Optimization of Turnaround Time for Group A *Streptococcus* PCR

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ABSTRACT The use of some nucleic acid amplification tests (NAATs) for the diagnosis of group A *Streptococcus* (GAS) pharyngitis allows laboratories to adopt single-tiered testing without reflex culture. However, centralization may delay the delivery of actionable information to the bedside, particularly in the outpatient setting. We describe two novel workflows at our institution and their effect on in-lab turnaround time (TAT) at a tertiary care microbiology lab. Laboratory records were extracted, and relevant data were analyzed after the implementation of qualitative *in vitro* diagnostic testing for GAS with the Xpert Xpress Strep A assay, performed using the GeneXpert Infinity-48s. Workflow optimization steps studied included: (i) direct specimen submission to the microbiology laboratory via the pneumatic tube system and (ii) autoverification of GAS NAAT results in the laboratory information system. Between April 2018 and October 2018, 2,595 unique specimens were tested for GAS by PCR. Of these, 2,523 were included in the final analysis. Linear regression established that the total in-lab TAT was significantly reduced by direct specimen submission to the microbiology laboratory, autoverification, and processing during the night shift. We describe two workflow optimization methods that reduced the in-lab TAT for GAS NAAT. Although microbiology labs historically use manual processes, the advent of total laboratory automation and the adoption of on-demand NAATs will allow for more streamlined processing of microbiology specimens. It may be beneficial to consider instrument interfacing and specimen processing optimization during the early phases of implementation planning for NAATs in the microbiology laboratory.

KEYWORDS NAAT, PCR, autoverification, group A *Streptococcus*, pharyngitis, specimen processing, turnaround time

Acute pharyngitis is a common cause of outpatient and urgent care visits in the United States (1). group A *Streptococcus* (GAS) is the most significant bacterial pathogen causing pharyngitis, and it is associated with the risk for a wide range of suppurative and nonsuppurative complications following untreated infection, particularly in pediatric patients (2). Due to the high volume of visits and the need for accurate diagnosis, considerable effort has been made by the medical and laboratory communities to streamline testing for this common chief complaint (3).

In recent years, molecular diagnostic methods have been cleared by the U.S. Food and Drug Administration for the laboratory diagnosis of GAS pharyngitis from throat swabs (4). Predicate laboratory workflows for microbial diagnosis of bacterial pharyngitis often relied on a two-tiered testing approach, where insensitive GAS rapid antigen detection tests (RADTs) were followed by sensitive bacterial culture reported at 24 or 48 h (5). In contrast, molecular methods have demonstrated excellent analytic sensitivity, eliminating the need for the additional step of conventional culture (6). Accordingly, institutions are migrating toward molecular testing for the laboratory diagnosis

Citation Durant TJS, Merwede J, Reynolds J, Peaper DR. 2019. Optimization of turnaround time for group A *Streptococcus* PCR. J Clin Microbiol 57:e00619-19. <https://doi.org/10.1128/JCM.00619-19>.

Editor Paul Bourbeau

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Received 13 April 2019

Returned for modification 4 May 2019

Accepted 13 June 2019

Accepted manuscript posted online 19 June 2019

Published 26 August 2019

of GAS pharyngitis to reduce the specimen-processing overhead and the regulatory challenges associated with RADT and culture without sacrificing sensitivity (7).

Although elimination of the two-tiered testing workflow is undoubtedly attractive, central laboratories must realize the predicate point-of-care (POC) RADTs provided clinically actionable test results at the bedside, if positive. Accordingly, in an effort to minimize patient wait-time, particularly in the emergency departments (EDs), optimization of in-lab specimen processing for GAS nucleic acid amplification testing (NAAT) was a primary consideration at our institution prior to implementation. With this in mind, we simultaneously implemented two novel workflows at our institution, including (i) direct specimen submission to the microbiology laboratory via the pneumatic tube system and (ii) autoverification of GAS NAAT results in the laboratory information system (LIS). In particular, we sought to determine the in-lab turnaround time (TAT) performance across shifts at a tertiary care microbiology lab in the setting of pneumatic tube system modifications and autoverification.

