COVID-19
ONLINE EDUCATIONAL PROGRAM

Update on SARS-CoV-2 Virological Diagnostic

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academic medical education

- Diagnosis: recommandations and alternatives
- Clinical significance of prolonged PCR positivity following recovery
- Update on seroprevalence and use of antibody testing as a public health tool

Common COVID-19 Diagnostic Methods

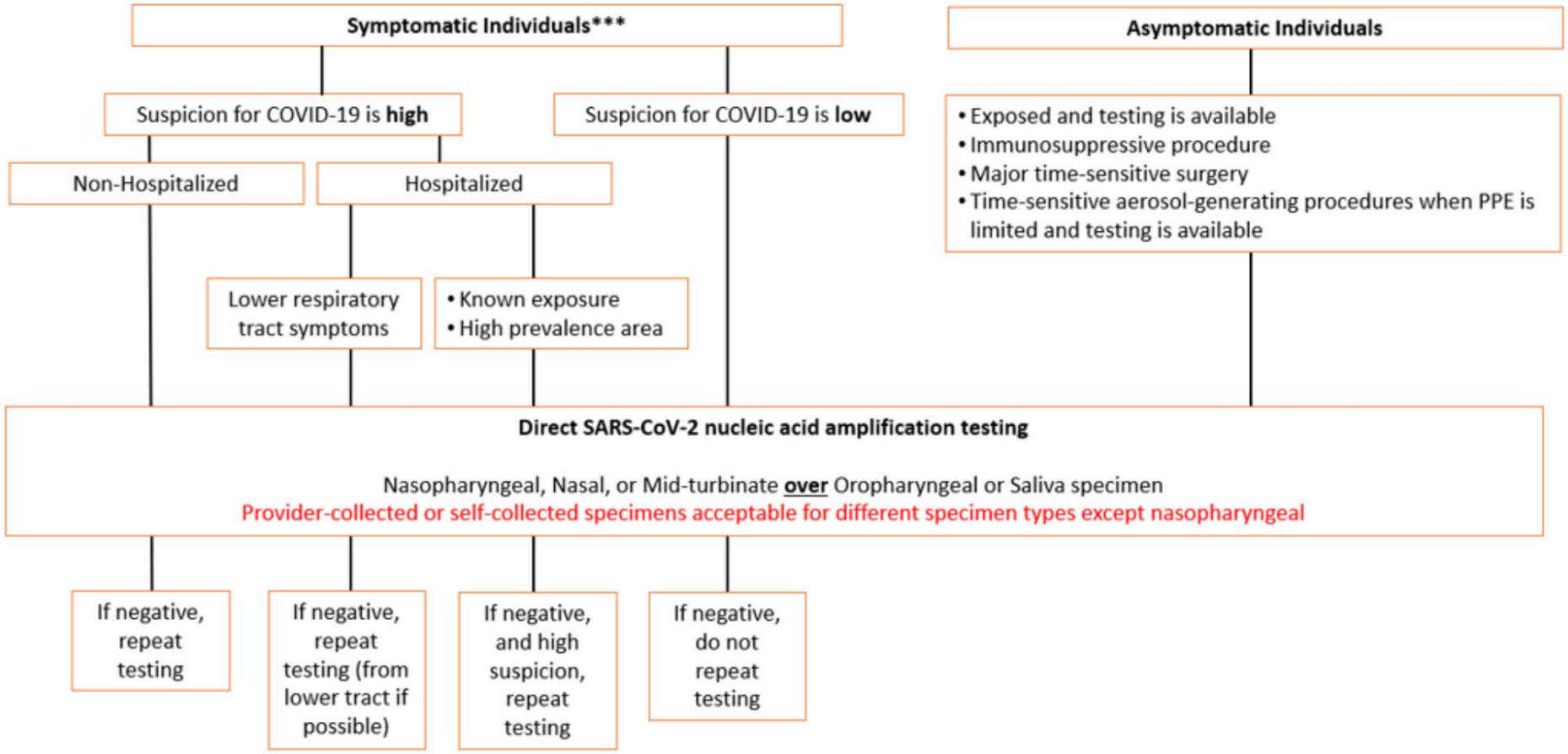
Test	Usually Indicates	Considerations
Viral nucleic acid*	Current infection	▪ Primary method for COVID-19 diagnosis with multiple RT-PCR kits available ▪ False negatives may result from improper sampling or handling, low viral load, or viral mutations ▪ SARS-CoV-2 RNA undetectable by ~ Day 14 following onset of illness in some cases
Serologic†	Past infection	▪ Provides a delayed but wider window of time for detection ▪ Useful for COVID-19 surveillance and identification of convalescent plasma donors ▪ False negative—sensitivity varies by platform ▪ False positive due to cross-reactivity ▪ Uncertain if positive read = immune protection if re-exposed

Typical specimen sources: *upper (eg, nasopharyngeal swabs or washes, oropharyngeal swabs, nasal aspirates) or lower (eg, sputum, bronchoalveolar lavage fluid, tracheal aspirates) respiratory tract, †blood serum or plasma.

