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Clinical Diagnostic Point-of-Care Molecular Assays for SARS-CoV-2



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KEYWORDS

- Rapid nucleic acid amplification tests (NAATs)
- Point-of-care testing
- COVID-19 pandemic
- SARS-CoV-2 molecular assays
- Method validation

KEY POINTS

- Several rapid, reliable point-of-care (POC) SARS-CoV-2 NAATs are available that provide rapid TATs with analytical performance comparable to traditional methods.
- Validation of POC methods must be done to ensure manufacturer performance specifications for the specific specimen collection and sample handling workflow implemented.
- Rapid POC SARS-CoV-2 NAATs represent a critical piece of the management of the pandemic, both at the POC but also to supplement in-laboratory testing.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic presented clinical laboratories with significant challenges in meeting the demands for molecular testing. These included: (1) the inability to implement laboratory-developed nucleic acid amplification tests (NAATs) during the state of emergency, (2) managing perpetual delays in the availability of Food and Drug Administration (FDA) emergency use authorization (EUA)-cleared assays due to supply-chain issues/reagent allocations, (3) the requirement to perform unprecedented volumes of molecular tests, and (4) all while meeting the expectation that turn-around-time (TAT) become increasingly short as the pandemic proceeded. When conventional severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) NAATs became available to clinical laboratories beyond the Centers for Disease Control and Prevention (CDC) and state-run facilities, it helped address the overall demand for testing in the health care setting. However, it quickly became apparent that these traditional testing formats could not meet one particular,

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and very important, aspect of the demand—the need for rapid TATs for specific patient groups. Such groups include urgent care and emergency room patients with respiratory symptoms compatible with SARS-CoV-2, expectant mothers in active labor, patients who had recovered from infection and were awaiting discharge to skilled nursing facilities or other congregant living settings, and patients of all types who needed to be admitted to hospitals or undergo a potential aerosol-generating procedure (eg, intubation) to ensure they did not infect staff or other patients. Although many institutions were able to implement workflow strategies to overcome the delays of conventional SARS-CoV-2 testing (eg, testing patients 72 hours before planned procedures), inevitably, these workflows would need to be supplemented by the addition of rapid molecular testing options. Point-of-care (POC) testing offered a solution, but it required careful implementation to ensure it was fit-for-purpose. Here, we present our experience implementing rapid POC SARS-CoV-2 NAATs for clinical diagnostic testing at the Brigham and Women’s Hospital (BWH) and Tufts Medical Center (Tufts) in Boston, MA, to highlight strategies used by laboratories to maintain and expand the diagnostic testing services that have been critical for rapidly identifying cases and preventing further spread of the virus.

Traditional Molecular Assays

Before the SARS-CoV-2 pandemic, most clinical laboratories were performing NAATs with conventional polymerase chain reaction (PCR) and reverse-transcriptase PCR (RT-PCR) assays. Examples include HIV and HCV viral loads, and HPV qualitative testing. These assays are FDA-cleared as Clinical Laboratory Improvement Amendments (CLIA) moderately/highly complex assays or laboratory-developed tests (LDTs) running on expensive laboratory-based instrumentation. They all have in common a TAT of several hours but are capable of testing large numbers of samples in that time frame. In the early stages of the pandemic, the laboratory-based SARS-CoV-2 NAATs developed by manufacturers were designed in similar fashion to these traditional molecular assays. A College of American Pathologists (CAP) SARS-CoV-2 proficiency testing (PT) survey from 2020 revealed that 93% of assays in use (13/14) at the time had analytical times that did not meet the requirements for rapid molecular testing.

POC NAATs for other respiratory viruses such as influenza A/B (Flu) and respiratory syncytial virus (RSV) have been commercially available for several years. These platforms are capable of providing rapid TAT results albeit with low throughput, and many are also CLIA-waived, which allows them to be performed in clinical settings by non-laboratory-trained individuals such as nurses and medical assistants. As the pandemic proceeded, these platforms were expanded to diagnose SARS-CoV-2 alone or as a multiplex panel, thus offering a pathway for using rapid molecular results in select patient populations. Accordingly, a significant increase was observed in the number of laboratories using rapid NAAT platforms as their primary method for SARS-CoV-2 diagnostics, from 680 in the 2020 CAP survey to 2739 laboratories in 2021. This is likely an underestimate given that CLIA-waived devices do not formally require participation in a PT program. There is a clear use case for many laboratories to supplement their large laboratory equipment with these single-use, cartridge-based methods within the laboratory itself.