MATERIALS AND METHODS

Testing. Qualitative *in vitro* diagnostic testing for the detection of *Streptococcus pyogenes* (group A beta-hemolytic *Streptococcus*) was done using an Xpert Xpress Strep A assay (Cepheid, Sunnyvale, CA) according to the manufacturer's instructions. Testing was performed on the GeneXpert Infinity-48s (Cepheid, Sunnyvale, CA) using throat swab specimens collected with a liquid Amies elution swab (ESwab; Copan, Murrieta, CA) collection and transport system. The Xpert Xpress Strep A assay was fully implemented in April 2018 and made available for clinical testing 24 h a day, 7 days a week. All testing was performed in the clinical microbiology laboratory. STAT (i.e., urgent) ordering was available to providers, and all testing was done as samples arrived in the laboratory without batching. Specimen processing shifts are defined as day (0730 to 1600), evening (1600 to 0030), and night (0000 to 0800).

The microbiology laboratory operates 24 h per day 7 days per week. The microbiology laboratory employs a total of 28.6 full-time equivalents, which covers testing for routine bacterial cultures, mycology, and acid-fast bacilli. In the fiscal year of 2019, the microbiology laboratory performed 55,000 blood and 63,000 urine cultures and 40,000 and 30,000 identifications and susceptibility tests, respectively. The microbiology laboratory services a tertiary care hospital with approximately 1,500 licensed beds, as well as 37,000 and 188,000 visits per year in the pediatric and adult EDs, respectively.

Autoverification. Initial installation of the GeneXpert Infinity-48s included the establishment of bidirectional interface connections with the laboratory middleware system (Data Innovations, South Burlington, VT) and the laboratory information system (LIS; Epic, Madison, WI). The laboratory middleware system is indirectly connected to the LIS via the institutional integration engine (Infor, New York, NY) over a private network. Messages between disparate systems are formatted according to American Society for Testing and Materials-1934 (ASTM-1934) and Health Level 7 (HL7) standards.

Autoverification rules were implemented in the LIS, with the laboratory middleware system or integration engine as pass-through touch points. Criteria for autoverification included the absence of instrument error flags and unexpected abnormality flags (e.g., "critical," "low critical," and "high critical"). In addition, autoverification would fail if the result was identified as a repeat test. If autoverification criteria were met, the associated result value was automatically released from the instrument and published to the patient chart, without the need for manual verification by a medical laboratory technician (MLT) (see Table S1 in the supplemental material). If autoverification criteria were not met, the result would be flagged on the instrument indicating the need for manual review by an MLT. The bidirectional instrument interface was active when testing was made clinically available (i.e., at go-live) in April 2018, but autoverification was not validated and active until July 2018.

Pneumatic tube system modification. Pneumatic tube system (PTS) input stations in the adult and pediatric EDs were programmed to allow submission directly to the microbiology lab. PTS input station routing typically relies on an input code to program the destination of the specimen. To optimize accessibility of this new function for staff, a preprogrammed button was implemented in the PTS control panel in the adult and pediatric EDs (see Fig. S1 in the supplemental material). ED personnel were educated prior to go-live that the microbiology routing option was available in an effort to minimize reliance on predicate routing practices to the core laboratory. Specimens submitted to the core laboratory which were then sent to the microbiology laboratory via a robotic courier will be subsequently referred to as the "core standard" method, while specimens submitted directly to the microbiology laboratory via the PTS will be referred to as the "direct to micro" method. In addition, specimens collected in the pediatric ED and submitted to the core laboratory were sent to the microbiology laboratory via the intralaboratory PTS rather than the robotic courier system; this is denoted as "core new."

Data collection. Data were retrospectively exported from the instrument management software for the GeneXpert Infinity-48s and the LIS. Data from the instrument provided timestamps for assay start and stop times, which are not routinely stored in the LIS. Data sets were combined using unique specimen identifiers. Data from the LIS included timestamps which represented relevant touch points for in-lab processing, including time received in the core-receiving section and the microbiology laboratory, and the time the result was final verified. In addition, LIS data included test utilization data such as specimen source, collection department, and patient demographic information.