Udugama. ACS Nano. 2020;14:3822. Lee. Front Immunol. 2020;11:879. Carter. ACS Cent Sci. 2020;6:591.

Slide credit: clinicaloptions.com

IDSA algorithm for RNA testing



***Note:

- Testing should be prioritized for symptomatic patients first.
- When resources are adequate, testing for selected asymptomatic individuals can also be considered.

https://www.idsociety.org/practice-guideline/covid-19-guideline-diagnostics/

Detection of viral RNA by RT-PCR

- RT-PCR is the current standard test for diagnosis of COVID-19

  - Using nasopharyngeal swabs or other respiratory tract specimens
  - A variety of RNA gene targets are used by different manufacturers, with most tests targeting 1 or more of the envelope (*env*), nucleocapsid (*N*), spike (*S*), RNA-dependent RNA polymerase (*RdRp*), and *ORF1* genes
  - After specimen collection, samples undergo RNA extraction followed by qualitative RT-PCR for target detection (results within 3-4 hours)
  - RT-PCR kits performed satisfactorily:
    - PCR efficiency (≥96%)

Nalla AK , et al. Comparative performance of SARS-CoV-2 detection assays using seven different primer/probe sets and one assay kit. *J Clin Microbiol*. 2020

Results of independent evaluation to verify the clinical performance of RT-PCR tests

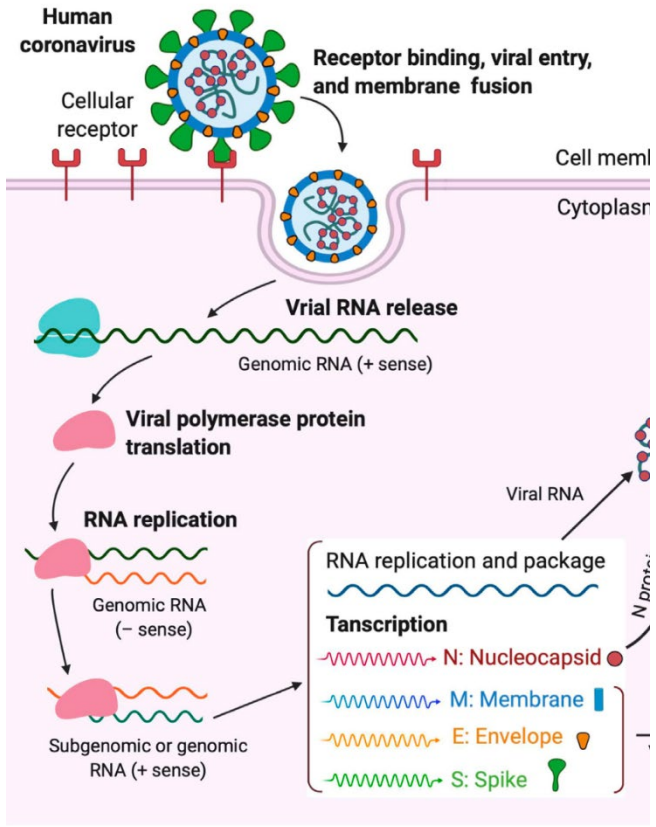
Developer	Name of the Kit	Gene	Clinical Sensitivity	Clinical Specificity	Limit of Detection LOD (Copies/Reaction)
altona Diagnostics	RealStar® SARS-CoV-2 RT-PCR Kit 1.0	*E*	92%	100%	1–10
		S	92%	100%	1–10
Atila BioSystems Inc.	Atila iAMP COVID-19 Detection (isothermal detection)	*ORF1ab*	100%	99%	20–100
		N	100%	100%	1–10
BGI Health (HK) Co. Ltd.	Real-time Fluorescent RT-PCR kit for detection 2019-nCOV (CE-IVD)	*ORF1*	100%	99%	1–10
bioMérieux	ARGENE® SARS-COV-2 R-GENE®	*N*	100%	100%	10–50
		RdRP	96%	100%	10–50

The research was carried out at the University Hospitals of Geneva

Robert Kubina, Arkadiusz Dziedzic. Diagnostics (Basel) 2020 Jun; 10(6): 434.

What do we need for a more fluid and rapid diagnosis?

- Simplifying tests procedure
  - Molecular POC that can be run everywhere
  - Virus antigen detection use
- Simplifying sampling
  - Saliva
- Samples pooling for large scale RT-PCR diagnostic ?



Rapid molecular tests

- Low-complexity, rapid (results within 1 hour)
- Most of the rapid tests evaluated used laboratory-developed RT-loop mediated isothermal amplification (RT-LAMP) technology
- Not available for clinical use in the US and conversely none of the EUA approved rapid tests use RT-LAMP technology
- The sensitivity and specificity of the rapid isothermal EUA compared to standard laboratory-based assays ranged between 75-94% and 99-100%, respectively
- Studies should be designed with a robust number of patients to define the clinical sensitivity and specificity of rapid and standard tests on the same patients
- No data on their feasibility in real life

Robert Kubina, Arkadiusz Dziedzic. Diagnostics (Basel) 2020 Jun; 10(6): 434.

