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Fondazione Policlinico Universitario A. Gemelli
Università Cattolica del Sacro Cuore

DOMENICA 27 FEBBRAIO 2022

08:45-10:45 Sala del Castello

Sessione 1
IL RUOLO DELLA MICROBIOLOGIA NELLE VARIE FASI DELLA PANDEMIA DA COVID-19 PER LE ATTIVITÀ DI PREVENZIONE E DI RICERCA
Sessione congiunta AMCLI - Ministero della Salute - ISS

Moderatori: **Pierangelo Clerici, Giovanni Rezza, Anna Teresa Palamara**

# Approcci diagnostici rapidi e molecolari: l'evoluzione in due anni di pandemia

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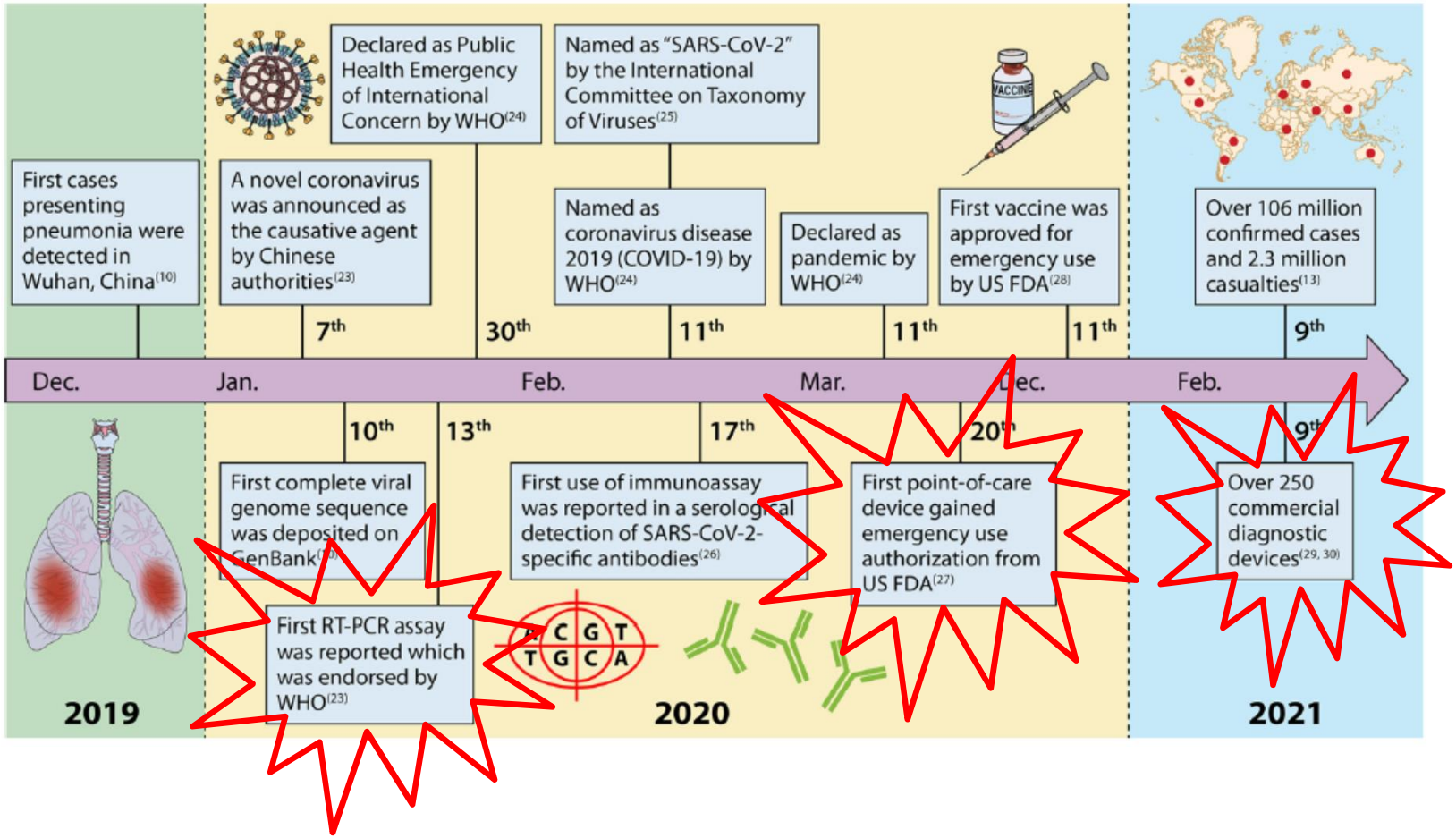
ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES




**Timeline of COVID-19 spread and the global response to it.** Of note, while SARS-CoV-2 was initially thought to have emerged from China in December 2020, there are data to suggest that it may have circulated more broadly earlier than initially recorded in other countries, and further studies are under way to investigate this possibility in other areas.

Safiabadi Tali et al., CMR, 2021

POLYMERASE CHAIN REACTION

## (PCR) TESTING

PROS: Most accurate
CONS: Longer processing time

## ANTIGEN TESTING

PROS: Rapid and inexpensive
CONS: Accuracy problems

## ANTIBODY

(SEROLOGY) TESTING

COVID-19 Te

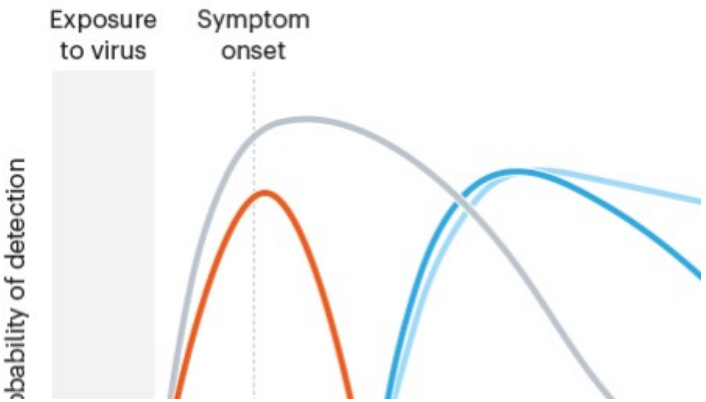
## CATCHING COVID-19

Different types of COVID-19 test can detect the presence of
SARS-CoV-2 virus or the body's response to infection. The pr
a positive result varies with each test before and after sympt

**PCR-based tests** can detect small amounts of viral gene
so a test can be positive long after a person stops being

**Rapid antigen tests** detect the presence of viral protein
return positive results when a person is most infectious.

**Antibody tests** detect the body's immune response to t
and are not effective at the earliest phase of infection.

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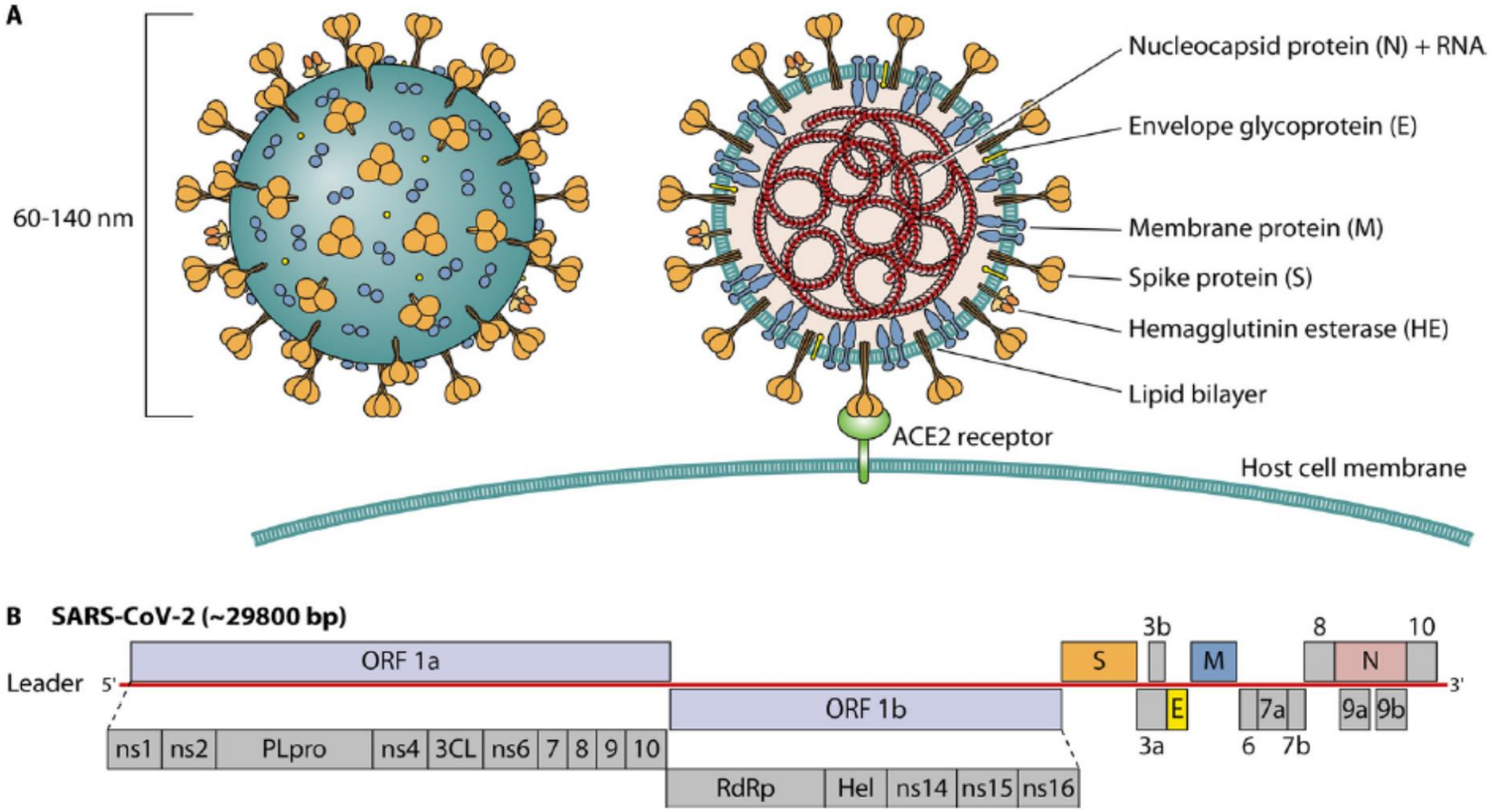


**Physical and genome structure of SARS-CoV-2.** (A) Diagram of the SARS-CoV-2 virion. (B) Genome organization and proteins with known or unknown functions.