FIG 1 Timestamps and associated time intervals for operational assessment of specimen processing: A “first received” timestamp denotes when a specimen was first scanned in the lab. A “track to micro” time stamp indicates when a specimen was scanned in the microbiology laboratory. A “PCR start” timestamp indicates when the GAS PCR assay began. A “PCR end” timestamp indicates when the GAS PCR assay was completed. A “verified” timestamp indicates when the result was finally verified and released to the electronic health record. (A) Time from first received to microbiology tracked; (B) time from microbiology received to PCR assay start; (C) assay run time; (D) time to result verification; (E) time from first received to assay start; (G) total in-lab turnaround time.

Inclusion criteria. All specimens submitted to the microbiology laboratory with an order for GAS testing by PCR between April 2018 and October 2018 were considered for inclusion in this study. TAT intervals associated with specimens were examined for outliers and excluded if testing required multiple runs for resolution, autoverification failure, intermediate result, patient/specimen identification error, or an instrument error.

Data analysis. Autoverification status for unique specimens is not accurately recorded as a discrete data element in the LIS or instrument management software. Accordingly, a surrogate data element which indicated test resolution status (i.e., whether single or multiple tests were required to resolve a verified test result for a unique specimen) was used to indicate autoverification status. If a specimen test result was resolved with a single instance of testing after autoverification go-live, it was considered to have autoverified. Comparisons between assay end time and sample verification time confirm this. Similarly, there is no discrete data element that delineates which specimens were routed directly to the microbiology laboratory. A tracking stop in the LIS indicates if a specimen was received in the core laboratory, and specimens were considered to be directly tubed to the microbiology laboratory if the specimens were collected in the pediatric or adult emergency department and received in microbiology first, rather than the core laboratory.

Timestamps for laboratory processing touchpoints were used to calculate seven clinically relevant specimen processing intervals, including in-lab TAT (Fig. 1). The distribution, mean, and standard deviation in minutes within each TAT interval were assessed and were not normally distributed. The median and interquartile ranges (IQR) for specimen processing time were calculated and stratified by experimental groups which included specimen submission method and autoverification. Median specimen processing time was further separated by shift to determine whether laboratory staffing levels affected TAT. Pairwise comparisons were assessed using the Mann-Whitney U test, and the Kruskal-Wallis test was used to compare the median TAT intervals between autoverification and specimen submission groups (8). Bonferroni corrections were used to account for multiple comparisons. Lastly, a linear regression was performed to evaluate the effect of variables on total in-lab TAT. Statistical analysis was performed using SPSS version 24 (IBM, Armonk, NY).

RESULTS

Test utilization and performance statistics. Between April 2018 and October 2018, 2,595 unique Eswab specimens were tested for GAS by PCR. Of these, 2,523 were included in the final analysis. Specimens were excluded ($n = 72$) for a variety of reasons, the most common of which was specimens requiring multiple assay runs for a final result and single runs which were not autoverified, reasons for which were not documented and unable to be surmised from associated specimen metadata on review in the LIS (Fig. 2).

Changes to the pneumatic tube system allowing direct submission to the microbiology laboratory went live in April 2018, and autoverification rules for the Xpert Xpress Strep A assay went live in July 2018. Of the complete cohort of specimens considered for inclusion in the analysis in the post-AV era, 97.8% ($n = 1175$) specimens successfully autoverified. Detection rates differed between pre- and post-AV cohorts, which was likely due to the seasonal prevalence of GAS. Expectedly, the majority of specimens were ordered from outpatient and pediatric practice locations, comprising 69.9% ($n = 1,731$) of the included unique specimens. Of the specimens included in the final analysis and collected in either the adult or pediatric ED, 24.3% ($n = 310$) were submitted to the core receiving and robotically sent to microbiology, 16.9% ($n = 216$) were submitted to core receiving and pneumatically tubed to microbiology, and

FIG 2 Diagram of samples considered for inclusion in final analysis with respect to autoverification cohorts. (A) Summary of specimens excluded from analysis and associated exclusion criteria. (B) Specimen cohorts pre- and postautoverification go-live subcategorized by specimen-processing shift.

58.8% ($n = 750$) were submitted directly to microbiology via the novel PTS workflow (Table 1).

