– Revista de –
MEDICINA DE LABORATORIO

www.revistamedicinadelaboratorio.es

Special Article

Usefulness and management of active infection detection tests (AIDTs)

Utilidad y manejo de las pruebas de detección de infección activa (PDIA)

Daniel Pineda Tenor[1], María S. Pacheco Delgado[2], Santiago Prieto Menchero[3], Laura Criado Gómez[4], Enrique Prada de Medio[5], Enrique Rodríguez Borja[6], Verónica Cámara Hernández[7]

[1]Laboratory Clinical Management Unit. Hospital de Antequera. Antequera, Málaga. Spain. Área de Gestión Sanitaria Norte de Málaga (AGSNM). Board of Directors, Asociación Española de Biopatología Médica – Medicina de Laboratorio (AEBM-ML). Quality, Management, Safety, and Evidence Committee (CCGSE), AEBM-ML. Madrid, Spain. [2]Clinical Laboratory Department. Hospital Universitario de Fuenlabrada. Fuenlabrada, Madrid. Spain. Board of Directors, Asociación Española de Biopatología Médica – Medicina de Laboratorio (AEBM-ML). Laboratory Reporting Committee, AEBM-ML. Madrid, Spain. [3]Clinical Laboratory Department. Hospital Universitario de Fuenlabrada. Fuenlabrada, Madrid. Spain. Board of Directors, Asociación Española de Biopatología Médica – Medicina de Laboratorio (AEBM-ML). Quality, Management, Safety, and Evidence Committee (CCGSE), AEBM-ML. Laboratory Reporting Committee, AEBM-ML. Madrid, Spain. [4]Clinical Laboratory Department. Hospital Universitario de Móstoles. Móstoles, Madrid. Spain. Quality, Management, Safety, and Evidence Committee (CCGSE), AEBM-ML. Madrid, Spain. [5]Clinical Laboratory Department. Hospital Virgen de la Luz. Cuenca, Spain. Board of Directors, Asociación Española de Biopatología Médica – Medicina de Laboratorio (AEBM-ML). Quality, Management, Safety, and Evidence Committee (CCGSE), AEBM-ML. Laboratory Reporting Committee, AEBM-ML. Madrid, Spain. [6]Clinical Laboratory Department. Hospital Clínico Universitario de Valencia. Valencia, Spain. Board of Directors, Asociación Española de Biopatología Médica – Medicina de Laboratorio (AEBM-ML). Laboratory Reporting Committee, AEBM-ML. Madrid, Spain. [7]Clinical Laboratory Department. Hospital Universitario de Getafe. Getafe, Madrid. Spain. Board of Directors, Asociación Española de Biopatología Médica – Medicina de Laboratorio AEBM-ML. Madrid, Spain

Received: 15/02/2022
Accepted: 15/02/2022

Correspondence: Daniel Pineda Tenor. Laboratory Clinical Management Unit. Hospital de Antequera. Avenida Poeta Muñoz Rojas, s/n. 29200 Antequera. Málaga, Spain
e-mail: daniel.pineda.sspa@juntadeandalucia.es

We submit an article requested within the board of directors of the *Sociedad Española de Biopatología Médica - Medicina de Laboratorio* (AEBM-ML) to answer multiple concerns reported by members and other professionals regarding the right usage of active infection detection tests for Covid-19. The article was written by the Quality, Management, Safety, and Evidence Committee, the Laboratory Reporting Committee, and Board members.

In order to facilitate reading, the paper is *submitted in infographic format.*

Keywords: COVID-19. SARS-CoV-2. RT-PCR. Rapid antigen detection test. Diagnosis. Screening.

Conflict of interest: the authors declare no conflict of interest.

DOI: 10.20960/revmedlab.00123

Pineda Tenor D, Pacheco Delgado MS, Prieto Menchero S, Criado Gómez L, Prada de Medio E, Rodríguez Borja E, Cámara Hernández V. Usefulness and management of active infection detection tests (AIDTs). Rev Med Lab 2022;3(1):25-28

ISSN (electrónico): 2660-7638 - ISSN (papel): 2660-7484
Rev Med Lab 2022;3(1):25-28

AEBM-ML Info-COVID-19 | Usefulness and management of active infection detection tests (AIDTs)

1/3

AEBM-ML Information Guide
Asociación Española de Biopatología Médica – Medicina de Laboratorio
Version 1
Date: January, 23rd, 2022

D Pineda Tenor[1,2], M Pacheco Delgado[1,3], S Prieto Menchero[1-3], L Criado Gómez[2], E Prada de Medio[1,2], E Rodríguez Borja[1,3], V Cámara Hernández[1].

