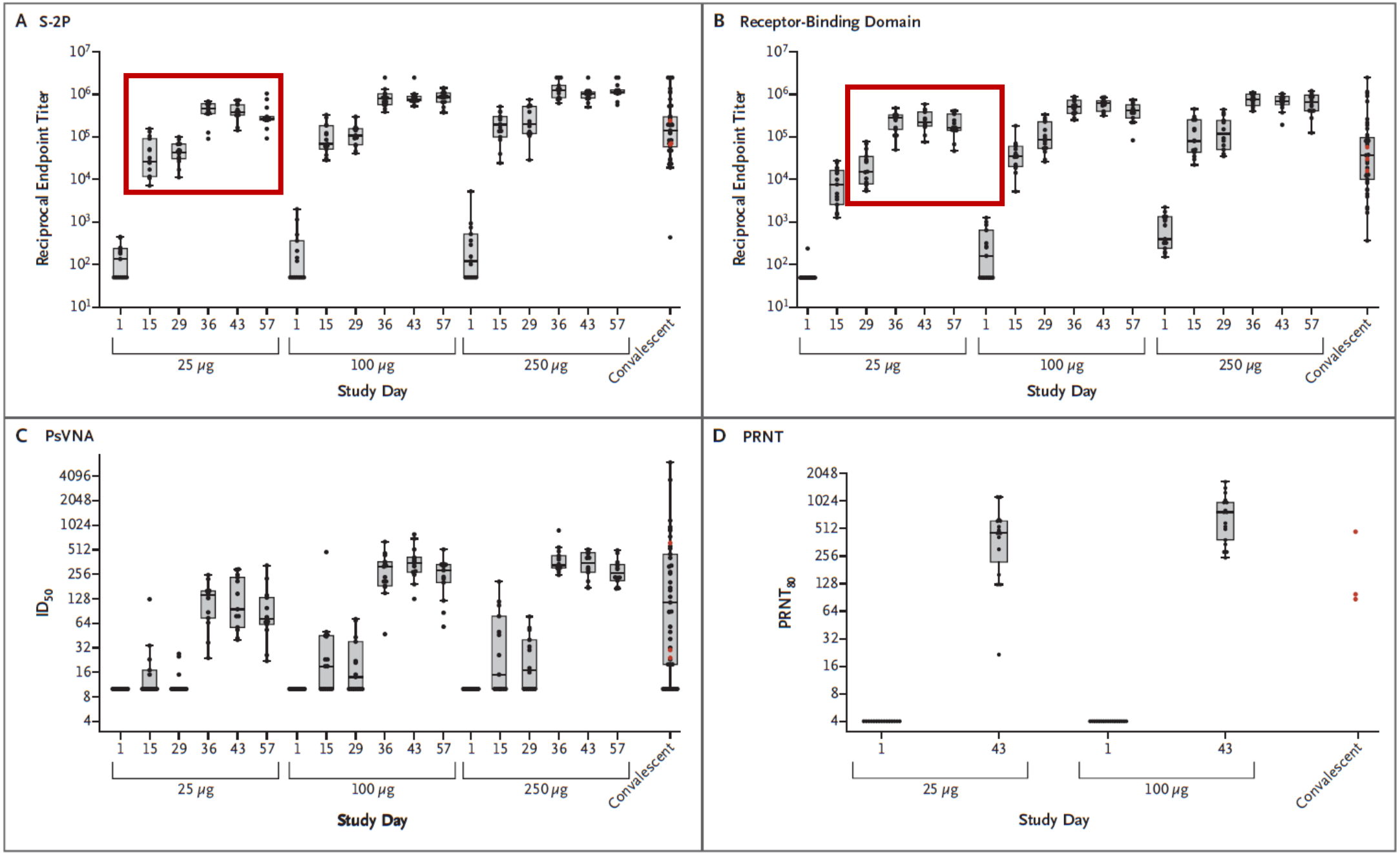



A
Negative
Rapid waning
Slow waning
Persistent
Delayed response
SVNT Inhibition (%)
B
y=17·7–0·0481 x, R^2_{adj} =0·06
y=63·1–0·306 x, R^2_{adj} =0·56
y=88·9–0·271 x, R^2_{adj} =0·63
y=96–0·0721 x, R^2_{adj} =0·23
y=56·1+0·232 x, R^2_{adj} =0·5
Days post-symptom onset
C
Negative
Rapid waning
Slow waning
Persistent
Avidity (%)
Days post-symptom onset

