SITA
Società Italiana di Terapia Antinfettiva Antibatterica Antivirale Antifungina

12° CONGRESSO NAZIONALE

CATANIA | 17-18 novembre 2022

Presidente SITA:
Prof. Matteo Bassetti

Comitato Organizzatore:
Prof. Carmelo Iacobello - Prof.ssa Stefania Stefani

Ruolo della virologia molecolare nella diagnostica della infezione da SARS-CoV-2

Guido Antonelli

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Microbiology and Virology Unit, University Hospital «Policlinico Umberto I»

Sapienza University of Rome

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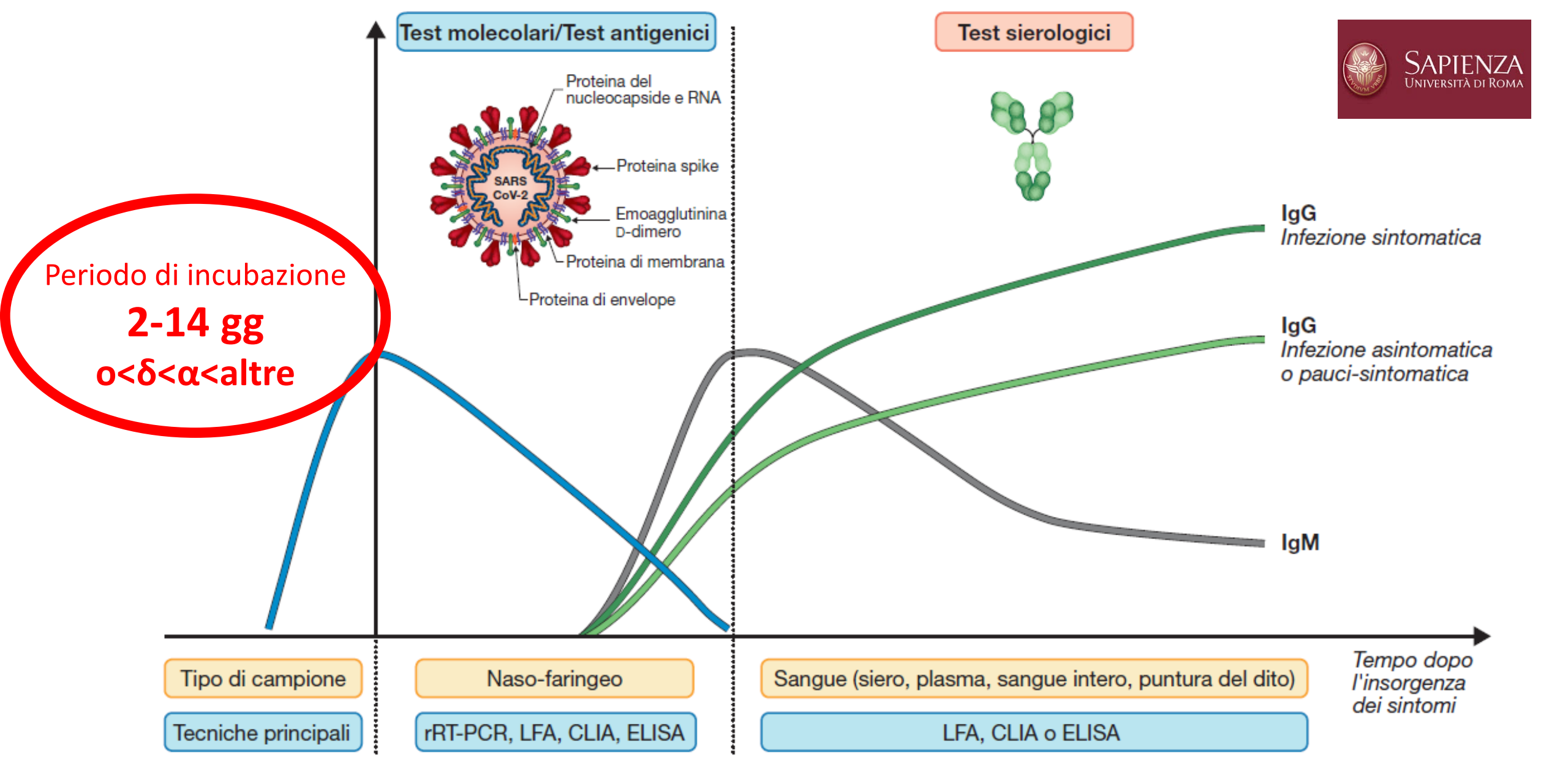
UMBERTO I
POLICLINICO DI ROMA



Harrison Principi di Medicina interna 20ª Edizione

La pandemia di COVID-19 in Italia

Giampiero Carosi, Roberto Cauda, Andrea Pession, Guido Antonelli

Edizione 03/08/2021

COVID-19 pandemics - diagnosis

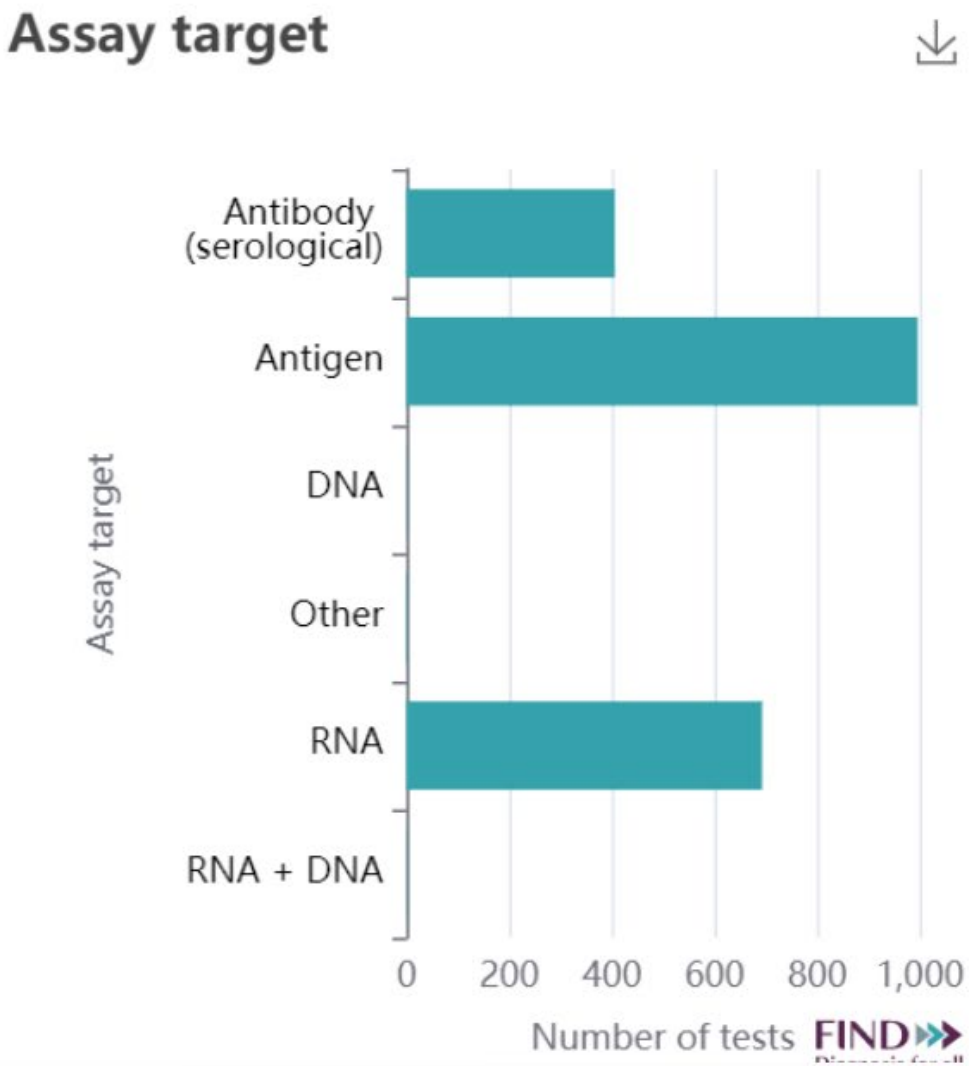
Rapid and accurate laboratory testing of SARS-CoV-2 has been (is) essential for:

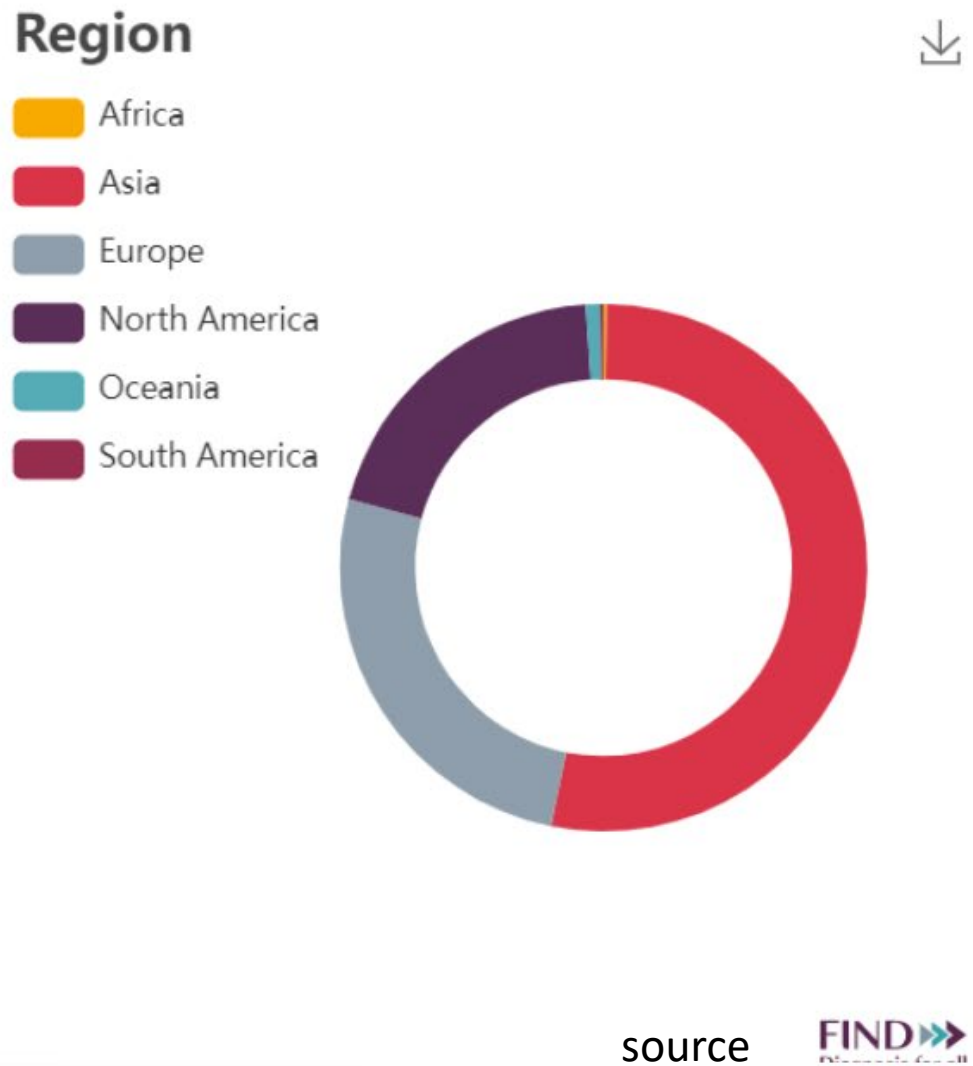
- early detection and then properly managing the patient;
- reporting and isolation of new cases;
- cutting of epidemic transmission

Testing of SARS-CoV-2 has been (is) important also to:

- estimate the effectiveness of community measures and social distancing;
- monitor a safe return to work, schools and universities;
- screen groups at risk, such as the elderly, patients with underlying medical conditions,
- screen professional groups with a low pre-test probability who are exposed to the virus while also posing a risk of spreading it (such as healthcare workers, emergency responders, essential workers)
- screen entire population to know the prevalence of the infection

TOTAL TESTS:
2099





source FIND

November 17, 2022

The tests must be considered differently, carefully and depending on the clinical settings.