Safiabadi Tali et al., CMR, 2021

**TABLE 1** Examples of the NAATs approved for emergency use by the U.S. FDA for detection of SARS-CoV-2 RNA[a]

| Device/assay (manufacturer) | Method | Target gene(s) | Specimen type(s) | Authorized setting(s) | Time/throughput | LoD[b] | Reference |
|---|---|---|---|---|---|---|---|
| cobas 6800/cobas SARS-CoV-2 (Roche Molecular Systems, USA) | RT-PCR | ORF1ab + E | NS, NPS, OPS | H, M, H-pooling | 3 h for the first-run results but 90 min per run in continuous mode/864 samples per 8 h | 46 copies/ml | 575 |
| Abbott m2000/RealTime SARS-CoV-2 (Abbott Diagnostics, USA) | RT-PCR | RdRp + N | NS, NPS, OPS, BAL fluid | H | 7 h per run/470 samples per 24 h | 100 copies/ml | 576 |
| NeuMoDx 288/NeuMoDx SARS-CoV-2 (NeuMoDx Molecular, USA) | RT-PCR | Nsp2 + N | NS, NPS, OPS, BAL fluid, saliva | H, M | 1.3 h per run/288 samples per 8 h | 150 copies/ml | 577 |
| Panther Fusion/Aptima SARS-CoV-2 (Hologic, USA) | TMA | ORF1ab | NS, NPS, OPS, MTS, NPW, NPA, NA | H, pooling | 2.4 h per run/500 samples per 8 h | 0.026 $TCID_{50}$/ml | 578 |
| Liaison MDX/Simplexa COVID-19 Direct (DiaSorin Molecular, Italy) | RT-PCR | ORF1ab + S | NS, NPS, NW, NA, BAL fluid | H, M | 1 h per run/8 samples per run | 500 copies/ml | 318 |
| FilmArray/BioFire Respiratory Panel 2.1 (BioFire Diagnostics, USA) | RT-PCR | S + M | NPS | H, M | 2-min hands-on time/1 h per run | 160 copies/ml | 579 |
| ePlex/ePlex SARS-CoV-2 (GeneMark Diagnostics, USA) | RT-PCR | N | NPS | H, M | 2-min hands-on time/1.5 h per run | 750 copies/ml | 319 |
| GeneXpert Xpress/Xpert Xpress SARS-CoV-2 (Cepheid, USA) | RT-PCR | E + N2 | NS, NPS, OPS, MTS, NW, NA | H, M, W | 1-min hands-on time/45 min per run | 0.02 PFU/ml | 580 |
| Accula Dock/Accula SARS-CoV-2 (Mesa Biotech, USA) | RT-PCR | N | NS, MTS | H, M, W | 5-min hands-on time/30 min per run | 150 copies/reaction | 320 |
| ID Now/ID Now COVID-19 (Abbott Diagnostics, USA) | NEAR | RdRp | NS, NPS, OPS | H, M, W | 2-min hands-on time/13 min per run | 125 copies/ml | 581 |
| SHERLOCK CRISPR SARS-CoV-2 kit (Sherlock Biosciences, USA) | RT-LAMP, CRISPR-Cas13 | ORF1ab + N | NS, NPS, OPS, NPW, NPA, NA, BAL fluid | H | 1 h per run | 6,750 copies/ml | 582 |
| SARS-CoV-2 DETECTR reagent kit (Mammoth Biosciences, USA) | RT-LAMP, CRISPR-Cas12 | N | NPS, OPS, MTS, ANS, NPW, NPA, NA | H | 45 min per run | 20,000 copies/ml | 583 |
| NovaSeq 6000/Illumina COVIDSeq test (Illumina Inc., USA) | Next-generation sequencing | 98 targets on the virus | NPS, OPS, MTS, ANS, NPW, NPA, NA, BAL fluid | H | 3,072 samples per 12 h | 500 copies/ml | 584 |

[a]The full list is available in reference 29. Abbreviations: RT-PCR, reverse transcription-PCR; TMA, transcription-mediated amplification; NEAR, nicking enzyme amplification reaction; RT-LAMP, reverse transcription–loop-mediated isothermal amplification; NS, nasal swab; NPS, nasopharyngeal swab; OPS, oropharyngeal (throat) swab; BAL, bronchoalveolar lavage; MTS, midturbinate nasal swab; NPW, nasopharyngeal wash; NPA, nasopharyngeal aspirate; NA, nasal aspirate; NW, nasal wash; ANS, anterior nasal swab; H, laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high-complexity tests; M, laboratories certified under CLIA, 42 U.S.C. §263a, that meet requirements to perform moderate-complexity tests; W, patient care settings operating under a CLIA certificate of waiver; $TCID_{50}$, median tissue culture infectious dose.

[b]The LoD (limit of detection) of each assay is the lowest LoD reported in the instructions for use for that assay, regardless of the specimen types.

Safiabadi Tali et al., CMR, 2021

RESEARCH LETTER

# Assessment of SARS-CoV-2 RNA Test Results Among Patients Who Recovered From COVID-19 With Prior Negative Results

JAMA Internal Medicine Published online November 12, 2020

We investigated RT-PCR retested positive nasal/oropharyngeal swab (NOS) samples from recovered patients with COVID-19 with prior negative results for the presence of replicative SARS-CoV-2 RNA

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Table. Testing Results for NOS Samples Obtained at COVID-19 Diagnosis or After COVID-19 Recovery in 32 Study Patients[a]

| Sample | COVID-19 samples tested | | | | | | | | | | | Days of recovery sampling since diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Diagnosis | | | | Recovery | | | | | | | |
| | Genomic RNA ($C_T$ value) | | | Subgenomic RNA ($C_T$ value) | Genomic RNA ($C_T$ value) | | | Subgenomic ($C_T$ value) | RNA load, copies/mL | Serology (positive or negative result) | | |
| Sample No. | *E* gene | *RdRP* gene | *N* gene | *E* gene | *E* gene | *RdRP* gene | *N* gene | *E* gene | *N* gene | IgG | IgA | |
| 1 | 31.6 | 31.3 | 31.2 | 34.5 | 29.3 | 30.7 | 31.2 | 39.1 | $1.2 \times 10^4$ | Positive | Positive | 39 |
| 2 | 27.0 | 26.9 | 30.0 | 36.0 | 30.0 | 30.5 | 31.2 | | $8.9 \times 10^3$ | Positive | Positive | 31 |
| 3 | 19.3 | 20.8 | 22.1 | 35.2 | 31.5 | 34.7 | 32.8 | | $3.3 \times 10^3$ | Positive | Negative | 44 |
| 4 | 21.6 | 22.0 | 22.9 | 36.4 | 31.8 | 31.4 | 32.3 | | $5.5 \times 10^3$ | Positive | Positive | 34 |
| 5 | 30.0 | 32.8 | 38.1 | 30.2 | 31.8 | 34.3 | 34.5 | | $3.2 \times 10^3$ | Positive | Positive | 62 |
| 6 | 20.8 | 20.9 | 22.3 | 37.3 | 32.2 | 32.8 | 34.1 | | $5.3 \times 10^3$ | Positive | Positive | 37 |
| 7 | 27.3 | 29.9 | 31.3 | 36.9 | 32.3 | 30.9 | 32.7 | | $6.4 \times 10^3$ | Positive | Positive | 39 |
| 8 | 26.9 | 27.0 | 31.2 | 38.1 | 35.0 | 34.4 | 36.1 | | $4.0 \times 10^2$ | Positive | Positive | 71 |
| 9 | 22.5 | 23.7 | 24.9 | 31.0 | 38.8 | 33.6 | 33.9 | | $2.6 \times 10^3$ | Negative | Negative | 42 |
| 10 | 21.3 | 21.4 | 28.9 | 38.9 | | 32.2 | 33.4 | | $1.2 \times 10^4$ | Positive | Positive | 56 |
| 11 | 26.6 | 26.9 | 28.1 | 33.0 | | 32.8 | 33.2 | | $1.3 \times 10^4$ | Positive | Positive | 54 |
| 12 | 22.8 | 24.2 | 25.3 | 31.0 | | 34.2 | 33.7 | | $6.9 \times 10^3$ | Positive | Positive | 55 |
| 13 | 25.8 | 25.8 | 26.1 | 39.8 | NA | 34.8 | 39.1 | | $3.0 \times 10^2$ | Positive | Positive | 36 |
| 14 | 20.8 | 20.4 | 21.1 | 32.0 | | 35.0 | 35.1 | | $1.9 \times 10^3$ | Positive | Positive | 56 |
| 15 | 29.4 | 30.1 | 32.2 | 37.0 | | 36.5 | 39.2 | | $3.2 \times 10^3$ | Positive | Positive | 36 |
| 16 | 27.9 | 29.1 | 31.1 | 32.0 | | 38.1 | 39.3 | | $1.6 \times 10^1$ | Positive | Positive | 77 |
| 17 | 30.6 | 29.9 | 31.8 | 32.1 | | | 35.7 | NA | $5.4 \times 10^3$ | Positive | Positive | 53 |
| 18 | 28.5 | 29.1 | 30.8 | 36.8 | | | 36.8 | | $2.9 \times 10^3$ | Positive | Positive | 43 |
| 19 | 26.9 | 22.2 | 26.1 | 30.1 | | | 37.5 | | $1.1 \times 10^3$ | Positive | Positive | 36 |
| 20 | 25.7 | 25.2 | 28.9 | 38.0 | | | 37.9 | | $2.6 \times 10^3$ | Positive | Positive | 48 |
| 21 | 27.0 | 29.0 | 30.2 | 32.3 | | | 38.1 | | $1.9 \times 10^3$ | Positive | Positive | 41 |
| 22 | 28.5 | 29.4 | 30.0 | 32.3 | | | 38.4 | | $4.9 \times 10^1$ | Positive | Negative | 76 |
| 23 | 27.1 | 28.6 | 29.3 | 36.1 | | | 38.9 | | $4.5 \times 10^2$ | Positive | Positive | 29 |
| 24 | 25.4 | 22.9 | 24.1 | 34.8 | | | 39.0 | | $5.6 \times 10^1$ | Positive | Positive | 70 |
| 25 | 28.7 | 29.5 | 31.4 | 37.3 | NA | | 39.1 | | $5.4 \times 10^3$ | Negative | Positive | 46 |
| 26 | 27.1 | 27.7 | 29.2 | 37.1 | | | 39.1 | | $1.9 \times 10^3$ | Positive | Positive | 34 |
| 27 | 26.7 | 27.7 | 29.6 | 39.2 | | | 39.2 | | $2.0 \times 10^3$ | Positive | Positive | 45 |
| 28 | 17.1 | 19.1 | 19.9 | 33.0 | | | 39.2 | | $8.5 \times 10^2$ | Positive | Positive | 40 |
| 29 | 27.0 | 28.9 | 30.0 | 32.1 | | | 39.3 | | $5.0 \times 10^1$ | Positive | Positive | 56 |
| 30 | 22.9 | 23.8 | 25.8 | 37.1 | | | 39.4 | | $1.6 \times 10^2$ | Positive | Positive | 55 |
| 31 | 28.6 | 30.4 | 30.9 | 33.0 | | | 39.6 | | $5.3 \times 10^2$ | Positive | Positive | 61 |
| 32 | 29.1 | 28.0 | 30.9 | 36.2 | | | 39.8 | | $3.4 \times 10^2$ | Positive | Positive | 53 |

Abbreviations: COVID-19, coronavirus disease 2019; $C_T$, cycle threshold; *E* gene, envelope gene; NA, not applicable; *N* gene, nucleocapsid gene; *RdRP*, RNA-dependent RNA polymerase; RT-PCR, real-time polymerase chain reaction.

[a] For RT-PCR testing, the Seegene Allplex 2019-nCoV and Clonit Quanty COVID-19 assays were used for total RNA detection and quantification, respectively, whereas replicative (*E* gene) RNA was detected by an in-house RT-PCR assay.[4] Results were expressed as $C_T$ values (<40 for positive detection) or quantified as RNA (*N* gene) copies per mL. NA indicates the absence of positive detection for the indicated gene. For serological testing, SARS-CoV-2 IgG/IgA Euroimmun enzyme-linked immunoassays were used, and positive and negative results were assessed using the 1.1 or greater or less than 1.1 times the manufacturer's cutoffs as reference IgG/IgA values, respectively.

Editor's Note

## Challenges in Testing for SARS-CoV-2 Among Patients Who Recovered From COVID-19

Mitchell H. Katz, MD

**Author Affiliation:** NYC Health and Hospitals, New York, New York.

**Published Online:** November 12, 2020. doi:10.1001/jamainternmed.2020.7575

- To avoid unnecessary quarantine for patients who have recovered from COVID-19, routine repeated PCR testing should not be done in the90days following infection.
- However, more complicated is what to do about patients who are symptomatic and have positive results on repeated PCR tests.
- Reinfection with SARS-CoV-2 has been documented (based on demonstration of different genetic differences between the viruses infecting the person on the first and second episode) but is rare.
- Until clinical laboratories have the capability to test for the reproductive capacity of coronavirus, interpretation of the epidemiologic significance of positive PCR results among recovered patients will remain challenging.