TAT statistical analysis. A Mann-Whitney U test was run to determine whether there were differences in TAT intervals between results which were autoverified and results manually verified. Median TAT intervals (Table S2) were statistically significant for intervals D, E, and G ($P < 0.0125$), with a Bonferroni correction for multiple comparisons (Fig. 3A), with an absolute reduction in median in-lab TAT of 8 min. A Kruskal-Wallis test was conducted to determine whether there were differences in TAT intervals between specimen routing groups, i.e., core standard ($n = 310$), core new ($n = 216$), and direct to micro ($n = 750$), which demonstrated statistically significant differences among the different specimen submission routes for median TAT intervals A ($\chi^2 = 872.43$, $P < 0.05$), B ($\chi^2 = 24.95$, $P < 0.05$), and G ($\chi^2 = 597.35$, $P < 0.05$) (Table S3), with an absolute reduction in median in-lab TAT of 33 min. Subsequent pairwise comparison were performed using Mann-Whitney U testing with a Bonferroni correction for multiple comparisons. This *post hoc* analysis demonstrated statistically significant differences as depicted in (Fig. 4A). The median in-lab TAT (interval G) for specimens that were autoverified and sent directly to the microbiology lab was 32 min (IQR, 28 to 39 min), and it was 80 min (IQR, 61 to 100 min) for specimens that were manually verified and submitted to the core laboratory via the standard method ($P < 0.0001$).

Lastly, linear regression established that total in-lab TAT was significantly reduced by: (i) specimen submission to the microbiology laboratory ($\beta = -32.26$; standard error [SE] = 1.35; $P < 0.001$) and (ii) processing during the night shift ($\beta = -8.21$; SE = 1.98; $P < 0.001$). The total in-lab TAT was significantly increased by (i) no autoverification ($\beta = 8.66$; SE = 1.14; $P < 0.001$) and (ii) standard specimen routing ($\beta = 3.92$; SE = 1.83; $P < 0.05$). Variables included in the model accounted for 24.5% of the variability of the total in-lab TAT with an adjusted R^2 of 0.244.

TABLE 1 Specimens included in final analysis summarizing basic demographic information, test utilization data, and specimen submission practices^a

Parameter	Pre-AV	Post-AV
No. of specimens	1,359	1,164
Mean age (yr) $\pm$ SD	24 $\pm$ 20	24 $\pm$ 20
Gender, no. (%)		
Male	535 (39.4)	476 (40.9)
Female	824 (60.6)	688 (59.1)
Result, no. (%)		
Not detected	1,047 (77)	1,005 (86.3)
Detected	312 (23)	159 (13.7)
Assay runs to resolution, no. (%)		
Single	1,360 (97.6)	1,175 (97.8)
Multiple	33 (2.4)	27 (2.2)
Ordering location, no. (%)		
Outpatient	593 (44.3)	463 (40.6)
Pediatric ED	371 (27.7)	304 (26.7)
Adult ED	311 (23.2)	290 (25.5)
Inpatient	63 (4.7)	82 (7.2)
Specimen transport from ED, no. (%)		
Direct to micro	418 (61.3)	332 (55.9)
Core standard	148 (21.7)	162 (27.3)
Core new	116 (17)	100 (16.8)

^aAbbreviations: AV, autoverification; ED, emergency department.

DISCUSSION

The added sensitivity of PCR-based assays for GAS detection comes with the added benefit of moving away from two-tiered testing algorithms seen with RADTs. However, for laboratories not implementing GAS NAAT in a POC manner, centralization may delay the delivery of actionable information to the bedside, particularly in the outpatient setting. Although GAS NAATs are available for POC implementation, there are barriers to their implementation in outpatient clinics and care environments with high-patient-acuity levels, such as EDs (3, 9, 10). Accordingly, as clinical microbiology laboratories seek to incorporate GAS NAAT within the central laboratory environment, methods for maintaining rapid in-lab TATs, particularly for the ED, should be considered prospectively.