Chen Y et al Cell 2020






A S-2P
Reciprocal Endpoint Titer
10^7
10^6
10^5
10^4
10^3
10^2
10^1
1 15 29 36 43 57 1 15 29 36 43 57 1 15 29 36 43 57
25 μg
100 μg
250 μg
Convalescent
Study Day
B Receptor-Binding Domain
Reciprocal Endpoint Titer
10^7
10^6
10^5
10^4
10^3
10^2
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250 μg
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ID_{50}
4096
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128
64
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250 μg
Convalescent
Study Day
D PRNT
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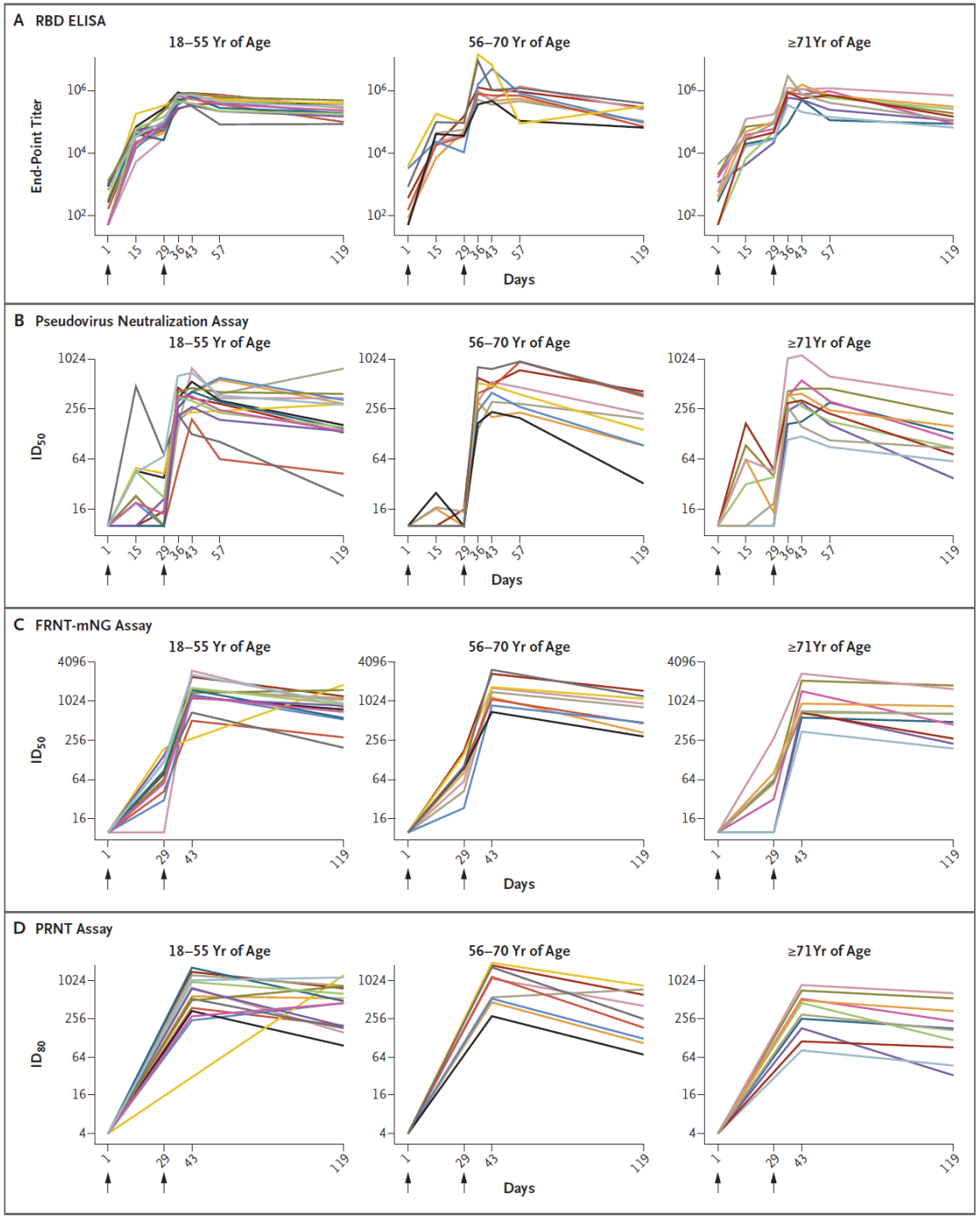
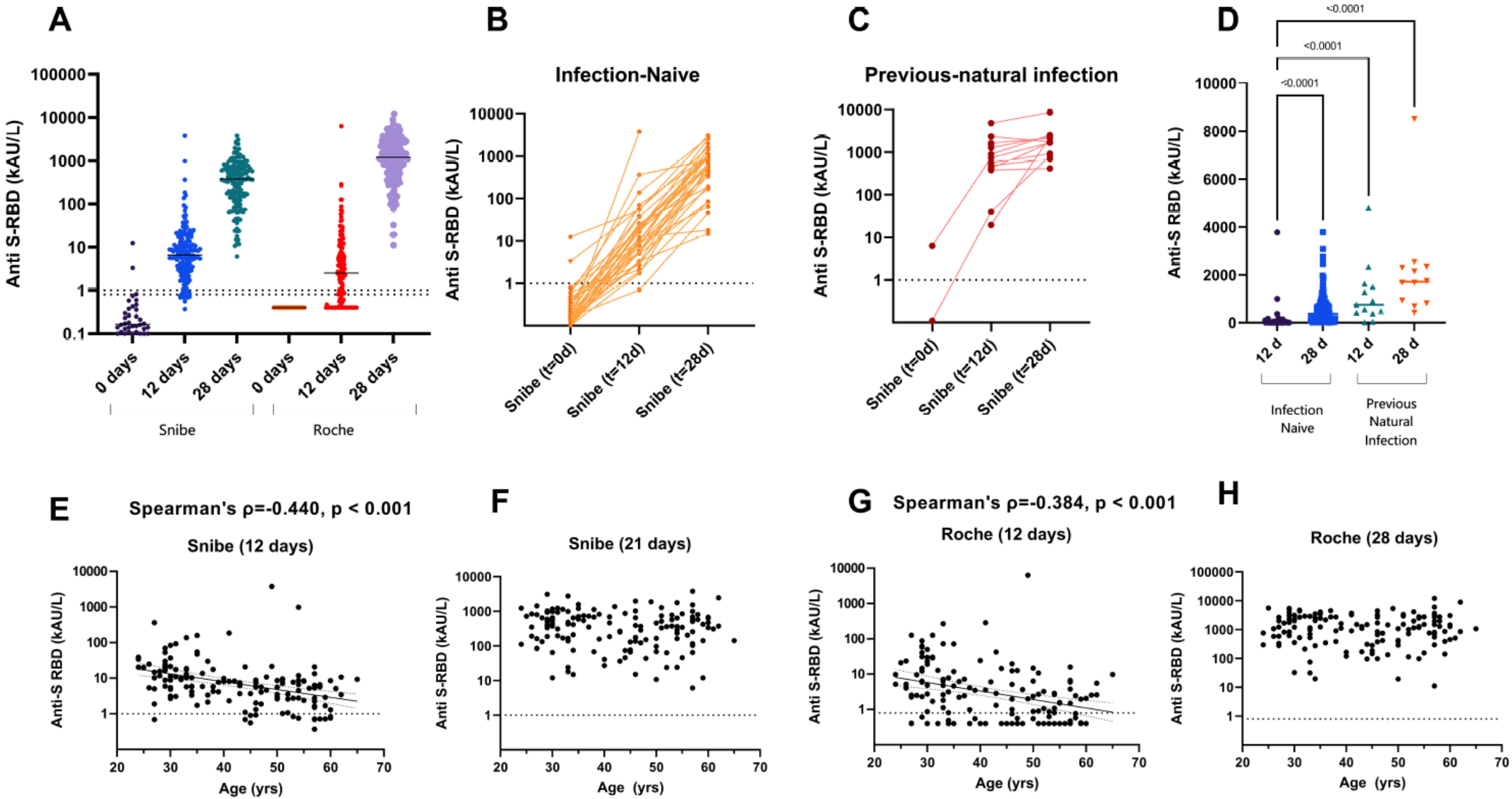
25 μg
100 μg
Convalescent
Study Day