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VIROLOGY

# Viral Culture Confirmed SARS-CoV-2 Subgenomic RNA Value as a Good Surrogate Marker of Infectivity

Marta Santos Bravo,[a] Carla Berengua,[b] Pilar Marín,[b] Montserrat Esteban,[b] Cristina Rodriguez,[a] Margarita del Cuerpo,[b] Elisenda Miró,[b] Genoveva Cuesta,[a] Mar Mosquera,[a] Sonsoles Sánchez-Palomino,[c] Jordi Vila,[a] Núria Rabella,[b] María Ángeles Marcos[a]

January 2022 Volume 60 Issue 1 e01609-21

**TABLE 4** Predictive performance of subgenomic RNA compared to viral culture as the reference standard[a]

| Parameter | Value | 95% CI |
|---|---|---|
| Sensitivity (%) | 96.9 | 91.8–100 |
| Specificity (%) | 90.2 | 79.9–100 |
| Positive predictive value (%) | 93.9 | 87.4–100 |
| Negative predictive value (%) | 94.9 | 86.7–100 |
| Positive likelihood ratio | 9.93 | 3.91–25.22 |
| Negative likelihood ratio | 0.03 | 0.01–0.14 |
| AUC | 0.94 | 0.88–0.99 |

[a]CI, confidence interval; AUC, area under the ROC curve.

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Letter to the Editor

## Evaluating the newly developed BioFire COVID-19 test for SARS-CoV-2 molecular detection

Flora Marzia Liotti [1,2,†], Giulia Menchinelli [1,2,†], Simona Marchetti [2], Grazia Angela Morandotti [2], Maurizio Sanguinetti [1,2,*], Brunella Posteraro [1,3,§], Paola Cattani [1,2,§]

- We analyzed the results of 120 N/OP samples (86 positive and 34 negative for SARS-CoV-2) tested with both the BioFire COVID-19 test against the Corman reference assay.
- The agreement between the BioFire COVID-19 test and the reference assay was 95.0% (114/120) for overall results and 100% (34/34) for negative results.
- Eighty (93.0%) of 86 positive samples yielded results with the BioFire COVID-19 test that matched those with the reference assay.
- Interestingly, virus loads (expressed as RNA copies/mL) of the six falsely negative samples were $2.20 \times 10^1$ to $1.60 \times 10^2$. However, these loads were below the limit of detection of $3.30 \times 10^2$ RNA copies/mL estimated for the BioFire COVID-19 test.
- Compared to the reference method, the BioFire COVID-19 test sensitivity, specificity, positive predictive value and negative predictive value (with their 95% confidence intervals) were 93.0 (85.4–97.4), 100.0 (89.7–100.0), 100.0 (95.5–100.0) and 85.0 (70.2–94.3), respectively.

# Comparison of a Point-of-Care Assay and a High-Complexity Assay for Detection of SARS-CoV-2 RNA

Bryan Stevens,[a,b,†] Catherine A. Hogan,[a,b,†] Malaya K. Sahoo,[a] ChunHong Huang,[a] Natasha Garamani,[a] James Zehnder,[a] Jason Kurzer,[a] and Benjamin A. Pinsky[a,b,c,*]

**Table 1. Comparison of the Cepheid Xpert Xpress SARS-CoV-2 assay and the Panther Fusion SARS-CoV-2 assay.**

| | | Panther Fusion | | |
|---|---|---|---|---|
| | | **Detected** | **Not Detected** | **Total** |
| Cepheid Xpert | Detected | 53 | 0 | 53 |
| | Not Detected | 1 | 50 | 51 |
| | Total | 54 | 50 | 104 |
| Positive Percent Agreement | | 98.1% | (95% CI: 90.1–100) | |
| Negative Percent Agreement | | 100% | (95% CI: 94.2–100) | |
| Overall Agreement | | 99.0% | (95% CI: 94.8–100) | |
| Cohen's Kappa Coefficient | | 0.98 | (95% CI: 0.94–1.0) | |

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; CI: confidence interval.

# Diagnostic or Screening Testing Using a Pooling Strategy General Guidance (1)

- Laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) can use a specimen pooling strategy to expand SARS-CoV-2 nucleic acid diagnostic or screening testing capacity when using a test authorized for such use by FDA.
- If a pooled test result is negative, then all specimens can be presumed negative with the single test. If the test result is positive or indeterminate, then all the specimens in the pool need to be retested individually. The advantages of this two-stage specimen pooling strategy include preserving testing reagents and resources, reducing the amount of time required to test large numbers of specimens, and lowering the overall cost of testing.
- A pooling strategy depends on the community prevalence of virus, and pool size will need to be adjusted accordingly. CDC recommends that laboratories should determine prevalence based on a rolling average of the positivity rate of their own SARS-CoV-2 testing over the previous 7–10 days. Laboratories should use a standardized methodology or calculator that factors in the sensitivity of the assay they are using and their costs of testing to determine when the positivity rate is low enough to justify the implementation of a pooling strategy.

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Interim Guidance for Use of Pooling Procedures in SARS-CoV-2 Diagnostic, Screening, and Surveillance Testing, CDC, 2020

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Original article

# Sample pooling for SARS-CoV-2 RT-PCR screening

Adolfo de Salazar [1,2,†], Antonio Aguilera [3,4,†], Rocio Trastoy [3,4], Ana Fuentes [1,2], Juan Carlos Alados [5], Manuel Causse [6,7], Juan Carlos Galán [8,9], Antonio Moreno [10], Matilde Trigo [11], Mercedes Pérez-Ruiz [2,12], Carolina Roldán [13], María José Pena [14], Samuel Bernal [15], Esther Serrano-Conde [1,2], Gema Barbeito [3,4], Eva Torres [5], Cristina Riazzo [6,7], Jose Luis Cortes-Cuevas [8], Natalia Chueca [1,2], Amparo Coira [3,4], Juan Manuel Sanchez-Calvo [5], Eduardo Marfil [6,7], Federico Becerra [8], María José Gude [10], Ángeles Pallarés [11], María Luisa Pérez Del Molino [3,4], Federico García [1,2,*]

**Table 3**

Performance characteristics of pooled analysis when major discordances were analysed

| Major discordance | Individual test (reference) | |
|---|---|---|
| | No. positive | No. negative |
| Pool test | | |
| Positive | 234 | 0 |
| Negative | 7 | 3268 |
| **Statistic** | **Value** | **95% CI** |
| Sensitivity (%) | 97.10 | 94.11 to 98.82 |
| Specificity (%) | 100.00 | 99.89 to 100.00 |
| PPV (%) | 100.00 | |
| NPV (%) | 99.79 | 99.56 to 99.90 |
| Accuracy (%) | 99.80 | 99.59 to 99.92 |
| Kappa (κ) | 0.984 | |

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

**Table 4**

Performance characteristics of pooled analysis when all discordances were analysed

| All discordance | Individual test (reference) | |
|---|---|---|
| | No. positive | No. negative |
| Pool test | | |
| Positive | 206 | 0 |
| Negative | 35 | 3268 |
| **Statistic** | **Value** | **95% CI** |
| Sensitivity (%) | 85.48 | 80.39 to 89.67 |
| Specificity (%) | 100.00 | 99.89 to 100.00 |
| PPV (%) | 100.00 | |
| NPV (%) | 98.94 | 98.57 to 99.22 |
| Accuracy | 99.00 | 98.62 to 99.30 |
| Kappa (κ) | 0.916 | |

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

Major discordance was defined as a negative pool result when at least one of the individual samples showed cycle threshold (Ct) values of <35 for one or more SARS-CoV-2 genes.

Minor discordance occurred when at least one individual sample had Ct > 35 in one or two of the SARS-CoV-2 genes assayed and the pool scored negative.

# Diagnostic or Screening Testing Using a Pooling Strategy General Guidance (2)

- Based on limited data, using a pooling testing procedure for SARS-CoV-2 has some limitations.
- In a pooling procedure, the laboratory cannot ensure the diagnostic integrity of an individual specimen because it is combined with other specimens before testing.
- Even if each individual specimen in a pool is adequate, the specimens in a pooled procedure are diluted, which could result in a low concentration of viral genetic material below the limit of detection of a given test. These limitations mean that monitoring the prevalence of disease and properly validating the assay and the instrumentation are important to limit the potential for false-negative results. In general, the larger the pool of specimens, the higher the likelihood of generating false-negative results.
- The prevalence of COVID-19 in a population also affects the efficiency of pooled testing strategies. In general, lower disease prevalence may enable a laboratory to use a larger optimal pool size.
- As the prevalence of COVID-19 increases, the cost savings of a pooling strategy decreases because more pooled tests will return positive results and those specimens will need to be retested individually.



Interim Guidance for Use of Pooling Procedures in SARS-CoV-2 Diagnostic, Screening, and Surveillance Testing, CDC, 2020

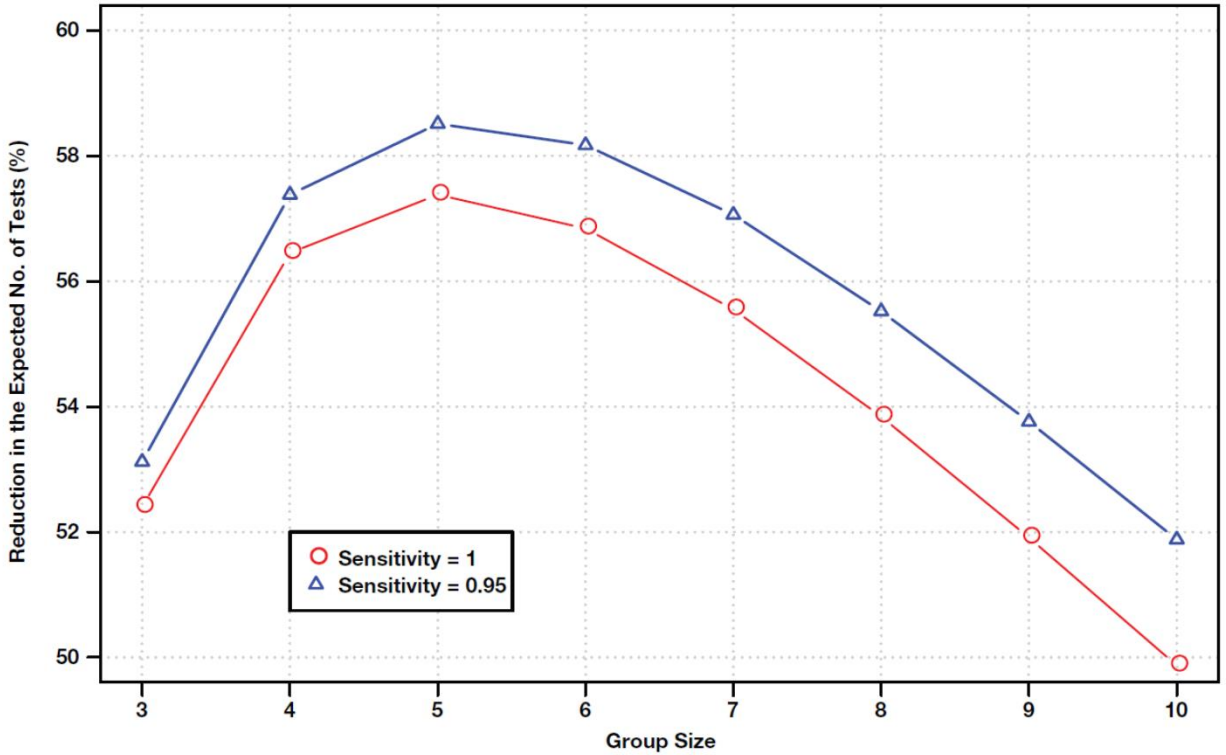
# Assessment of Specimen Pooling to Conserve SARS CoV-2 Testing Resources

*Baha Abdalhamid, MD, PhD,[1,2] Christopher R. Bilder, PhD,[3,*] Emily L. McCutchen, MS,[1,2] Steven H. Hinrichs MD,[1] Scott A. Koepsell, MD,[1,*] and Peter C. Iwen, PhD[1,2]*

From the [1]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha; [2]Nebraska Public Health Laboratory, University of Nebraska Medical Center, Omaha; and [3]Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE.

