



Optimal Timing of Repeat Multiplex Molecular Testing for Respiratory Viruses

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ABSTRACT Determining whether and when multiplex nucleic acid amplification tests (NAATs) for respiratory viruses should be repeated is difficult. We analyzed 5 years of results for a multiplex NAAT targeting 14 respiratory viruses, to determine how often repeat tests were ordered and the time period in which results were likely to change. Results for NAATs performed on nasopharyngeal specimens and repeated within 90 days after initial testing were analyzed. Logistic regression models were used to compare time periods between tests with respect to the odds of a change in the sample result. During the study period, 21,819 nasopharyngeal specimens from 16,779 individuals were submitted. Of these, 8,807 samples (40%) were positive for at least one viral pathogen. Among this cohort, 2,583 specimens (12%) collected from 1,473 patients (9%) were repeat tests performed within 90 days after an initial test. If repeated within 90 days, 71% of tests (1,833 tests) did not have a change in result. Initially negative tests typically remained negative, whereas initially positive tests mostly remained positive until 11 to 15 days. The odds of result change plateaued after 20 days. The odds of result change for tests repeated within 20 days were only 0.52 times the odds (95% confidence interval, 0.43 to 0.62) for those repeated at 21 to 90 days ($P < 0.001$). Multiplex tests for respiratory viruses that are repeated within short periods lead to redundant results at additional costs. Repeat testing of nasopharyngeal specimens before 20 days demonstrates little change. These results provide a vital component for use in laboratory stewardship to curtail unnecessary respiratory viral testing.

KEYWORDS laboratory stewardship, NAAT, community-acquired pneumonia, influenza, multiplex PCR, pneumonia, repeat testing, respiratory syncytial virus, respiratory viral panel, respiratory viruses

Respiratory tract infections account for substantial proportions of inpatient, outpatient, and emergency department visits, with an estimated \$17.3 billion in direct annual costs (1, 2). Viruses are recognized as the most common group of pathogens identified in cases of upper and lower respiratory tract disease in both children and adults (3, 4). As a result, advancements in viral detection have markedly improved our ability to quickly identify key agents causing respiratory illness (5). Multiplex nucleic acid amplification tests (NAATs) are now commonly employed for the diagnosis of respiratory tract illness, due to their short turnaround times, high throughput, and ability to simultaneously identify multiple pathogens with high sensitivity and specificity (6). Testing with these platforms has been associated with reduced hospital stays, decreased antibiotic use, and more timely infection prevention interventions (7, 8).

The global market for multiplex assays targeting genetic markers or microbial pathogens was estimated at \$2.17 billion for 2017 and is projected to reach upwards of \$3.35 billion in 2023 (9). To date, over 25 multiplex nucleic acid-based tests have

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been cleared or approved by the FDA for the simultaneous detection of multiple viral species (10). The estimated cost per test (not including capital purchases) to perform a multiplex respiratory assay ranges from approximately \$45 to \$121; variables include reagent costs, test volume, hands-on time, labor costs, and frequency of quality control (11). Medicare payments for molecular tests have consistently decreased since 2014, including a 37% decrease between 2015 and 2016 (12). Additionally, the Centers for Medicare and Medicaid Services (CMS) recently concluded that multiplex respiratory viral panels do not meet Medicare's "reasonable and necessary" standard for the diagnosis and treatment of medical illness. The CMS would like to see more evidence to support multiplex screening's nonsyndromic approach, fixed nature, and inclusion of additional pathogens of low clinical relevance and epidemiological prevalence (13).

The higher costs and reimbursement challenges associated with molecular testing underscore the need to avoid inappropriate use. While there are numerous reports evaluating the clinical utility and accuracy of multiplex NAATs for the initial diagnosis of respiratory tract illness, we are unaware of guidelines defining the optimal frequency of subsequent pathogen testing for patients with prolonged or progressing symptoms (14, 15). As a result, unnecessary testing may be repeated for patients over short periods. This overuse places a financial burden on patients and health care systems when not reimbursed. We analyzed multiplex viral panel results for children and adults presenting at the Cleveland Clinic over a 5-year period, to determine the time when a change in results would be expected.