Clinical diagnostic tests

- are designed for use with symptomatic/hospitalized people
- to confirm diagnosis, where necessary
- require high analytic and clinical sensitivity to return a definitive clinical diagnosis
- do not need necessarily to be low-cost and easy to perform.

"Non clinical" diagnostic tests can be used in effective surveillance regimens intended to reduce the population prevalence of a respiratory virus

- should be sufficiently inexpensive and easy to execute to allow frequent testing (multiple times per week)
- need to return results quickly to limit asymptomatic spread

The commercially available tests must be considered differently, carefully and depending on the clinical settings.

Clinical diagnostic tests

- are designed for use with symptomatic/hospitalized people
- to confirm diagnosis, where necessary
- require high analytic and clinical sensitivity to return a definitive clinical diagnosis
- do not need necessarily to be low-cost and easy to perform.

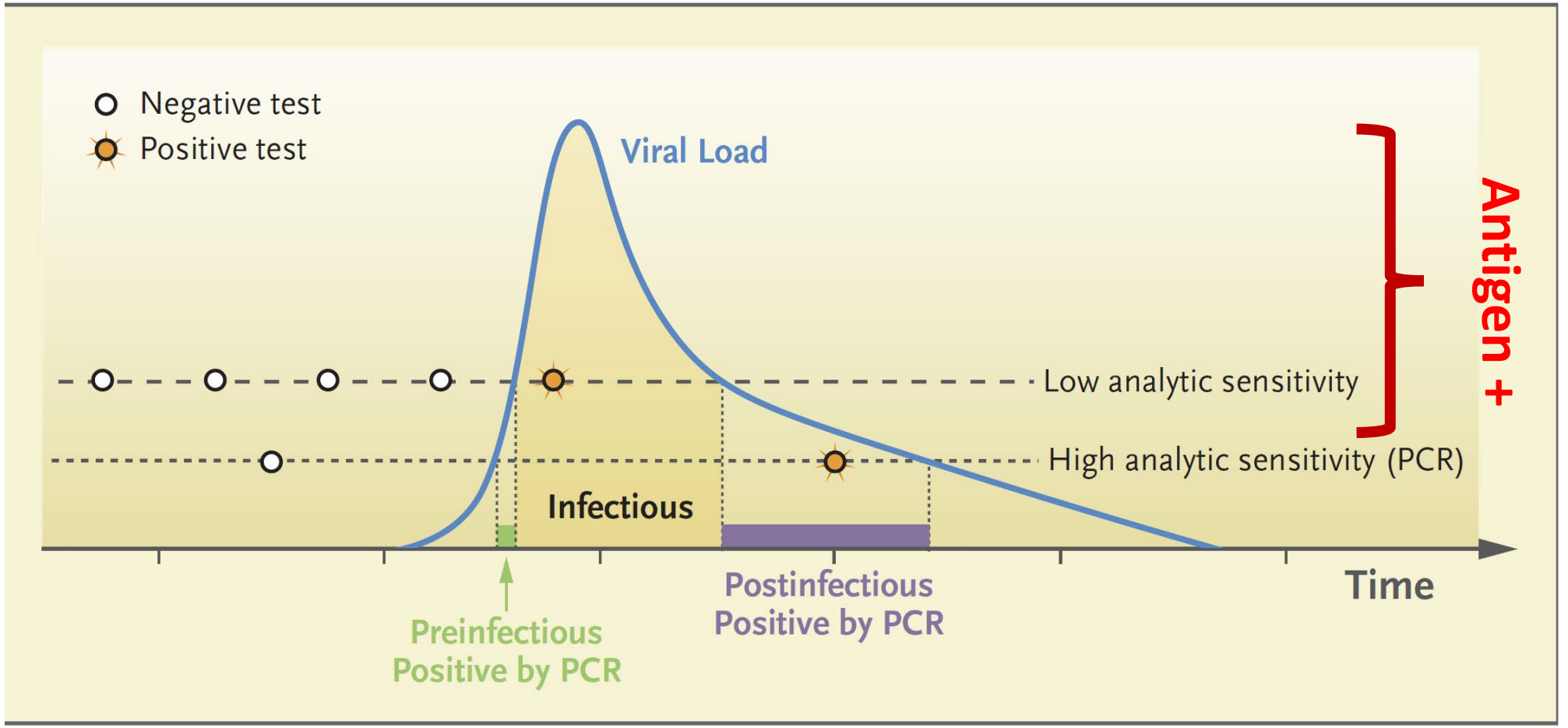
"Non clinical" diagnostic tests can be used in effective surveillance regimens intended to reduce the population prevalence of a respiratory virus

- should be sufficiently inexpensive and easy to execute to allow frequent testing (multiple times per week)
- need to return results quickly to limit asymptomatic spread

Surveillance testing regimens that can sever transmission chains to reduce community spread should complement, not replace, our current clinical diagnostic tests

Ruolo della virologia molecolare nella diagnostica della infezione da SARS-CoV-2

Antigenic assay vs molecular assay



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

Test rapidi e ravvicinati, anche se meno sensibili, possono, <u>in alcune situazioni</u>, essere migliori di test molto sensibili (i.e molecolari) ma dilazionati nel tempo;

Michael J. Mina et al.

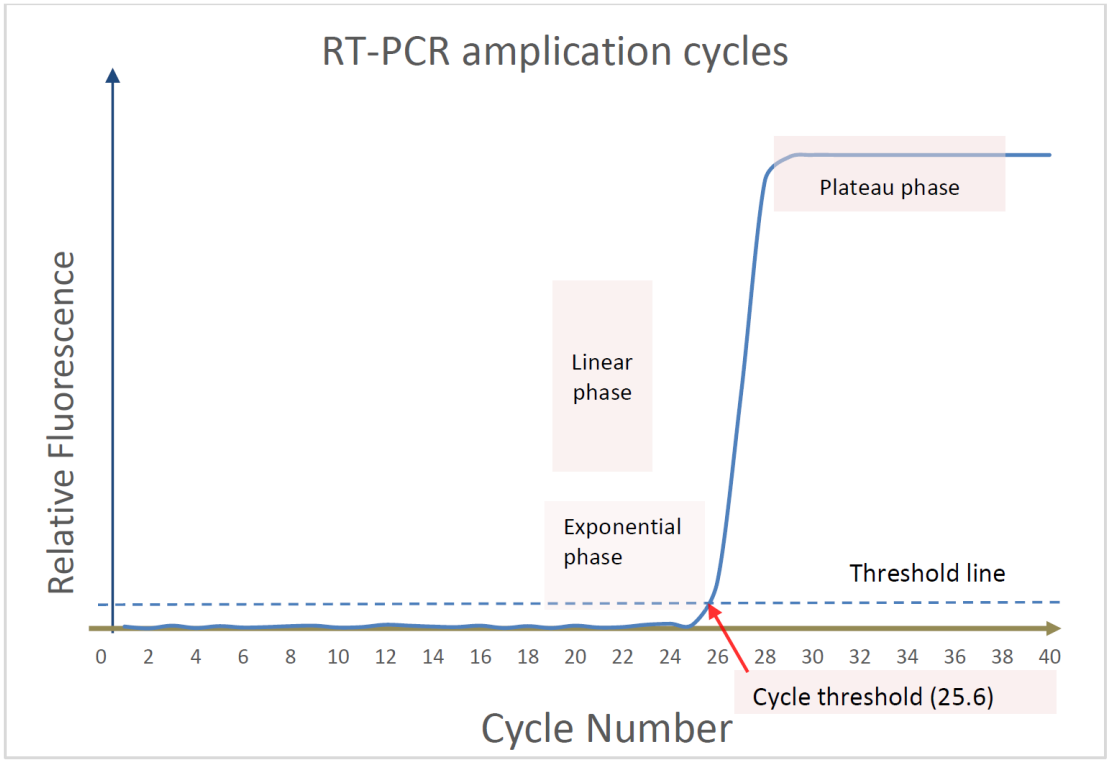
Ruolo della virologia molecolare nella diagnostica della infezione da SARS-CoV-2

The Ct value

CT Values - Definition

CT value refers to the number of cycles needed for the PCR to determine a positive result.

For RT-PCR tests, the more viral RNA present in your body, the fewer cycles (low CT value) are needed for the machine to detect the virus. Whereas, if you have very little to no virus in your body, more cycles (high CT value) are required to detect the virus.

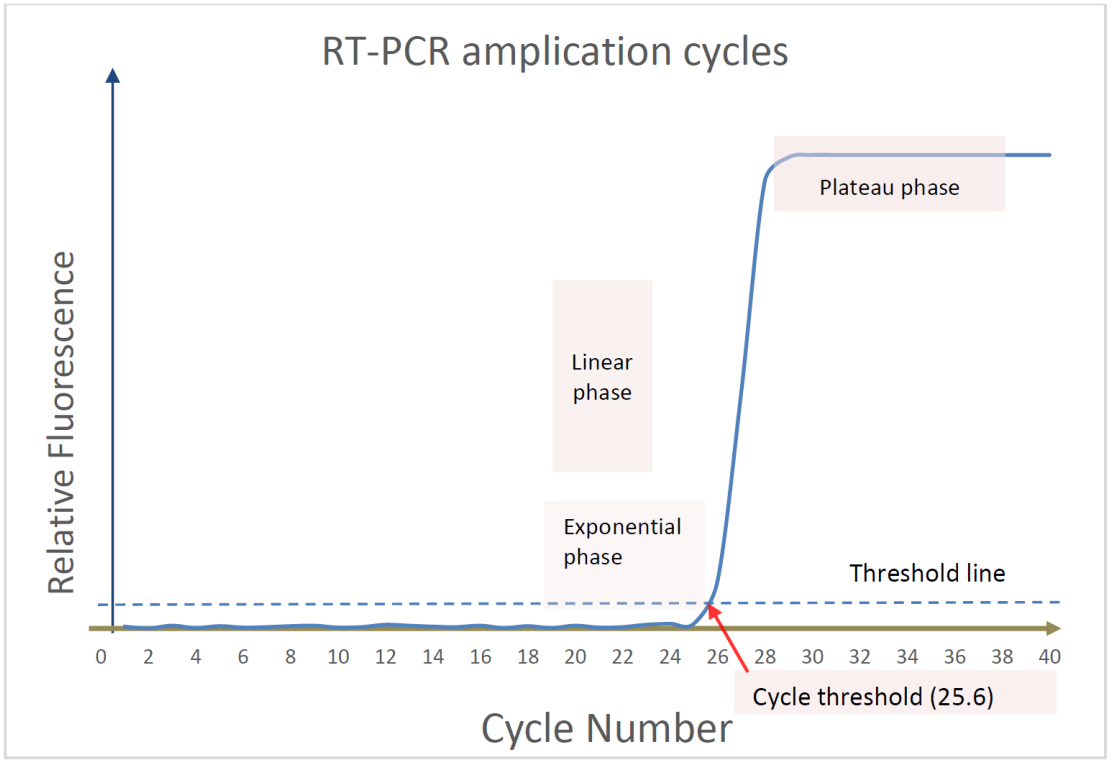


CT Values - Summary

Ct values cannot be direclty compared between different assays

While high Ct values are probably associated with reduced **infectivity**, to what extent this indicates the absence of potential transmission is still to be established.