The NEW ENGLAND JOURNAL of MEDICINE

CORRESPONDENCE

Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination



Padoan A et al. Clin Chim Acta 2021

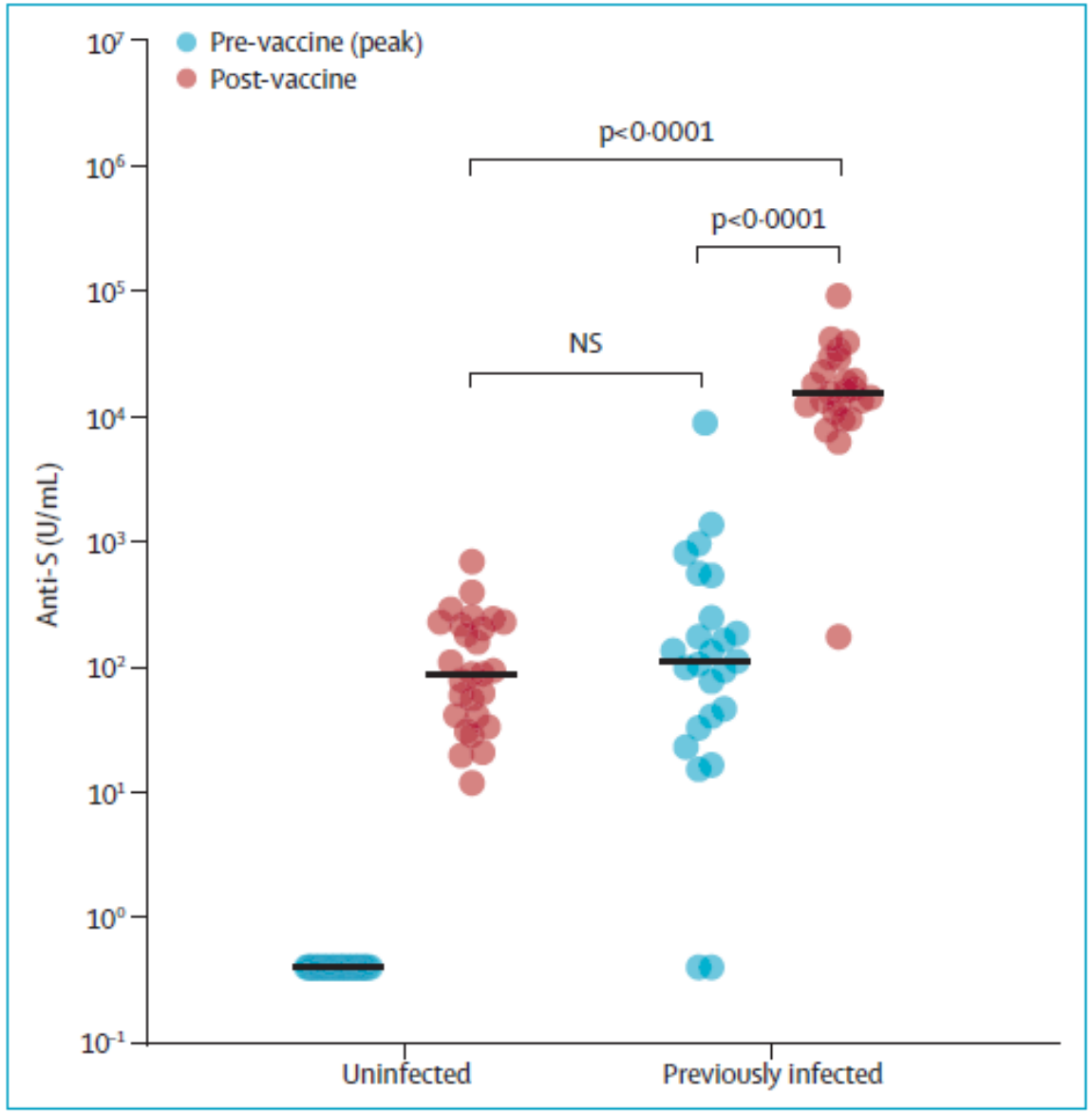
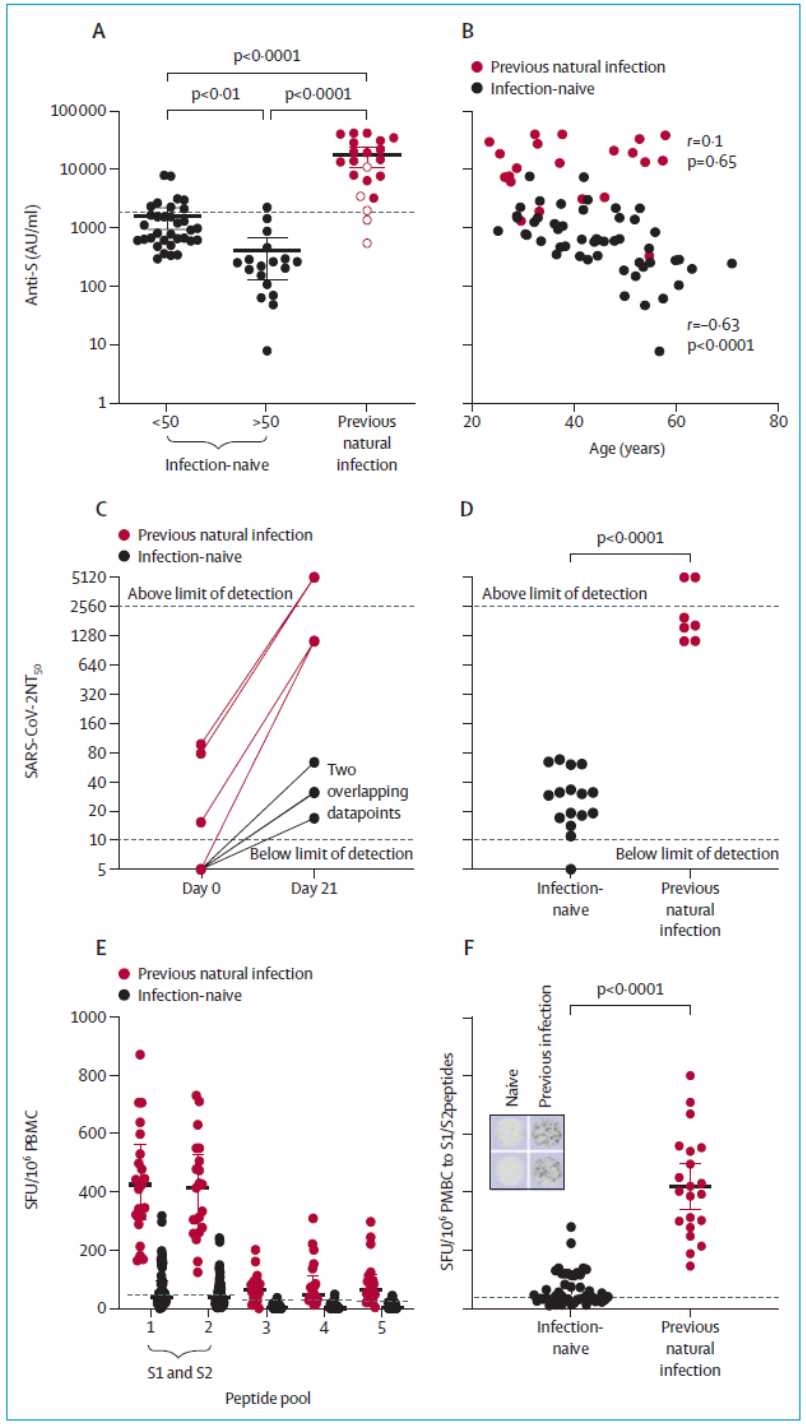