Viral antigen detection

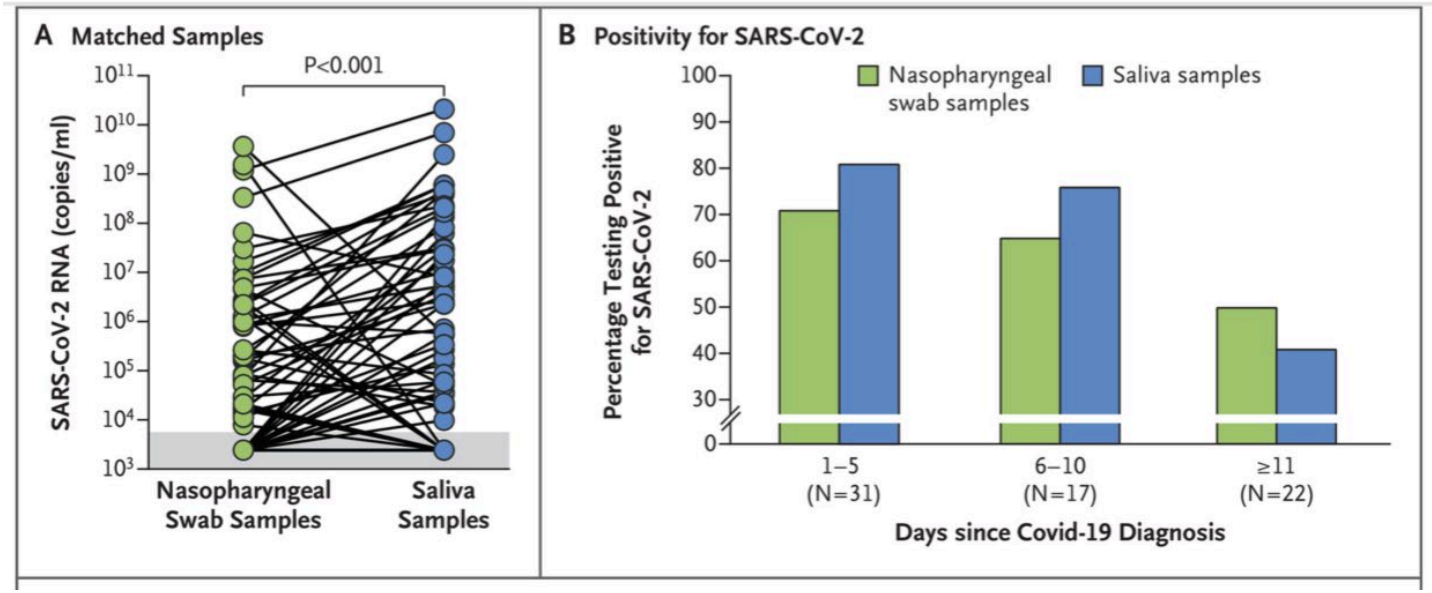
- Lateral flow immunoassay
- May be performed using swabs similar to those currently used in RT-PCR
- Would be quick to run (< 15 minutes) and could be used at the point-of-care (no need for a lab)
- Need high enough presence of the surface proteins to be detectable – means they have a higher chance of false negatives than PCR tests
- Would need to be implemented with clear guidance on correct interpretation

Developer	Test	Sensitivity:	Specificity:	Sample Size	Time (min)
Coris BioConcept	COVID-19 Ag Respi-Strip	60%	98–100%	100 µL extract	15
RapiGEN, Inc.	BIOCREDIT COVID-19 Ag	89.4	98%	90–150 µL extract	5–8
SD BIOSENSOR,	STANDARD Q COVID-19 Ag Test	84%	100%	10 µL extract	15–30

Robert Kubina, Arkadiusz Dziedzic. Diagnostics (Basel) 2020 Jun; 10(6): 434.

Alternative diagnostic samples

- Samples (NP and self collected saliva) were obtained from 70 hospital inpatients who had a diagnosis of Covid-19



More SARS-CoV-2 RNA copies in the saliva specimens (mean log copies per milliliter, 5.58) than in the NP swab specimens (mean log copies per milliliter, 4.93)

higher percentage of saliva samples than NP swab samples were positive up to 10 days after the Covid-19 diagnosis

Saliva specimens and NP swab specimens have at least similar sensitivity in the detection of SARS-CoV-2 during the course of hospitalization