A molecular swab taken at single point does not provide information about the **subsequent course of illness**. The clinical significance of single positive result with high Ct are difficult to interpret without considering the clinical context.

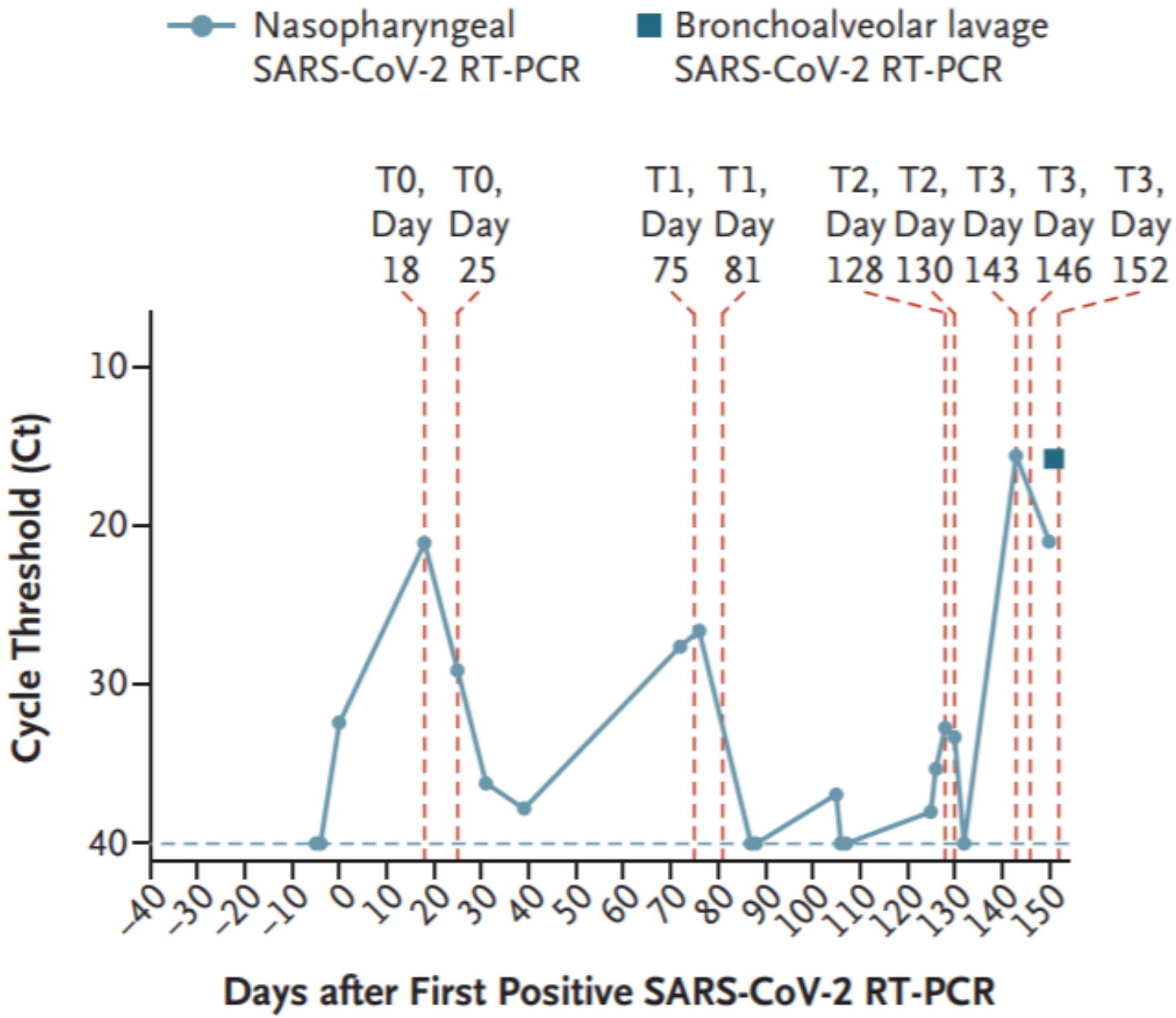


Ruolo della virologia molecolare nella diagnostica della infezione da SARS-CoV-2

There is a need to distinguish SARS-CoV-2:

Initial phase of infection or recovery

Persistence or Reinfection


Nasopharyngeal SARS-CoV-2 RT-PCR
Bronchoalveolar lavage SARS-CoV-2 RT-PCR
T0, Day 18
T0, Day 25
T1, Day 75
T1, Day 81
T2, Day 128
T2, Day 130
T3, Day 143
T3, Day 146
T3, Day 152
Cycle Threshold (Ct)
10
20
30
40
-40 -30 -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Days after First Positive SARS-CoV-2 RT-PCR

ARTICLE IN PRESS

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journal homepage: www.clinicalmicrobiologyandinfection.com

Letter to the Editor

Virological and clinical rebounds of COVID-19 soon after nirmatrelvir/ritonavir discontinuation

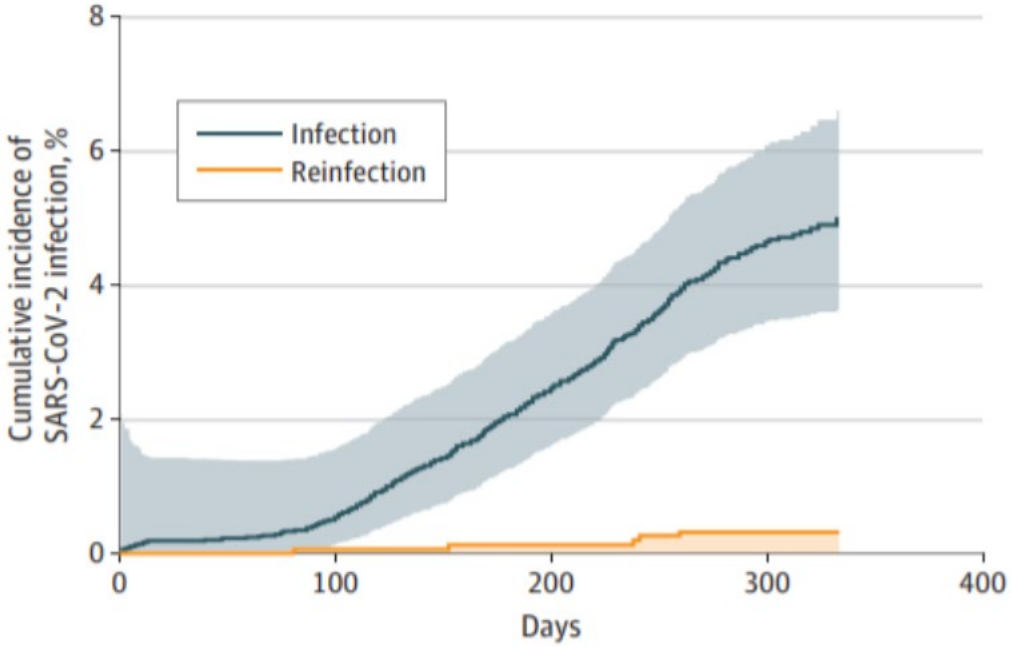
Guido Antonelli [1], Daniele Focosi [2,*], Ombretta Turriziani [1], Marco Tuccori [3,4], Rossella Brandi [5], Silvia Fillo [5], Camilla Ajassa [6], Florigio Lista [5], Claudio M. Mastroianni [6]

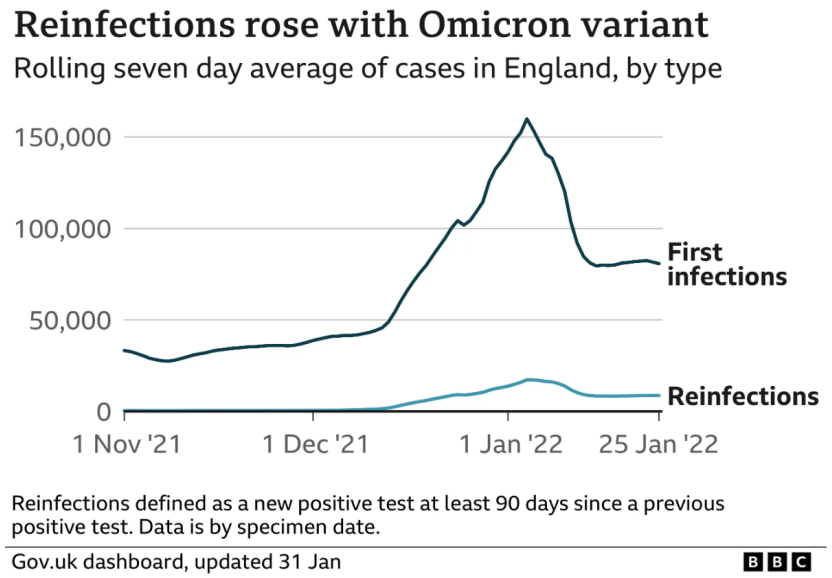
Article history:
Received 4 May 2022
Received in revised form
9 June 2022
Accepted 28 June 2022
Available online xxx