**Key Words:** SARS CoV-2; COVID-19; Group testing

*Am J Clin Pathol* June 2020;153:715-718

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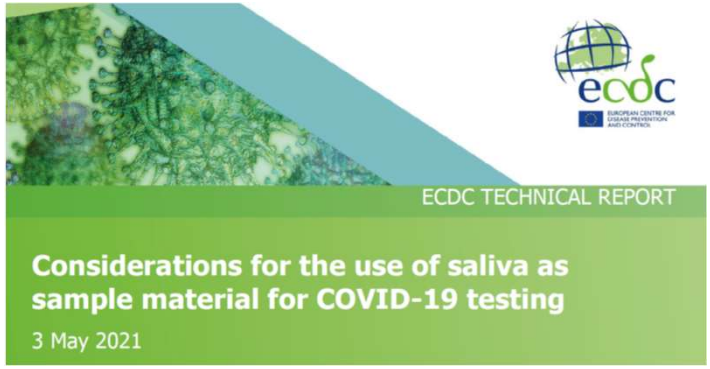



- Although nasopharyngeal swabs remain the gold standard for diagnostic testing of SARS-CoV-2, saliva sampling can contribute to timely identification of infectious individuals in the community. Saliva is an easy to collect, non-invasive, well accepted method of specimen collection for both health and non-healthcare professionals, as well as lay individuals.
- Overall, study results are variable and often showed that the sensitivity of detection of viral RNA in saliva was lower than that of nasopharyngeal or oropharyngeal swabs performed on the same day of the salivary collection from the same patient, although some studies even showed a slightly higher sensitivity of the saliva samples. However, during the period of highest viral load, the sensitivity is comparable and sufficient to detect infectious individuals reliably.
- **Although the sensitivity of RT-PCR tests using saliva as a diagnostic specimen for SARS-CoV-2 detection is often lower to nasopharyngeal specimen in several studies, the benefits of saliva testing may outweigh the loss in sensitivity and make it an attractive alternative as a screening tool, especially when nasopharyngeal samples cannot be collected.**
- **Overall, the evidence supports the conclusion that saliva can be used as alternative sample material for RT-PCR testing, when nasopharyngeal swabs cannot be collected in the following scenarios: in symptomatic patients and for repeated screening of asymptomatic individuals.**

Research

JAMA Internal Medicine | Original Investigation

# Comparison of Saliva and Nasopharyngeal Swab Nucleic Acid Amplification Testing for Detection of SARS-CoV-2

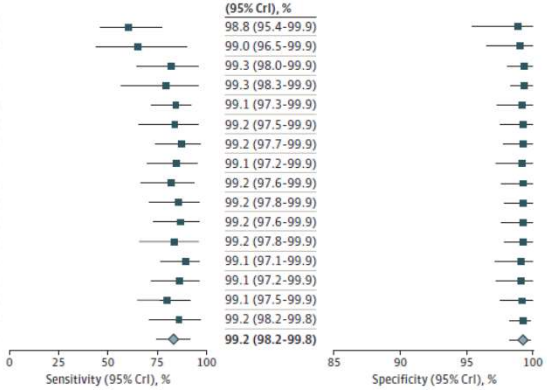
## A Systematic Review and Meta-analysis

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

- Eight peer-reviewed studies and 8 preprints were included in the meta-analyses (5922 unique patients).
- Fifteen studies included ambulatory patients, and 9 exclusively enrolled from an outpatient population with mild or no symptoms.
- In the primary analysis, the saliva NAAT pooled sensitivity was 83.2%(95% credible interval [CrI], 74.7%-91.4%) and the pooled specificity was 99.2%(95%CrI, 98.2%-99.8%). The nasopharyngeal swab NAAT had a sensitivity of 84.8%(95%CrI, 76.8%-92.4%) and a specificity of 98.9% (95%CrI, 97.4%-99.8%). Results were similar in secondary analyses.
- 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.

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)** |



# Rapid Antigen Testing for SARS-CoV-2

- Antigen tests are immunoassays that detect the presence of a specific viral antigen, which implies current viral infection.
- Antigen tests are currently authorized to be performed on nasopharyngeal or nasal swab specimens placed directly into the assay's extraction buffer or reagent.
- The "gold standard" for clinical diagnostic detection of SARS-CoV-2 remains RT-PCR. Thus, it may be necessary to confirm a rapid antigen test result with a nucleic acid test, especially if the result of the antigen test is inconsistent with the clinical context.
- The sensitivity of rapid antigen tests is generally lower than RT-PCR. The first antigen tests to have received FDA EUAs demonstrate sensitivity ranging from 84.0%-97.6% compared to RT-PCR. Antigen levels in specimens collected beyond 5-7 days of the onset of symptoms may drop below the limit of detection of the test. This may result in a negative test result, while a more sensitive test, such as RT-PCR, may return a positive result.
- The specificity of rapid antigen tests is generally as high as RT-PCR – the first antigen tests that have received FDA EUAs have reported specificity of 100% – which means that false positive results are unlikely.



Interim Guidance for Rapid Antigen Testing for SARS-CoV-2, CDC, 2020

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Trusted evidence.
Informed decisions.
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[Diagnostic Test Accuracy Review]

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

Jacqueline Dinnes[1,2], Jonathan J Deeks[2,3], Sarah Berhane[2], Melissa Taylor[4], Ada Adriano[3], Clare Davenport[2,3], Sabine Dittrich[5], Devy Emperador[5], Yemisi Takwoingi[2,3], Jane Cunningham[6], Sophie Beese[3], Julie Domen[7], Janine Dretzke[3], Lavinia Ferrante di Ruffano[3], Isobel M Harris[3], Malcolm J Price[3], Sian Taylor-Phillips[8], Lotty Hooft[9], Mariska MG Leeflang[10], Matthew DF McInnes[11], René Spijker[12,13], Ann Van den Bruel[7], Cochrane COVID-19 Diagnostic Test Accuracy Group[2]

## Antigen tests

Forty-eight studies reported 58 evaluations of antigen tests. Estimates of sensitivity varied considerably between studies. There were differences between symptomatic (72.0%, 95% CI 63.7% to 79.0%; 37 evaluations; 15530 samples, 4410 cases) and asymptomatic participants (58.1%, 95% CI 40.2% to 74.1%; 12 evaluations; 1581 samples, 295 cases). Average sensitivity was higher in the first week after symptom onset (78.3%, 95% CI 71.1% to 84.1%; 26 evaluations; 5769 samples, 2320 cases) than in the second week of symptoms (51.0%, 95% CI 40.8% to 61.0%; 22 evaluations; 935 samples, 692 cases). Sensitivity was high in those with cycle threshold (Ct) values on PCR ≤25 (94.5%, 95% CI 91.0% to 96.7%; 36 evaluations; 2613 cases) compared to those with Ct values >25 (40.7%, 95% CI 31.8% to 50.3%; 36 evaluations; 2632 cases). Sensitivity varied between brands. Using data from instructions for use (IFU) compliant evaluations in symptomatic participants, summary sensitivities ranged from 34.1% (95% CI 29.7% to 38.8%; Coris Bioconcept) to 88.1% (95% CI 84.2% to 91.1%; SD Biosensor STANDARD Q). Average specificities were high in symptomatic and asymptomatic participants, and for most brands (overall summary specificity 99.6%, 95% CI 99.0% to 99.8%).

At 5% prevalence using data for the most sensitive assays in symptomatic people (SD Biosensor STANDARD Q and Abbott Panbio), positive predictive values (PPVs) of 84% to 90% mean that between 1 in 10 and 1 in 6 positive results will be a false positive, and between 1 in 4 and 1 in 8 cases will be missed. At 0.5% prevalence applying the same tests in asymptomatic people would result in PPVs of 11% to 28% meaning that between 7 in 10 and 9 in 10 positive results will be false positives, and between 1 in 2 and 1 in 3 cases will be missed.

RESEARCH

# Comparative sensitivity evaluation for 122 CE-marked rapid diagnostic tests for SARS-CoV-2 antigen, Germany, September 2020 to April 2021

Heinrich Scheiblauer[1], Angela Filomena[1], Andreas Nitsche[2], Andreas Puyskens[2], Victor M Corman[3,4], Christian Drosten[3], Karin Zwirglmaier[5], Constanze Lange[6], Petra Emmerich[7], Michael Müller[8], Olivia Knauer[1], C Micha Nübling[1]

Euro Surveill. 2021;26(44):pii=2100441.

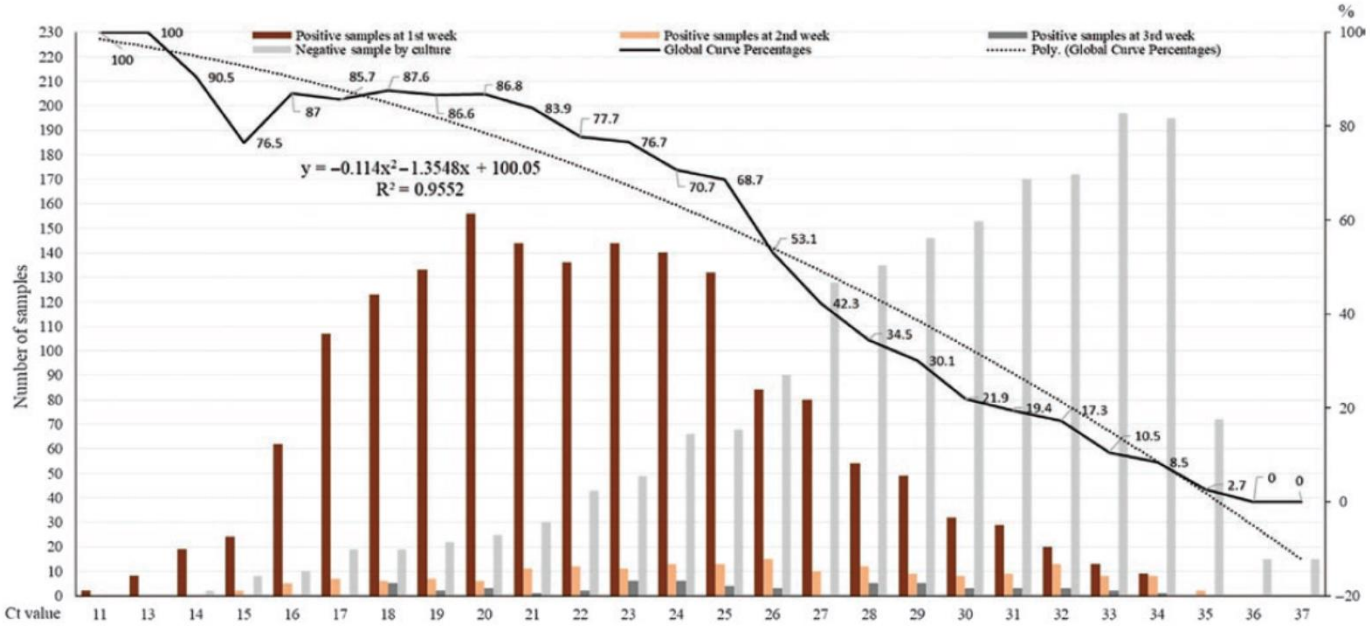
- The authors performed an independent head-to-head evaluation of the sensitivity of SARS-CoV-2 Ag RDT offered in Germany.
- Minimum sensitivity of 75% for panel specimens with a PCR quantification cycle (Cq) ≤25 was used to identify Ag RDT eligible for reimbursement in the German healthcare system.
- The sensitivity of different SARS-CoV-2 Ag RDT varied over a wide range. The sensitivity limit of 75% for panel members with Cq ≤25 was met by 96 of the 122 tests evaluated; 26 tests exhibited lower sensitivity, few of which failed completely.
- This comparative evaluation succeeded in distinguishing less sensitive from better performing Ag RDT. Most of the evaluated Ag RDT appeared to be suitable for fast identification of acute infections associated with high viral loads.
- Market access of SARS-CoV-2 Ag RDT should be based on minimal requirements for sensitivity and specificity.

