SARS-CoV-2 testing

Comparison of rapid RT-LAMP and rapid RT-PCR methods for the detection of SARS-CoV-2 RNA

Key messages

- Rapid molecular tests with performance comparable to lab-based PCR are an essential tool for prevention of COVID-19 transmission.
- RT-PCR and RT-LAMP are different methods to amplify nucleic acid for the detection of SARS-CoV-2.
- The Thermo Fisher Scientific™ Accula™ SARS-CoV-2 Test is a rapid RT-PCR test used at the point of care that delivers comparable performance to lab-based PCR [1].

Introduction

One of the major challenges during the COVID-19 pandemic has been the development and scaling of reliable methods for SARS-CoV-2 molecular detection. Nucleic acid amplification tests (NAATs) have been widely deployed and are the primary tool for SARS-CoV-2 diagnosis.

NAAT formats vary from complex tests performed in a laboratory to those that can be performed at the point of care (POC) with a Clinical Laboratory Improvement Amendment (CLIA) Certificate of Waiver. The high specificity of the reverse-transcription polymerase chain reaction (RT-PCR) and sensitivity due to its ability to generate billions of copies of a nucleic acid target make this amplification method the current gold standard for the detection of SARS-CoV-2. However, this lab-based RT-PCR test requires well-equipped laboratories and highly trained operators.

Furthermore, although lab-based RT-PCR tests have a total run time of 3–5 hours, in practice, the time from sample collection to result can span days due to the proximity of samples to the testing site.

POC tests with performance characteristics comparable to the lab-based RT-PCR test are a key tool for the rapid detection of nucleic acid from SARS-CoV-2 (which causes COVID-19) and to aid in prevention of community transmission. Two tests that have emerged to meet this need are rapid RT-PCR and rapid reverse-transcription loop-mediated isothermal amplification (RT-LAMP). They are often categorized together as “molecular tests” or “NAATs” (nucleic acid amplification tests). Furthermore, the differences between the approaches may be understated by marketing language used for commercialized products, such as when a LAMP assay is described as “PCR quality”, or “just like PCR” [2,3]. Both RT-PCR and RT-LAMP are methods to exponentially amplify RNA. While the methods may share similar steps in sample preparation (e.g., reverse transcription of viral RNA into complementary DNA) and detection (Figure 1), the power and difference between the methods derive from the approach to amplification, and associated implications for performance and effective use. This white paper focuses on the key differences between these two amplification technologies in order to understand their best application for SARS-CoV-2 detection at the POC.

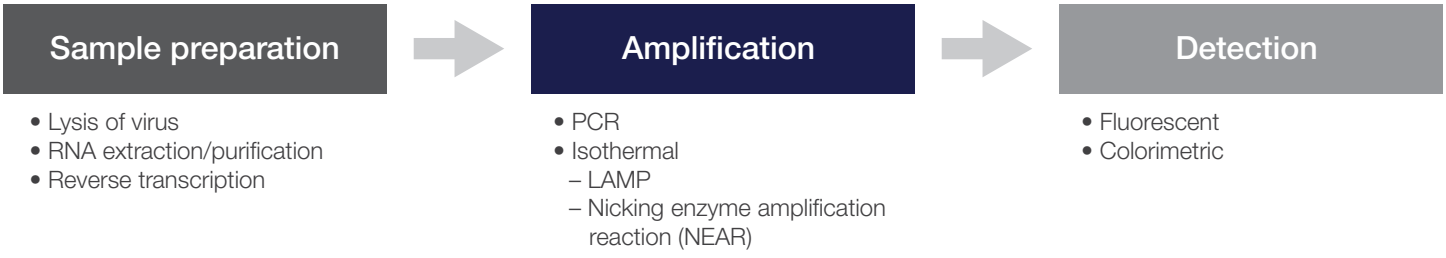

Figure 1. Process steps for the detection of SARS-CoV-2 by NAAT.

Amplification technologies

PCR

Developed in 1983, PCR is a technique for obtaining large amounts of a specific DNA sequence from a double-stranded DNA template. It is broken down into three phases: denaturation, annealing, and extension. The products of each synthesis step serve as a template for the subsequent phases, enabling exponential amplification. Reactions are subjected to repetitive temperature changes in a thermal cycler in which the temperature can shift quickly and precisely from 0–100°C by the Peltier effect.

The denaturation step at a raised temperature, generally 94–96°C, separates the double-stranded template DNA into single strands so that primers can bind to the target region. Annealing of primers to single-stranded DNA is carried out at a temperature generally between 40°C and 70°C. The primers are short single-stranded sequences complementary to regions that flank the DNA to be amplified. Synthesis of the complementary strand takes place during extension, generally at a temperature of 72°C. A thermostable *Taq* polymerase binds to primed single-stranded DNAs and catalyzes replication using the deoxyribonucleoside triphosphates present in the reaction mixture. It takes 20–40 cycles to synthesize an analyzable amount of DNA (about 0.1 µg).

LAMP

Compared to PCR, LAMP is a relatively new technique, developed in 2000 [4]. Unlike PCR, DNA amplification is accomplished via auto-cycling under isothermal conditions, typically 60–65°C; a thermal cycler is not required. While novel implementations of isothermal amplification continue to be developed, LAMP is the best characterized and most widely applied iteration.

LAMP utilizes 4–6 primers to target 6 distinct regions of a target DNA sequence and a DNA polymerase with strong strand displacement activity, such as *Bst* polymerase. There are constraints on the distances between priming sites and the requirements on the free energy of primer binding. LAMP amplification begins by the first set of primers complexing with the target DNA, followed by initiation of DNA synthesis from the DNA polymerase that simultaneously displaces a single strand of DNA. A dumbbell-like DNA structure is formed that serves as the template for subsequent exponential amplification. The annealing and displacement cycles repeat throughout the reaction, and the amplified product grows to form long concatemers. DNA can be amplified up to 10^9-fold within an hour.