Wyllie et al. NEJM 2020

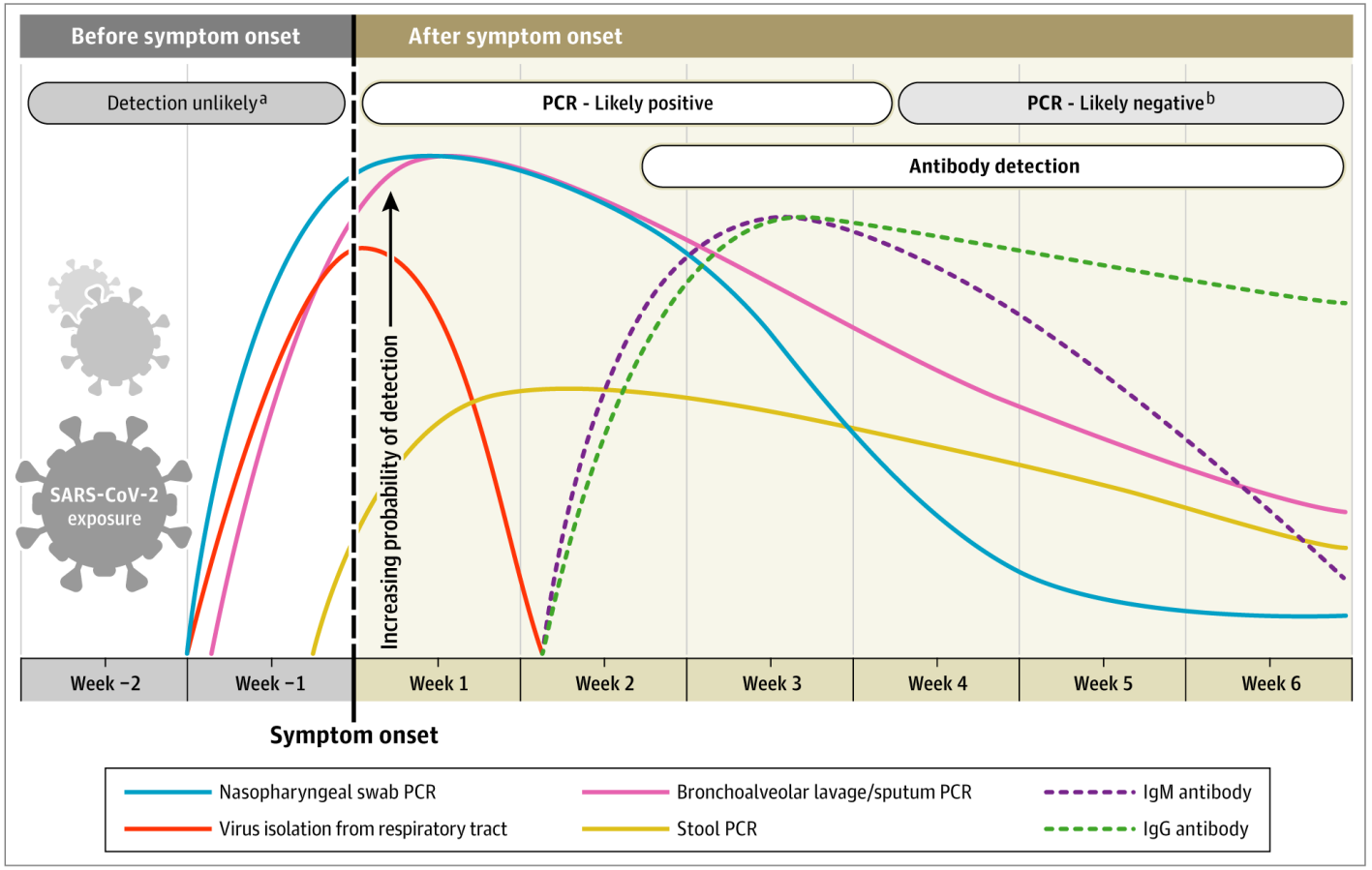
Saliva as an alternative for screening asymptomatic HCW for SARS-CoV-2

- 495 asymptomatic HCW screened: RT-PCR on NP and saliva
- 13/495 saliva positive
  - 9/13 with NP and 7 were tested negative at the same time or later confirmed

- Interests of saliva:
  - Collection by patients themselves
    - Negates the need for direct interaction between HCW and patients (source of testing bottlenecks and risk of nosocomial infection)
    - Alleviates demands for supplies of swabs and personal protective equipment

Given the growing need for testing, these findings provide support for the potential of saliva specimens in the diagnosis of SARS-CoV-2 infection

Kinetics of virological markers



In most individuals with symptomatic COVID-19 infection, viral RNA in the NP swab as measured by the cycle threshold (Ct) becomes **detectable as early as day 1 of symptoms and peaks within the first week of symptom onset.**

This positivity starts to decline by week 3 and subsequently becomes undetectable.

JAMA. Published online May 06, 2020. doi:10.1001/jama.2020.8259

Clinical significance of prolonged PCR positivity following recovery

- Studies suggested that around 15% of COVID-19 patients re-tested positive for SARS-CoV-2 RNA after discharge, during 14 days of strict quarantine

- Why?
  - 2 PCR neg: false negative?
    - sampling procedures, quality of sampling, sample storage temperature and time, transportation process
  - positive signal of viral RNA might be from the “dead” viruses or viral gene fragments without active viral replication
  - delay virus clearance in some patients
    - glucocorticoid therapy, comorbidities, > 65 years

Kang et al. J Med Virol 2020

Are these patients infectious?