Figure: Serological response to one dose of the BNT162b2 mRNA COVID-19 vaccine in individuals with and without laboratory-confirmed previous SARS-CoV-2 infection

Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals

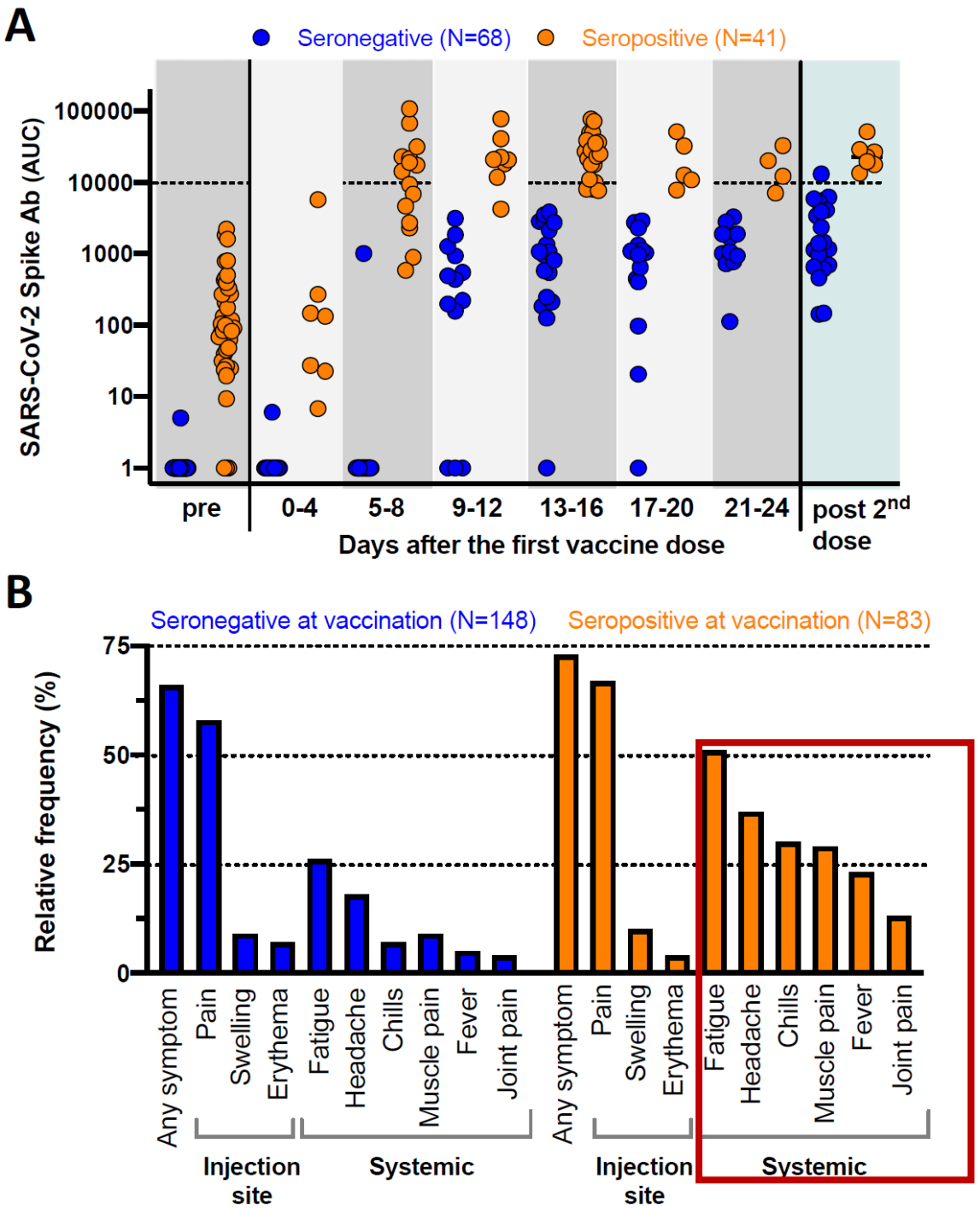
Manisty C et al.Lancet 2021



Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine

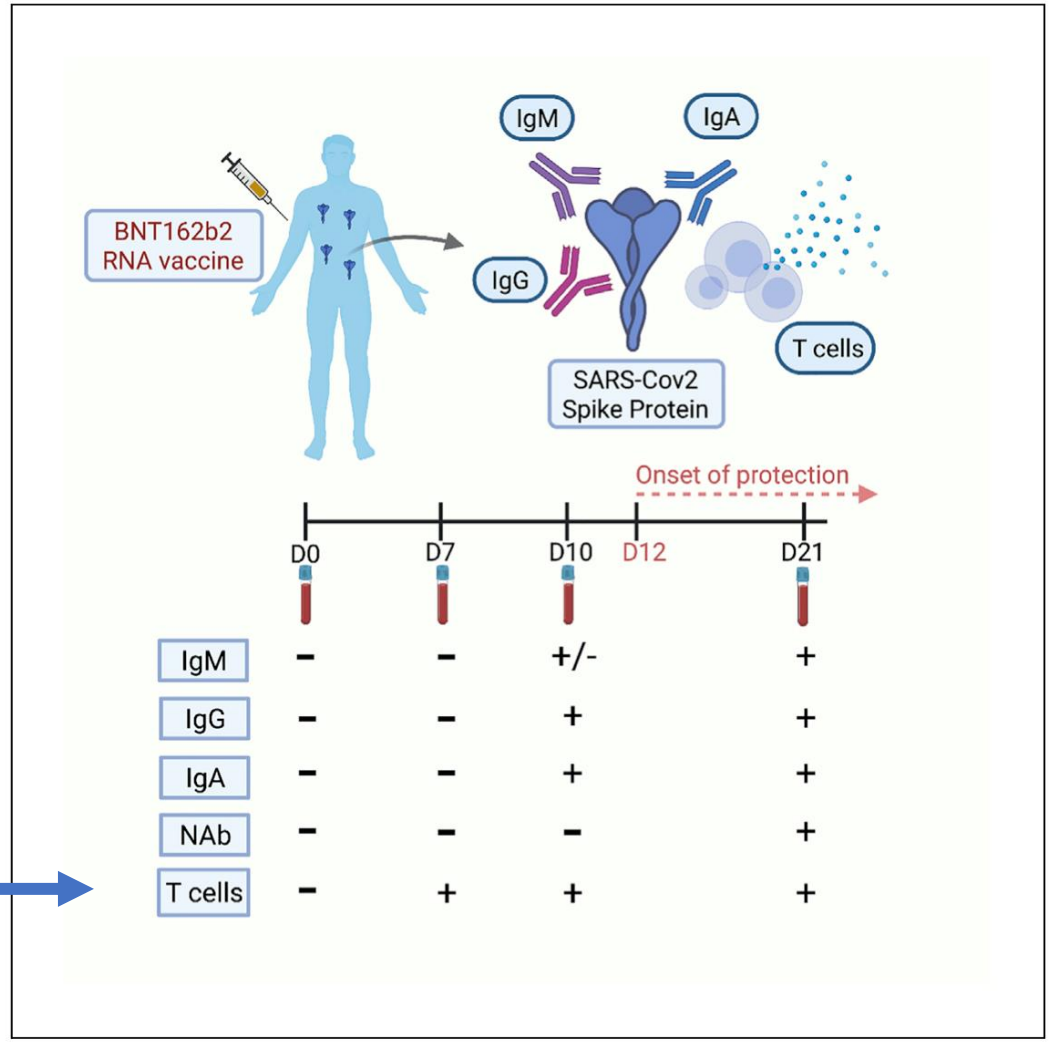
Prendecki M et al Lancet 2021