MATERIALS AND METHODS

Sample collection. Results for nasopharyngeal specimens submitted to the Cleveland Clinic between 1 November 2013 and 6 June 2018 for respiratory pathogen testing using the GenMark real-time PCR assay (GenMark Diagnostics, Carlsbad, CA) were analyzed. Patient specimens originated from outpatient clinics, inpatient wards, and emergency departments, at the discretion of the on-service physicians; the specimens were placed in universal viral transport medium and transported to the central laboratory for testing. The GenMark real-time PCR assay includes targets for 14 viruses associated with upper and lower respiratory tract disease, including respiratory syncytial virus A and B, parainfluenza virus 1, 2, and 3, human metapneumovirus, rhinovirus, adenovirus types B, C, and E, influenza A types H1, H1N1(2009), and H3, and influenza B (GenMark Diagnostics).

Initial and subsequent viral panel results for patients who had more than one sample submitted within 90 days were reviewed. Subsequent sample calculations were made on a rolling basis (i.e., the days from the initial specimen to a subsequent specimen were calculated from the dates of each repeat specimen and the most recent prior specimen). Specimens with indeterminate results and those originating from sources other than the nasopharynx were excluded. The change in sample result was defined as a change from overall positive or overall negative, with positive indicating any positive result for any of the 14 viral targets. The change in any virus was defined as a change in sample result in addition to any identification of a new or additional viral pathogen (or pathogens).

Statistical methods. Data were described using medians and quartiles for continuous variables and counts and percentages for categorical variables. Associations of specimen results, collection of subsequent specimens, and changes in results versus specimen, subject, and test characteristics were assessed using Wilcoxon rank sum tests for continuous and ordinal characteristics and chi-square or Fisher's exact tests for categorical characteristics. Logistic regression models were used to compare time intervals between tests with respect to the odds of a change in test result. All analyses were performed on a complete-case basis. All tests were two-tailed and performed at a significance level of 0.05. SAS 9.4 software (SAS Institute, Cary, NC) was used for all analyses. Graphs were constructed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

RESULTS

Between 1 November 2013 and 6 June 2018, 21,819 nasopharyngeal specimens, originating from 16,779 individuals, were submitted to the Cleveland Clinic microbiology laboratory with an order for respiratory virus detection by multiplex NAAT. Of these, 8,807 samples (40%) were positive for at least one viral pathogen, while 13,012 (60%) were negative. Approximately one-third of the specimens (7,736 specimens [35%]) were from children, including 2,980 (39%) from infants <1 year of age, whereas 14,083 specimens (65%) originated from adults (≥ 18 years of age). Samples from children were more than twice as likely to be positive, compared with samples from adults (62% versus 28%; $P < 0.001$).

Among these specimens, 2,583 nasopharyngeal specimens (12%) from 1,473 pa-

TABLE 1 Characteristics of subsequent samples taken within ≤90 days, according to prior sample test result and age group

Factor	No. (%)			P	No. (%)		P
	Total (n = 2,583)	Negative initial result (n = 1,695)	Positive initial result (n = 888)		Adult (n = 1,561)	Child (n = 1,022)	
Days from baseline				0.062 ^a			0.20 ^a
0–1	179 (7)	148 (9)	31 (3)		127 (8)	52 (5)	
2–5	103 (4)	80 (5)	23 (3)		52 (3)	51 (5)	
6–10	235 (9)	138 (8)	97 (11)		138 (9)	97 (9)	
11–15	262 (10)	167 (10)	95 (11)		145 (9)	117 (11)	
16–20	210 (8)	123 (7)	87 (10)		125 (8)	85 (8)	
21–25	199 (8)	123 (7)	76 (9)		113 (7)	86 (8)	
26–30	178 (7)	119 (7)	59 (7)		100 (6)	78 (8)	
31–45	420 (16)	287 (17)	133 (15)		260 (17)	160 (16)	
46–60	311 (12)	202 (12)	109 (12)		191 (12)	120 (12)	
61–75	250 (10)	155 (9)	95 (11)		160 (10)	90 (9)	
76–90	236 (9)	153 (9)	83 (9)		150 (10)	86 (8)	
Change or not in sample result				<0.001 ^b			0.001 ^b
Change	750 (29)	376 (22)	374 (42)		417 (27)	333 (33)	
No change	1,833 (71)	1,319 (78)	514 (58)		1,144 (73)	689 (67)	
Change in sample result				<0.001 ^b			<0.001 ^b
Remain negative	1,319 (51)	1,319 (78)	0 (0)		922 (59)	397 (39)	
Become positive	376 (15)	376 (22)	0 (0)		199 (13)	177 (17)	
Remain positive	514 (20)	0 (0)	514 (58)		222 (14)	292 (29)	
Become negative	374 (14)	0 (0)	374 (42)		218 (14)	156 (15)	
Change in any virus result				<0.001 ^b			<0.001 ^b
No change	1,590 (62)	1,319 (78)	271 (31)		1,073 (69)	517 (51)	
Change	993 (38)	376 (22)	617 (69)		488 (31)	505 (49)	