*Clinical Infectious Diseases*

CORRESPONDENCE

## Correlation Between 3790 Quantitative Polymerase Chain Reaction–Positives Samples and Positive Cell Cultures, Including 1941 Severe Acute Respiratory Syndrome Coronavirus 2 Isolates

Rita Jaafar,[1,2] Sarah Aherfi,[1,2] Nathalie Wurtz,[1,2] Clio Grimaldier,[1,2] Van Thuan Hoang,[1,3,4] Philippe Colson,[1,2] Didier Raoult,[1,2] and Bernard La Scola[1,2]




| Ct value | 11 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of samples (N = 3790) | 2 | 8 | 21 | 34 | 77 | 133 | 153 | 164 | 190 | 186 | 193 | 210 | 225 | 217 | 192 | 218 | 206 | 209 | 196 | 211 | 208 | 220 | 213 | 74 | 15 | 15 |
| Total positive (n = 1941) | 2 | 8 | 19 | 26 | 67 | 114 | 134 | 142 | 165 | 156 | 150 | 161 | 159 | 149 | 102 | 90 | 71 | 63 | 43 | 41 | 36 | 23 | 18 | 2 | 0 | 0 |
| **Week 1**: number of positives (n = 1700) | 2 | 8 | 19 | 24 | 62 | 107 | 123 | 133 | 156 | 144 | 136 | 144 | 140 | 132 | 84 | 80 | 54 | 49 | 32 | 29 | 20 | 13 | 9 | 0 | 0 | 0 |
| (% by total positive) | (100) | (100) | (100) | (92.3) | (92.5) | (93.9) | (91.8) | (93.7) | (94.5) | (92.3) | (90.7) | (89.4) | (88.1) | (88.6) | (82.4) | (88.9) | (76.1) | (77.8) | (74.4) | (70.7) | (55.6) | (56.5) | (50.0) | (0) | (-) | (-) |
| **Week 2**: number of positives (n = 187) | 0 | 0 | 0 | 2 | 5 | 7 | 6 | 7 | 6 | 11 | 12 | 11 | 13 | 13 | 15 | 10 | 12 | 9 | 8 | 9 | 13 | 8 | 8 | 2 | 0 | 0 |
| (% by total positive) | (0) | (0) | (0) | (7.7) | (7.5) | (6.1) | (4.5) | (4.9) | (3.6) | (7.1) | (8.0) | (6.8) | (8.2) | (8.7) | (14.7) | (11.1) | (16.9) | (14.3) | (18.6) | (22.0) | (36.1) | (34.8) | (44.0) | (100) | (-) | (-) |
| **Week 3**: number of positives (n = 54) | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 2 | 3 | 1 | 2 | 6 | 6 | 4 | 3 | 0 | 5 | 5 | 3 | 3 | 3 | 2 | 1 | 0 | 0 | 0 |
| (% by total positive) | (0) | (0) | (0) | (0) | (0) | (0) | (3.7) | (1.4) | (1.8) | (0.6) | (1.3) | (3.7) | (3.8) | (2.7) | (2.9) | (0) | (7.0) | (7.9) | (7.0) | (7.3) | (8.3) | (8.7) | (5.6) | (0) | (-) | (-) |
| Negative cultured (n = 1849) | 0 | 0 | 2 | 8 | 10 | 19 | 19 | 22 | 25 | 30 | 43 | 49 | 66 | 68 | 90 | 128 | 135 | 146 | 153 | 170 | 172 | 197 | 195 | 72 | 15 | 15 |

Percentage of positive viral cultures of severe acute respiratory syndrome coronavirus 2 polymerase chain reaction–positive nasopharyngeal samples from coronavirus disease 2019 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve. Abbreviations: Ct, cycle threshold; Poly., polynomial.

| Cycle threshold (Ct, number) | Viral Load (VL, copies/ml) | Hypothetical contagiousness[§] | Sensitivity° of RAT | Sensi of RT |
|---|---|---|---|---|
| >37.2 | $<10^3$ | Negligible | 0% | Accep |
| 37.2 – 33.5 | $10^3$-$10^4$ | Very low | 0% | Good |
| 33.5 – 29.8 | $10^4$-$10^5$ | **Significant*** | **Very low*** | Excel |
| 29.8 – 26.1 | $10^5$-$10^6$ | **High*** | **Low*** | Excel |
| 26.1 – 22.4 | $10^6$-$10^7$ | High | Acceptable | Excel |

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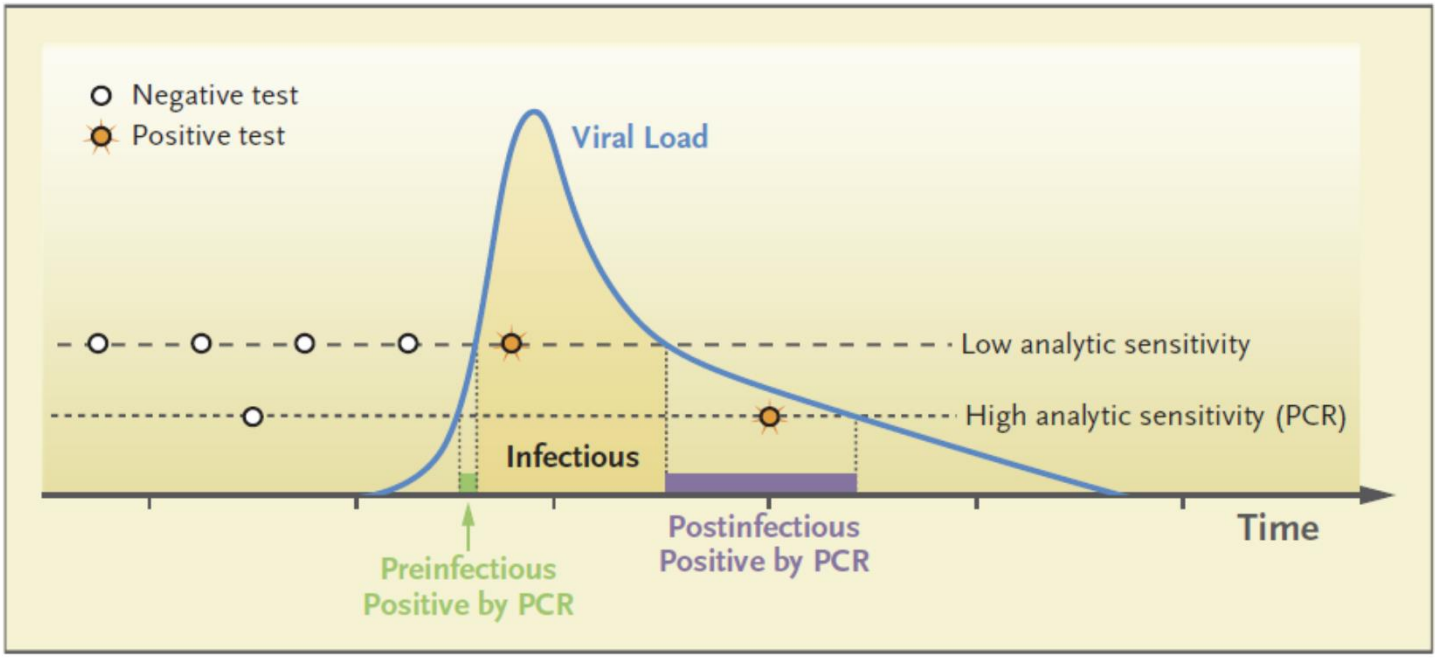
Gilbert Greub, ESCMID Web Symposia 2021

Perspective
NOVEMBER 26, 2020

# 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.

N ENGL J MED 383;22 NEJM.ORG NOVEMBER 26, 2020




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

biomolecules

MDPI

Article

# COVID-19 Rapid Antigen Test as Screening Strategy at Points of Entry: Experience in Lazio Region, Central Italy, August–October 2020

Francesca Colavita [1], Francesco Vairo [1], Silvia Meschi [1], Maria Beatrice Valli [1], Eleonora Lalle [1], Concetta Castilletti [1], Danilo Fusco [2], Giuseppe Spiga [2], Pierluigi Bartoletti [3], Simona Ursino [4], Maurizio Sanguinetti [5], Antonino Di Caro [1], Francesco Vaia [1], Giuseppe Ippolito [1] and Maria Rosaria Capobianchi [1,*]

**Table 1.** Percentage of confirmed FIA-positive results by SARS-CoV-2 RT-PCR according to the COI values ($n$ = 603, as in yellow box of Figure 1).

| COI | RT-PCR Positive/N (% TP) | % FP by FIA |
|---|---|---|
| ≥1 | 207/603 (34.3%) | 65.7% |
| ≥3 | 186/228 (81.6%) | 18.4% |
| ≥5 | 175/188 (93.1%) | 6.9% |
| ≥8 | 159/165 (96.4%) | 3.6% |
| ≥10 | 152/154 (98.7%) | 1.3% |
| ≥15 | 138/139 (99.3%) | 0.7% |
| ≥20 | 127/127 (100%) | 0.0% |

Abbreviation: COI, cut-off index; TP, true positive; FP, false positive.

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Article

# SARS-CoV-2 Antigen Detection to Expand Testing Capacity for COVID-19: Results from a Hospital Emergency Department Testing Site

Giulia Menchinelli [1,2,†], Giulia De Angelis [1,2,†], Margherita Cacaci [1,2], Flora Marzia Liotti [1,2], Marcello Candelli [3], Ivana Palucci [1,2], Rosaria Santangelo [1,2], Maurizio Sanguinetti [1,2,*], Giuseppe Vetrugno [4,5], Francesco Franceschi [3,6] and Brunella Posteraro [1,7]

*VS*

Rapid antigen assay was performed for all subjects admitted to the emergency department

**COVID-19 zone**

RT-PCR assay was also performed

- for all subjects admitted to this zone

**Non-COVID-19 zone**

RT-PCR assay was performed

- to confirm a positive antigenic result

OR

- for all subjects that required hospitalisation

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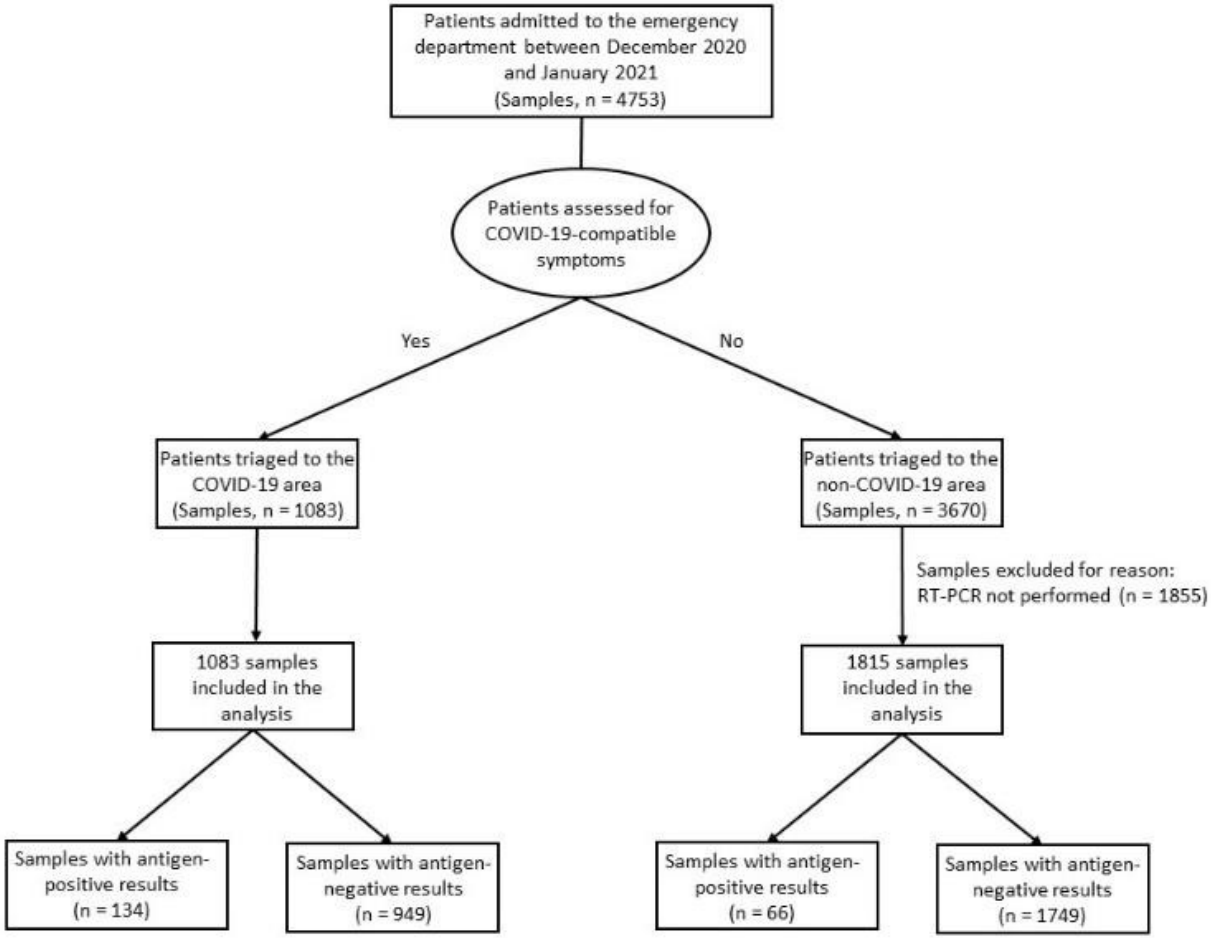
SARS-CoV-2 Antigen Detection to Expand Testing Capacity for COVID-19: Results from a Hospital Emergency Department Testing Site

