Repeat Molecular Testing for Respiratory Pathogens: Diagnostic Gain or Diminishing Returns?

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Background: Upper respiratory tract infections are common, and the ability to accurately and rapidly diagnose the causative pathogen has important implications for patient management.

Methods: We evaluated the test-ordering practices for 2 commonly utilized nucleic acid amplification tests (NAATs) for the detection of respiratory pathogens: the Xpert Flu Assay for influenza A/B (Flu assay) and the Biofire FilmArray respiratory panel assay (RP assay), which detects 20 different targets. Our study examined repeat testing; that is, testing within 7 days from an initial test.

Results: Our study found that repeat testing is common for each of the individual assays: 3.0% of all Flu assays and 10.0% of all RP assays were repeat testing. Of repeat testing, 8/293 (2.7%) of repeat Flu assays and 75/1257 (6.0%) of RP assays resulted diagnostic gains, i.e., new detections. However, for the RP assay, these new detections were not always clinically actionable. The most frequently discrepant organisms were rhinovirus/enterovirus (28/102, 27.5%), followed by respiratory syncytial virus (12/102, 11.8%) and coronavirus OC43 (11/102, 10.8%). Furthermore, there were 3,336 instances in which a patient was tested using both a Flu assay and RP assay, of which only 44 (1.3%) had discrepant influenza results.

Conclusions: Our findings suggest opportunities exist to better guide ordering practices for respiratory pathogen testing, including limiting repeat testing, with the goal of optimization of clinical yield, and diagnostic stewardship.

IMPACT STATEMENT

We demonstrate low clinical value of repeating respiratory multiplex or molecular influenza testing within a 7-day interval. These results inform approaches to diagnostic stewardship for this testing.
INTRODUCTION

Upper respiratory tract infections are common and it can be challenging for healthcare providers to assess whether anti-infective treatment is needed based solely on clinical signs and symptoms. Rapid molecular assays that can detect common causes of upper respiratory tract infection have the potential to reduce this diagnostic uncertainty and allow optimization of directed therapy. Molecular testing for respiratory pathogens may impact patient care and outcomes through early discontinuation of unnecessary antibiotics, decreased length of hospital stay, and reduction in unnecessary infection prevention precautions (1–3). However, these assays can be costly and data on the utility of serial molecular testing, or simultaneous testing on multiple specimen types, is sparse.

Our goal was to evaluate the test ordering practices for respiratory pathogen testing at Barnes-Jewish Hospital (St. Louis, MO), a tertiary-care, academic medical center. We sought to examine the overlap between targeted influenza testing and multiplex respiratory pathogen testing, including the frequency and yield of repeat molecular testing. We performed a retrospective analysis of 2 commonly utilized nucleic acid amplification tests (NAATs) for detection of respiratory pathogens: (1) an assay for influenza A/B and (2) a multiplexed respiratory pathogen assay.

MATERIALS AND METHODS

Following approval from the Human Research Protection Office at Washington University in St. Louis, an electronic query was performed from March 2013 to September 2015 for all test results from the influenza A/B assay (Xpert Flu Assay, Cepheid) and Respiratory Pathogen assay (FilmArray Respiratory Panel BioFire) (4–7). For the remainder of the paper, the Xpert Flu Assay for influenza A/B is referred to as the Flu assay and the Biofire FilmArray respiratory panel assay as the RP assay. The electronic query assessed patients from Barnes-Jewish Hospital, an urban, tertiary-care, academic medical center.

Any test performed within 7 calendar days from a previous test on the same patient was considered a repeat test. This included any test irrespective of specimen type. During the time frame queried, the ordering for both assays was unrestricted and available to all clinical services within the hospital. Each assay was performed according to the manufacturer’s instructions. The Flu assay was validated by the performing laboratory for nasopharyngeal swabs, whereas the RP assay was validated for nasopharyngeal swabs and lower respiratory tract specimens. The turnaround time published by the laboratory for the Flu assay was 4 hours during the local influenza season, and 8 hours the rest of the year. The published turnaround time for the RP assay was 8 hours throughout the year. These turnaround time estimates are based on competing tasks within the laboratory. Both assays were offered 24/7. A separate specimen was required and obtained for each assay, even if both were ordered simultaneously. The Flu assay detects and differentiates influenza A, influenza A 2009 H1N1, and influenza B viruses. A breakdown of the organisms detected using the RP assay is included in the Supplemental Information (Supplemental Table 1). Briefly, the assay detects 20 targets consisting primarily of viruses, with 3 bacterial targets. It differentiates influenza A H1 2009, influenza A H3, influenza A (nontypable), and influenza virus type B.

