

SARS-CoV-2 RNA Amplification Tests Available at YNHH- May 2021 Update

Nucleic acid amplification tests (NAAT) for viral RNA are essential for diagnosis of acute SARS CoV-2 infections, and are much more sensitive than antigen tests. Due to limited supplies and unreliable delivery of reagents during the pandemic, laboratories have offered **multiple NAAT “platforms”** to remain operational and meet the need for increased COVID-19 test availability (Table, page 2).

Test differences: Tests vary in methods, viral genes targeted, instrumentation, skill level and labor required, as well as the results displayed in EPIC. Result terminology, i.e. “Detected or Not detected” versus “Positive or Negative”, and the use of “Inconclusive”, vary according to the manufacturer’s protocol and instructions.

Cycle threshold (Ct) values: The cycle threshold (Ct) value is the cycle of amplification in real-time PCR that the signal crosses the threshold to positive. Ct value is inversely correlated to viral load. The lower the Ct value (i.e. the fewer cycles required to cross the threshold to positive), the higher the viral load in the sample. In general Ct values <22 are very strong positives, Ct values 22-30 are moderate positives, and Ct values >30 are low positive results. However, **Ct values between assays are not directly comparable due to different sample input, gene targets, reagents, assay parameters, and instruments**, and one patient may be tested serially with multiple different assays. Sample type, quality, collection method, timing and handling are variable and also affect the viral load. **Ct values should not be used for clinical decision making without expert ID or IP input, including removal from COVID isolation.**

Only real-time polymerase chain reaction (PCR) tests provide a cycle threshold (Ct) value. Panther transcription mediated amplification (TMA) and the BioFire respiratory panel do not provide Ct values. (Table, page 2)

Test selection: Since test reagents may be inadequate to meet demand, priorities are set by the YNHHS Test Stewardship Committee. The laboratory staff follow the committee’s recommendations. Thus, samples are routed to different test platforms according to patient population, sample type, turnaround time required, test reagent inventory, test supplies, and available staff trained to perform the test. Routing to the correct platform is reliant on correct test ordering by the provider, which leads to an icon on the sample label.

Unusual sample types: The CDC lab-developed assay is reserved for **sputum, tracheal aspirates, and saliva** as these sample types are not approved for testing by other platforms used at YNHH. Due to the low numbers of these samples, the CDC assay is now done only once a day and often not on weekends.

Tests offered may change over time and communication is strongly encouraged. Inquiries and feedback may be directed to Marie Landry, MD, David Peaper MD, PhD, or Maureen Owen at 203-688-3524 or Maureen.owen@ynhh.org.

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Table. SARS CoV-2 Test Platforms Available as of May, 2021

Test ^a <i>Go live</i>	Method ^b	Gene targets	Samples	Time to result ^c	Workflow	Features	Utility	Patient population
CDC modified (Lab developed) 3/13/20	Singleplex Real time RT-PCR	N1, N2	NP, OP, nasal, mid-turbinate, BAL, sputum ^d , saliva ^e	24-48 hrs	Batched, 15-23 samples 3 PCRs per sample Labor intensive	Gold standard Manual, low throughput High skill level	BAL, tracheal aspirate, sputum^d, or saliva^e Ct value available	Uncommon sample types Backup to other tests if needed
GeneXpert (Cepheid) 3/29/20	Multiplex Real time RT-PCR	N2, E	NP, OP, nasal, mid-turbinate	2 hrs	Sample inoculated into single cartridge and placed on instrument	Automated test, Single sample Simple to train	Rapid result 24/7 capability Ct value available	Prioritized list of indications
TaqPath (ThermoFisher) 5/7/20 96 well 8/11/20 384 well	Multiplex Real time-RT-PCR	ORF1ab, N, S	NP, OP, nasal, mid-turbinate	12-72 hrs	Batched, pooled 1:4, 384-1536 samples per run. Positive pools retested single samples.	High throughput Many critical steps in procedure High skill level	Large batches Ct value available Day and evening shifts only	Outpatients
Panther Aptima (Hologic) 5/14/20	Transcription mediated amplification (TMA)	ORF1ab, 2 regions	NP, OP, nasal, mid-turbinate	6 hrs	Samples placed on instrument singly or in batches	Up to 250-300 samples per shift	24/7 capability No Ct value	Inpatient and outpatient
Simplexa (Diasorin)* 5/24/20	Multiplex Real time RT-PCR	ORF1ab, S	NP, nasal	2-3 hrs	Samples inoculated into wells in 8-well wheel; 1.5 hrs to run on instrument	Automated, batched Simple to train 1-8 samples/ batch	Rapid result 24/7 capability Ct value can be obtained	Back up to GeneXpert
GeneXpert SARS-CoV-2/Flu/RSV [4-plex] 11/20	Multiplex Real time-RT-PCR	E, N2	NP or nasal swab	1 hr	Sample inoculated into single cartridge and placed on instrument	Automated test Simple to train	Rapid result 24/7 capability Ct value available	Inpatients
Cobas SARS CoV-2 (Roche 6800) 1/14/21	Multiplex Real time-RT-PCR	ORF1ab, E	NP, OP, nasal, mid-turbinate	4-24 hrs	Batched, 94 samples	Automated Up to 250-300 samples per shift	24/7 potential Ct value	Inpatient and outpatient
BioFire Respiratory RP2.1 Panel (bioMerieux) 4/5/21	Nested Multiplex RT-PCR, endpoint melting curve	S, M	NP swab	1 hr	Sample inoculated into single pouch and placed on instrument	Automated test Simple to train	24/7 potential No Ct value Detects 15 viruses and 4 bacteria	New admission or immunocompromised outpatient when both SARS CoV-2 & RP ordered
Panther Fusion SARS CoV-2 (Hologic) 6/21	Multiplex Real time-RT-PCR	ORF1ab, 2 regions	NP, OP, nasal, mid-turbinate	6 hrs	Samples tested singly or in batches	Automated test Up to 250-300 samples per shift	24/7 capability Ct value available	Inpatients

EUA, Emergency Use Authorization obtained from the FDA to allow testing

“Throughput “refers to the amount of testing completed in a given time period (e.g. 3 tests per hour, versus 50 tests per hour)

a, Samples are received in Virology. Due to the volume of testing and need for multiple platforms, tests may be performed in the Virology, Microbiology or COVID Testing Laboratories.

b, “Multiplex” indicates that multiple PCRs to detect multiple gene targets are combined in a single vial to simplify workflow

c, Time is from arrival in lab, not from sample collection

d, Sputum preferred for retesting if NP or mid-turbinate swab is negative.

e, If sputum negative and suspicion still high, saliva can be tested. Saliva is not recommended for initial screen. NP swab is the preferred sample, especially on hospitalized patients.

Note: BD Max SARS CoV-2 PCR has been discontinued.