Rapid SARS-CoV-2 NAAT POC Methods

Manufacturers developed SARS-CoV-2 NAATs for systems that were already on the market, with the major examples being the Abbott ID NOW COVID-19,¹ the Cepheid GeneXpert Xpress SARS-CoV-2,² and the Roche LIAT.³ These methods leveraged

existing technologies, and either substituted the primer-probe sets with those specific to SARS-CoV-2 or added them to the influenza/RSV sets to offer a respiratory virus panel. As detailed in **Table 1**, these methods are classified as rapid molecular assays that incorporate either traditional RT-PCR or isothermal nucleic acid amplification and are capable of producing results in less than 60 min. They are cartridge-based methods that incorporate the reagents necessary for amplification of the complementary DNA template of the viral RNA contained within the respiratory sample as well as an internal control to ensure against false-negative results with assay malfunctions. These methods have predominately targeted the RNA-dependent RNA polymerase (RdRP), nucleocapsid N2, and envelope E genes. They have been approved for use in laboratories with high- (H) and moderate- (M) CLIA certificates as well as patient care settings operating under a CLIA Certificate of Waiver (W). Throughout the pandemic, these assays have been available as SARS-CoV-2 only tests but 2 of the 3 major manufacturers we highlight have also incorporated other targets to create a respiratory panel test.

An interesting approach to providing rapid and inexpensive NAATs for SARS-CoV-2 detection at the POC has been the adaptation of reverse transcription loop-mediated isothermal amplification (RT-LAMP) technology.^{4,5} In contrast to conventional RT-PCR, which requires multiple cycles of heating and cooling (thermocycling) to amplify target RNA (**Fig. 1A**), LAMP assays are isothermal. RT-LAMP significantly reduces the amplification time typically required for thermocycling of the primers that extend the DNA template (**Fig. 1B**). Typical RT-LAMP assays can be completed in under an hour, and in some cases as quickly as 15 minutes. One commercial assay, the Lucira CHECK-IT COVID-19 Test Kit,⁶ received FDA EUA for home use with a physician's order and then later, for over-the-counter (OTC) and direct-to-consumer (DTC) use. This method can provide positive results in as little as 11 minutes; however, it has yet to be widely adopted for clinical diagnostic use because of concerns over sensitivity compared to traditional RT-PCR.⁷⁻⁹

Validation

In most US states, tests categorized as CLIA-waived do not require local laboratory validation; they can be used based entirely on the data submitted to the FDA for

Entity, Diagnostic	Date of EUA	Attributes	Authorized Settings
Abbott ID NOW SARS-CoV-2	03/27/2020	RT, Isothermal amplification (≤ 13 min)	H,M,W
Cepheid Xpert Xpress SARS-CoV-2	03/20/2020	Real-time RT-PCR (56 min)	H,M,W
SARS-CoV-2/Flu/RSV	09/24/2020	Multi-Analyte (38 min)	H,M,W
Roche LIAT SARS-CoV-2	6/17/2021	Real-time RT-PCR (~ 20 min)	H,M,W
SARS-CoV-2/Flu	9/14/2020	Multi-Analyte (~ 20 min)	H,M,W

Abbreviations: H, high-complexity testing; M, moderate-complexity testing; W, patient care settings operating under a CLIA Certificate of Waiver.

Summarized from: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-molecular-diagnostic-tests-sars-cov-2#individual-molecular>, Accessed: 07/01/2021

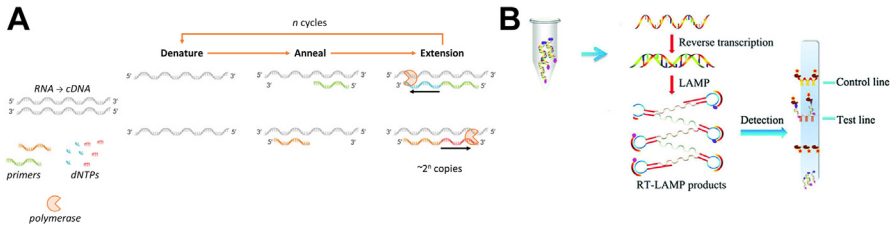


Fig. 1. Comparison of the mechanism of (A) traditional RT-PCR requiring 3 to 5 hours and thermocycling equipment to (B) RT-LAMP technology requiring 10 to 60 minutes performed isothermally. (A) Author's Original; (B) Adapted from Huang et al⁴ with permission from *Frontiers in Microbiology* (2018).

ensuring the test performance characteristics. These methods have been determined by the FDA as simple to perform and have low risk for erroneous results. To maintain this distinction, sites using CLIA-waived tests must strictly follow all of the manufacturer's instructions. Simply put, this classification allows sites with a CLIA waiver to simply plug in the device, train the operators, and follow the instructions to perform patient testing. However, in our experience, this system of FDA review does not necessarily ensure that performance expectations are met when deployed for clinical diagnostic testing, particularly during a crisis on the scale of the COVID-19 pandemic.