Giulia Menchinelli [1,2,†], Giulia De Angelis [1,2,†], Margherita Cacaci [1,2], Flora Marzia Liotti [1,2], Marcello Candelli [3], Ivana Palucci [1,2], Rosaria Santangelo [1,2], Maurizio Sanguinetti [1,2,*], Giuseppe Vetrugno [4,5], Francesco Franceschi [3,6] and Brunella Posteraro [1,7]

# Flowchart of nasopharyngeal swab samples from patients triaged to COVID-19 or non-146 COVID-19 areas of the ED

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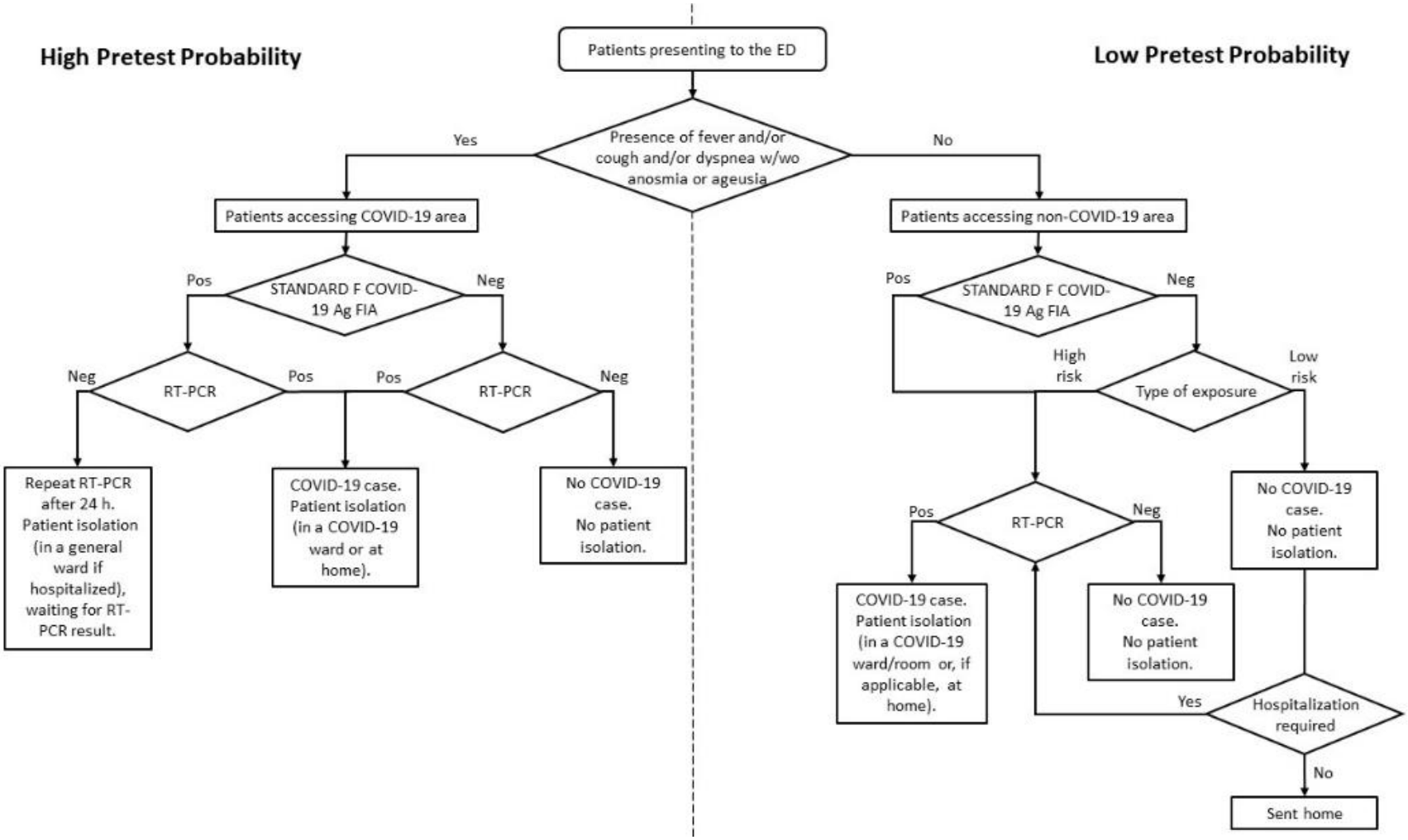
Testing algorithm for diagnosing SARS-COV-2 infection in patients who presented to the 133 ED from December 2020 to January 2021



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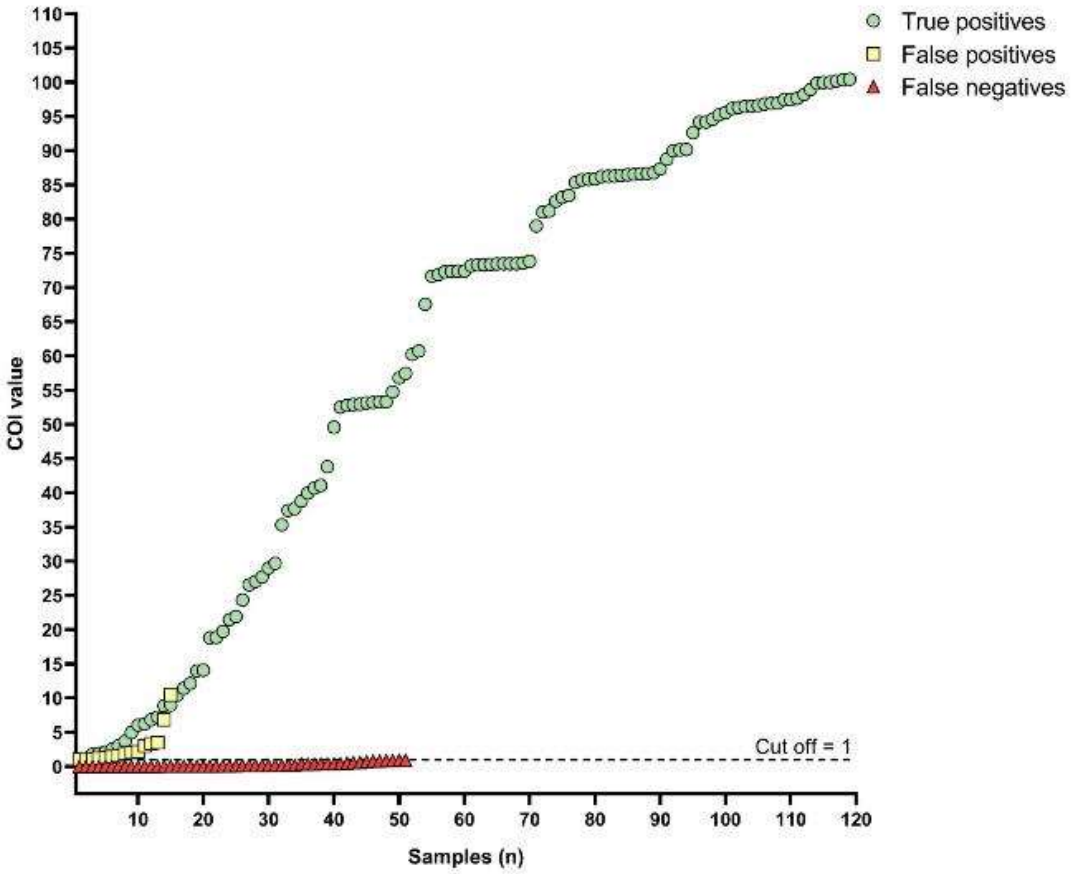
Giulia Menchinelli [1,2,†], Giulia De Angelis [1,2,†], Margherita Cacaci [1,2], Flora Marzia Liotti [1,2], Marcello Candelli [3], Ivana Palucci [1,2], Rosaria Santangelo [1,2], Maurizio Sanguinetti [1,2,*], Giuseppe Vetrugno [4,5], Francesco Franceschi [3,6] and Brunella Posteraro [1,7]

# COVID-19 zone

| STANDARD F COVID-19 Ag FIA assay[a] | |
|---|---|
| True positives/RT-PCR positives | 119/170 |
| False negatives/RT-PCR positives | 51/170 |
| True negatives/RT-PCR negatives | 893/908 |
| False positives/RT-PCR negatives | 15/908 |
| **Sensitivity** | **70.0% (95% CI 62.5–76.8)** |
| **Specificity** | **98.3% (95% CI 97.3–99.1)** |
| Overall accuracy | 93.8% (95% CI 93.1–95.8) |
| Cohen's kappa value | 0.74 (95% CI 0.69–0.81), substantial |

[a]STANDARD F COVID-19 Ag FIA assay's results were compared to RT-PCR assay's results

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Article

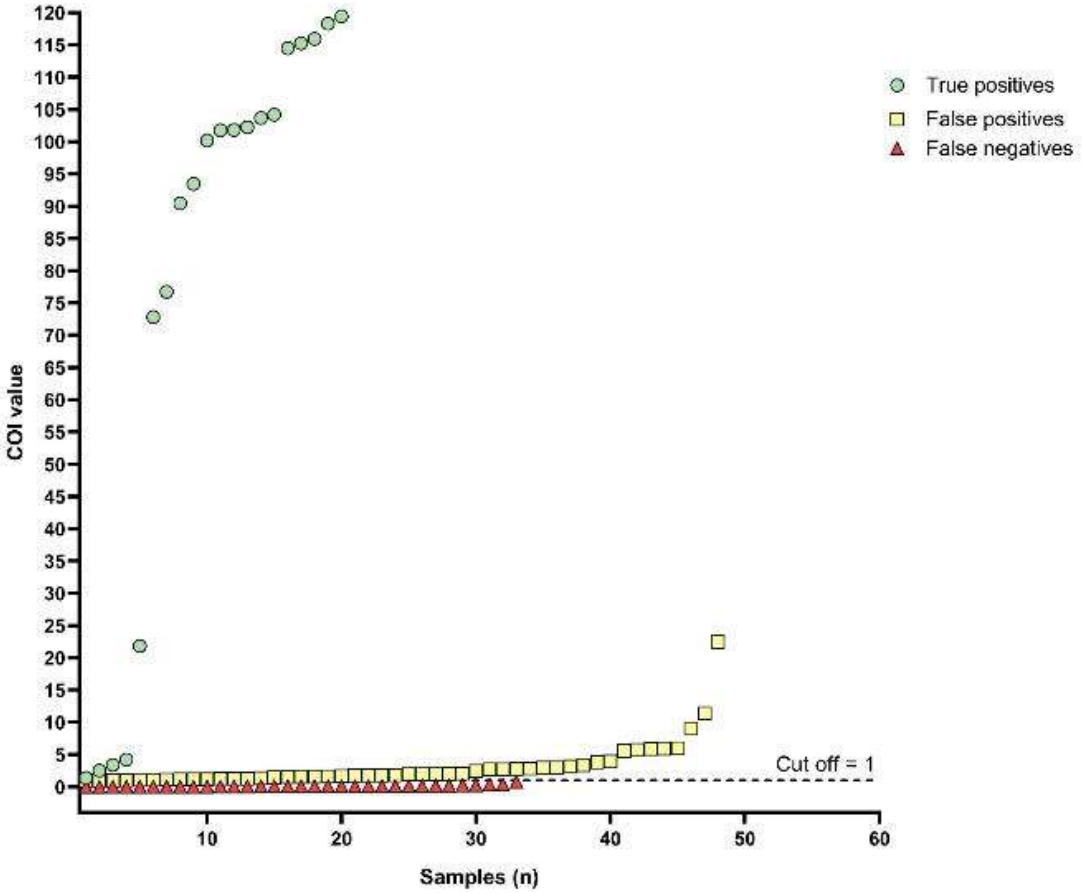
SARS-CoV-2 Antigen Detection to Expand Testing Capacity for COVID-19: Results from a Hospital Emergency Department Testing Site