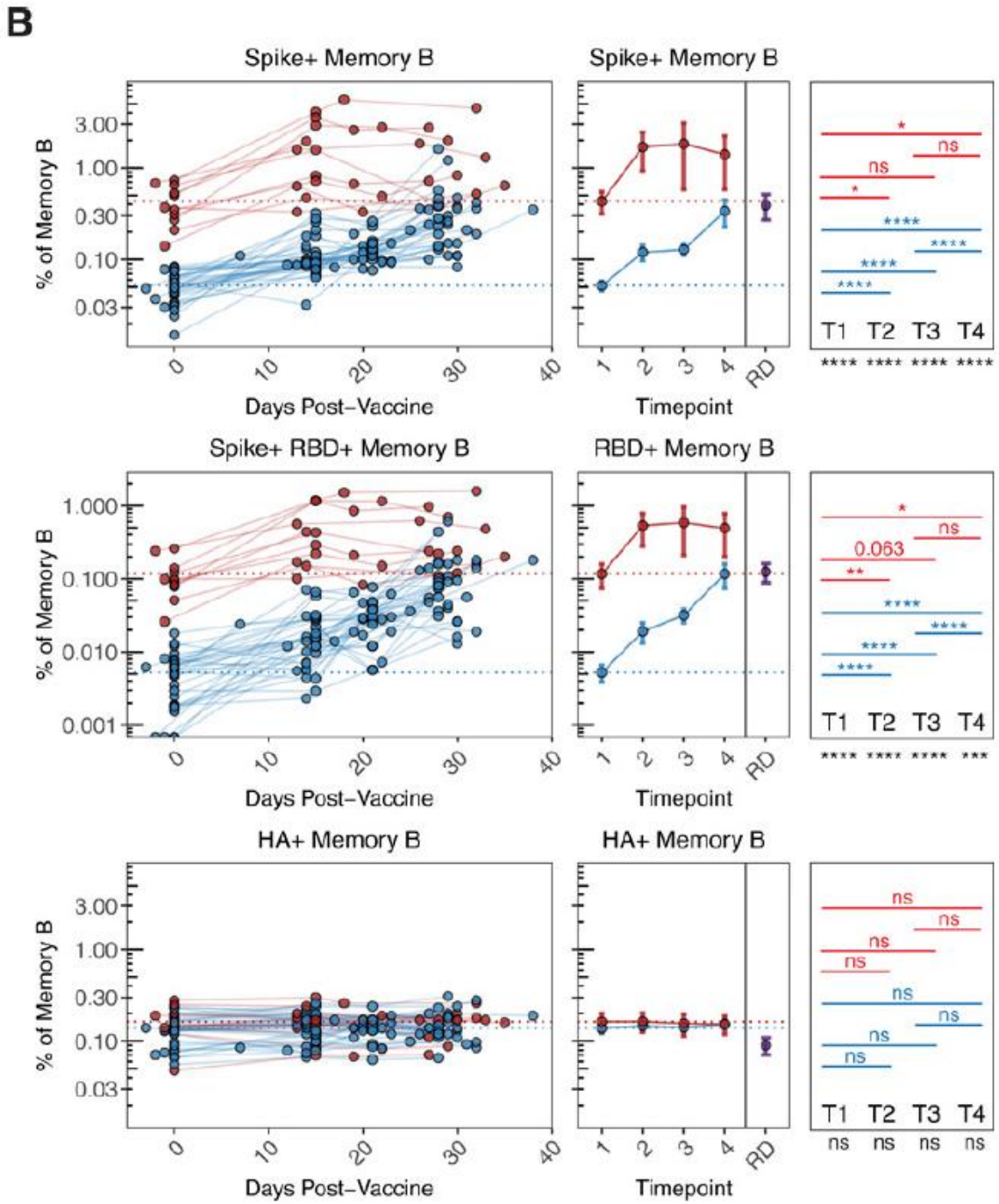
T-cells responses to spike peptides



Krammer F et al. Robust spike antibody responses and increased reactogenecity in seropositive individuals after a single dose of SARS-CoV-2 mRNA vaccine medRxiv 2021

EARLY T CELLS AND BINDING ANTIBODY RESPONSES INDUCE EARLY PROTECTION AGAINST COVID-19





…..demonstrate robust serological and cellular priming by RNAm vaccines and revealed distinct