1. Board of Directors AEBM-ML; 2. Quality, Management, Safety, and Evidence Committee; 3. Laboratory Reporting Committee

What types of tests may be used to detect active infection with SARS-CoV-2?

The diagnostic approach to **COVID-19** (**CO**rona**VI**rus **D**isease 20**19** is currently based on active infection detection tests (AIDTs) for SARS-CoV-2, and include both molecular tests and rapid antigen detection tests:

1 Molecular techniques. Nucleic acid amplification

Viral RNA detection using nucleid acid amplification tests (NAATs), which include TMA (Transcription-Mediated Amplification), RT-LAMP (Reverse Transcriptase - Loop-Mediated Isothermal Amplification), and **RT-PCR** (Reverse Transcripción Polymerase Chain Reaction). The **latter represents the reference test given its high sensitivity and specificity.** Most common gene amplification targets include E (envelope), S (spike), N (nucleocapsid), and ORF (open reading frame) 1ab.

Results are provided as a positivity cycle threshold (Ct). A sample's genetic material amplification is exponential over a number of cycles that repeat sequentially. A Ct indicates the number of cycles where the fluorescent signal crosses the threshold of detection. This value represents a semi-quantitative measure that is inversely related to RNA copies in the sample, hence it provides an indirect estimation of viral load.

Sensitivity and specificity of RT-PCR as related to Ct

RT-PCR Ct	Study references	Samples	Cases	Mean sensitivity, % (95 % CI)	Mean specificity of RT-PCR, % (95 % CI)
Global	r	4,537	1,973	**95.5 (91.5-97.7)**	**98.8 (98.3-99.2)**
Ct ≤ 30	6	204	204	**100 (98.2-100)**	-
Ct > 30	6	149	149	**95.6 (55.7-99.7)**	-

Dinnes et al. Cochrane Library. 2021; 3(3)

RT-PCR may be considered the technique of choice to identify active infection as it offers the highest sensitivity and specificity. Positive results may be seen days before symptom onset, and persist throughout the course of disease. Its high methodological complexity requires specialist laboratories and qualified personnel, and involves long processing times with results being reported 12-72 hours after the test was requested. Notably, detection of viral RNA does not necessarily entail the presence of infectious viruses in clinical samples. Similarly, given the wide spread of the Omicron variant and current vaccination schedule, healthy SARS-CoV-2 carriers may also be found.

Rapid Antigen Detection Tests 2

Sensitivity of antigen tests as related to RT-PCR Ct

RT-PCR Ct	Study references	Samples	Cases	Mean sensitivity of antigen test, % (95 % CI)
Ct ≤ 25	36	2,613	2,632	**94.5 (91.0-96.7)**
Ct > 25	36	2,632	2,632	40.7 (31.8-50.3)
Ct > 33/32	15	346	346	8.9 (3.3-21.7)

Dinnes et al. Cochrane Library. 2021; 3(3)

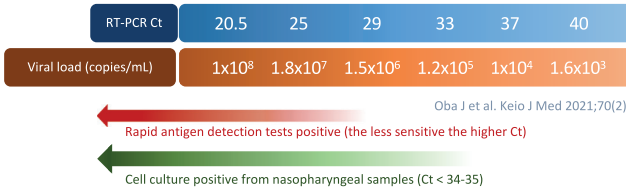
Rapid antigen detection tests **(RADT).** Detection using lateral flow immunochromatographic assays, with most common targets being protein N (nucleocapsid) or S (spike). **Results are qualitative (presence or absence of antigen)** with inferior **detection window, sensitivity, and specificity when compared to RT-PCR.** Its primary advantage is test speed, as it can be performed within 15-30 minutes.

The WHO recommends that antigen tests display a sensitivity ≥ 80 % and specificity ≥ 97 %.

Sensitivity and specificity data as supplied by the manufacturers are usually obtained under ideal rather than actual clinical conditions. Antigen tests have an adequate detection capacity in patients **with higher viral loads**. **Positivity as related to RT-PCR Ct value is variable according to the various studies and tests, and is deemed to be adequate in patients with Ct ≤ 25.** Test specificity is usually very high regardless of viral load. Given their ease of use and shorter response times these tests are particularly useful for initial screenings (with RT-PCR being recommended for high-suspicion, negative patients) and in cases where RT-PCR is unavailable or has response times longer than 36 hours.

How do the results of these tests relate?