Giulia Menchinelli [1,2,†], Giulia De Angelis [1,2,†], Margherita Cacaci [1,2], Flora Marzia Liotti [1,2], Marcello Candelli [3], Ivana Palucci [1,2], Rosaria Santangelo [1,2], Maurizio Sanguinetti [1,2,*], Giuseppe Vetrugno [4,5], Francesco Franceschi [3,6] and Brunella Posteraro [1,7]

# Non-COVID-19 zone

| STANDARD F COVID-19 Ag FIA assay[a] | |
|---|---|
| True positives/RT-PCR positives | 20/53 |
| False negatives/RT-PCR positives | 33/53 |
| True negatives/RT-PCR negatives | 1724/1772 |
| False positives/RT-PCR negatives | 48/1772 |
| **Sensitivity** | **37.7% (95% CI 24.8–52.1)** |
| **Specificity** | **97.3% (95% CI 96.4–98.0)** |
| Overall accuracy | 95.6% (95% CI 94.9–96.8) |
| Cohen's kappa value | 0.31 (95% CI 0.19–0.42), fair |

[a]STANDARD F COVID-19 Ag FIA assay's results were compared to RT-PCR assay's results

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Journal of Clinical Medicine

MDPI

Article

**Saliva is a valid alternative to nasopharyngeal swab in chemiluminescence-based assay for detection of SARS-CoV-2 antigen**

Alessandra Amendola[1*], Giuseppe Sberna[1*], Eleonora Lalle[1], Francesca Colavita[1], Concetta Castilletti[1], Giulia Menchinelli[2,3], Brunella Posteraro[3,4], Maurizio Sanguinetti[2,3], Giuseppe Ippolito[5], Licia Bordi[1*], Maria Rosaria Capobianchi[1] on behalf of INMI COVID-19 study group

In revision

**Table 5.** Performance of Lumipulse G SARS-CoV-2 Ag vs reference molecular test (Simplexa™ COVID-19 Direct) on fresh saliva samples

| | | Lumipulse G SARS-CoV-2 Ag | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| Simplexa™ COVID-19 Direct | Positive | 22 | 20 | 42 |
| | Negative | 5 | 80 | 85 |
| | Total | 27 | 100 | 127 |

| | Proportion# | Percentage (95% CI) |
|---|---|---|
| Sensitivity | 22/42 | 52.4% (36.4%- 68.0%) |
| Specificity *vs* RT-PCR reference test | 80/85 | 94.1% (86.8% - 98.1%) |
| Specificity *vs* stage of infection | 45/45 | 100% (92.1%-100.0%) |

# n. pos./N. Tot.

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Clin Chem Lab Med 2021; 59(8): 1468–1476 DE GRUYTER

Giulia Menchinelli, Licia Bordi, Flora Marzia Liotti, Ivana Palucci, Maria Rosaria Capobianchi, Giuseppe Sberna, Eleonora Lalle, Lucio Romano, Giulia De Angelis, Simona Marchetti, Maurizio Sanguinetti*, Paola Cattani and Brunella Posteraro

# Lumipulse G SARS-CoV-2 Ag assay evaluation using clinical samples from different testing groups

**Table 2:** Positive detections of Lumipulse antigen and subgenomic RNA compared with those of RT-PCR for SARS-CoV-2 in different testing groups.[a]

| Ct values (no. of results) | Results according to indicated RT-PCR Ct values for: | | | | p-Value[d] |
|---|---|---|---|---|---|
| | Lumipulse antigen detection[b] | | Subgenomic RNA detection[c] | | |
| | No. of results (including gray-zone results) | Percent agreement (95% confidence interval) | No. of results | Percent agreement (95% confidence interval) | |
| **Diagnostic group** | | | | | |
| <18 (15) | 15 (0) | 100.0 (78.2–100.0) | 13 | 86.7 (59.5–98.3) | 0.14 |
| 18–<25 (20) | 20 (0) | 100.0 (83.2–100.0) | 20 | 100.0 (83.2–100.0) | NA |
| 25–<30 (18) | 17 (1) | 94.4 (72.7–99.9) | 10 | 55.6 (30.8–78.5) | 0.009 |
| 30–<35 (10) | 6 (5) | 60.0 (26.2–87.8) | 0 | 0.0 (0.0–30.8) | 0.003 |
| 35–40 (6) | 2 (0) | 33.3 (4.3–77.7) | 1 | 16.7 (0.4–64.1) | 0.52 |
| All (69) | 60 (6) | 87.0 (76.7–93.9) | 44 | 63.8 (51.3–75.0) | 0.002 |
| **Screening group** | | | | | |
| <18 (15) | 15 | 100.0 (78.2–100.0) | 15 | 100.0 (78.2–100.0) | NA |
| 18–<25 (17) | 17 | 100.0 (80.5–100.0) | 16 | 94.1 (71.3–99.9) | 0.30 |
| 25–<30 (10) | 8 (5) | 80.0 (44.4–97.5) | 2 | 20.0 (2.5–55.6) | 0.007 |
| 30–<35 (8) | 3 (2) | 38.0 (8.5–75.5) | 0 | 0.0 (0.0–36.9) | 0.05 |
| 35–40 (3) | 0 | 0.0 (0.0–70.6) | 0 | 0.0 (0.0–70.6) | NA |
| All (53) | 43 (7) | 81.1 (68.0–90.6) | 33 | 62.3 (47.9–75.2) | 0.03 |
| **Monitoring group** | | | | | |
| <18 (8) | 8 | 100.0 (63.1–100.0) | 8 | 100.0 (63.1–100.0) | NA |
| 18–<25 (12) | 12 (1) | 100.0 (73.5–100.0) | 10 | 83.3 (51.6–97.9) | 0.13 |
| 25–<30 (12) | 12 (4) | 100.0 (73.5–100.0) | 5 | 41.7 (15.2–72.3) | 0.002 |
| 30–<35 (30) | 14 (7) | 46.7 (28.3–65.7) | 1 | 3.3 (0.1–17.2) | <0.001 |
| 35–40 (10) | 6 (4) | 60.0 (26.2–87.8) | 0 | 0.0 (0.0–30.8) | 0.003 |
| All (72) | 52 (16) | 72.2 (60.4–82.1) | 24 | 33.3 (22.7–45.4) | <0.001 |

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Clin Chem Lab Med 2021; 59(8): 1468–1476 DE GRUYTER

Giulia Menchinelli, Licia Bordi, Flora Marzia Liotti, Ivana Palucci, Maria Rosaria Capobianchi, Giuseppe Sberna, Eleonora Lalle, Lucio Romano, Giulia De Angelis, Simona Marchetti, Maurizio Sanguinetti*, Paola Cattani and Brunella Posteraro

# Lumipulse G SARS-CoV-2 Ag assay evaluation using clinical samples from different testing groups

**Table 3:** Characteristics of 194 RT-PCR positive samples that tested positive or negative for the presence of SARS-CoV-2 subgenomic RNA.[a]

| Characteristic[b] | Samples with a subgenomic RNA positive result (n=101) grouped as | | Samples with a subgenomic RNA negative result (n=93) grouped as | | p-Value[c] |
|---|---|---|---|---|---|
| | RT-PCR positive/ Antigen positive (n=101) | RT-PCR positive/ Antigen negative (n=0) | RT-PCR positive/Antigen positive (n=54) | RT-PCR positive/Antigen negative (n=39) | |
| RT-PCR Ct, mean value ± SD | 20.3 ± 4.8 | NA[d] | 29.9 ± 4.8 | 33.8 ± 2.4 | <0.001 |
| Testing from COVID-19 diagnosis, mean days ± SD | 1.6 ± 3.3 | NA[d] | 6.1 ± 7.0 | 7.7 ± 8.6 | <0.001 |

[a]All samples were from diagnostic (n=69), screening (n=53), or monitoring (n=72) testing groups (see Table 2). Testing for SARS-CoV-2 subgenomic RNA was performed using an in-house RT-PCR assay to assess the presence of replicative (*E* gene) RNA. [b]The time period between SARS-CoV-2 RT-PCR (to which Ct values refer) used to diagnose COVID-19 and testing for SARS-CoV-2 subgenomic RNA (and antigen) ranged from 0 days in the diagnostic or screening groups to 32 days in the monitoring group. Only in the last group, consequently, two temporally different samples were tested. [c]For comparisons between the RT-PCR positive/Antigen positive groups herein listed. [d]NA, not applicable.

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# Another false-positive problem for a SARS-CoV-2 antigen test in Japan

*To the Editor,*

A recent article in your journal states that the antigen test for SARS-CoV-2 is insufficiently sensitive and of little clinical significance [1]. The ongoing use of the quantitative antigen test in Japan since June 2020 is increasingly causing serious clinical problems because of its high incidence of false positives.

The quantitative antigen test Lumipulse G SARS-CoV-2 Ag (Fujirebio, Tokyo, Japan) has a 91.7 % (22/24 cases) and 97.3 % (293/301 cases) positive and negative concordance rate compared with RT-PCR, respectively. Eight of the 30 antigen-positive patients were reported to have negative RT-PCR results. [2]. Although it is true that the sensitivity of RT-PCR for SARS-CoV-2 is insufficient [3], RT-PCR is the current golden standard in clinical practice. Therefore, although difficult to accurately evaluate, some false-positive results can be expected with this antigen test. In addition, because the Japanese MHLW permits definitive diagnosis of COVID-19 without PCR if the antigen test is positive [4], concerns arise about the potential for false-positive patients to be admitted to the same medical room as patients with a true COVID-19 diagnosis.

A patient with a false-positive result for COVID-19 was recently admitted to our facility. The patient was a 96-year-old woman who tested positive on the Lumipulse G SARS-CoV-2 Ag, had a general malaise, and had no abnormal lung shading on chest computed tomography. However, in accordance with MHLW policy, the local health authorities recognized her as a confirmed COVID-19 case. Furthermore, because all COVID-19 cases in Japan require hospitalization, the patient was admitted to our hospital. We considered the possibility of false-positive result for this patient and decided to admit her to a private room isolated from true COVID-19 cases. After two subsequent RT-PCR tests performed more than 24 hours apart were negative, we determined that the patient's condition was not COVID-19, and we discharged her. If instead—with no suspicion of a false-positive result—had the patient been admitted to the same room as a true COVID-19 patient, then she could have been at risk for a nosocomial infection.

Because Lumipulse G SARS-CoV-2 Ag is a quantitative test, raising the cutoff value could prevent the occurrence of false positives. If the cutoff were raised to 100 pg/mL of antigen volume, the results would be 38.7 % (12/31 cases) with a positive concordance rate and 99.6 % (293/294 cases) with a negative concordance rate, resulting in 1 RT-PCR negative result for 13 positive antigen tests, meaning fewer false positives [2]. However, the sensitivity of the test would be significantly lower. The use of antigen tests in Japan that are prone to false positives, coupled with the current MHLW policy, is creating a situation in which false-positive patients who are not infected with SARS-CoV-2 are at risk of nosocomial infection. Since Fujirebio has initiated the clinical trial to gain U.S. Food and Drug Administration approval [5], this test will be used outside of Japan. Because the characteristics of antigen tests vary among products, we believe it is important to fully understand their use.