responses based on prior SARS-CoV-2 exposure, whereby COVID-19 recovered subjects may only require a single vaccine dose to achieve peak antibody and memory B cell responses

Goel RR et al. Sci Immunol 2021

WHAT ABOUT ANTIBODY PERSISTENCE AFTER VACCINATION ?

Estimated decay rates of neutralisation titres in vaccination and convalescence

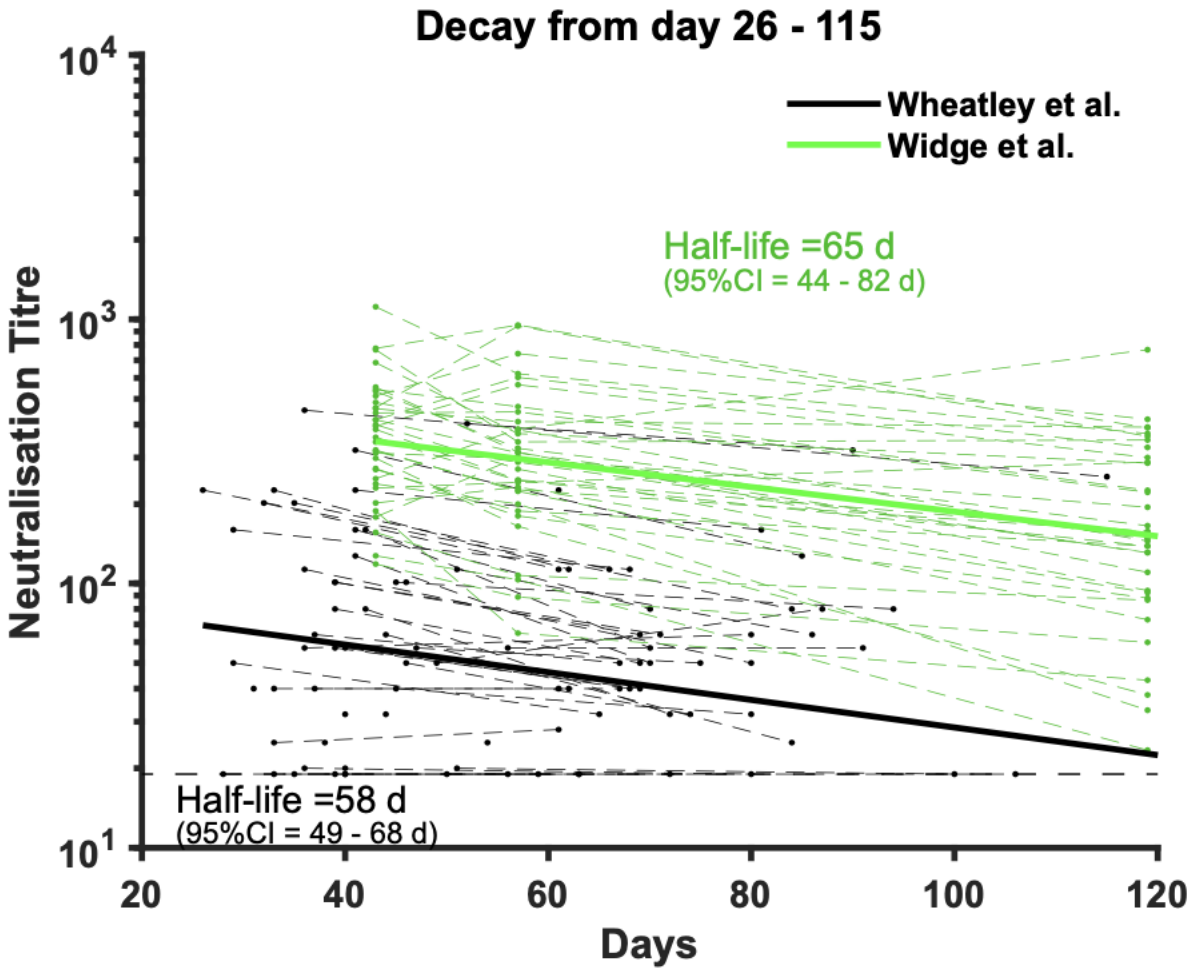
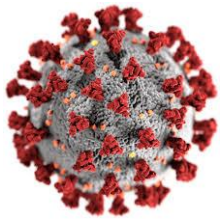