- 13 discharged patients with retest positive for viral RNA in Guangdong province on 25 March 2020
  - follow-up results demonstrated no new infected cases from 104 close contacts to the original patients.

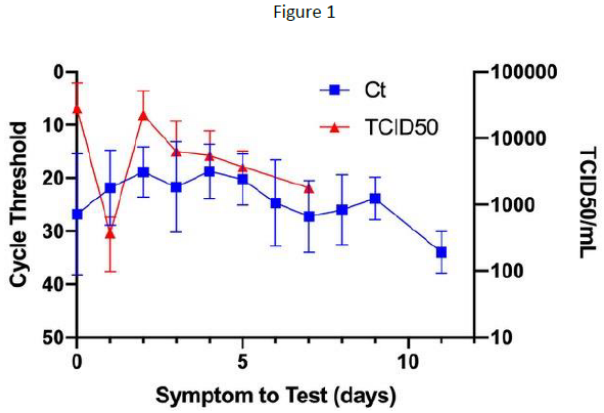
- No single family member being infected by the 4 recovered COVID-19 patients with retest positive for SARS-CoV-2 RNA, who were discharged from Zhongnan Hospital of Wuhan University

No clinical evidence of infectivity of those recovered patients with retest positive for viral RNA

Kang et al. J Med Virol 2020

SARS-CoV2 viral load and infectivity

- SARS-CoV-2 RT-PCR performed on nasopharyngeal (NP) or endotracheal (ETT) samples in Canada
- Culture on Vero cells in P4 lab
- 90 samples analyzed
  - Patients' median age 45 y (30-59)
  - 49% from males
- SARS-CoV-2 successfully cultivated from 26/90 (28.9%) samples
  - Ranging from D0 to D21 post symptoms onset
  - Positive cultures:
    - Only observed up to day 8 post symptom onset, peaking at 3 dpo
    - Only if VL was high: median Ct=17 [16-18] vs Ct=27 [22-33] in non-culturable samples, p<0.001
    - Odds of ⊕ culture ↓ by 32% for every one unit ↑ in Ct
- In total: PCR ≠ from infectivity



Bullard J. et al. Clin Infect Dis. 2020 May 22 : ciaa638. Published online 2020 May 22. doi: 10.1093/cid/ciaa638
Confirms Wölfel R et al. Nature. doi:10.1038/s41586-020-2196-x

Clinical significance of prolonged PCR positivity following recovery

Viral culture is only rarely positive for low viral load (Ct values above 25 to 30) and after 8 to 10 days after symptom onset

Viral culture is not positive for feces sample

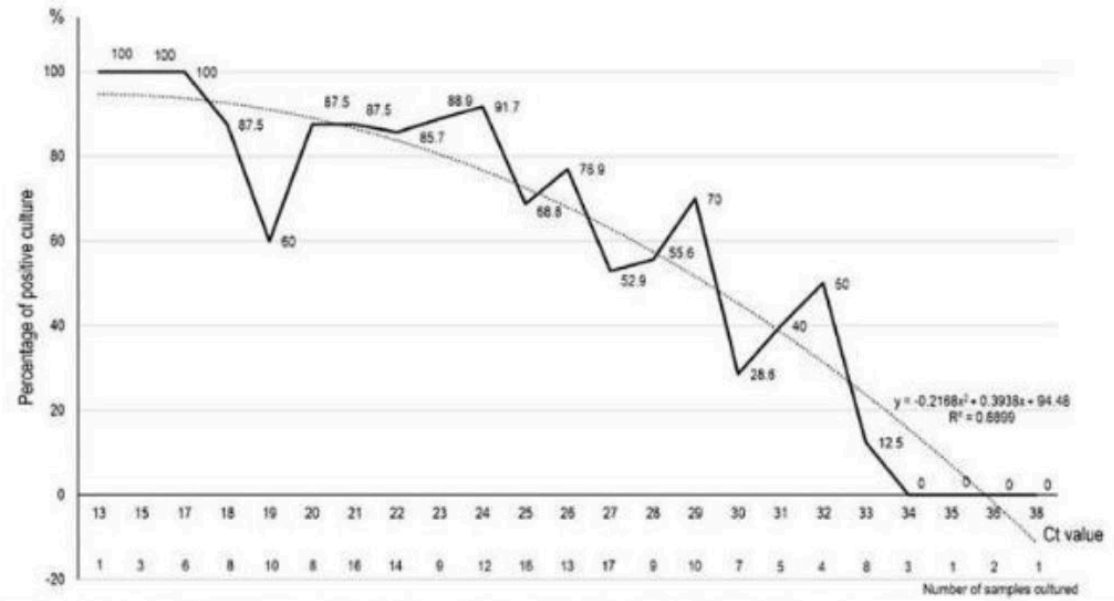


Fig. 1 Percentage of positive viral culture of SARS-CoV-2 PCR-positive nasopharyngeal samples from Covid-19 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve

Patients with Ct above 35? are not contagious and thus can be discharged from hospital care or strict confinement for non-hospitalized patients

Aarons NEJM May 2020

The New York Times

Aug. 29, 2020

https://www.nytimes.com/2020/08/29/health/coronavirus-testing.html

Your Coronavirus Test Is Positive. Maybe It Shouldn't Be.