Although certain states do not require formal validation studies for CLIA-waived tests, many laboratories still choose to perform validations. The POC SARS-CoV-2 NAATs implemented in clinical laboratories in the United States, just like the CLIA moderately and highly complex counterparts, were EUA-cleared as qualitative tests, which simplifies the validation studies. When reporting qualitatively, the validation of sample precision, accuracy, analytical specificity, and sensitivity are the major focus. Sample precision, or repeatability through replicate testing, typically requires samples with known concentrations—either patient samples with quantitative results determined by a reference method, quality control (QC) material, or calibration standards with assigned values. Ideally, these should be a mix of positive and negative samples near the threshold and not simply at either extreme of the assay results. Accuracy studies are performed to demonstrate the qualitative comparability of results to a reference method. Without knowing the viral copy number or cycle threshold (Ct) values of the positive samples of the samples included in the validation, it would be quite possible for laboratories to miss differences in performance. As shown in **Fig. 2A**, the qualitative comparison of the Roche LIAT was performed against the laboratory-based Panther Fusion and Cepheid GeneXpert methods. Here, 28 residual frozen samples were selected over a wide range of Ct values, which are inversely proportional to viral copy numbers. Only a single sample with an original Ct value of 39, near the positive threshold of 42 on the Panther Fusion, was found to be negative after thawing and running by LIAT. The sample was repeated on the Cepheid GeneXpert and was confirmed to be negative. The analytical specificity, or interference, can largely be inferred from the manufacturer's FDA submission studies and peer-reviewed literature. For the LIAT validation, 10 SARS-CoV-2 negative samples that were positive for commonly circulating pathogens were evaluated and were shown to be 100% concordant with the reference methods.

As it pertains to SARS-CoV-2, many health care providers and infection control specialists have found utility in reviewing Ct values to differentiate patients with high viral loads from those who have a small amount of residual RNA remaining in the setting of resolved infection.^{10,11} The reporting of Ct values is considered a modification of the

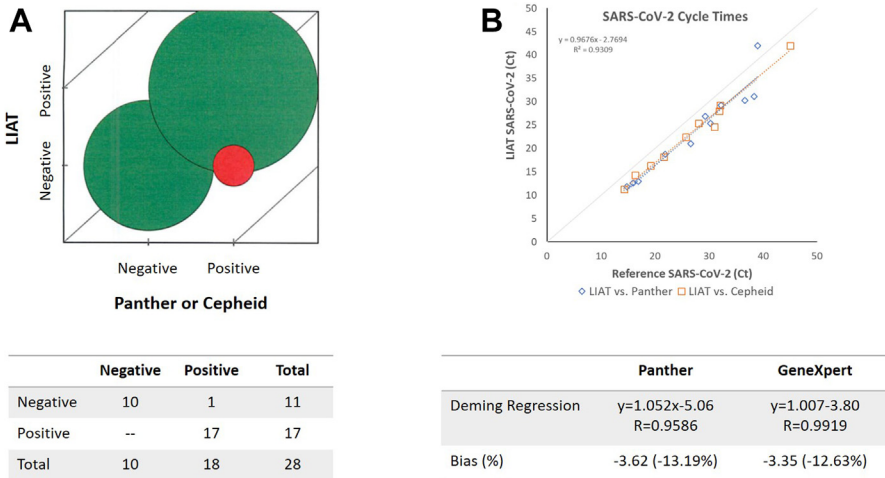


Fig. 2. SARS-CoV-2 LIAT accuracy validation against the traditional Panther Fusion and Cepheid GeneXpert NAAT methods for (A) qualitative and (B) quantitative reporting of cycle time (Ct value).

EUA-cleared method and requires additional validation of quantitative precision to ensure reproducible results and verifying the comparability between methods. Studies for quantitative interassay (between day) precision are typically conducted using QC material, as outlined earlier, with Ct values at multiple points throughout the reportable range to ensure the variability (measured as the coefficient of variation) is clinically acceptable. With respect to quantitative accuracy, Fig. 2B shows excellent correlation in Ct values between the LIAT method and the 2 reference methods that were clinically available at the time, the Panther Fusion and Cepheid GeneXpert. There was a minor systematic bias as the LIAT method is shown to run, on average, 3.5 cycles (12.9%) lower, which is consistent with findings reported in a 2020 letter to the editor of *Clinical Infectious Disease*, in which the CAP Microbiology Committee warned of the risks of interpreting Ct values of SARS-CoV-2 molecular assays.¹² However, aside from the Abbott m2000, the remaining 7 methods analyzed in the letter had median Ct results with relatively good agreement using the same batch of PT material, across multiple manufacturers' methods and various gene targets. Therefore, it would seem reasonable to be able to use this datum to reliably differentiate high versus low Ct values in the clinical context of a given patient's presentation. However, the Ct ranges observed for patients without symptomatic disease or with low levels of virus require additional studies beyond those currently available¹²⁻¹⁴; the details of which are beyond the scope of this article. Regardless, if a laboratory were to choose to report Ct values, the comparability across clinically reported methods must be evaluated and the results accompanied by sufficient interpretation, by way of automatic result comments or other means of appropriately interpretive comments.