## Funding

None.

## Declaration of Competing Interest

The authors report no declarations of interest.

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# Key messages

- Rapid antigen tests can contribute to overall COVID-19 testing capacity, offering advantages in terms of shorter turnaround times and reduced costs, especially in situations in which RT-PCR testing capacity is limited.
- Test sensitivity for rapid antigen tests is generally lower than for RT-PCR.
- Rapid antigen tests perform best in cases with high viral load, in pre-symptomatic and early symptomatic cases up to five days from symptom onset.
- ECDC agrees with the minimum performance requirements set by WHO at ≥80% sensitivity and ≥97% specificity.
- ECDC recommends that EU Member States perform independent and setting-specific validations of rapid antigen tests before their implementation.
- The use of rapid antigen tests is appropriate in high prevalence settings when a positive result is likely to indicate true infection, as well as in low prevalence settings to rapidly identify highly infectious cases.
- Rapid antigen tests can help reduce further transmission through early detection of highly infectious cases, enabling a rapid start of contact tracing.

Clinical Infectious Diseases

IDSA GUIDELINES

IDSA Infectious Diseases Society of America

The Infectious Diseases Society of America Guic
the Diagnosis of Coronavirus Disease 2019 (CO
Antigen Testing

## IDSA Guidelines COVID-19 Rapid Antigen Testing

- **High specificity, low to modest senstivity** vs. reference NAAT
- Sensitivity depends on viral load (symptomatic vs. asymptomatic), time since onset of symptoms
- **Rapid or lab-based NAAT remain the method of choice**
- Helpful when molecular testing is not available

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# Patients with signs & symptoms of COVID-19: Rapid Antigen testing

| *Recommendation* | *Strength of recommendation ** | *Overall certainty of the evidence *** |
|---|---|---|
| In patients with **mild and moderate COVID-19**, we suggest the use of laboratory-based NAAT versus rapid antigen detection testing for diagnosis of COVID-19. | Weak against | Very low |
| In patients with **severe or critical COVID-19**, we recommend the use of laboratory-based NAAT versus rapid antigen detection testing for diagnosis of COVID-19. | **Strong against** | Very low |
| In patients with signs and symptoms compatible with COVID-19, we suggest the use of laboratory based NAAT in **nasopharyngeal samples** versus rapid antigen detection testing in nasopharyngeal samples for diagnosis of COVID-19. | Weak against | Very low |
| In patients with signs and symptoms compatible with COVID-19, we suggest the use of laboratory based NAAT in **saliva samples** versus rapid antigen detection testing in saliva samples for diagnosis of COVID-19. | Weak against | Very low |
| In patients with signs and symptoms compatible with COVID-19, we suggest the use of laboratory based NAAT in **samples other than nasopharyngeal and saliva** samples versus rapid antigen detection testing in samples other than nasopharyngeal and saliva samples for diagnosis of COVID-19. | Weak against | Very low |
| In patients with signs and symptoms compatible with COVID-19 **of equal or less than 7 days-onset**, we suggest the use of laboratory-based NAAT versus rapid antigen detection testing for diagnosis of COVID-19. | Weak against | Very low |
| In patients with signs and symptoms compatible with COVID-19 of **more than 7 days-onset**, we suggest the use of laboratory-based NAAT versus rapid antigen detection testing for diagnosis of COVID-19. | Weak against | Very low |
| In **children <12 years old** with signs and symptoms compatible with COVID-19, we suggest the use of laboratory-based NAAT versus rapid antigen detection testing for diagnosis of COVID-19. | Weak against | Very low |
| In **patients ≥ 12 years old** with signs and symptoms compatible with COVID-19, we suggest the use of laboratory-based NAAT versus rapid antigen detection testing for diagnosis of COVID-19 | Weak against | Very low |
| In patients with signs and symptoms compatible with COVID-19 **at risk for severe illness**, we recommend the use of laboratory-based NAAT versus rapid antigen detection testing for diagnosis of COVID-19. | **Strong against** | Very low |

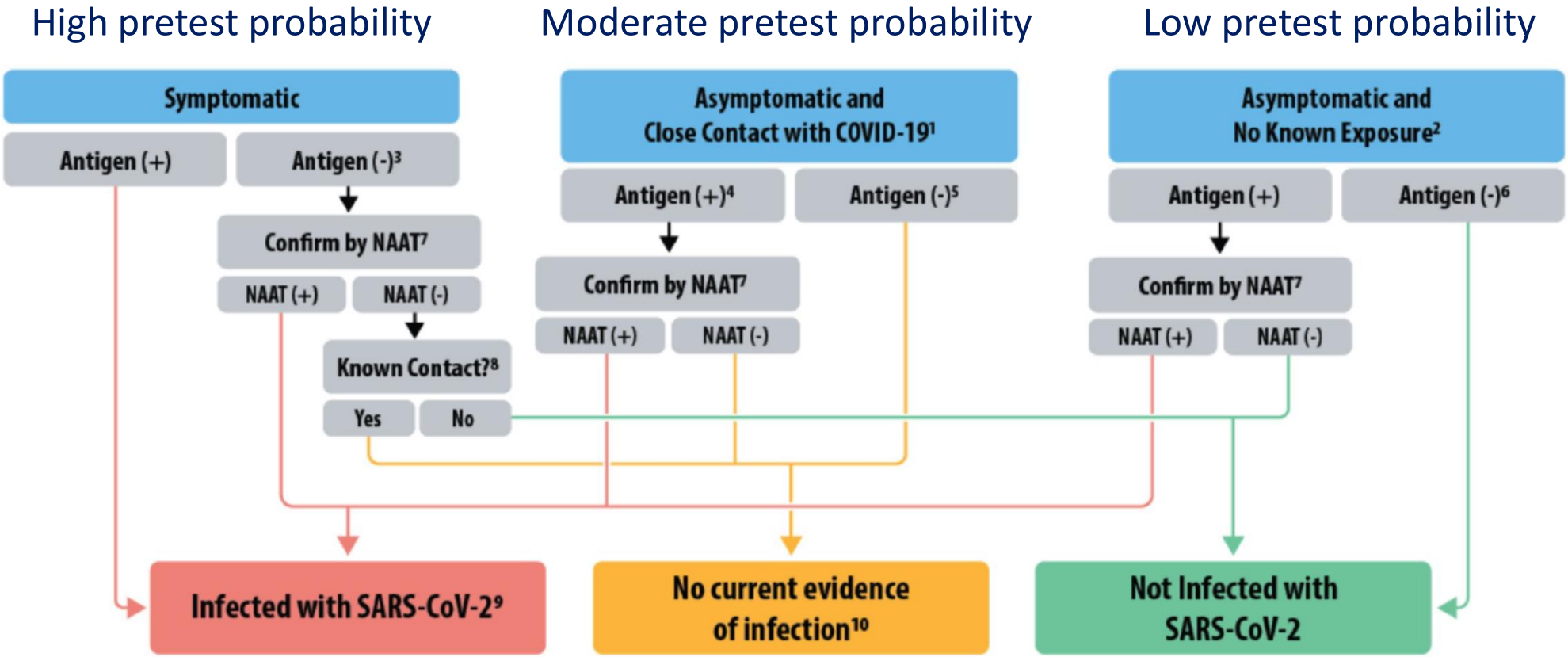
* Strength of recommendation (strong against, weak against, in research only, weak for, strong for).
** Overall certainty of the evidence (high, moderate, low, very low).

CDC Centers for Disease Control and Prevention

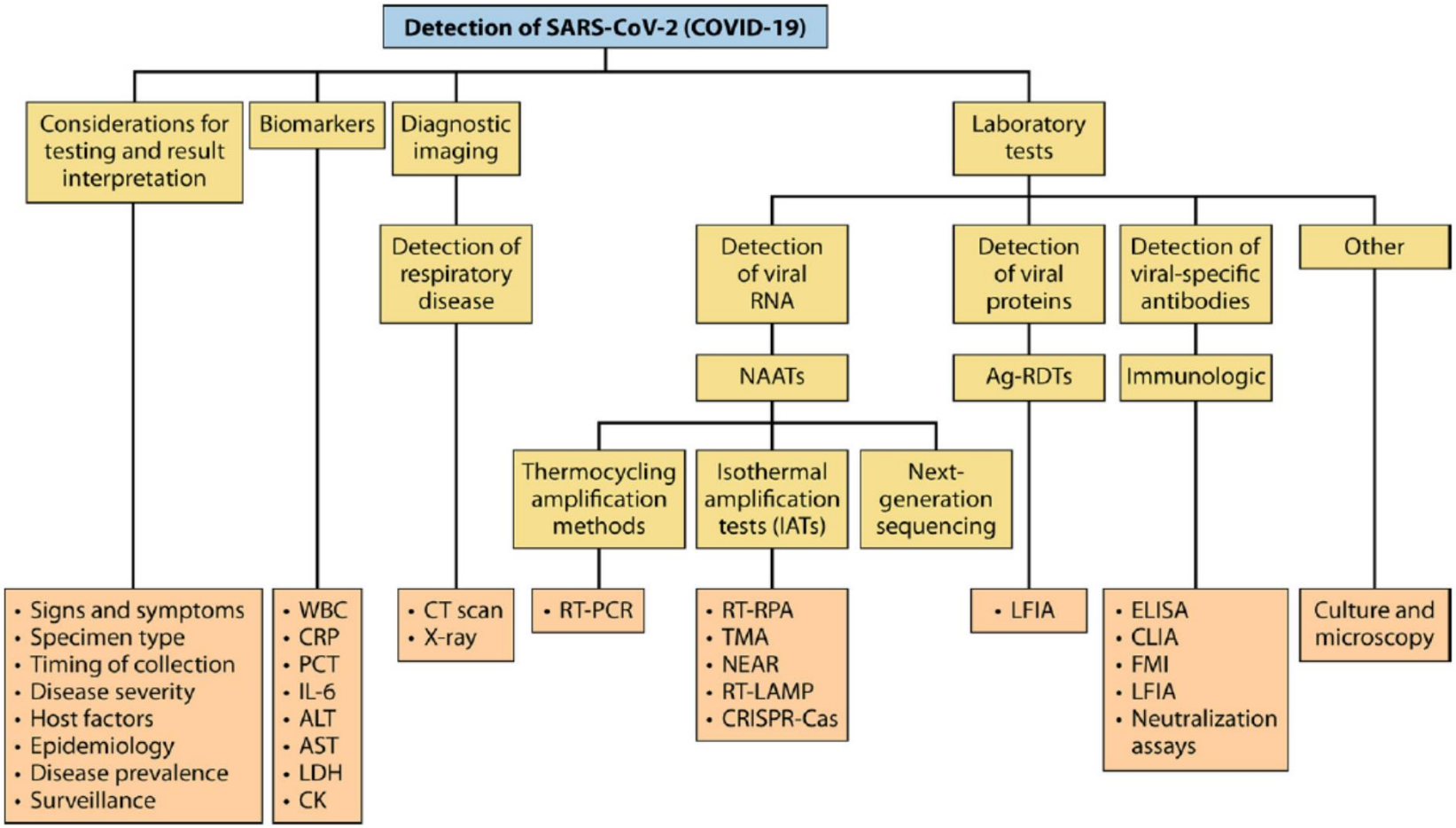
COVID-19

Interim Guidance for Antigen Testing for SARS-CoV-2

Updated Dec. 16, 2020



# Methods used for SARS-CoV-2 detection or identification of COVID-19




Safiabadi Tali et al., CMR, 2021

Perché non investire su una rete di diagnostica microbiologica nazionale e invece demandare una parte importante della diagnostica alle Farmacie???

Il Microbiologo Clinico, il vero «Sistema Esperto» per la gestione delle patologie infettive

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Thank you to Cecilia, Ivana, Lia, Flora, Giulia and all the persons still active in the Microbiology Lab of the «A. Gemelli» Hospital who still work 24 hours/day and 7 days/week to provide in «real-time» the results of SARS-CoV-2 diagnostic tests!!!

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Rome, March 27th, 2:20 AM