Performance of rapid NAATs for SARS-CoV-2 detection

Several commercial technologies based on RT-PCR and RT-LAMP amplifications have been granted the FDA Emergency Use Authorization (EUA) for POC (Table 1). The majority of these offerings have been evaluated for limit of detection (LOD) against the FDA SARS-CoV-2 Reference Panel and can thus be readily compared for analytical sensitivity [5].

Table 1. Rapid, POC RT-PCR and isothermal tests for SARS-CoV-2 [1,5]

Manufacturer	Test	Amplification technology	FDA Reference Panel LOD (NDU/mL)*	Sensitivity limitation noted in IFU**
Thermo Fisher Scientific	Accula SARS-CoV-2	RT-PCR	475 [1]	
Cepheid	Xpert Xpress SARS-CoV-2	RT-PCR	5,400	
Roche Molecular Systems	cobas SARS-CoV-2 & Influenza A/B	RT-PCR	5,400	
BioFire Diagnostics	BioFire Respiratory Panel 2.1	RT-PCR	6,000	
Cue Health	Cue COVID-19 Test	Isothermal	60,000	Negative results in an asymptomatic individual are presumptive [3]
Abbott Diagnostics	ID NOW COVID-19	RT-NEAR† (isothermal)	300,000	Negative results are presumptive [6]
Lucira Health	Lucira CHECK-IT COVID-19 Test Kit	RT-LAMP (isothermal)	Not reported	Negative results are presumptive [2]

* NAAT detectable units per mL.

** IFU: Instructions for Use.

† RT-NEAR: reverse transcription nicking enzyme amplification reaction.

The Accula SARS-CoV-2 Test from Thermo Fisher Scientific relies on a proprietary version of PCR technology to achieve high performance in the POC setting. Oscillating Amplification Reaction (OSCAR™) PCR reduces the absolute temperature requirements for amplification such that there is a smaller temperature differential between denaturing and annealing/extension steps, oscillating over a temperature range of about 20°C with reduced cycling times (Figure 2). The fully automated, sample-to-answer test uses a palm-sized dock to control the reaction temperatures, timing, and fluid movements within a self-contained test cassette.

The FDA Reference Panel LODs for the rapid RT-PCR–based tests are the lowest among the EUA rapid NAATs, with a >100-fold difference in performance between the most sensitive rapid RT-PCR and isothermal tests (Table 1). While FDA Reference Panel evaluation has not been reported for the Lucira™ RT-LAMP test, two clinical studies are described in the package insert [2]. In a study of symptomatic subjects, the Lucira test demonstrated 94% positive percent agreement (PPA) with a high-sensitivity EUA RT-PCR test and 98% negative percent agreement (NPA). In asymptomatic subjects, the test showed 90% PPA and 98% NPA with the RT-PCR comparator. It is noted that the majority of discrepant negative Lucira tests were derived from samples with high C_t values (>37.5) when tested by the comparator assay, suggesting a lower LOD for the comparator RT-PCR.

Notably, all EUA rapid isothermal tests, including RT-LAMP, indicate in their respective IFUs that negative test results should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, should be confirmed with different authorized or cleared molecular tests. This limitation to the intended use is recommended by the FDA in order to mitigate the risk of false negative results [7] and has important implications for the effective use of these tests.

For example, molecular testing guidelines from the Infectious Diseases Society of America state that isothermal assays are an acceptable testing option when rapid RT-PCR or standard lab-based NAAT is not available, but negative isothermal test results in persons with a high suspicion of SARS-CoV-2 infection should be confirmed with standard NAAT or rapid RT-PCR [8]. Similarly, the United States Centers for Disease Control and Prevention (CDC) advises that diagnostic professionals understand test performance characteristics to recognize potentially false-negative or false-positive test results and to direct additional confirmatory testing and management of the patient or person. In a recent guidance [9], they note that it may be necessary to confirm an antigen test result with a NAAT, especially if the result of the antigen test is inconsistent with the clinical context. In this guidance, the CDC further advises that "POC NAATs that generate presumptive results are not appropriate for use in confirmatory testing" [9].

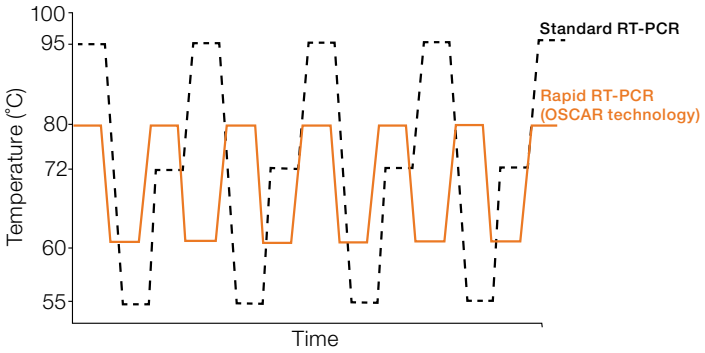

Figure 2. Comparison of standard RT-PCR and rapid RT-PCR (OSCAR technology).

Conclusions

POC NAATs are a promising approach to achieve an expansion in testing, and a diverse range of POC tests based on RT-PCR and isothermal amplification such as RT-LAMP offer rapid, decentralized options. A rapid RT-PCR test such as the Accula SARS-CoV-2 Test is an ideal solution that addresses the requirements for timely performance among the lowest LOD found by the FDA Reference Panel Study [1,5].

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This test has not been FDA cleared or approved but has been authorized for emergency use by FDA for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high, moderate, or waived complexity tests. The test is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.