Editor: L. Kaiser

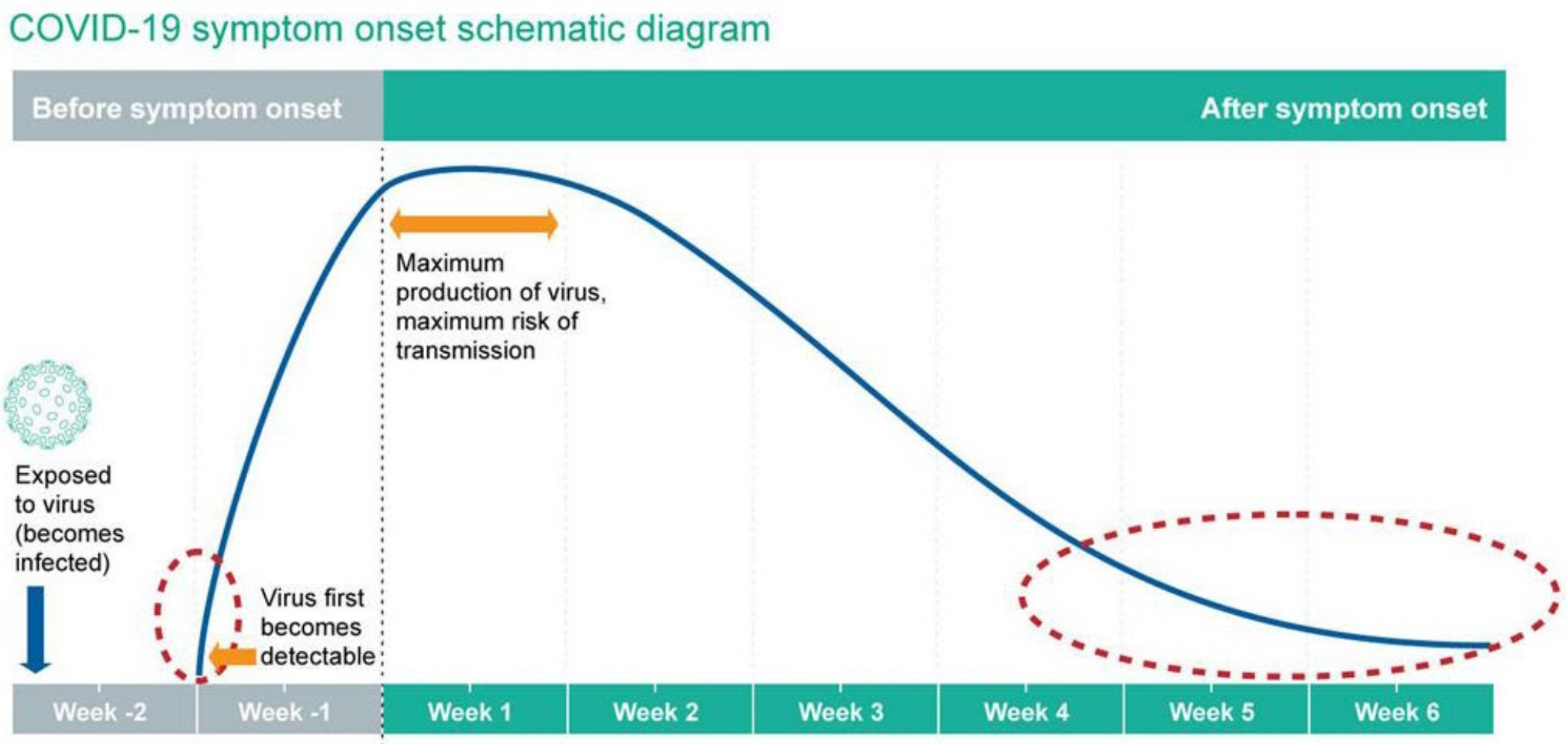
Case 1 was a 63-year-old male patient vaccinated with 3 doses of BNT162b2 (last dose on October 2021) and exposed to a COVID-19 patient on 28 February 2022. On 5 March, he developed mild pharyngodynia and fever and tested positive for SARS-CoV-2 on nasopharyngeal swab (NPS) at both rapid antigen assay (cutoff/index = 121; COVID-19 Ag FIA-SD BIOSENSOR, Gyeonggi-do, Republic of Korea) and RT-PCR (cycle threshold (Ct) RdRp/N = 21; FTD SARS-CoV-2, Siemens Healthineers, Erlangen, Germany). Being at risk of progression because of age, previous acute myocardial infarction, systemic arterial hypertension, and first-grade obesity, he started nirmatrelvir/ritonavir treatment the same day, which was discontinued on 10 March. On 8 March, symptoms resolved and the antigen assay showed lower reactivity, and on 11 March both antigen and molecular assays were negative (RdRp/N Ct = 37). On 17 March, following sudden rhinorrhea, a repeat RT-PCR was again positive (RdRp/N Ct = 17). On 19 March, RT-PCR in peripheral blood was negative, but on 22 March, RT-PCR was still positive on NPS. On 25 March, the rapid antigen assay turned negative, as well as the RT-PCR on NPS on 29 March (after 24 days from the original onset of the symptoms). Whole-genome sequencing of SARS-CoV-2 at baseline and relapse showed full identity (Omicron BA.2 sub-lineage), excluding treatment-emergent mutations or reinfection from a different lineage. Serum anti-trimeric Spike IgG (CLIA, Liaison, DiaSorin, Saluggia, Italy) gradually increased from 2750 BAU/mL, after 17 days of infection, to 4690 BAU/mL, 23 days after the onset of the symptoms. Anti-N antibodies (Elecsys Anti-SARS-CoV-2, Roche, Basel, Switzerland) were negative 17 days after infection and became positive 25 days after the onset of the symptoms.

[Risk of reinfection after seroconversion to SARS-CoV-2: A population-based propensity-score matched cohort studyexternal icon](). **Leidi et al. Clinical Infectious Diseases (May 27, 2021).**





Initial phase of infection or recovery



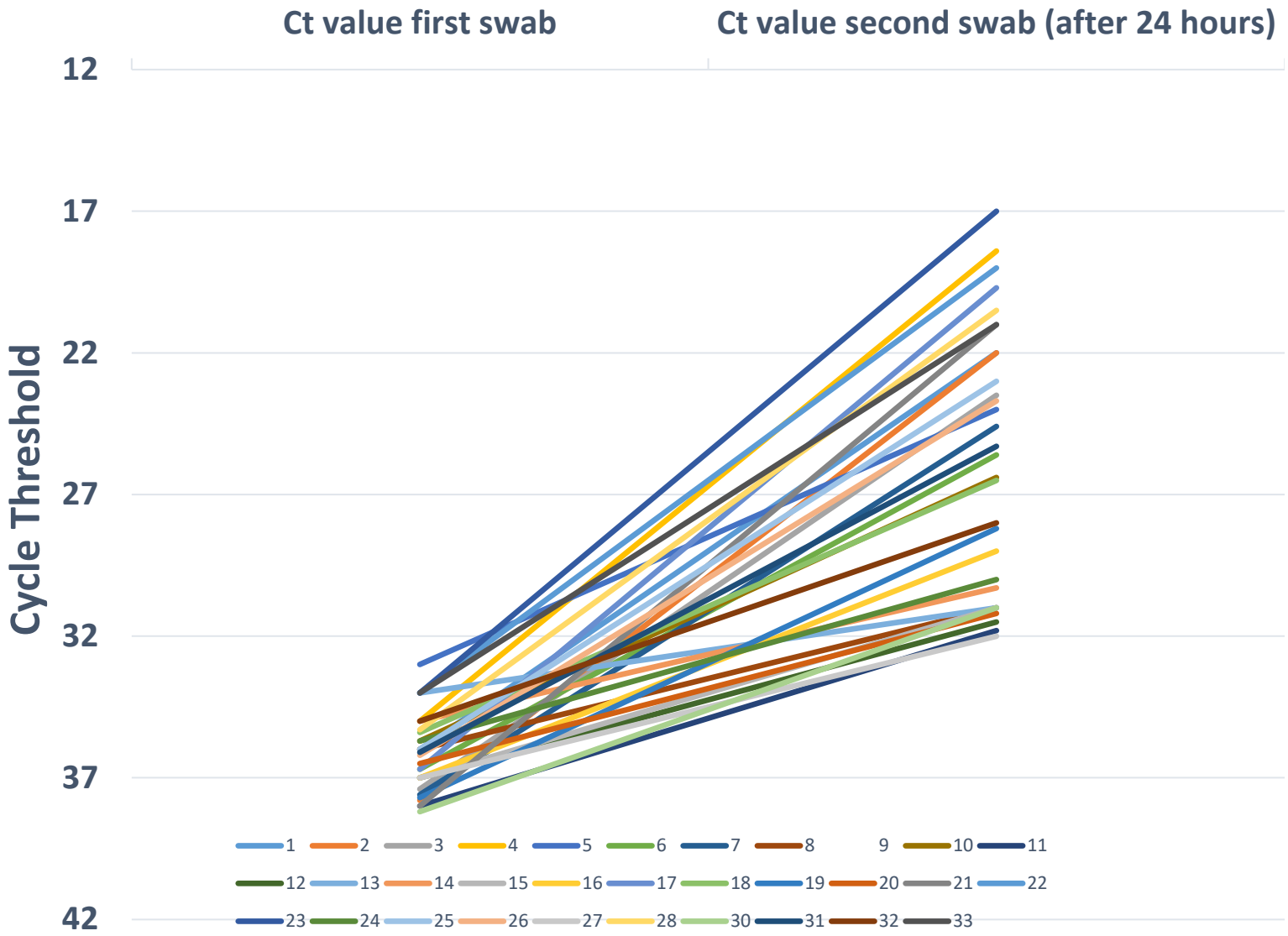
Modified from Public Health England

Understanding cycle threshold (Ct) in SARS-CoV-2 RT-PCR

Published October 2020

Initial phase of infection or recovery

32/338 **«Suspected infections» confirmed as «initial phase»** of infection (9/32 HP + 23/32 pts) (6 months of observation)



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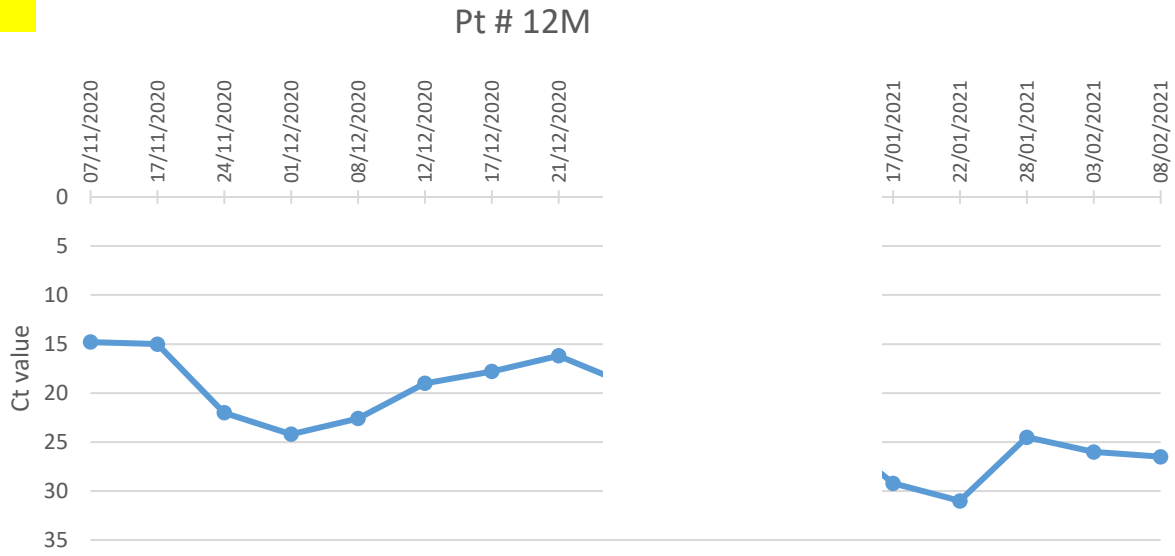
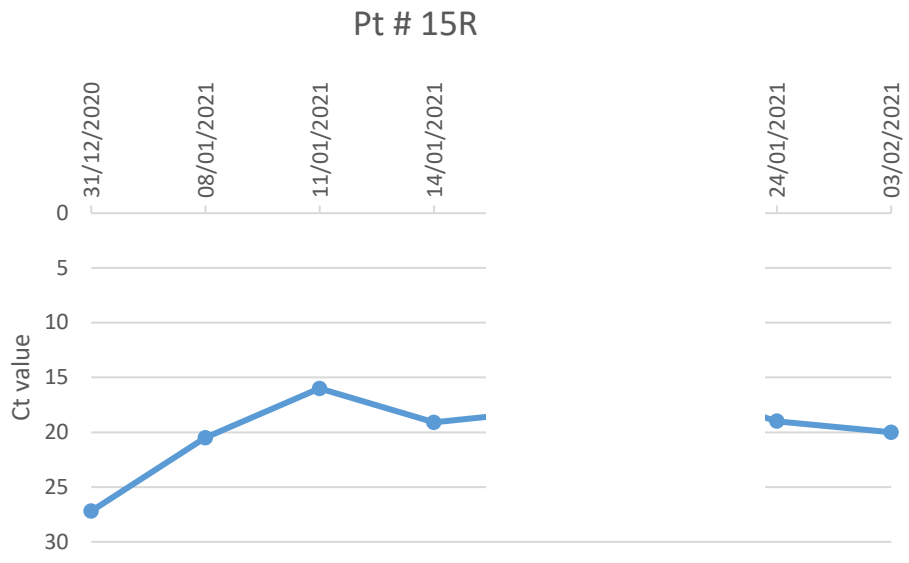
Mazzuti et al. submitted