Figure S3: Neutralisation titres reported in vaccined and convalescent individuals over time.

medRxiv preprint doi: https://doi.org/10.1101/2021.03.09.21252641;



CATEGORIES OF VARIANTS

VARIANTS

That arise when SARS-Cov-2 replicates in people (D614G, B.1.1.7)

VARIANTS MORE CONCERNING

that arose under neutralising antibody selection pressure (e.g. B.1.351)
In particular, high levels replication for prolonged periods in immunocompromised individuals

Moore JP, JAMA 2021

Mutations in the SARS-CoV-2 RBD portion are highly suggestive of escape from neutralization: the case of South Africa Variant

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China National Center for Bioinformation
2019 Novel Coronavirus Resource (2019nCoVR)

Showing 1 to 5 of 863 entries

Previous 1 2 3 4 5 ... 173 Next

Search

- B.1.335 (79)
- B.1.336 (114)
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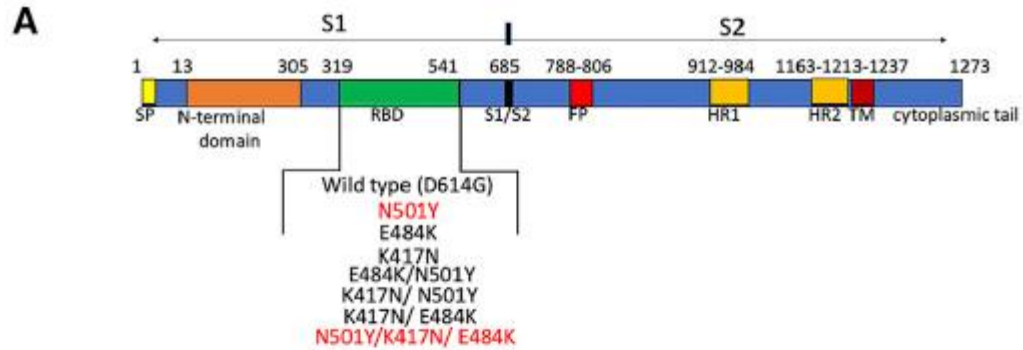
- B.1.342 (8)
- B.1.343 (26)
- B.1.344 (60)
- B.1.346 (164)
- B.1.348 (51)
- B.1.349 (354)
- B.1.350 (236)
- B.1.351 (764)
- B.1.354 (15)
- B.1.355 (32)
- B.1.356 (434)
- B.1.357 (74)
- B.1.358 (19)
- B.1.359 (75)

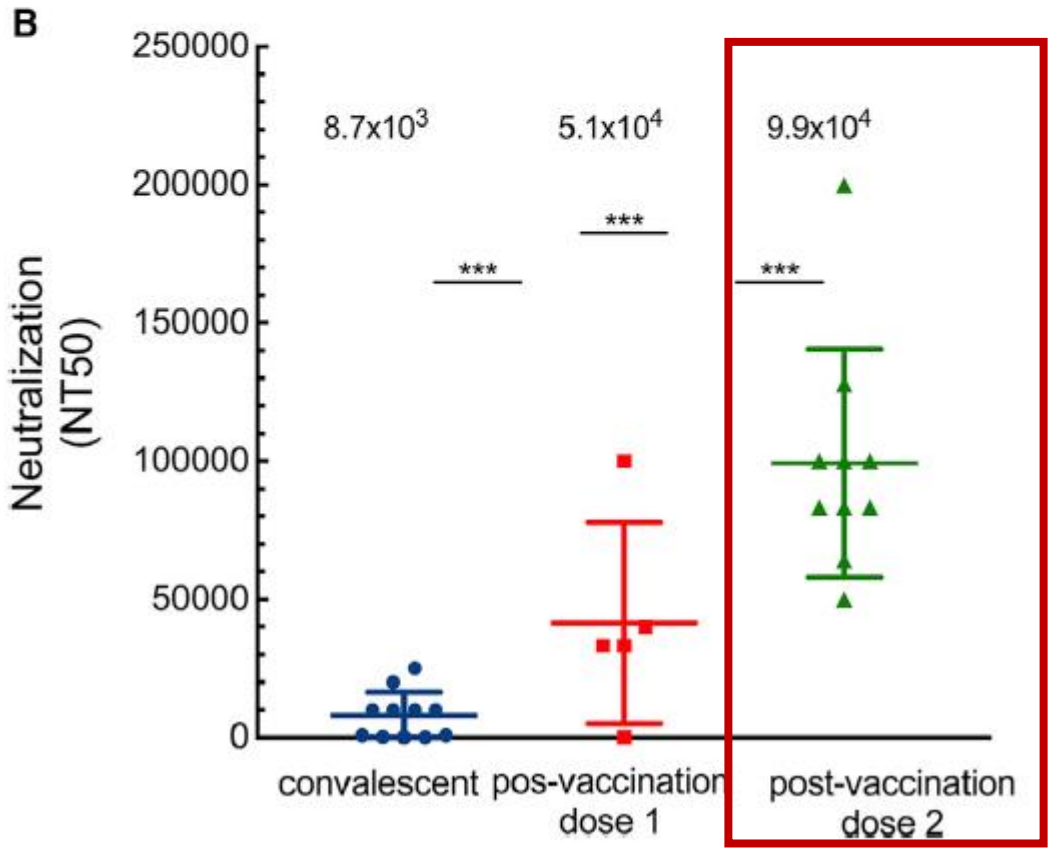
Sampling date distribution | Sampling country distribution | Virus samples list | **Variants**