^aWilcoxon rank sum test.^bPearson's chi-square test.

tients (9%) were collected and rescreened within 90 days after the initial test. These repeat specimens included 1,561 specimens (60%) from adults (median age, 57 years), while 1,022 specimens (40%) originated from children (median age, 2 years), of whom 265 (26%) were infants. In total, 779 samples (30%) were repeated within 15 days, 1,366 (53%) within 30 days, and 2,097 (81%) within 60 days after the initial sample (Table 1). The median time from baseline to repeat testing was 28 days (27 days for children and 30 days for adults; $P = 0.22$).

Overall, we found that repeat multiplex NAATs for respiratory viruses were unlikely to demonstrate a change in respiratory viral panel test results if the specimens were collected within 90 days after the initial specimen (Table 1 and Fig. 1D). Only 750 (29%) of all repeat specimens obtained within 90 days had a change in test result. This change in any test result increased over time, with discrepant findings in only 3% of repeat tests obtained within 1 day but in 36% of tests repeated between 76 and 90 days (see Table S1 in the supplemental material). When initial specimens tested negative for respiratory viruses, subsequent test results frequently remained negative throughout the 90-day follow-up period (Fig. 1A). Conversely, positive samples continued to screen positive until 11 to 15 days, after which the percentage of samples that continued to test positive converged with an increasing percentage of samples that tested negative (Fig. 1B). Still, 58% of initially positive specimens remained positive for the same virus when testing was repeated during the 90-day follow-up period. Interestingly, identification of any new or additional pathogens through repeat testing was infrequent within 20 days after the initial screening (Fig. 1C). Changes in any virus result were seen for 993 specimens (38%) during the study period. Result changes were disproportionately identified in the pediatric population, in which greater percentages of repeat specimens became positive from negative baseline results and repeat specimens became positive for new or additional viral pathogens.

Because multiplex PCR testing is used very differently in the inpatient setting versus

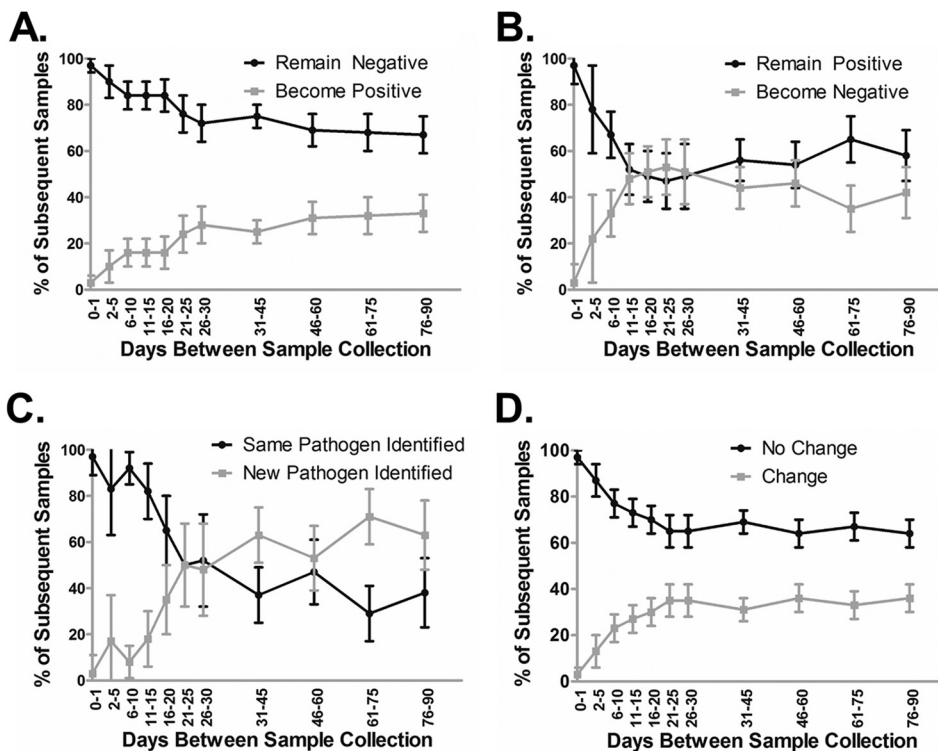


FIG 1 Change or persistence of subsequent multiplex viral panel results over time, compared with baseline specimens. (A) Subsequent results for previously negative specimens ($n = 1,695$). (B) Subsequent results for previously positive specimens ($n = 888$). (C) Identification of the same or new viral pathogens for specimens previously identified with a viral pathogen ($n = 514$). (D) Overall changes in results ($n = 2,583$). Error bars represent 95% CIs of proportions.