Persistence or Reinfection





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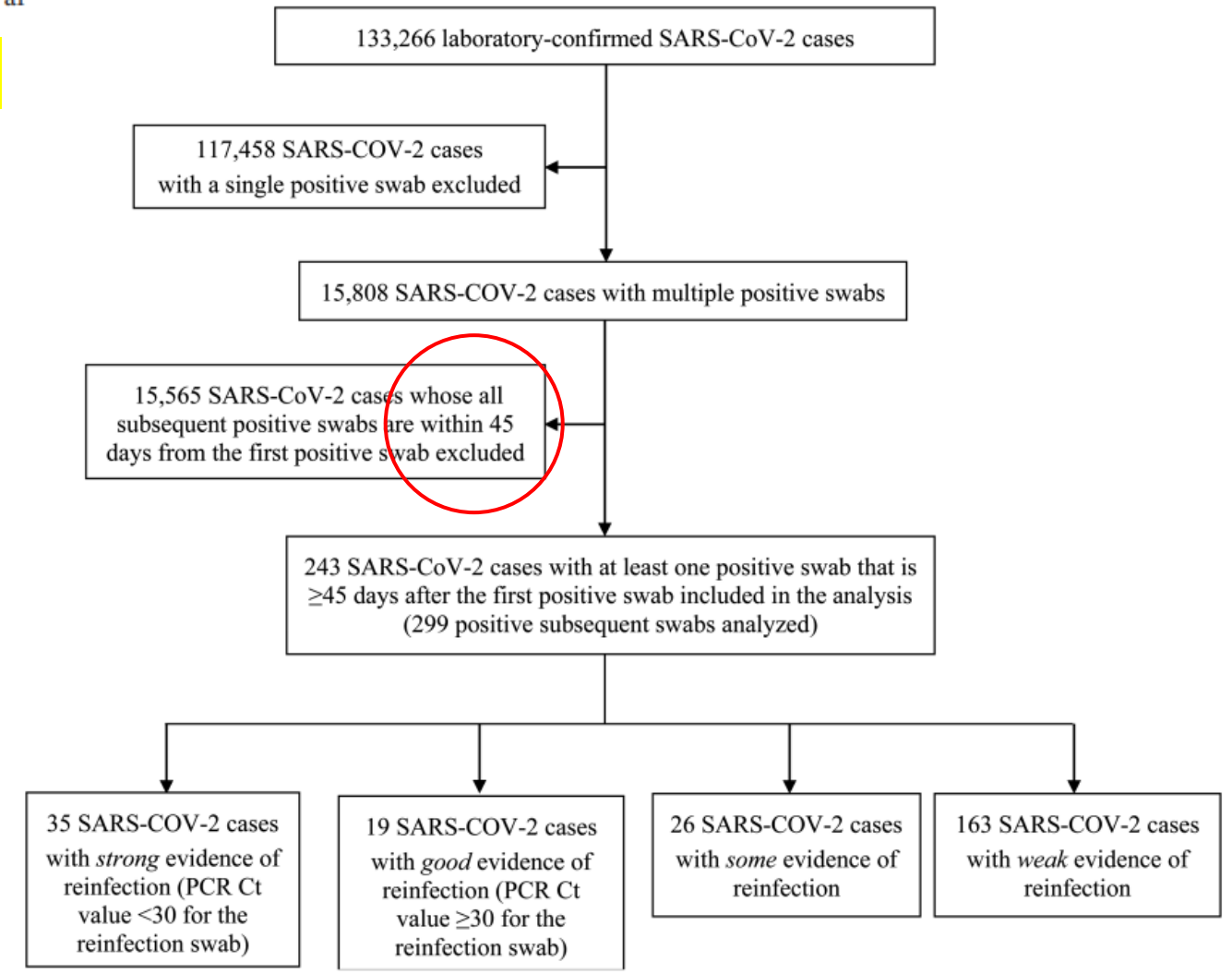


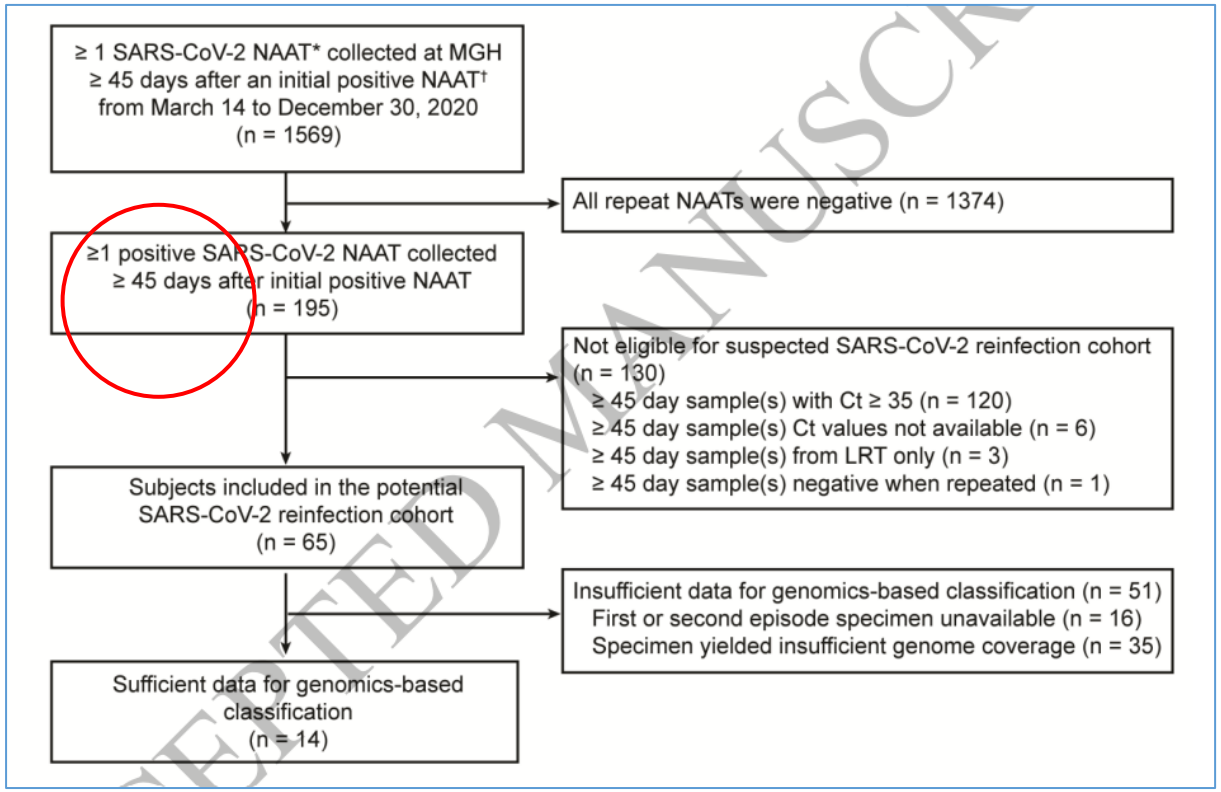
Ct <30

Ct > 30 with a supporting context

Ct > 30 without a supporting context

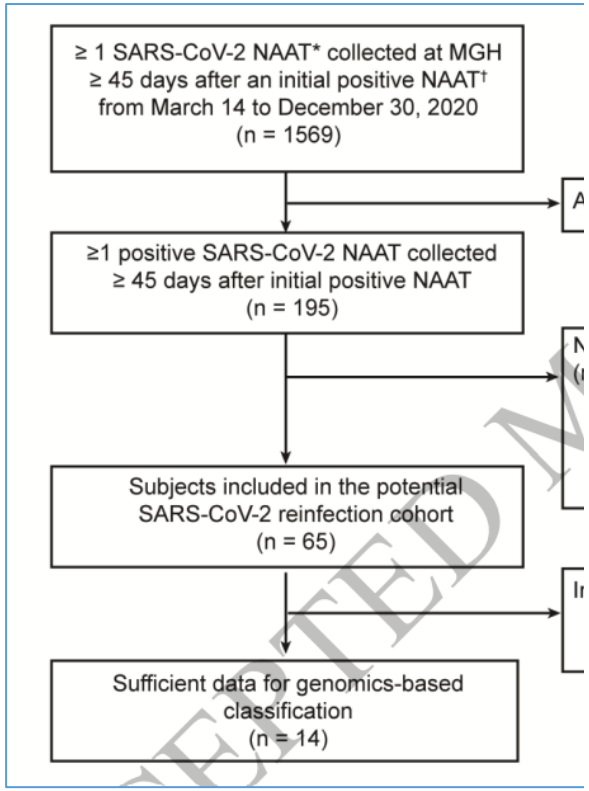
Ct >30 with a poor infection control of the first infection

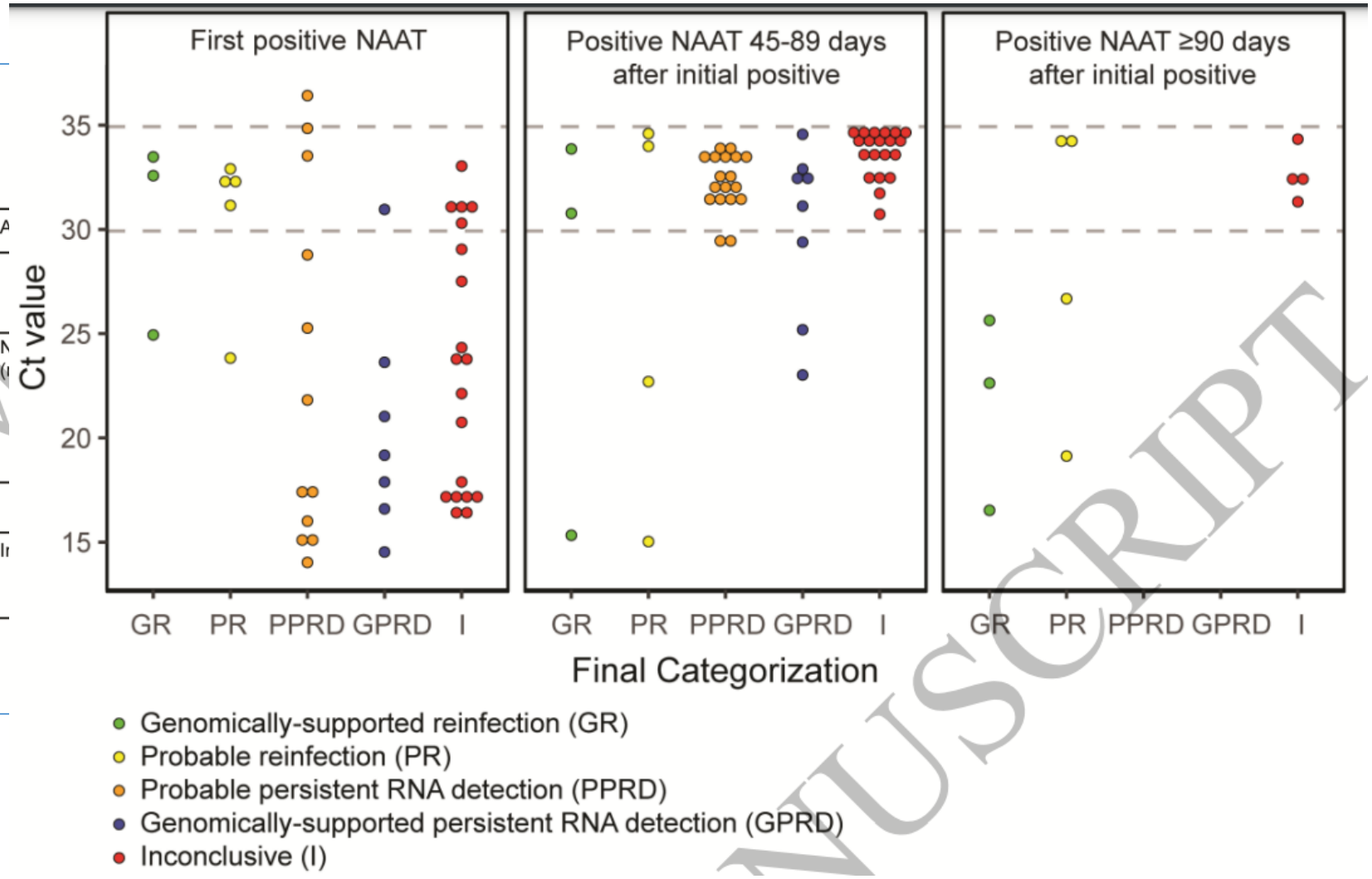
Persistence or Reinfection



Clin Infect Dis . 2022 Oct 21;ciac830. doi: 10.1093/cid/ciac830.
Online ahead of print. **Sarah E Turbett** et al.