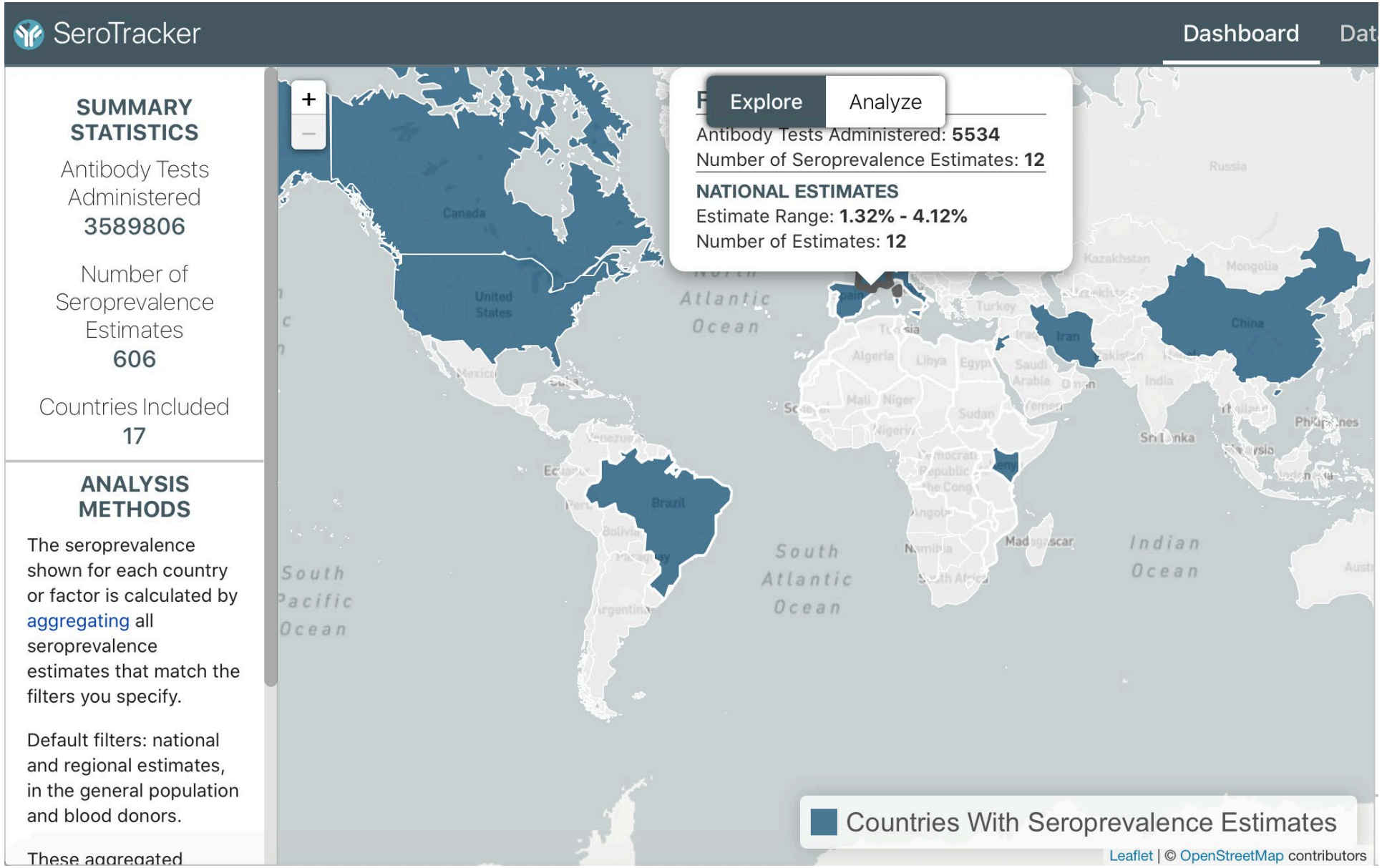
The usual diagnostic tests may simply be too sensitive and too slow to contain the spread of the virus.

Officials at the Wadsworth Center, New York's state lab, have processed, and analyzed their numbers at The Times's request. In July, the lab identified 794 positive tests, based on a threshold of 40 cycles.

With a cutoff of 35, about half of those tests would no longer qualify as positive.

About 70 percent would no longer be judged positive if the cycles were limited to 30.