We evaluated test ordering practices by patient service. The location from which an assay was ordered was categorized into one of 7 categories. For testing with both the Flu and RP assay, detection of different viruses is defined as “Additional Detection.” For both assays, for the same pathogens, a “Discrepant” result is defined
as either a prior negative followed by a positive test, or a prior positive followed by a negative test. Statistical analyses were performed using an “n-1” chi-squared test (8, 9). For calculating the statistical significance of the number of tests ordered for each assay, for each month, an exact Clopper–Pearson interval was used (10). SPSS Statistics 25 Desktop was used for calculating the aforementioned statistics.

RESULTS

Summary of Findings

A total of 22,734 test results were identified, corresponding to 11,754 unique patients. Of these, 10,198 Flu assays were performed, of which 1,047 (11.2%) were positive, the majority for influenza A (n = 884, 83.4% of positives) (Supplemental Table 2). Out of the 12,536 total RP assays performed, 2,745 (21.9%) were positive. However, the major driver of this positivity was the high rate of detection of rhinovirus/enterovirus, often of unclear clinical significance, and commonly found among both asymptomatic and symptomatic patients (11–15). Eliminating rhinovirus/enterovirus from this analysis reduced the number of positive results to 1,593 (12.7%). The ordering practices and results for both the Flu and RP assays are summarized graphically by each month in Supplemental Fig. 1 and detailed numerically in Supplemental Tables 3 and 4. Both the Flu and RP assay orders (and positive results) declined during the summer months of June, July, and August (P < 0.001 for both).

Repeat Testing

For both assays, we evaluated the number of repeat tests within 7 calendar days from a prior test, and the time interval between repeat tests (Table 1). There were 293 instances of repeat testing out of a total of 10,198 Flu assays performed (3.0%). The majority of patients with repeat Flu testing (284; 96.9% of all repeat tests) had 2 influenza assays performed in total, while there were a small number of instances (9; 3.1%) in which there were 3 total Flu assays performed. For the RP assay, there were 1,257 instances of repeat testing out of a total of 12,536 assays performed (10.0%). The frequency of repeat RP testing was more variable than Flu testing, with 1,023 (84.4% of all repeat tests) instances of 2 RP assays, 182 (14.5%) instances of 3 RP assays, and 52 (4.1%) instances having between 4 and 10 tests. Of note, many of the repeat tests were performed within the same calendar day: 91 (30.0%) for the Flu assay, and 468 (29.8%) for the RP assay.

The mean/median intervals for repeat testing were 60/29 hours for the Flu assay and 59/40 hours for the RP assay. For all repeat Flu assays, 10.8% were ordered within 4 hours (the reported turnaround time in Flu season) and 25.4% were ordered within 8 hours (the turnaround time for the remainder of the year). For the RP assay, 19.9% of all repeat orders were placed within the turnaround time of 8 hours.

While the Flu assay only used nasopharyngeal swabs, the RP assay could be ordered on both upper and lower respiratory specimens. Of all the repeat RP assays ordered, 289 of 1,257 (23.0%) of repeat testing had the same specimen types. For RP assays of the same specimen type, 17.3% of repeat testing occurred within the published turnaround time of 8 hours.