Persistence or Reinfection





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Ruolo della virologia molecolare nella diagnostica della infezione da SARS-CoV-2

Molecular diagnostic testing using a pooling strategy

The experience at COVID-19 lab in AOU PUI/ Sapienza University

COVID-19 pandemics - diagnosis

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Testing of SARS-CoV-2 has been (is) important also to:

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- screen entire population to know the prevalence of the infection

Such efforts needed (needs) the support of an efficient and high capacity laboratory diagnostics

Strategies that can be used to increase the number of the tests during epidemic/pandemic period:

Public health, and policy:

- Centralization of the process in public health laboratories
- Conversion of research laboratories into clinical laboratories
- Production of reagents and disposable in house
- Increase in automation

Laboratory

- Diversification of platforms
- Maximizing number of samples per plate
- Applying innovative technologies to COVID-19 diagnostics
- Pooling

The Annals of Mathematical Statistics, (1943) 14, 436-440.

NOTES

This section is devoted to brief research and expository articles, notes on methodology and other short items.

THE DETECTION OF DEFECTIVE MEMBERS OF LARGE POPULATIONS

By Robert Dorfman

Washington, D. C.

In 1943 the economist Robert Dorfman first developed the theory and practice of pool testing to detect syphilis (based on the serological Wasserman test) in US soldiers during World War II.

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JOURNAL ARTICLE

Group testing with a dilution effect

F. K. HWANG

Biometrika, Volume 63, Issue 3, December 1976, Pages 671–680,

Journal of Medical Virology 49:218–222 (1996)

Evaluation of a Pooling Method for Routine Anti-HCV Screening of Blood Donors to Lower the Cost Burden on Blood Banks in Countries Under Development

Zaida García, Lizeth Taylor, Alcira Ruano, Lizeth Pavón, Esperanza Ayerdis, Ronald B. Luftig, and Kirsten A. Visoná

Louisiana State University-International Center for Medical Research and Training (Z.G., L.T., K.A.V., Costa Rica, R.B.L., New Orleans,) and Red Cross Blood Banks from El Salvador, Honduras, and Nicaragua (A.R., L.P., E.A.), Louisiana State University-International Center for Medical Research and Training, San José, Costa Rica

Feasibility and efficacy of routine PCR screening of blood donations for hepatitis C virus, hepatitis B virus, and HIV-1 in a blood-bank setting

Willi Kurt Roth, Marijke Weber, Erhard Seifried

REGIONE LAZIO

Sapienza University

Hospital "Policlinico Umberto I"

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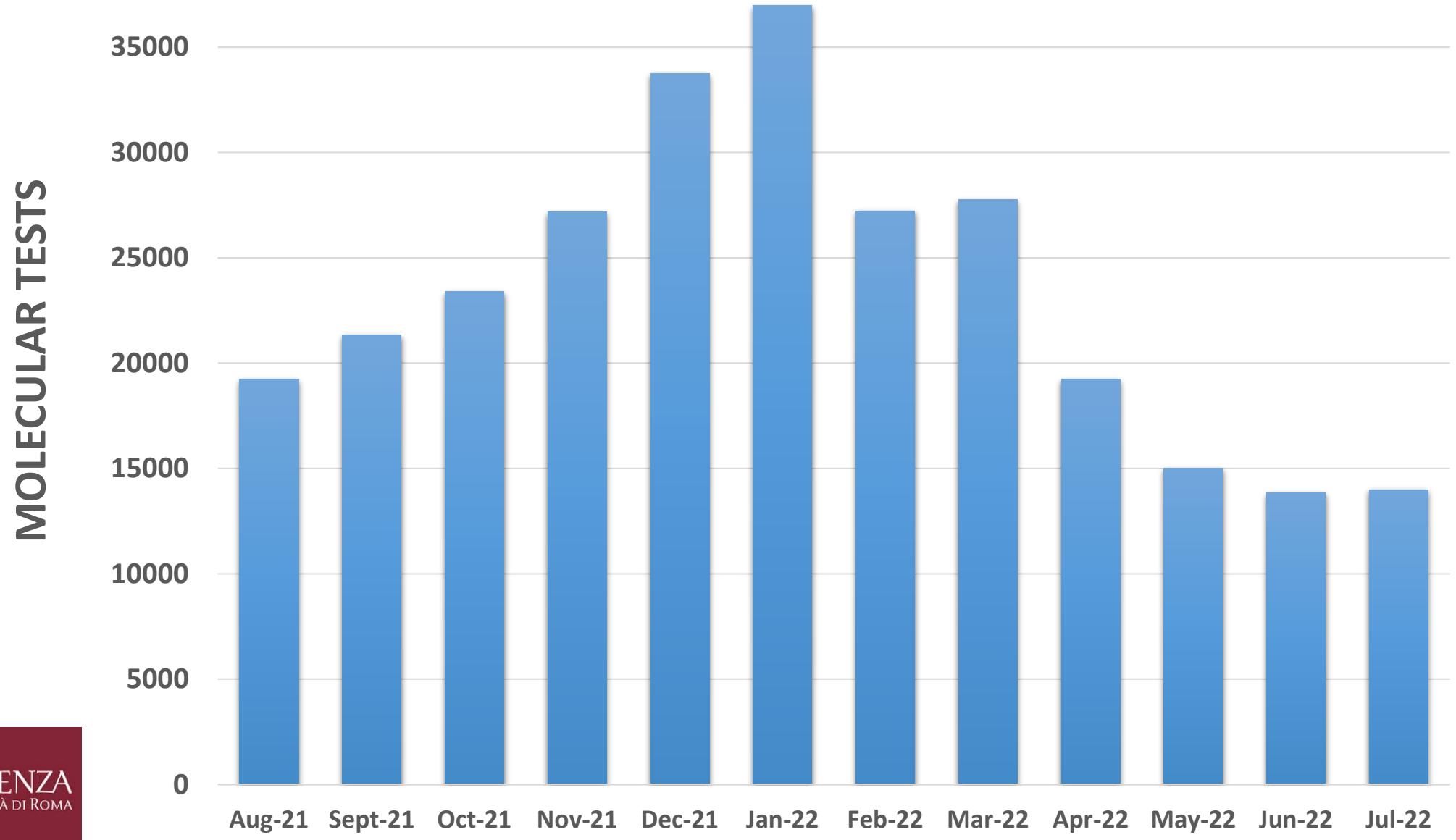
Microbiology and Virology Unit

COVID-19 Laboratory

During the period march 2020 - july 2022 more than 550,000 nasopharyngeal swabs were processed by Lab COVID-19 at the Sapienza University Hospital.

Number of Molecular test on NPS: **from August 2021 and July 2021**)
(about 280.000 NPS)



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Single sample ID

Pool preparation by Opentrons system

AltoStar SARS-CoV-2 RT-PCR

Negative Pool

Automatically sent to LIS as individual sample

Single sample ID

Pool preparation by Opentrons system

AltoStar SARS-CoV-2 RT-PCR

Positive Pool

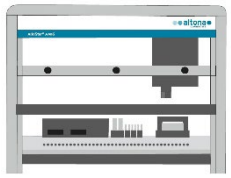
Samples run individually

AltoStar SARS-CoV-2 RT-PCR

Automatically sent to LIS as individual sample

RESULTS

POOLING (10x) performed for SCREENING purposes by MOLECULAR TESTS on:
-HEALTH WORKERS HOSPITAL «POLICLINICO» AND
- SAPIENZA University STUDENTS.
(01 August 2021- 31 July 2022)



AltoStar
SARS-CoV-2 RT-PCR

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Period	Results	N.	N. of pools tested	Positive pools (%)
Aug-21	Negative	26	29	10%
	Positive	3		
Sept-21	Negative	548	567	3%
	Positive	19		
Oct-21	Negative	596	609	2%
	Positive	13		
Nov-21	Negative	865	916	6%
	Positive	510		
Dec-21	Negative	769	880	13%
	Positive	111		
Jan-22	Negative	802	1129	29%
	Positive	327		
Feb-22	Negative	519	682	24%
	Positive	163		
Mar-22	Negative	502	705	29%
	Positive	203		
Apr-22	Negative	398	567	30%
	Positive	169		
May-22	Negative	451	573	21%
	Positive	122		
Jun-22	Negative	310	447	31%
	Positive	137		
Jul-22	Negative	238	385	38%
	Positive	147		

Number of POOLS performed during the period August 2021- July 2021) **and savings**

1.08.2021 - 31.07.2022	Results	N.	N. of pools tested	Positive pools (%)
POOLS	Negative	6024	7489	19.6 %
	Positive	1465		

Number of swab to be processed	Euro/swab	Total	
74890	10	748900,00 EUROS	
versus			
Number of pooling on 7489 swab			
7489	10	74890	89540,00 EUROS
+20% repetitions	+	+	
1465	10	14650	

More than 85% of money savings

Major concern on «Pooling strategy»

Even if each individual specimen in a pool is adequate, the specimens in a pooled procedure are diluted, which could result in a concentration of viral genetic material below the limit of detection of a specific test.
Then, **pooling could reduce test sensitivity.**

RESULTS

POOLING (10x) performed for SCREENING purposes by MOLECULAR TESTS on:
-HEALTH WORKERS HOSPITAL «POLICLINICO» AND
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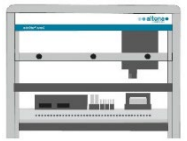