Relationship between RT-PCR Ct, viral load, RADT positivity, and growth in cell culture



Oba J et al. Keio J Med 2021;70(2)

A RT-PCR Ct value should not be used for viral load quantification. However, an inverse relationship has been reported between Ct and viral load, as well as with the probability of infectious virus recovery in cell cultures.
Viral RNA detection does not necessarily entail the presence of infectious viruses in a clinical sample.

A RT-PCR Ct value is related to viral growth capacity in a cell culture, which is considered an indicator of infectious capacity. The presence or absence of symptoms does not affect such growth capacity.
Although variability may be found in the literature, a Ct value of 34 is deemed to be equivalent to approximately 10^6 viral particles in 10 mL of either a nasopharyngeal or saliva sample. Here no viral growth in cell culture may be observed. Higher viral loads of 10^5-10^6 are required for positivity in an antigen detection test.

AEBM-ML Info-COVID-19 | Usefulness and management of active infection detection tests (AIDTs)

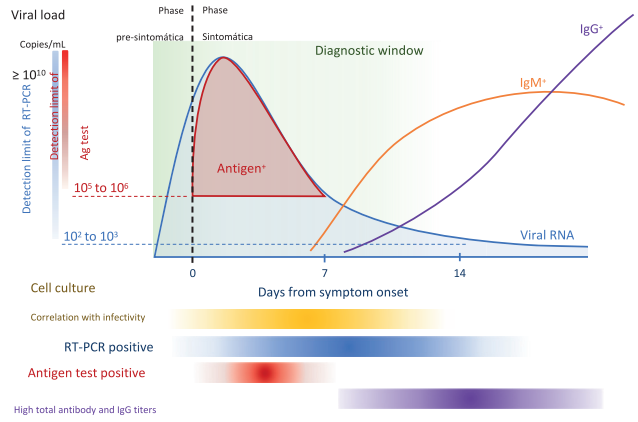
2/3

D Pineda Tenor[1,2], M Pacheco Delgado[1,3], S Prieto Menchero[1-3], L Criado Gómez[2], E Prada de Medio[1,2], E Rodríguez Borja[1,3], V Cámara Hernández[1].
1. Board of Directors AEBM-ML; 2. Quality, Management, Safety, and Evidence Committee; 3. Laboratory Reporting Committee

AEBM-ML Information Guide
Asociación Española de Biopatología Médica – Medicina de Laboratorio
Version 1
Date: January, 23rd, 2022

When is it useful to administer tests for detecting active infection with SARS-CoV-2?

Timeline for the proper use of different diagnostic tests and patient response



Drain et al. N Eng J Med 2022;386(3) y Peeling et al. Lancet December 20 2021

The times to positivity for the various tests included in the graph above correspond to general conditions but may vary. Patients may be found with RT-PCR Cts detectable for several weeks after symptom clearance, as well as antigen tests that remain positive beyond the first week after symptom recovery.

Interpreting RT-PCR Ct has limitations including expression with non-linear values, dependence on sample collection type and quality (wrong pre-analytical conditions may result in false negative results), and presence of intratest or intertest variability. Furthermore, RT-PCR detects the presence of viral RNA but not its viability. Therefore, interpretation must fit the patient's clinical/epidemiological context.

Any interpretation of rapid antigen test results must be cautious. False negative results may occur when sample collection is inadequate or assessment lies outside the detection window. False positive results are common when testing is flawed by using other diluents (water, juice...) rather than the appropriate pH buffer during antigen binding.

1. COVID-19 symptoms develop at 2 to 14 days after exposure, with a mean of 3 to 5 days after infection. It has been reported that in 1 % of patients symptoms may develop after day 14, while asymptomatic individuals also exist.

2. **RT-PCR** is able to identify the virus at **around 3 days before symptom onset,** once viral load exceeds 10^2-10^3 copies/mL, and remains positive throughout the course of infection. Viral load peaks before symptom onset, within the first 3-5 days, and then decreases to become eventually undetectable (mean replication time is around 17 days, and may become longer over time).

3. **Rapid antigen tests** may be positive one or two days prior to symptom onset, and have been reported to display adequate diagnostic sensitivity within the **first week after symptom onset.** Detection requires viral loads greater than 10^5-10^6 copies/mL.

Relationship between symptoms and antigen test positivity

Days from symptom onset	Study references	Samples	Cases	Mean sensitivity of antigen test, % (95 % CI)
Week 1	**26**	**5,769**	**2,320**	**78.3 (71.1-84.1)**
Week 2	22	1,581	692	51.0 (40.8-61.0)
Symptom-free	12	1,581	295	58.1 (40.2-74.1)

Dinnes et al. Cochrane Library. 2021; 3(3)

4. **Antibody serology tests** may be positive during acute infection and after infection resolution, and are associated with presence of only IgG against past infection or vaccination. At any rate, **they are not considered useful to identify active infection** but rather as tools to evaluate a patient's epidemiological status.