the outpatient setting, a subanalysis was performed based on patient location of the initial sample (Tables S2 and 3). Subsequent testing of inpatient samples ($n = 1,799$) demonstrated less change in overall sample results, compared to outpatient samples (28% versus 32%; $P < 0.04$). This difference was more pronounced when changes to any virus result were compared between inpatients and outpatients (34% versus 49%; $P < 0.001$), likely owing to reduced exposure to additional viral pathogens during hospital admission. Changes in subsequent results were more common among pediatric patients for both inpatient and outpatient samples (Fig. 2; also see Table S4).

As estimated by a logistic regression model, the odds of any result change plateaued after 20 days, compared to the odds of any result change seen at the end of the follow-up period (odds ratio [OR] for repeat specimens in 16 to 20 days versus 76 to 90 days, 0.61 [95% confidence interval [CI], 0.41 to 0.88]) (Fig. 3). Noting this, a subanalysis was then performed on repeat specimens collected within 20 days after the initial specimens; of 656 baseline-negative specimens, 576 (88%) remained negative, while only 80 (12%) became positive. Similarly, of 333 initially positive specimens with repeat testing performed within 20 days, 205 (62%) remained positive, whereas 128 (38%) became negative. The odds of a change in result for tests repeated within 20 days were only 0.52 times the odds (95% CI, 0.43 to 0.62) for those repeated between 21 and 90 days ($P < 0.001$) (Table 2). The odds of an overall change in result before 20 days were slightly higher for adults than for children (0.58 versus 0.44).

DISCUSSION

The benefits offered by multiplex NAATs for the initial diagnosis of respiratory tract pathogens are well established (16). In addition to high diagnostic sensitivity and rapid turnaround, viral pathogen testing may lead to significant reductions in the utilization of hospital resources and the use of antimicrobials (8). Furthermore, improved