AltoStar SARS-CoV-2 RT-PCR
Gene: E and S

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Period	Results	QNT	Total Pool tested	Positive - Total Pool tested ratio
Aug-21	Negative	26	29	10%
	E Gene Positive	2		
	S Gene Positive	1		
	Positive	0		
Sept-21	Negative	548	567	3%
	E Gene Positive	2		
	S Gene Positive	2		
	Positive	15		
Oct-21	Negative	596	609	2%
	E Gene Positive	0		
	S Gene Positive	1		
	Positive	12		
Nov-21	Negative	865	916	6%
	E Gene Positive	3		
	S Gene Positive	3		
	Positive	45		
Dec-21	Negative	769	880	13%
	E Gene Positive	5		
	S Gene Positive	0		
	Positive	106		
Jan-22	Negative	802	1129	29%
	E Gene Positive	19		
	S Gene Positive	11		
	Positive	297		
Feb-22	Negative	519	682	24%
	E Gene Positive	12		
	S Gene Positive	8		
	Positive	143		
Mar-22	Negative	502	705	29%
	E Gene Positive	9		
	S Gene Positive	5		
	Positive	189		
Apr-22	Negative	398	567	30%
	E Gene Positive	12		
	S Gene Positive	15		
	Positive	142		
May-22	Negative	451	573	21%
	E Gene Positive	7		
	S Gene Positive	14		
	Positive	101		
Jun-22	Negative	310	447	31%
	E Gene Positive	11		
	S Gene Positive	5		
	Positive	121		
Jul-22	Negative	238	385	38%
	E Gene Positive	12		
	S Gene Positive	6		
	Positive	129		
Total	Negative	6024	7489	20%
	E Gene Positive	94		
	S Gene Positive	71		
	Positive	1300		

Analysis (AltoStar RT-PCR) performed on about **400 positive pools** recorded during two months period

Median values of Ct (cycle Threshold)

	S GENE (POOL)	S GENE (SAMPLE)
MEDIAN	27.3	27.5
IQ1	22.2	21.5
IQ3	33.3	33.6

	E GENE (POOL)	E GENE (SAMPLE)
MEDIAN	28.0	28.4
IQ1	23.1	22.2
IQ3	34.3	34.8

In our experience pooled specimens with low viral loads were detected thus suggesting that the detection of samples with lower CT values (high viral loads) was not impaired in 10x pooling when the assay is sensitive enough. **Also initial phase of infections can be detected**

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Key issue: Sensitivity vs efficiency and pool size.

- In our hands (HCW) a 10X sample pool, with a complete traceability of the sample, is effective in savings money, time and human resources.

- In our hands (10 X sample pool) the detection of samples with high CT values (low viral loads) was not impaired.

Pool-testing strategies can be focused to detect:

Antigen (low sensitivity)

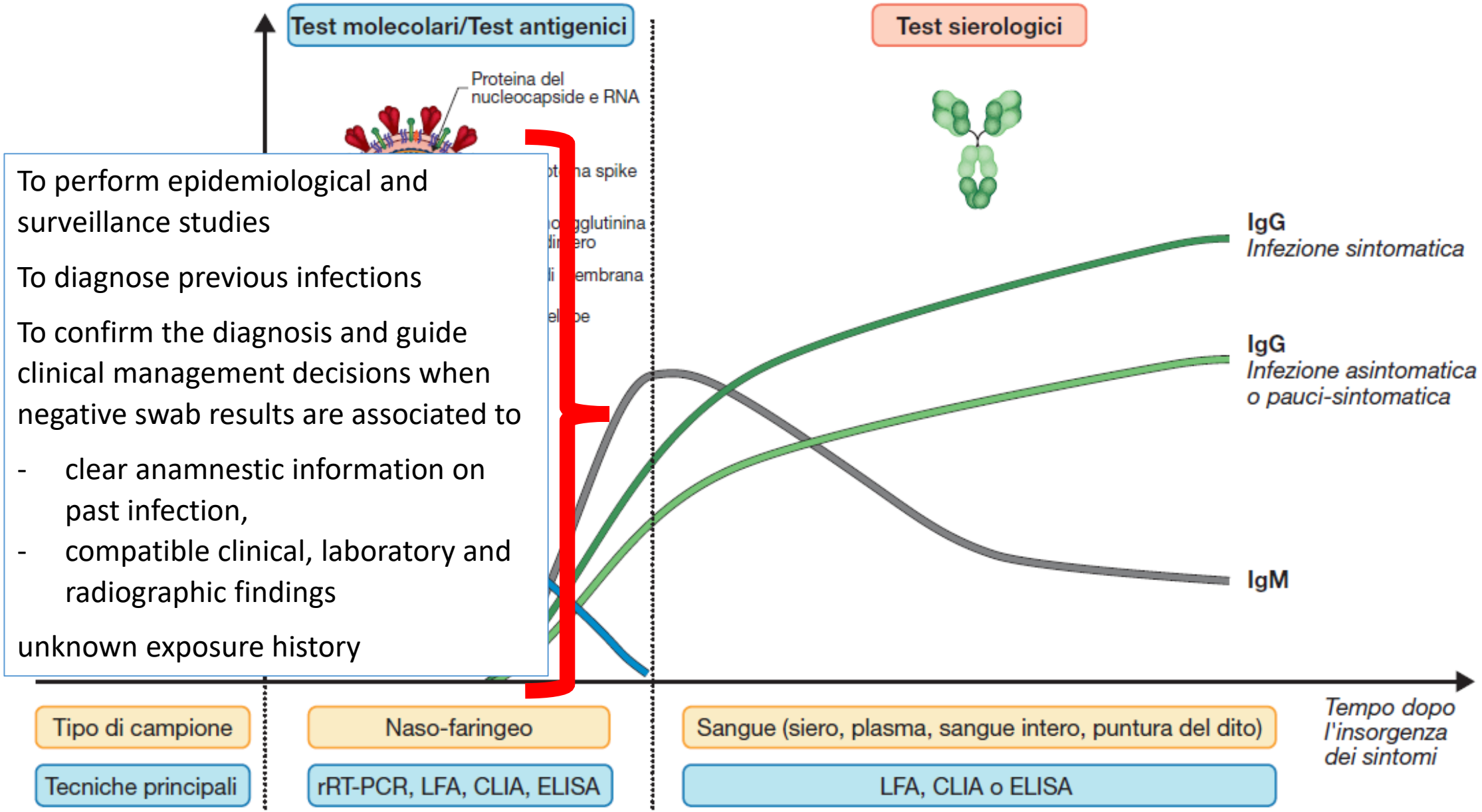
Antibody (low sensitivity)

Nucleic acids (high sensitivity)

The type of assay to be used on the pool-testing strategies should be chosen depending on the scope of the survey and/or the natural history of the infection (i.e. symptomatic vs asymptomatic or Transmitters vs Non-Trasmitters)

Ruolo della virologia molecolare nella diagnostica della infezione da SARS-CoV-2

Detection of antibodies vs detection of nucleic acids



To perform epidemiological and surveillance studies

To diagnose previous infections

To confirm the diagnosis and guide clinical management decisions when negative swab results are associated to

- clear anamnestic information on past infection,
- compatible clinical, laboratory and radiographic findings

unknown exposure history

CORRESPONDENCE | VOLUME 2, ISSUE 5, E178, MAY 01, 2021

Asymptomatic individuals positive for anti-SARS-CoV-2 antibodies negative on molecular swab

Guido Antonelli • Emanuela Anastasi • Fabrizio Ciprani • Rodolfo J Riveros Cabral • Cristiano Ialongo • Maria R Capobianchi • Ombretta Turriziani • Antonio Angeloni • Show less

Open Access • Published: May, 2021 • DOI: https://doi.org/10.1016/S2666-5247(21)00083-5

THE LANCET Microbe

Anti-SARS-CoV-2 antibody positive asymptomatic subjects result negative on molecular swab.

Guido Antonelli et al.

Table. Past SARS-CoV-2 infection in specific groups of subjects and rate of positivity at molecular swab of seropositive subjects.

Group of subjects	Number of subjects examined	Number positive for antibodies (%)^	Number positive at molecular swab/number examined (%)^^
Police officers	6,731	129 (1.9)	1/129 (0.8)
Workers from different areas (see text)	10,646	83 (0.7)	0/83 (0)

……such a procedure is redundant in the context of an epidemiological survey in which all participants are asymptomatic…..

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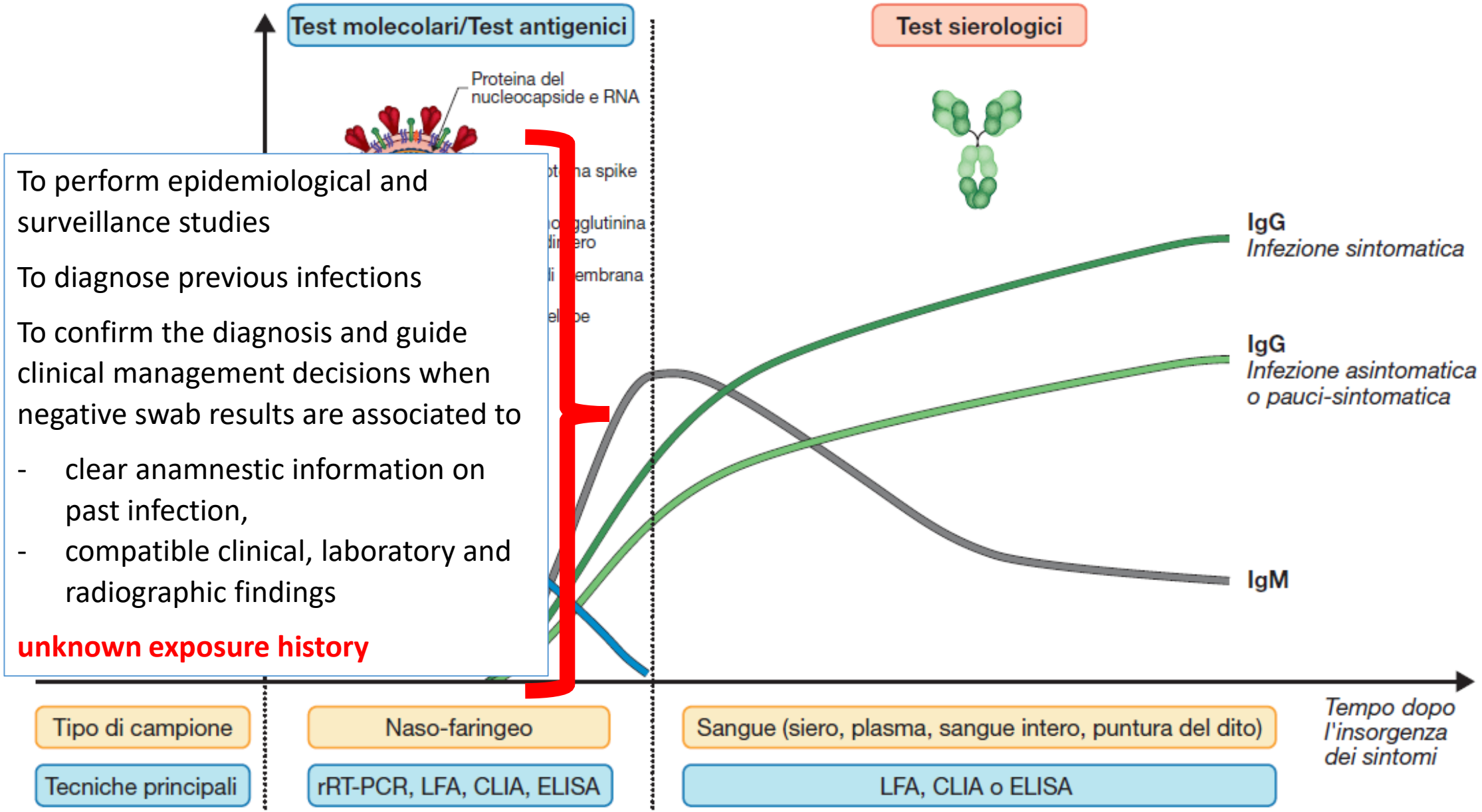