Show 20 entries Search: download

Genome position	Base change	gene	Amino acids change	Samples number	Ratio of sample (%)
22488	A -> G	S	E309G	1	0.13%
22509	C -> T	S	S316F	1	0.13%
22675	C -> T	S	S371	1	0.13%
22713	C -> T	S	P384L	1	0.13%
22813	G -> T	S	K417N	121	15.84%
22987	C -> T	S	A475		
23012	G -> A	S	E484K		
23063	A -> T	S	N501Y		
23131	T -> A	S	T523	1	0.13%

highly suggestive of escape from neutralization (South Africa Variant)
(Wibmer et al. https://doi.org/10.1101/2021.01.18.427166, bioRxiv preprint)





Kuzmina A et al. Cell Host & Microbe 2021

VACCINATION and NEUTRALIZATION

…….these findings imply that the ***second dose*** of vaccination is essential to achieve ***high neutralizing titers*** against wild-type SARS-CoV-2 pseudoviruses, relative to the first-dose or to convalescent sera.

Wild-type or UK-N501Y SARS-CoV-2spike pseudoviruses were comparably neutralized by sera from the second post-vaccination dose.

However, ***SA-N501Y/K417N/E484K*** spike pseudovirus ***partly resisted*** to neutralization by post-vaccinated sera, exhibiting 6,8 fold decrease in mean NT_{50} relative to wild-type SARS-CoV-2 spike pseudoviruses.

Resistance to neutralization seemed to be driven by the ***E484K***, and to a lesser extent, on the ***K417N mutations***

Kuzmina A et al. Cell Host & Microbe 2021

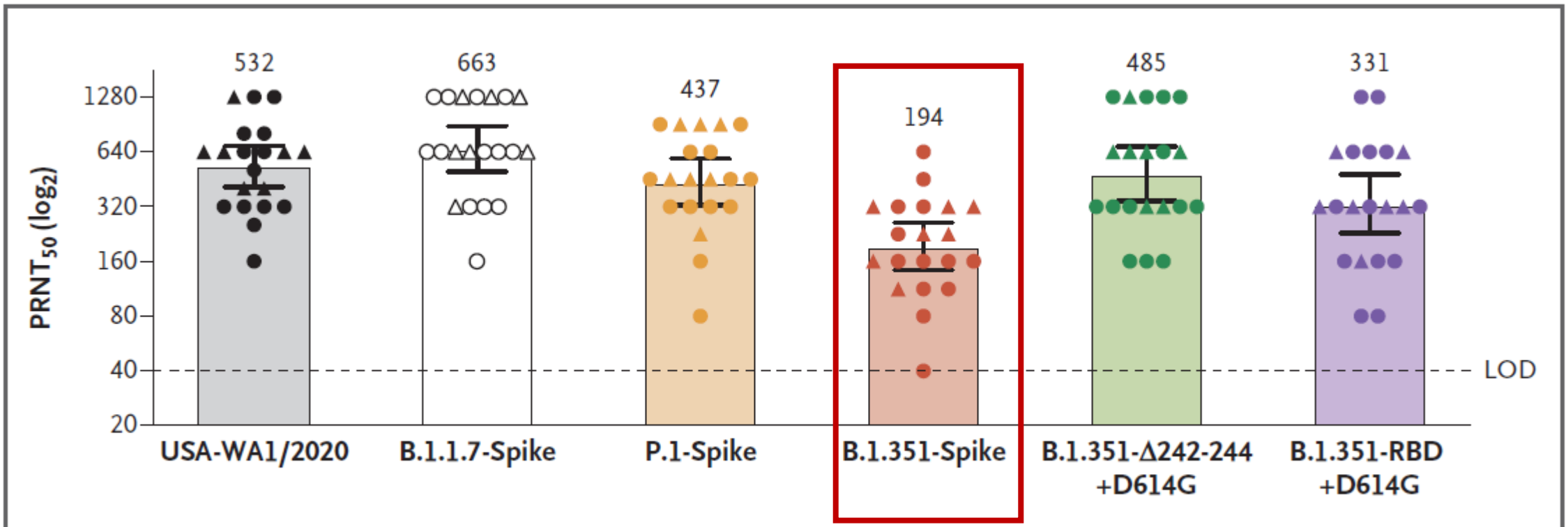


Figure 1. Serum Neutralization of Variant Strains of SARS-CoV-2 after the Second Dose of BNT162b2 Vaccine.

Liu Y et al. N Engl J Med 2021

VISIBILITY

The **WHO** message «***TEST, TEST, TEST***» was really important from a population perspective.

QUICKNESS

However, a ***rapid communication*** of a ***wrong result*** is even ***worse !***

«**NO TEST** IS BETTER THAN A **BAD** TEST»

Gray N et al. PlOS ONE 2020