Beyond these components of the validation, even for qualitatively reported assays, it is particularly important to verify the manufacturer's claims of performance in terms of the analytical sensitivity and limit of detection (LOD). The traditional method of assessing the analytical sensitivity of NAATs is to prepare a series of reference standard dilutions, which are then run in replicate. The conventional definition of the LOD represents the lowest concentration that can be detected at least 95% of the time (eg, 20 of 21 replicates). Most laboratories cannot run such extensive numbers of

replicates, but they can make a reasonable assessment of the LOD with fewer replicates. As shown in **Fig. 3A**, multiple dilutions of a SARS-CoV-2 standard (5336 copies/mL, Accuplex SARS-CoV-2 Reference Material, SeraCare, Milford, MA) confirmed that the Cepheid assay detected concentrations down to roughly 60 copies/mL. An alternative approach to determining analytical sensitivity, which is perhaps more informative, is to compare clinical specimens against a previously validated, highly sensitive comparison method. As shown in the top panel of **Fig. 3B**, 47 patient samples with estimated copy numbers (log copies/mL) determined using the Abbott m2000 were evaluated by the Cepheid Xpert Xpress, which in this case is the “test” method. The LOD of the Abbott m2000 method had previously been formally confirmed using a reference standard, as described earlier. With this set of clinical specimens, the lowest positive concentration that the Cepheid could determine (ie, with no false-negative results) was approximately 80 copies/mL, which was roughly equivalent to the previous study using the externally purchased standard material. Examples of similar comparisons of 2 other SARS-CoV-2 NAATs are reflected for an LDT method (**Fig. 3C**) and a traditional NAAT (**Fig. 3D**). Neither of these could reliably detect concentrations as low as the Cepheid, with their cut-off limits being roughly 180 and 8500 copies/mL, respectively.

In the absence of a standard curve relating Ct values to viral RNA copy numbers, many studies were reported comparing positive and negative percent agreements (PPA and NPA) with highly sensitive NAATs, the method required by the FDA EUA authorization template for molecular tests.^{15,16} These reports used a wide range of comparator methods and patient populations, but they still provide useful information. **Table 2** shows a compilation of the data from a meta-analysis of the ID NOW,¹⁷ indicating that it has excellent NPA but a suboptimal PPA of approximately 70% (with 30% false-negative results).

An interesting aspect of the Lucira package insert is that it provided clinical performance data as a function of viral RNA concentration.⁶ In 2 studies in community

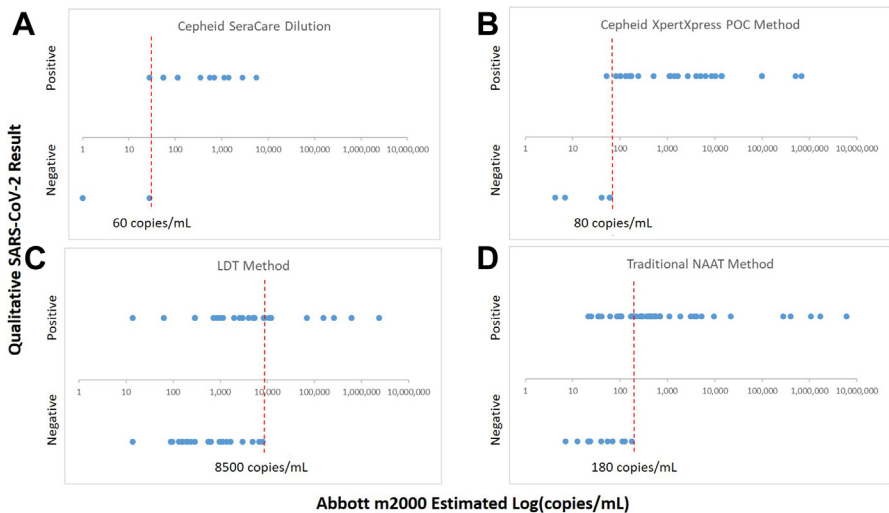


Fig. 3. Analytical sensitivity determination using (A) a standard with known viral copy numbers (SeraCare: 5663 copies/mL) and (B–D) three sets of real patient samples as compared to the estimated copy numbers determined by a highly-sensitive reference method, Abbott m2000, for 3 SARS-CoV-2 NAAT methods. The limit of detection is shown for each method by the dashed vertical line.