We also examined whether repeat testing within 7 days resulted in additional information. Table 2 summarizes the results of repeat testing. For the Flu assay, the majority of repeat testing was consistent with initial results: 276 (94.2%) of assays on the same patient were always negative, while 4 (1.4%) tests remained positive with repeat testing. There were only 13 (4.4%) instances in which repeat testing with the Flu assay had discordant results: of the discordant results, there were 5 instances in which the first test was positive followed by a negative, and 8 instances in which the first test was negative followed by a positive.
For the RP assay, 950 (75.5%) of repeat tests were consistently negative, while 161 (12.8%) remained positive with the same organisms. There were discordant results for 138 (11.7%) repeat RP tests: of these discordant results, 62 were initially positive followed by negative, 75 were initially negative followed by a positive result, and there was 1 instance in which both tests were ordered simultaneously.

### Flu and RP Assays Ordered Together within a 7-Day Interval

We compared the results of the Flu and RP assays performed on the same patient within 7 calendar days (Fig. 1). There were 3336 instances in which a patient received both a Flu and RP assay within this time frame. When considering any positive result for the RP assay, 675 (20.2%) of patients had additional detections. Considering only results for influenza A/B, 48 (1.4%) patients had discordant results between the Flu and RP assays. Of these discordant results, 44 (91.7%) were positive only on the RP assay, while 4 (8.3%) were positive only on the Flu assay.

### Test Yield by Specimen Type for the RP Assay

The RP assay could be ordered on 5 different specimen types, summarized in Table 3. The most common specimen type received was nasopharyngeal swabs (64.3%). With regards to positivity, sputum samples had the highest positivity (33.3%), while bronchoalveolar lavages had the lowest (13.6%). Supplemental Table 5 summarizes the targets identified for each specimen type. For all specimen types, rhinovirus/enterovirus was the most commonly identified target, ranging from 29.2 to 48.2% of all positive results.

| Table 1. Summary of repeat tests within a 7-day period for both the Flu and RP assays for a single patient. Counts and percentages of all repeat tests, calendar days between each repeat test for a single patient, and the time between repeat test orders. |
|----------------------------------------------------------|
| **Total tests for a single patient** | **Flu assay** | **RP assay** |
| | **N** | **Percentage** | **N** | **Percentage** |
| 2 | 284 | 96.9 | 1023 | 81.4 |
| 3 | 9 | 3.1 | 182 | 14.5 |
| 4–10 | 0 | 0.0 | 52 | 4.1 |
| **Total Instances** | 293 | 100.0 | 1257 | 100.0 |
| **Calendar days from previous test** | **N** | **Percentage** | **N** | **Percentage** |
| 0 | 91 | 30.0 | 468 | 29.8 |
| 1 | 67 | 22.1 | 298 | 19.0 |
| 2 | 15 | 5.0 | 179 | 11.4 |
| 3 | 22 | 7.3 | 145 | 9.2 |
| 4 | 24 | 7.9 | 128 | 8.1 |
| 5 | 24 | 7.9 | 115 | 7.3 |
| 6 | 36 | 11.9 | 108 | 6.9 |
| 7 | 24 | 7.9 | 129 | 8.2 |
| **Total** | 302 | 100.0% | 1571 | 100.0% |
| **Time between orders** | **Mean/median (hour)** | **Min/max (hour)** | **Mean/median (hour)** | **Min/max (hour)** |
| | 61/59 | 0.02/187 | 58/56 | 0.00/180 |
We identified 1018 instances, corresponding to 891 patients, in which a single patient had 2 separate RP assays performed within 7 calendar days of each other. Figure 2 and Supplemental Table 6 summarizes the specimen combinations seen within this patient cohort, as well as the number of discrepant results within each combination. Supplemental Table 7 provides additional breakdown of the discrepant organisms identified in each combination. In 752 of these instances (74.0%), the assays were performed on different specimen types. Among all discrepant results, rhinovirus/enterovirus made up 28/101 (27.7%) of all identified organisms. Furthermore, pathogens typically warranting direct antimicrobial treatment (influenza and bacterial pathogens) accounted for only 19/101 (18.8%) of total discrepant results and 52/1257 (4.2%) of total repeat testing.

### Test Ordering Patterns by Patient Care Service Line

Supplemental Tables 8 and 9 summarize the ordering trends and positivity rates for each assay across different clinical locations in our study, as well as the locations from which repeat tests were ordered. The RP assay was more frequently ordered by the Intensive Care Units, Bone Marrow Transplant & Oncology, and General Medicine Units than the Flu Assay.