In this study, we describe two workflow optimization methods that significantly reduced the in-lab TAT for GAS NAAT in the microbiology laboratory. While the microbiology lab is historically oriented around manual processes, the advent of total laboratory automation and the increasing adoption of on-demand molecular methods will allow for more streamlined processing of microbiology specimens (11). As seen in the results of this study, implementing autoverification resulted in an appreciable reduction in the median in-lab TAT (interval G) throughout staffing shifts, demonstrating an 8-min differential between autoverification groups (Table S2). Accordingly, it may be beneficial for microbiology managers and directors to consider instrument interfacing with middleware or the LIS, as well as autoverification, during early phases of implementation planning for PCR-based assays in the microbiology laboratory.

When considering the effect of shift on specimen processing time, our *a priori* hypothesis was that processing during the night shift would result in the longest TAT due to lower staffing. However, we found that TAT was longest on the evening shift and appreciably shorter during the night shift. In retrospect, longer a TAT during the evening shift is likely because this shift has the highest test to staffing ratio due to the delivery of specimens from outpatient clinics. Accordingly, visual analysis and linear regression demonstrate the night shift has the lowest TAT, which is likely due to lower specimen volume and limited service responsibilities. Notably, the night shift is only

FIG 3 (A) Median autoverification times for specimens in the pre- and postautoverification cohorts with IQRs represented by error bars. Pairwise comparisons between pre- and postautoverification cohorts were performed using the Mann-Whitney U test (***, $P < 0.0125$). (B) Median autoverification times for specimens in the pre- and postautoverification cohorts with IQR error bars subgrouped by shift. Interval: D, time to result verification; E, time from first received to assay start; G, total in-lab turnaround time.

responsible for blood cultures, STAT and priority specimens, and molecular testing, with responsibilities for plating or reading routine cultures if time permits.

Specimen transport optimization, particularly for chief complaints which rely on fast and accurate diagnostic information, can also be considered during instrument implementation planning. As seen in the results of this study, PTS transport options that allow specimen submission directly to the microbiology laboratory can result in a much more significant decrease for in-lab TAT. It should be noted, however, that implementation of this change in ED workflow required significant coordination with hospital facility staff to implement and test the changes to the PTS, as well as education of the nursing staff to ensure use of the new specimen routing option.

The logistical overhead of implementing changes to the PTS may not be feasible for all laboratories, depending on competing priorities of the managers and directors. An alternative strategy for specimen submission optimization would be to instruct the core-receiving laboratory to handle STAT GAS NAAT specimens differently, such as, in our case, to receive specimens and then subsequently tube the specimen directly to the microbiology laboratory rather than relying on human or robotic couriers. However,

FIG 4 (A) Median autoverification times for specimens in specimen transport cohorts with IQRs represented by error bars. Pairwise comparisons between pre- and postautoverification cohorts were performed using the Kruskal-Wallis test (***, $P < 0.0125$). (B) Median autoverification times for specimens in specimen transport cohorts with IQR error bars separated by shifts. Interval: A, time from first received to microbiology tracked; B, time from microbiology received to Cepheid assay start; G, total in-lab turnaround time.

results from this study did not demonstrate a significant reduction with in-lab TAT when this specimen-receiving workflow was used.

Limitations of this study include a lack of randomization of specimens into autoverification and specimen submission cohorts. While this may have allowed more equal distribution of specimens across cohorts, we believe the overall effect to be minimal given the large number of specimens involved and their distribution across the relevant comparator groups. In addition, autoverification cohorts included periods of time with different GAS prevalences. The effect of seasonal prevalences is likely negligible, since none of our interventions depended on the test result, and there was a minimal difference of 65 additional tests per month in the preautoverification cohort.

With microbiology trending toward automation and high-throughput testing, the need and opportunities exist for optimization of current specimen handling and result reporting processes. With the move from molecular testing for acute pharyngitis, our hospital implemented and leveraged direct specimen submission and autoverification for optimization of the in-lab TAT for GAS PCR testing. Although AV is commonly used in other areas of laboratory medicine, experience with this method in clinical microbiology is not well described or familiar to practicing laboratorians. Future research will seek to determine the clinical impact of STAT GAS NAAT compared to a two-tiered approach.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/JCM.00619-19>.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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