Study #	TP	FN	FP	TN	Total	PPA (%)	NPA (%)
1	12	15	0	60	87	44.4	100.0
2	17	14	1	69	101	54.8	98.6
3	12	1	0	169	182	92.3	100.0
4	139	47	2	336	524	74.7	99.4
5	4	2	0	46	52	66.7	100.0
6	33	13	0	15	61	71.7	100.0
7	94	31	0	73	198	75.2	100.0
8	90	6	0	0	96	93.8	UTC
9	65	23	0	25	113	73.9	100.0
10	8	7	0	167	182	53.3	100.0
11	50	7	0	50	107	87.7	100.0
12	0	1	0	116	117	0.0	100.0
13	16	1	1	95	113	94.1	99.0
14	23	0	0	35	58	100.0	100.0
Total	563	168	4	1256	1991	77.0	99.7

Abbreviations: FN, false negative; FP, false positive; NPA, negative percent agreement; PPA, positive percent agreement; TN, true negative; TP, true positive; UTC, unable to calculate.

Summarized from Tu et al. eLife 2021; 10:e65726. DOI: <https://doi.org/10.7554/eLife.65726>. Accessed: 07/01/2021

settings, the Lucira NAAT showed an overall PPA of 92% on 404 specimens from symptomatic and asymptomatic patients, including 10 specimens with very low viral RNA concentrations. Excluding these specimens, whose Ct values were greater than 37.5, the assay was shown to be 98% accurate in comparison to high-sensitivity laboratory assays.

The ability to maintain a single sample type was challenging throughout the pandemic without reliable sources of collection swabs and transport media. Furthermore, experts were concerned about the loss of sensitivity as samples deviated from the preferred nasopharyngeal (NP) swabs. In addition to traditional NP swabs, 3 major POC methods used in clinical laboratories did receive EUA for some combination of anterior nares (nasal) swabs, nasal midturbinate, oropharyngeal, and in some cases, nasal wash/aspirate collection types. A few studies have also suggested reliable results from alternate specimen types including oral fluid,¹⁸ stool, and ocular secretions,^{19,20} but outside of saliva, these are not in widespread use. To ensure comparability in method performance across sample types, it is essential for laboratories to separately validate alternate collection materials/conditions, with a particular focus on specimens with low viral RNA copy numbers.

Implementation

The actual implementation of assays categorized by the FDA as waived and designed to be performed at the POC requires additional considerations beyond assay performance.

Assays that are CLIA-waived have a major advantage in that they have reduced educational requirements for the testing personnel and can be performed by

laboratory accessioning staff, medical assistants, nurses, and other nontechnical staff. This offers the opportunity for implementing these assays within the clinical laboratory itself for triaging specimens with rapid TAT requests, but without requiring the same technologists who meet the educational requirements of moderate- and high-complexity testing. Furthermore, although federal CLIA and state requirements apply whether performing the test in the laboratory or truly at the POC, the laboratory setting is much more conducive to the rigorous laboratory quality essentials as compared with the clinical settings like the emergency department, which are staffed by personnel focused on patient care.

When one considers the workflows affecting the TAT of NAATs used in the laboratory setting, one needs to take into account many factors beyond the analytical time of the assay. These include the time required for:

- Preparing the specimen for transport to the laboratory
- Transporting the specimen, often in a batched process
- Receiving and/or accessioning the specimen into the laboratory information system
- Preanalytical processing, which depends on the NAAT used
- Resulting and transmitting the result to the ordering provider and clinical team.

If the analytical time is measured in hours, these other factors may represent a relatively small fraction of the overall TAT. However, when they are all combined, they lead to significant additional delays compared with implementing these rapid methods at the POC (Fig. 4). Although POC NAATs cannot compete with traditional NAATs within the laboratory, in terms of providing high-throughput testing volumes on a daily basis, they can outperform these tests in providing reliable, actionable results within minutes to an hour of specimen collection (depending on the workflow). In the best-case scenario, sample transport and receipt into the laboratory would be limited to 1 hour, there would be no batching delays and specimens would be tested as they were received, analytical times would be no longer than 2 hours and the instrument would be interfaced with results reporting into the electronic record without significant delay in the provider reviewing the results. With these parameters, the laboratory testing workflow could be as short as 3 hours from the time of collection (see Fig. 4). However, this certainly is not the case for all clinical laboratories and may not even be consistent throughout the week, or even a given day (Fig. 5). Therefore, from a purely TAT standpoint, implementing POC rapid NAATs in the clinic at the point of specimen collection, rather than the laboratory, reduces the TAT to less than 60 min and is mostly reliant on the analytical time of the method.

POC tests can be performed by operators with limited laboratory skills as compared with the highly trained and qualified laboratory personnel required for most traditional NAATs. Indeed, the same person collecting the specimen can run the test, while wearing the same personal protective equipment. To protect staff, laboratories that perform infectious disease testing, whether it be culture or NAAT, often use biologic hoods for specimen preparation. However, for SARS-CoV-2 testing, the use of biologic hoods is not required by the EUAs covering POC NAATs. Arguably, the greatest risk of acquiring SARS-CoV-2 for staff at the POC relates more to their direct interactions with patients, and specifically specimen acquisition, as opposed to specimen processing on the assay. Thus, the personal protective equipment requirements for specimen collection are sufficient for testing personnel to guard against any potential aerosolizing steps (eg, vortexing) of the testing procedure.