To perform epidemiological and surveillance studies

To diagnose previous infections

To confirm the diagnosis and guide clinical management decisions when negative swab results are associated to

- clear anamnestic information on past infection,
- compatible clinical, laboratory and radiographic findings

unknown exposure history

J Infect. 2021 Oct; 83(4): e9–e10.
Published online 2021 Aug 9. doi: 10.1016/j.jinf.2021.08.012
PMCID: PMC8351117
PMID: 34384812

Serological markers of SARS-CoV-2 infection; anti-nucleocapsid antibody positivity may not be the ideal marker of natural infection in vaccinated individuals

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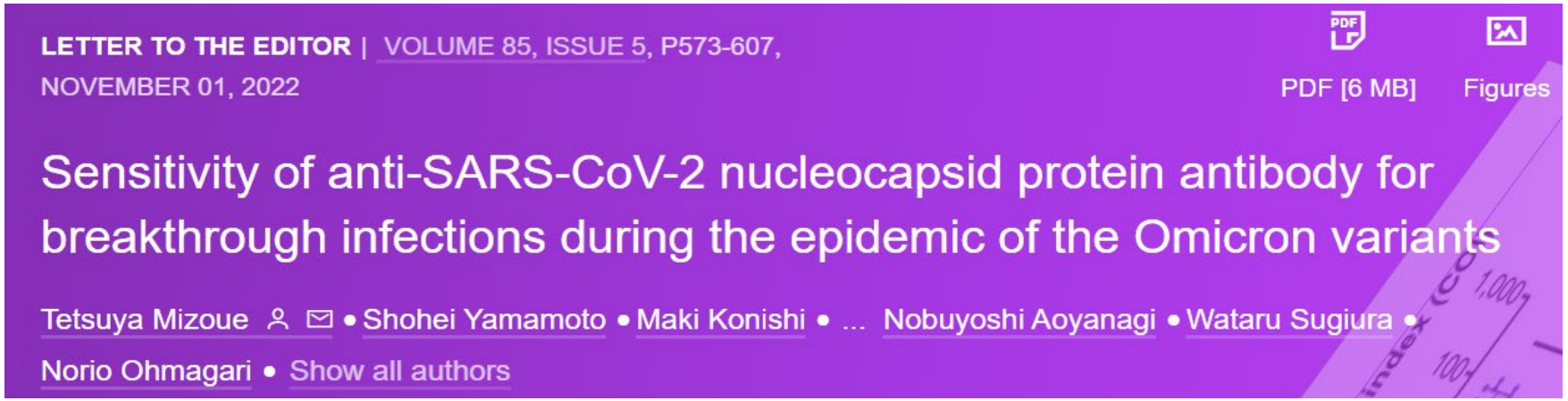


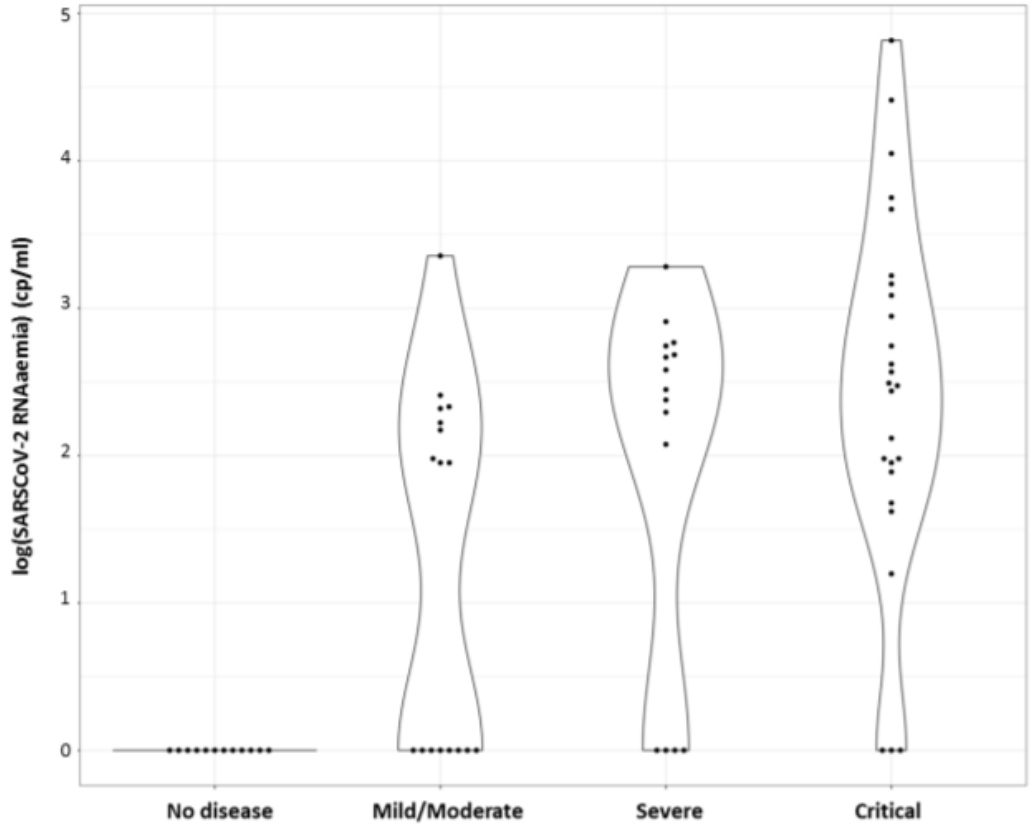
- **Not every natural infection induces production of anti-nucleocapsid (or, “anti-N”) antibodies. These antibodies are not reliable markers for a prior SARS-CoV-2 infection**
- **Vaccinated individuals may neutralize incoming viruses early during infection, thus preventing and/or limiting their ability to develop antibodies against nucleocapsid protein**

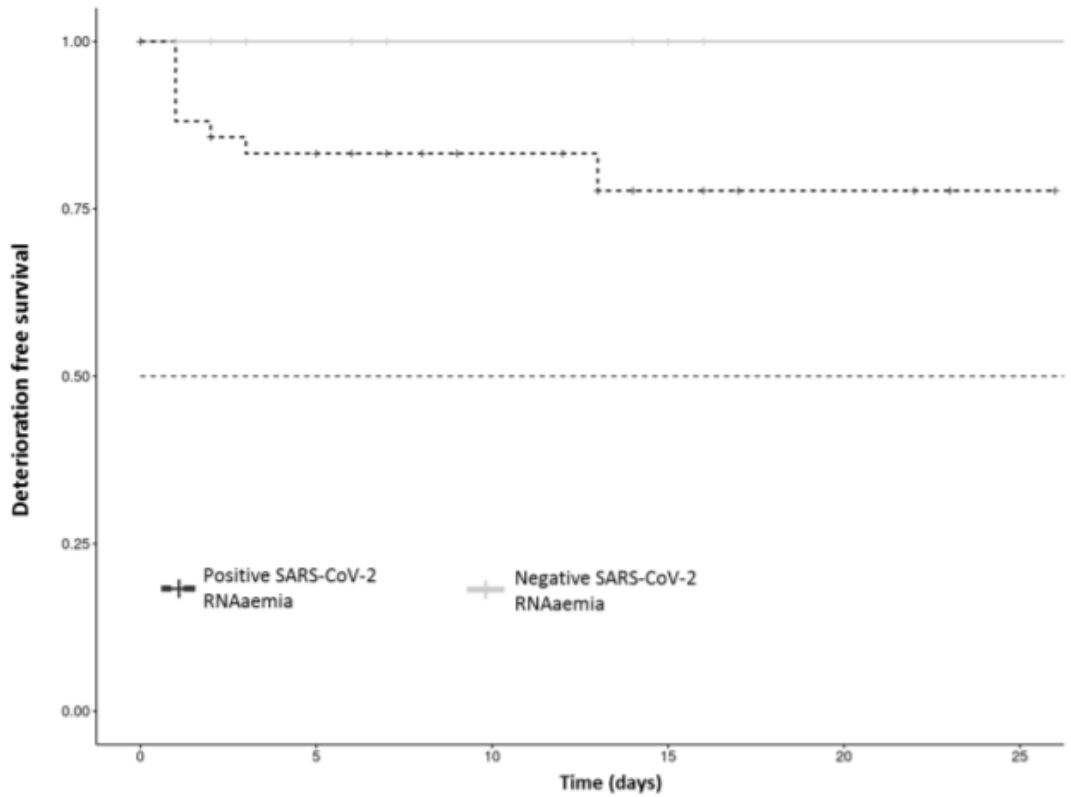
Ruolo della virologia molecolare nella diagnostica della infezione da SARS-CoV-2

Molecular detection in different districts

Additional studies are needed to determine the significance of viral dynamics and detection in different districts and whether the degree of SARS-CoV-2 viral load within compartments (including blood) different from the respiratory tract may predict disease outcomes.

SARS-CoV-2 RNA was detected in 75% of plasma samples, and level of plasma viral RNA (termed RNAaemia) was associated with severity of COVID-19.





It is likely that application of such single copy assays to blood samples from patients with COVID-19 will increase the frequency of SARS-CoV-2 RNA detection in severe disease and possibly less severe disease

Veyer D, Kerneis S, Poulet G, et al. Clin Infect Dis 2020;

Che cosa abbiamo imparato dalla pandemia: Novembre 2022:

Virologia/Ricerca

- *Il virus evolve… forse più di quanto atteso*
- *Ulteriore dimostrazione dell'importanza dell'immunopatogenesi/ autoimmunità come determinanti di malattia e dell'influenza di alcuni fattori (es. età, comorbidità, polimorfismi genetici) sulla severità della malattia virale*
- *Potenzialità di sviluppo rapido di un vaccino insieme alla conferma della necessità assoluta di definire i correlati di efficacia*
- *La ricerca su COVID-19 ha prodotto un enorme quantità di studi inadeguati, duplicati e dispendiosi (Nature, 13 May 2021)*

Sanità Pubblica

- *Controllo della attendibilità della risposta – verifica dei risultati – attivazione di reti regionali*
- *Il Laboratorio di Microbiologia Clinica, e in particolare del Laboratorio di Virologia Clinica, ha fortemente suscitato l'interesse della sanità e delle diverse organizzazioni di stakeholders.*
- *Il laboratorio di Microbiologia, e in particolare di Virologia, viene riconosciuto come essenziale ai fini di strategie di sanità pubblica (diagnosi, isolamento e contact tracing)*
- *La società non era (/è) pronta ad affrontare da un punto di vista medico, politico, sociale e di ricerca.*

Laboratorio di Virologia Clinica/Unità di Microbiologia e Virologia

- ***Nuovi determinanti della scelta dei saggi diagnostici (approvvigionamento, rapidità di refertazione, necessità di alti volumi, disponibilità di piattaforme esistenti etc.)***
- ***Ulteriore conferma della necessità di porre attenzione alla potenziale differenza tra sensibilità/specificità clinica vs sensibilità/specificità analitica***
- ***Importanza della differenza tra saggi dedicati alla diagnosi o allo screening***
- ***Importanza della procedura di pooling (da eseguire solo in particolari settings)***
- ***Ulteriore dimostrazione dell'importanza diagnostica della rilevazione del virus in diversi distretti (upper and lower respiratory tract, blood etc.)***
- ***Attenzione al flusso delle indagini microbiologiche durante l'emergenza COVID-19***

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