Seroprevalence estimates

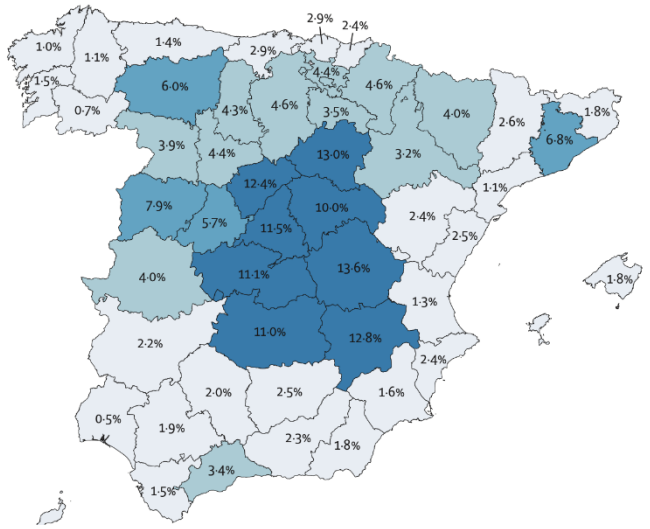


https://serotracker.com/Dashboard

Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study

*Marina Pollán, Beatriz Pérez-Gómez, Roberto Pastor-Barriuso, Jesús Oteo, Miguel A Hernán, Mayte Pérez-Olmeda , Jose L Sanmartín, Aurora Fernández-García, Israel Cruz, Nerea Fernández de Larrea, Marta Molina, Francisco Rodríguez-Cabrera, Mariano Martín, Paloma Merino-Amador, Jose León Paniagua, Juan F Muñoz-Montalvo, Faustino Blanco, Raquel Yotti, on behalf of the ENE-COVID Study Group**

- 51958 participants: questionnaires + IgG serology
- Seroprevalence was 4·6% (4·3–5·0) by immunoassay
- No differences by sex
- Lower seroprevalence in children younger than 10 years
- Substantial geographical variability, with higher prevalence around Madrid (>10%) and lower in coastal areas (<3%)



The majority of the Spanish population is seronegative to SARS-CoV-2 infection, even in hotspot areas. These results emphasise the need for maintaining public health measures to avoid a new epidemic wave.

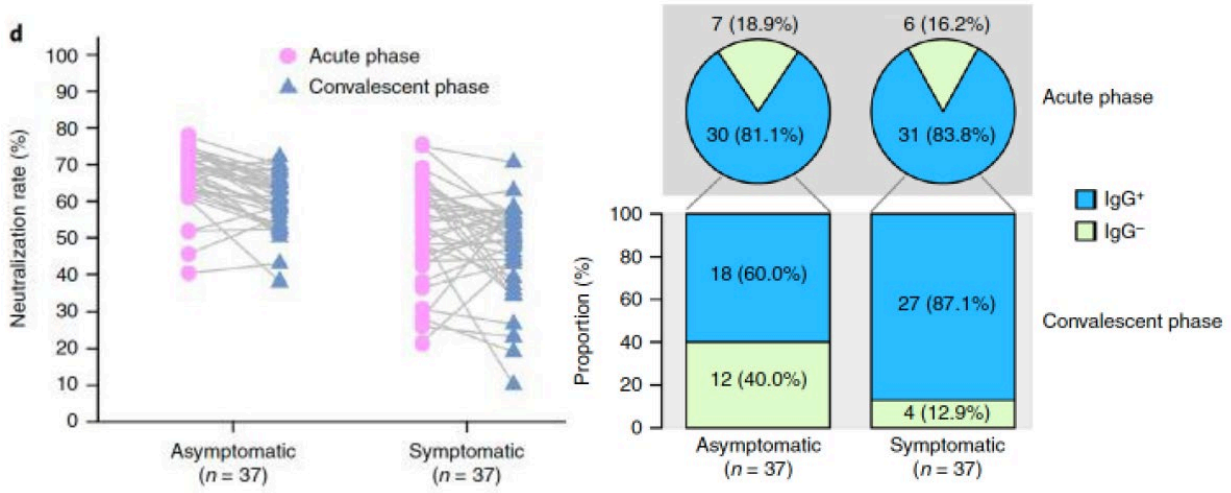
Pollan et al. Lancet 2020

Decline of humoral response

IgG and IgM, 8 weeks after exposure (convalescent phase)

- A decline of IgG is observed among >90% of patients
- 40% and 13% of asymptomatic individuals IgG+ at the acute phase became seronegative