We took advantage of a number of these aspects at our respective institutions. At BWH, LIATs were deployed in the microbiology laboratory, as well as in the laboratory

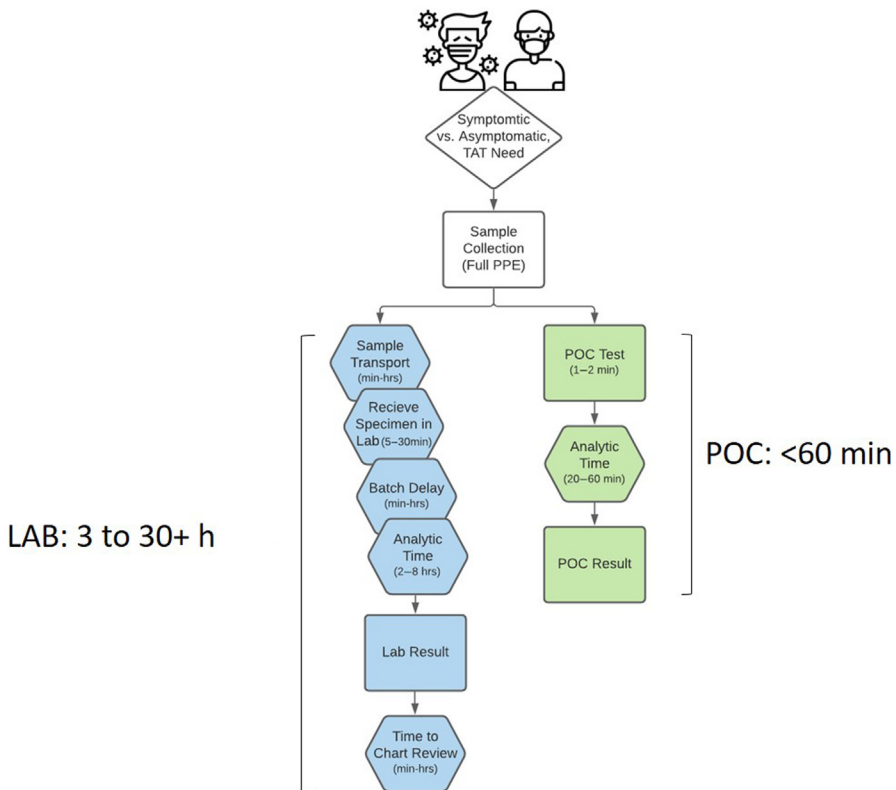


Fig. 4. Comparison of SARS-CoV-2 laboratory-based testing with the POC workflow. Traditional laboratory-based workflows typically incur delays from (1) sample transport, (2) batch delays, and (3) longer analytical time as compared with POC workflows requiring less than 60 minutes and are highly dependent on analytical assay time, which is much shorter.

of an affiliate urgent care clinic, separate from the main hospital. Although not truly implemented at the POC, the laboratories found great value in offering this rapid, cartridge-based method to meet the TAT needs of hospital-based urgent testing (eg, asymptomatic patients presenting to the ED, preprocedural, admit/discharge) and reduce the time for results from the off-site urgent care clinic testing symptomatic patients, that would otherwise incur long-delays in batched transport back to the main campus laboratory. This workflow allowed the laboratory to provide a TAT of approximately 1 hour from sample collection by reducing the analytical time to approximately 20 min on the Roche LIAT (see [Table 1](#)). At Tufts, LIATs and GeneXpert methods were deployed in the central laboratory to achieve the same goal, and the use of these methods was not restricted to just patients in the emergency room, but was expanded, as reagent supplies allowed, to any patient for whom the infectious diseases consulting physicians needed rapid results. In addition, LIATs were deployed as genuine POC devices at 4 individual affiliated urgent care sites, where results were reported in real-time. The total test volumes peaked at roughly 600 per week and often exceeded the volumes of rapid NAATs performed by the laboratory (approximately 250 per week), indicating that they were providing an extremely valuable service in the communities they served.