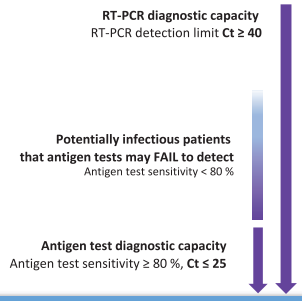
Is it possible to stratify contagiousness risk based on these tests?

As was described above, a patient's contagiousness depends on viral load, which is proportional to infectious virus recovery and inversely proportional to RT-PCR Ct. Anyway, transmissibility is a complex phenomenon with no validated techniques to measure it.

The **Sociedad Española de Médicos de Atención Primaria (SEMERGEN)** has published a review according to which a Ct ≥ 37 is associated with viral loads low enough for patients to be deemed not contagious, whereas patients with Ct lower than 30 have a high contagiousness risk because of association with high viral loads.

Contagion risk according to RT-PCR Ct value

Serrano-Cumplido et al. SEMERGEN 2021;47(5)



Not contagious
Does not require isolation
Use of standard protective measures

Indecision zone
Essential social activity with strict protective measures
Repeat RT-PCR in a few days
Monitor for symptom onset

Moderately contagious
Avoid social gatherings
Self-isolation

Highly contagious
Immediate strict isolation
Follow-up of contacts

A correct interpretation of this risk diagram requires consideration of the patient's clinical/epidemiological context since high Ct values may be seen in patients with infection in the process of being resolved and also early in the course of infection.
Furthermore, Ct values are variables as they depend on pre-analytical conditions (which may result in erroneously high Ct values), analysis platform characteristics, and detection methods (differences in cycles between different assessment systems).

However, the **Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC)** points out that **using Ct to infer patient contagiousness may be inappropriate,** since this value is dependent upon both prenalytical conditions (sample type and means of transportation, sample quality, storage conditions, vortex time) and analytical process characteristics (extraction method and conditions, platform used for viral RNA amplification and detection, targets, amplification techniques, probe fluorophores, channel baselines, and experiment cycles).

Based on the available evidence, the **Sociedad Española de Biopatología Médica - Medicina de Laboratorio (AEBM-ML)** recommends that any assessment of contagiousness risk based on AIDTs should consider the following:

1. Patients with RT-PCR Ct < 30 or positive results in a rapid antigen test may be contagious. Assigning a risk level has however limitations.

2. RT-PCR Ct ≥ 30 or negative results in a rapid antigen test do not rule out contagiousness and should be evaluated within the patient's clinical/epidemiological context.

Info-COVID-19 | Usefulness and management of active infection detection tests (AIDTs)

3/3

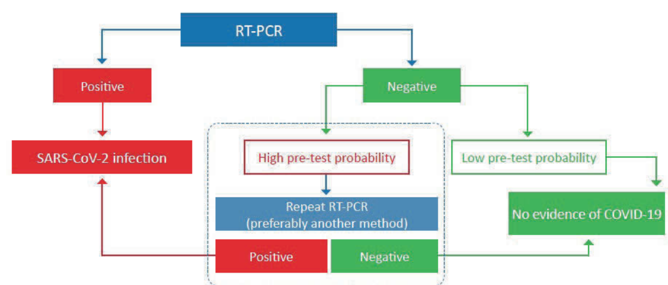
D Pineda Tenor[1,2], M Pacheco Delgado[1,3], S Prieto Menchero[1-3], L Criado Gómez[2], E Prada de Medio[1,2], E Rodríguez Borja[1,3], V Cámara Hernández[1].
1. Board of Dire [illegible] laboratorio

AEBM-ML Information Guide
Asociación Española de Biopatología Médica – Medicina de Laboratorio
Version 1
Date: January, 23rd. 2022

How are tests administered to detect active SARS-CoV-2 infection?