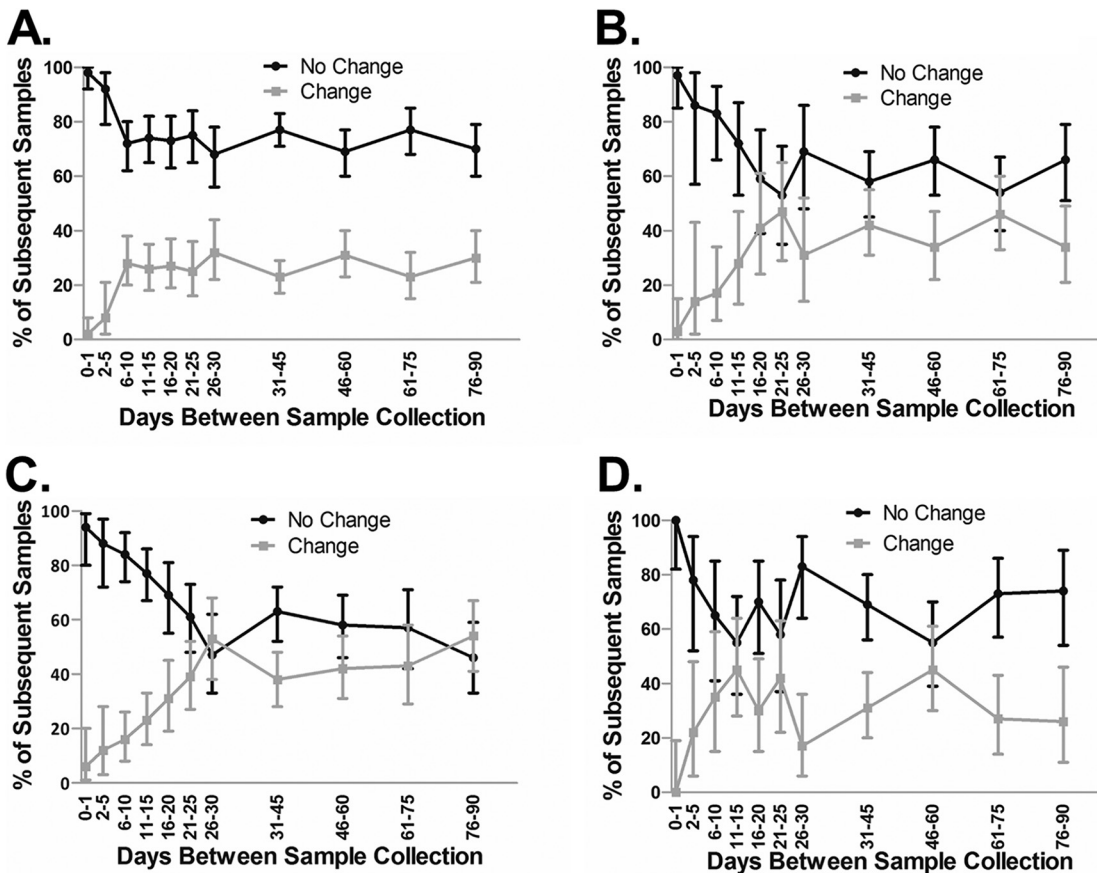


FIG 2 Change or persistence of subsequent viral panel results for inpatient or outpatient baseline specimens. (A) Subsequent results for inpatient adult samples ($n = 1,125$). (B) Subsequent results for outpatient adult samples ($n = 436$). (C) Subsequent results for inpatient pediatric samples ($n = 674$). (D) Subsequent results for outpatient pediatric samples ($n = 348$). Error bars represent 95% CIs of proportions.

physician-patient satisfaction with testing has been reported (5). The use of multiplex viral NAATs is emphasized in guidelines for initial diagnosis for infants and pediatric patients presenting with community-acquired pneumonia (17). In contrast, adult guidelines for community-acquired pneumonia limit the recommendation for viral testing to

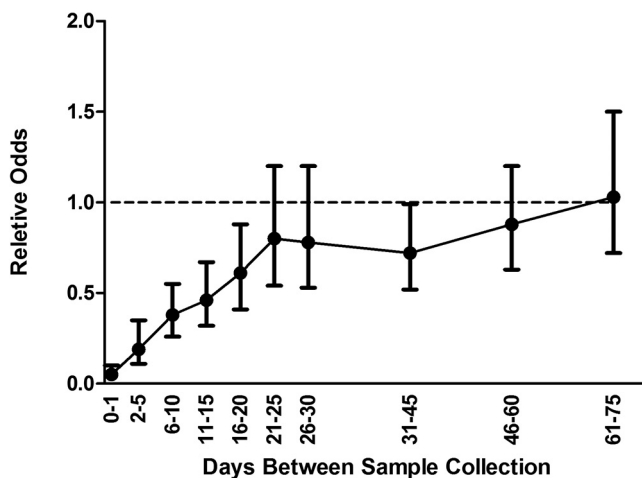


FIG 3 ORs from a logistic regression model for changes in sample results for given specimen collection intervals, compared to specimens retested within 76 to 90 days. Error bars represent 95% CIs. The dashed line represents an OR of 1 versus the reference group of 76 to 90 days between specimen collections.

TABLE 2 ORs for overall changes in sample results at 0 to 20 days versus 21 to 90 days

Age group	Day of follow-up sample	OR for change (Wald 95% CI) ^a	P
Total	0–20 vs 21–90	0.52 (0.45 ± 0.74)	<0.001
Adult	0–20 vs 21–90	0.58 (0.45 ± 0.74)	<0.001
Child	0–20 vs 21–90	0.44 (0.33 ± 0.58)	<0.001

^aEstimated via logistic regression models.

influenza alone, primarily to allow timely initiation of anti-influenza therapy. However, the same recommendations, along with large-scale epidemiological surveys, report that substantial proportions of adults admitted to the hospital have evidence of noninfluenza viral pathogens. (3, 18, 19). In our review, 21% of adult nasopharyngeal specimens (3,023/14,083 specimens) had evidence of at least 1 noninfluenza virus.

To our knowledge, this is the first investigation reporting the likely time period during which a change in result for a repeat multiplex respiratory viral NAAT is likely to occur. In our study, this practice of repeating screening at 15, 30, and 60 days accounted for 3.7%, 6.4%, and 9.7%, respectively, of total respiratory viral panel tests performed at our institution. Oftentimes, subsequent testing did not produce an overall change from the previous result. While still low, the odds of obtaining a change in test results for a specimen were optimized if retesting was delayed more than 20 days after the initial result. It is important to note that the likelihood of a result change was highly dependent on the result for the original specimen. Specimens that initially screened negative frequently remained negative for the entire 90-day follow-up period, whereas specimens that were initially positive for a viral infection equalized between remaining positive and becoming negative after 11 to 15 days. Lastly, the presence of a new viral pathogen is unlikely to be identified with further testing within 20 days when a viral pathogen was previously identified. Together, our findings demonstrate an overuse of these diagnostic screens, with unlikely changes in overall results.