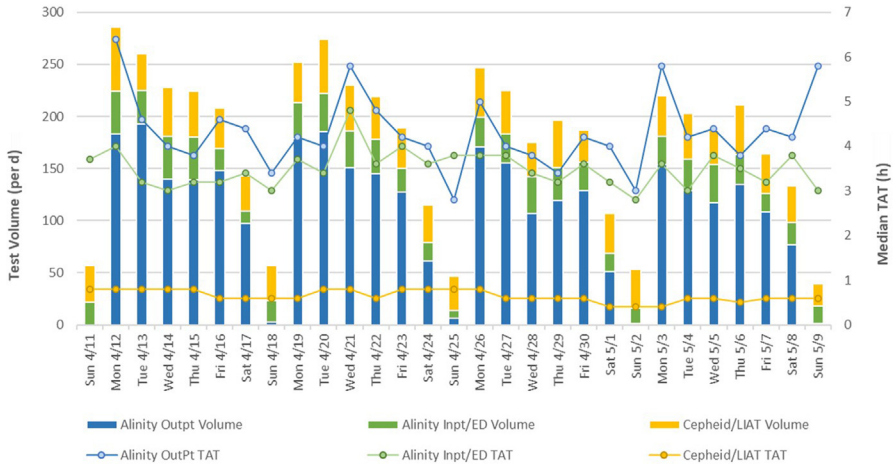


Fig. 5. Daily test volume and median TAT (from receipt in laboratory to result) for the laboratory-based Abbott Alinity method (2-hour analytical time, used for outpatient and inpatient/ED) and Cepheid/LIAT POCT assays performed at Tufts Medical Center. TAT goals were established as: Alinity outpatient: ≤ 6 hours; Alinity inpatient/ED: ≤ 4 hours; and Cepheid/LIAT: ≤ 1 hour.

As previously discussed, attention to specimen types and specimen acquisition is important for all SARS-CoV-2 NAATs. However, this deserves special emphasis for POC testing, where nontechnical staff perform the tests and may be less vigilant about observing requirements. Only the types of specimens covered by the EUA and validated by the laboratory should be processed. Early in the pandemic, only properly collected NP swabs could be used. Midturbinate, anterior nasal, and other specimen types were easier to collect but represented different matrices and likely had different nucleic acid concentrations depending on where the individual was on their viral replication curve. As these other specimen types were added to POC EUAs, they could be used but required in-house validation and clear communication to clinicians as to the potential for reduced performance (see above).

Once collected, specimen handling issues become paramount. Whether testing is performed at the POC or in a traditional laboratory, appropriate labeling of specimens is critical, but the temptation to be more lax with this requirement is far greater at the POC. In most cases, specimen swabs have to be placed in viral transport media, at which point specimen stability for testing is assured for suitable lengths of time. In some cases, however, specimens need to be tested directly within an hour of collection (eg, Abbott IDNOW). In all cases, the testing process must follow the manufacturer's instructions and laboratory validated protocols to remain unmodified and CLIA-waived.

Once testing is complete, it is critical not only to inform the patient's caregivers but also to enter the results into the medical record and to report them to local public health authorities. One of the advantages of POC testing is that informing the patient's caregivers is usually straightforward, especially if the testing is completed while the patient and caregivers are still present. But it is critically important that the information be accurately captured in the medical record, particularly for patient precautions, but to also ensure reporting to public health authorities. Many of the POC devices are capable of being interfaced with laboratory information systems, a feature whose importance should not be understated. The effort required to build and maintain this

interface must also not be underestimated. Manual transcription of results is not as reliable and requires error-checking for accuracy, something that can be challenging to enforce at the POC, particularly when testing volumes are high. As compared with other POC methods (eg, rapid Streptococcal antigen tests), relying on a physician's note to capture SARS-CoV-2 NAAT results severely limits the ability to interface with state reporting mechanisms, challenges the ability for local institution tracking, and managing patient precautions for the safety of other healthcare providers.

Beyond the qualitative result reporting, simply as "positive" or "negative" (or "detected" or "not detected"), the assay performance limitations must be communicated to the ordering provider. At the onset of COVID-19, clear statements of assay limitations were required by the EUA to include disclaimers indicating, for example, that negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other patient management decisions. As time progressed, these statements of limitations were maintained in the EUA but there were no longer requirements for this disclaimer to accompany each result report in the medical record. However, clear understanding and communication of assay performance differences remain.

DISCUSSION

During the initial phases of the COVID-19 pandemic, it was widely recognized that unprecedented numbers of NAATs needed to be performed to monitor and control the spread of the virus. Traditional RT-PCR assays were well suited to addressing this need, with individual instruments capable of performing thousands of tests per day. Even so, demand far surpassed capacity. Clinically significant delays in reporting results (often exceeding 1–2 days and sometimes as long as 5–7 days) occurred at clinical laboratories, public health laboratories, and ultimately even at large commercial laboratories. These delays caused by demand were soon exacerbated by reagent and other supply shortages. As important as it was to provide large numbers of reliable results, there was also a need to deliver faster TATs to manage certain populations of patients, particularly those in urgent care settings and emergency rooms who would require admission to hospitals to determine bed placement and precautions for staff. This was a niche that could be filled extremely well by POC rapid NAATs. The analytical times, almost always less than an hour, were the most attractive aspect of this strategy. One downside of these assays is throughput, which can be made mitigated by limiting testing to those patients who need it most.