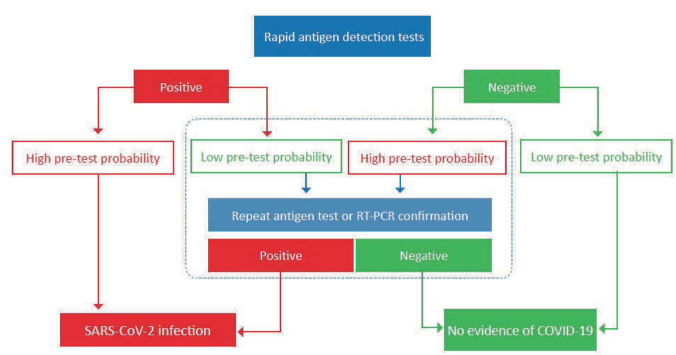
1 RT-PCR

- It is considered the reference technique because of its high sensitivity and specificity.
- A positive results may be assessed based on its Ct, as this value informs about time of infection and and contagion risk.
- A negative result in the presence of high pre-test probability does not rule out infection as it may result from inappropriate sample collection, transportation or preservation, or isolated errors in the analytical phase. Along the same lines, the test may also have been performed early during infection, with still undetectable viral load. In both cases a second RT-PCR is recommended, if possible using a different method.



2 Rapid Antigen Detection Tests

- Less sensitive and specific than RT-PCR. However, given their ease of use and rapid results, they are useful whenever molecular techniques are unavailable, have a response time above 36 hours, or immediate screening is required for an at-risk group.
- A positive result indicates active SARS-CoV-2 infection with high specificity. However, pre-test handling must have been appropriate since use of pH-altering substances or of the wrong or degraded substances may result in false positive results. When pre-test probability is low, a second antigen test or confirmation molecular technique may be used.
- A negative result must be interpreted within the patient's clinical/epidemiological context. COVID-19 patients, whether contagious or otherwise, may have negative results because of concentrations below the test's limit of detection (in association with low viral loads). Similarly, appropriate sample collection is key to ensure result reliability. In cases with high pre-test probability, confirmation with a second antigen test or preferably a molecular technique is recommended.



Pre-test probability is considered to be high when:
- There is clinical evidence of infection, whether symptoms or laboratory, imaging results
- The patient had a close contact with a high probability of contagion

Pre-test probability is considered to be low when:
- The patient is symptom-free or has no suggestive manifestations
- The patient has no close contacts or contacts are low-risk (low exposure, adequate preventive measures, open spaces, vaccinated individuals)

References

1. Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, et al. Rapid, point-of-care antigen and molecular-based test for diagnosis of SARS-CoV-2 infection. Cochrane database Syst Rev [Internet] 2021;3(3).
2. Greub G, Caruana G, Schweitzer M, Imperiali M, Muigg V, Risch M, et al. Multicenter Technical Validation of 30 Rapid Antigen test for the Detection of SARS-CoV-2 (VALIDATE). Microorganisms [Internet] 2021;9(12):2589.
3. Serrano-Cumplido A, Ruiz Garcia A, Segura-Fragoso A, Olmo-Quintana V, Micó Pérez RM, Barquilla-García A, et al. Application of the PCR number of cycle threshold value (Ct) in COVID-19. Semergen [Internet] 2021;47(5):337-41.
4. Drain PK. Rapid Diagnostic Testing for SARS-CoV-2. Solomon CG, editor. N Engl J Med [Internet]. 2022;386(3):264-72.
5. Oba J, Taniguchi H, Sato M, Takamatsu R, Morikawa S, Nakagawa T, et al. RT-PCR Screening test for SARS-CoV-2 with Saliva Samples in Asymptomatic People: Strategy to Maintain Social and Economic Activities while Reducing the Risk of Spreading the Virus. Keio J Med [Internet] 2021;70(2):35-43.
6. Scheiblauer H, Filomena A, Nitsche A, Puyskens A, Corman VM, Drosten C, et al. Comparative sensitivity evaluation for 122 CE-marked rapid diagnostic tests for SARS-CoV-2 antigen, Germany, September 2020 to April 2021. Euro Surveill. 2021;26(44):2100441.
7. Peeling RW, Heymann DL, Teo Y-Y, Garcia PJ. Diagnostics for COVID-19: moving from pandemic response to control. Lancet 2021;20:S0140-6736(21)02346-1.
8. Ministerio de Sanidad. Instituto de Salud Carlos III. Estrategia de detección precoz, vigilancia y control de COVID-19. Actualizado a 22 de Diciembre de 2021. Disponible en: https://www.sanidad.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov/documentos.htm
9. Observaciones SEIMC en relación con la interpretación de la Ct- PCR de SARS-CoV-2. https://www.seimc.org
10. Posicionamiento de la sociedad española de enfermedades infecciosas y microbiología clínica (SEIMC) ante la situación actual (7 de febrero 2022) de la pandemia de COVID-19 en España. https://www.seimc.org