



THE CORONA VIRUS PCR TEST: FACTS AND MYTHS WHY TEST RESULTS VARY AND MAY NOT MATCH CLINICAL SUSPICION

BACKGROUND

Polymerase chain reaction testing aims to amplify a target region of genetic material. For SARS-CoV-2, a reverse transcriptase PCR is performed in order to identify if the viral genetic material (RNA) is present or absent. There are several factors that can have impact on this test, and there are some questions regarding variability in test results (between labs and/or inconsistent with clinical picture).

Unlike most other routine laboratory tests where testing conditions and parameters are well standardized, all current SARS-CoV-2 testing are performed under Emergency Use Authorization (an "EUA") from the FDA which requires that performance data on sensitivity and specificity be submitted to and reviewed by the FDA. Although appropriate controls are run and limited validations and interlaboratory comparisons are performed, there are still some issues that arise in this testing.

INTERPRETATION OF RESULTS

- The overall *sensitivity* of the test is still low (60-80% rate). **A negative test does not rule out Coronavirus infection.**
- *Specificity* is relatively very high - and hence a **positive test is very meaningful.**
- When only one of the target genetic regions is identified (as opposed to 2 or more), the result may be resulted as indeterminate or presumptive positive. This should be interpreted within the clinical context of the patient's presentation.

WHY TEST RESULTS MAY VARY OR NOT MATCH CLINICAL SUSPICION:

Preanalytic test variables:

- Collection technique: [See this helpful UTHSC video on best practice collection technique.](#) for nasopharyngeal swabs
- Anatomic source of collection: Nasopharyngeal, nasal turbinate/deep nasal or anterior nares (unilateral, bilateral) and oropharyngeal sites may all have variable viral loads.
- Type of swab: Flexible shaft NP swab vs rigid shaft routine swab, and even the type of swab tip can affect the quality of sample obtained and test sensitivity.
- Type of viral specimen transport media: Viral or universal transport media, saline, or other transport media can affect how long the virus survives or RNA target is stable in the sample.
- Patient: Symptomatic vs asymptomatic presentations, as well as the phase and severity of disease.

NOTE: there are critical supply shortages for many swabs and transport media types that result in substitution of sub-optimal alternatives that may not have been validated for use in the testing process.



Analytic test variables:

- There is marked variability in viral primer and probes utilized in various tests (see attached listing); although there are some common targets, there are significant variations among EUA approved tests.
- Variability in nucleic acid amplification methods: although most assays are RT-PCR based, some use a lower efficiency isothermal amplification method (e.g. rapid Abbott ID NOW)

Post- Analytic test variables:

- Laboratories may define different criteria and thresholds for calling positivity: Limits of detection vary between tests and when multiple targets are used, the significance of a single positive target varies. In some settings, a single positive probe may called presumptive positive, in others, the same scenario may be called inconclusive.

Note that because of these variables, the actual clinical sensitivity and specificity for these assays is not clearly established, and no large-scale diagnostic accuracy comparison studies have been performed to date.

Manufacturer	Diagnostic (Letter of Authorization)	Technology	Targets
Abbott Diagnostics Scarborough, Inc.	ID NOW COVID-19	Molecular	RdRp segment
Thermo Fisher Scientific, Inc.	TaqPath COVID-19 Combo Kit	Molecular	ORF1ab gene, N protein, S protein
Roche Molecular Systems, Inc. (RMS)	cobas SARS-CoV-2	Molecular	ORF1 and structural protein envelope E-gene for pan-Sarbecovirus detection
Centers for Disease Control and Prevention's (CDC) – [USED IN THE UTHSC TEST]	CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)	Molecular	N1 and N2 regions of virus nucleocapsid gene

Document prepared by:

Drs. Vickie Baselski, Lauren King, Corrie Bosworth, Mahul B. Amin
Department of Pathology and Laboratory Medicine, UTHSC