Similar observations were made for neutralizing antibodies

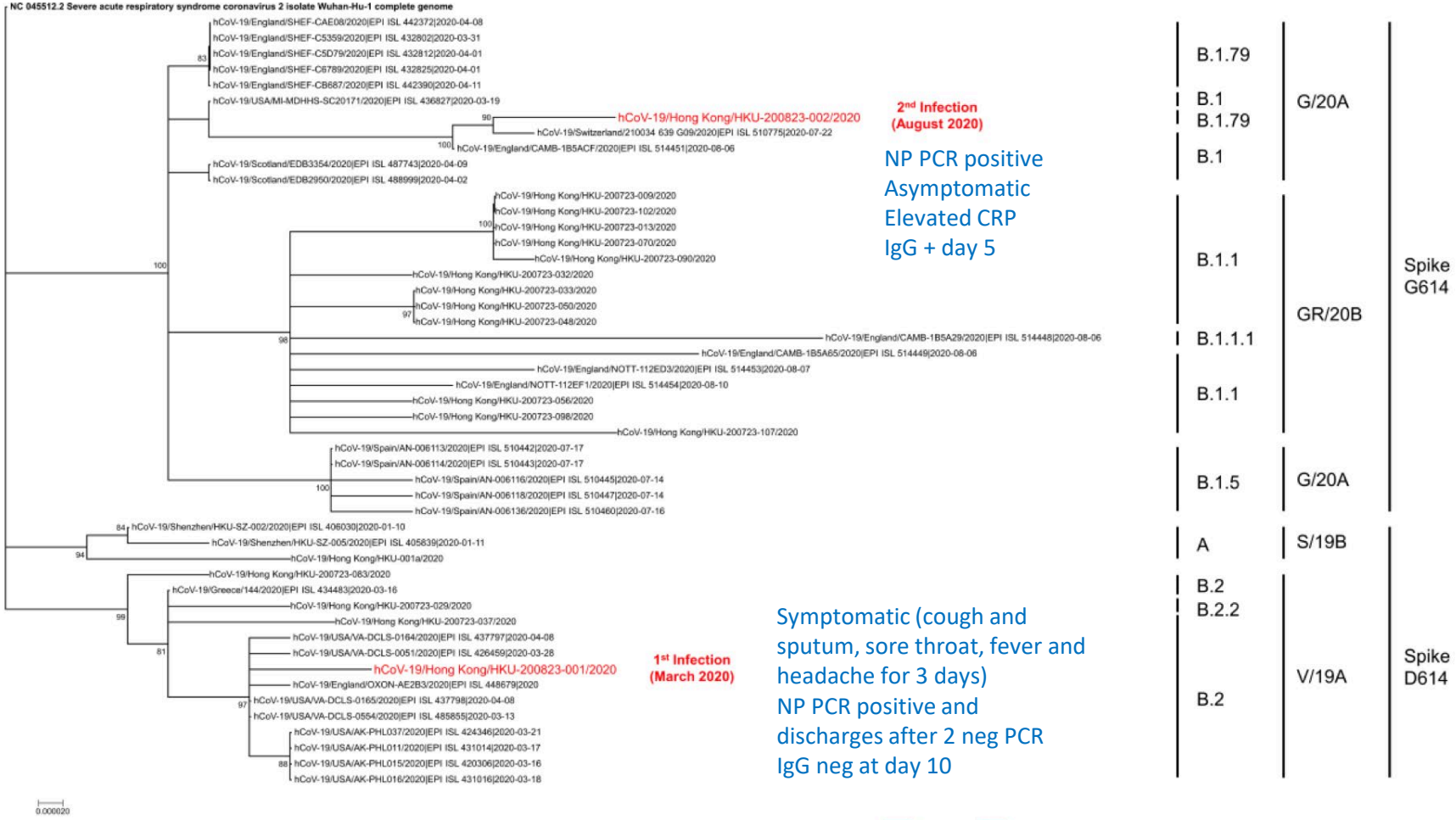


The relatively low seroprevalence and its decrease within 2-3 months after infection highlights the potential limits of serology for diagnostic and the need of timely serosurvey.

Long QX et al. Nat Med june 2020

Case of reinfection

- 33 years old man, immunocompetent, Hong Kong



Re-infection instead of persistent viral shedding from first infection

Kai-Wang To et al. CID 2020

Conclusions

- Diagnostic
  - RT-PCR is the recommended test for diagnosis of COVID-19
    - Symptomatic and close contacts
      - *Testing, tracking and tracing*
    - Nasopharyngeal swabs
  - Growing need for testing
    - Alernative tests: RT-LAMP, antigen
    - Alternative samples: saliva
  - Diagnostic vs large scale screening
    - Tolerate less sensitivity if more convenient, rapid
    - Complementarity

Conclusions

- Clinical significance of prolonged PCR positivity following recovery
  - Around 15%
  - No clinical or *in vitro* evidence of infectivity
  - Patients with Ct > 35 could be discharged from hospital care or strict confinement for non-hospitalized patients
  - Particular case: imunocompromised patients

Conclusions

- Use of antibody testing as a public health tool
  - Seroepidemiological studies are useful to evaluate circulation of the virus and herd immunity
  - Limits
    - Decrease seroprevalence and neutralizing antibodies within 2-3 months after infection
    - Need of timely serosurvey
  - Risk of reinfection
  - Need for maintaining public health measures