While the clinical impact of molecular testing for the identification of respiratory viruses is well described, the economic costs of such testing are high. Several studies reported that the use of NAATs led to reductions in antimicrobial use (7, 8, 20). However, Oosterheert et al. performed a randomized trial that demonstrated that neither costs nor use of antimicrobials differed between patients who had PCR testing and those who received conventional diagnostic screening (21). In our investigation, repeat testing within 20 days after the initial test accounted for 4.5% of all tests performed during the 5-year review period. The 2017 Medicare reimbursement of \$571.72 for CPT code 87633 (tests with 12 to 25 targets) demonstrates the economic benefit that can be realized from even a modest reduction in respiratory viral screening. Mitigation of repeat testing will require both educational campaigns for ordering providers and active laboratory stewardship efforts, with adjustments to electronic ordering systems.

Hard and soft stops are commonly used in electronic ordering systems to minimize unnecessary tests. Based on our data, we have implemented an extended hard stop intervention at the Cleveland Clinic for duplicate respiratory pathogen panel orders within 14 days after a previous study. Hard stops at the Cleveland Clinic are electronic interventions that cannot be overridden at the computer terminal used for order entry; however, this electronic intervention can be overridden by requesting an override through laboratory client services, if the additional test is deemed to be truly medically necessary. Laboratory client services is staffed to handle such requests and to provide guidance. If excessive override requests occur, then the reasons for the override requests are determined and additional guidance is provided as necessary. If additional override requests occur, then one option is to convert the intervention to a soft stop. The soft stop intervention allows the provider to override the intervention at the point of order entry. Hard stops have been shown to be more effective laboratory stewardship interventions for duplicate orders that are usually unnecessary, but soft stops may be a more acceptable approach in certain settings (22).

There are notable limitations to our study. Because this was a retrospective analysis, specimens were obtained at the discretion of clinical providers. As a result, we analyzed viral result data in aggregate and did not focus on specific clinical scenarios for individual patients. Commonly, reasons for repeat viral testing are not reported in the medical record. Factors that often influence additional testing include intensive care unit stays, complex underlying medical conditions, and hospitalization beyond 7 days (23). Additionally, excessive ordering of tests is influenced by defensive behavior and fear of uncertainty (24). The utility of repeat testing is highly dependent on the patient's circumstances and the clinical reasoning employed. Motivations for repeat respiratory testing include acute changes in clinical status, the desire for removal from inpatient isolation, the need to institute immunosuppressive therapy, and clearance for hematological or solid organ transplantation. Still, there were not large changes in overall results for viral multiplex NAATs repeated within 20 days after the initial test, especially in the inpatient setting. This finding should prompt reflection regarding the utility of subsequent testing within that time frame. Further studies looking at the reasons for repeat viral testing and the usefulness of follow-up testing in higher-risk populations, such as patients who require intensive care, those who have underlying immunocompromise, or those who have pulmonary comorbidities, are warranted. Lastly, this study evaluated results obtained with the FDA-approved GenMark platform, which detects 14 viral pathogens. Other platforms, including the Luminex and BioFire FilmArray platforms, have expanded panels that include additional viruses (such as bocavirus and coronaviruses) that are not detected by GenMark analysis (6). Still, the respiratory pathogens detected in our study include many of those commonly associated with severe respiratory illnesses, accounting for over 75% of the viral pathogens seen in both children and adults (3, 4), and the sensitivity and specificity were comparable to those of other multiplex arrays (6). It is important to note that several viruses (e.g., rhinovirus) have prolonged shedding times, which would affect the time for a patient's follow-up specimen to become negative (25). Further studies analyzing subsequent testing according to specific virus type would aid in refining decisions regarding timing for repeat testing.

In conclusion, multiplex respiratory virus NAATs are commonly repeated within a short period, often leading to redundant results at additional costs. There is little change in overall results if repeat testing is ordered within 20 days after the initial result. Recent policies of nonreimbursement place additional economic strains on diagnostic laboratories and hospital systems. These results provide evidence for use in the development of diagnostic algorithms to curtail repeat testing and to improve laboratory stewardship while still ensuring optimal patient care.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.05 MB.

SUPPLEMENTAL FILE 2, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 3, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 4, PDF file, 0.1 MB.

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