In our experience, the analytical sensitivity of POC NAATs is often adequate and in some cases, even better than some conventional laboratory-based RT-PCR NAATs. However, it is critical that they be validated by the laboratory rather than rely solely on manufacturer data. Although the FDA template for EUA for these POC CLIA-waived assays mandated comparison to highly sensitive methods, it did not prescribe that a range of viral concentrations be included in those assessments. Because of differences in the comparison method used, in the populations tested, and in the specimen types used, the NPA and PPA might be excellent even for an assay with less than optimal analytical sensitivity. As one example, the Abbott ID NOW assay was determined by many independent investigators to have lower sensitivity than other NAATs, with differences large enough to be potentially clinically significant. Abbott ultimately updated the ID NOW package insert, indicating that tests could only be done in patients whose symptoms were compatible with COVID-19 and within 7 days of symptom onset, the time frame when viral concentrations in upper respiratory specimens are highest. This does not suggest that the ID NOW is a

poor test. Rather, it means its use should be limited to patients who meet their defined criteria for testing.^{17,21}

With respect to implementation, several aspects of CLIA-waived POC rapid NAATs should be noted. The first is the reduced educational requirements for testing personnel, allowing the tests to be performed by nontechnical staff within the laboratory and at the POC. As a result, workflows can be optimized, allowing for TATs of less than an hour from specimen collection. Second, especially at sites outside the laboratory, vigilance is required in maintaining adherence to specimen types and specimen handling. The third is the importance of capturing results in the medical record and transmitting them to public health authorities, requiring sufficient support and resources to implement and maintain electronic interfacing.

One of the more interesting aspects of POC SARS-CoV-2 NAATs is their costs. In general, the actual cost to produce results with a POC test is far more than the cost to do the same test in the central laboratory. This is often related to the reagent costs and inability to leverage economies of scale like in large central laboratories, as well as incremental labor and capital equipment costs. It was our experience that the costs associated with POC SARS-CoV-2 NAATs were often comparable to their laboratory-based counterparts, unless one pooled specimens or developed one's own assay, which the overwhelming majority of laboratories did not do. Regardless, even if POC NAATs clearly did cost more, their beneficial impact in terms of added safety to the patient, staff, and other patients in hospitals and residents in community living settings arguably more than offset those costs.

It is important to point out that rapid POC NAATs existed pre-pandemic. A particularly good example is for influenza testing. At a time when rapid antigen tests were not sensitive enough to reliably rule out influenza, it was strongly suggested that all negative tests performed at the POC be confirmed by NAAT. As most tests were negative, this translated into excessive delays to get definitive results on most specimens with additive costs for both assays. POC NAATs for flu provided an excellent solution to the problem and enhanced patient care, providing real-time patient-physician interaction and ability to prescribe antiviral medication when indicated. Looking to the future, one wonders whether we can provide POC NAATs for other diseases, like HIV or HCV, where many patients are lost to follow-up before they can be connected to care.²²

In summary, POC SARS-CoV-2 NAATs, properly validated and implemented, represent extremely valuable resources, for POC sites as well as traditional laboratories. They should not be seen, as many POC assays are, as inferior and more expensive counterparts to moderately and highly complex traditional laboratory assays. Central laboratories can, and should, assist with validating and implementing these assays, helping to ensure they are fit for purpose. In doing so, they may find that these assays can play a vital role in addressing their own needs for reliable, appropriately sensitive, rapid turnaround time assays. POC SARS-CoV-2 NAATs represent another example of the continuum of laboratory testing, and it is vital that traditional laboratories embrace them in helping us meet our role in delivering the highest level of quality patient care.

CLINICS CARE POINTS

- Although certain states do not require formal validation of CLIA-waived POC SARS-CoV-2 NAATs, it is highly recommended that laboratories evaluate the method performance.
 - Even when reporting results qualitatively, it is particularly important to determine the limit of detection/analytical sensitivity using specimens with known cycle threshold (Ct) values that span the clinical range.

- Additional considerations exist if Ct values are made available to treating clinicians, including the need to:
 - Determine assay precision (variability within sample)
 - Perform method comparison (variability across methods)
 - Establish the clinical acceptability of the assay for differentiating very low viral copies (residual RNA) from high copies (acute infection, increasing over time)
- POCT methods offer several advantages but implementation and regulatory compliance can be challenging.
 - CLIA-waived methods have reduced educational requirements for operators and can be run by non-laboratory-trained staff.
 - Although not well-suited for high-throughput demands, POC SARS-CoV-2 NAATs can offer rapid TAT at the POC but also when implemented within-laboratory.
 - It can be particularly challenging to maintain compliance with assay and regulatory requirements at the POC, particularly with regards to:
 - Staying within the clinical indications of use (eg, including testing only symptomatic individuals, and/or within a certain number of days since symptom onset)
 - Testing only approved specimen types and following sample handling requirements
 - Communication of assay limitations to treating clinicians and maintaining state/federal reporting requirements

DISCLOSURE

The authors have nothing to disclose.

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