In some instances, the original test may have been ordered from a different patient care location. This applied to 59.4% of the Flu assays and 25.9% of the RP assays. For the Flu assay, there were a total of 302 repeat test orders, originating from 4 locations, General Medicine Units, Intensive Care Units, “Other Inpatient Units,” and Bone Marrow Transplant & Oncology Units. In contrast, there were 1571 repeat orders for the RP assay, of which 906 (57.7%) came from Intensive Care Units.

### DISCUSSION

Herein, we have evaluated test ordering practices and yield of a molecular assay for influenza and a multiplexed respiratory pathogen assay over a 31-month period. The most common organisms identified from the RP assay were rhinovirus/enterovirus, parainfluenza virus type 3, parainfluenza virus type 1, and parainfluenza virus type 2.
and respiratory syncytial virus, consistent literature reports on the relative prevalence of these targets (16–19). The least commonly detected targets were the bacterial pathogens on the panel: the prevalence of all 3 were low (20–25).

There was a difference in positivity rates of the RP assay by specimen types. There have been previous studies examining the differences in specimen types with the RP assay, although these have been limited to comparing 2 or 3 specimen types within a single study (26–28). Of note, the positivity rates for noninvasive specimen types were higher than invasive specimen types. This may be due to differences in obtaining the specimens from the patients, sampling protocols, or higher viral loads for the specimen types.

Another interesting finding was that in patients who received 2 separate nasopharyngeal swabs for the RP assay, 11.4% of results were discordant. Collection adequacy of a nasopharyngeal swab is user dependent, which may affect results. Collection adequacy may also explain the small amount of discordant influenza results between RP and Flu testing, and the variation in positivity between different specimen types; for example, a nasopharyngeal swab and bronchoalveolar lavage (which saw 8.8% discrepant results). Our results are in contrast to the previous work by Azadeh and colleagues (27), who demonstrated a 23% discordance rate between nasopharyngeal swabs and bronchoalveolar lavages with the RP assay.

The frequency at which repeat tests were ordered before the reported turnaround time was another significant finding. While not a perfect metric for the availability of test results, the turnaround time serves as a proxy for us to identify when clinicians may have actionable information from a previous test. Our results suggest a significant portion of testing is being reordered before the results are available in the EMR. Another finding was that repeat testing appears to be more closely associated with initial negative results

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**Table 3. Summary of RP assays ordered for each specimen site, as well as the total positivity rate.**

| Specimen                      | Tests ordered (n) | Tests positive (n) | Tests positive excluding rhinovirus/enterovirus (n) |
|-------------------------------|-------------------|--------------------|---------------------------------------------------|
| **Noninvasive specimen**      |                   |                    |                                                   |
| Nasopharyngeal swab           | 8041              | 2196 (27.3%)       | 1237 (15.4%)                                      |
| Sputum                        | 206               | 67 (33.3%)         | 38 (18.4%)                                        |
| **Invasive specimen**         |                   |                    |                                                   |
| Bronchoalveolar lavage        | 2246              | 305 (13.6%)        | 186 (8.3%)                                        |
| Bronchial washing             | 992               | 164 (16.5%)        | 85 (8.6%)                                         |
| Tracheal aspirate             | 1026              | 168 (16.4%)        | 119 (11.6%)                                       |

**Fig. 2. Differences in specimens retrieved from bronchoalveolar lavages (BAL) and nasopharyngeal swabs (NP). Comparison of RP assay results for patients who received both a BAL and NP swab during a single visit.**
This may explain some of the clinical rationale behind the ordering practice (i.e., not trusting a negative value).

Our study also looked at service line placing test orders. The Flu assay was commonly ordered from all Inpatient Units, with relatively few from outpatient settings. In contrast, the RP assay was ordered frequently within the ICU, Bone Marrow Transplant & Oncology Units, General Medicine Units, as well as Outpatient Units. The broader ordering range of the RP assay might make it more appealing to clinicians in settings where critically ill or immunocompromised patients are present, where a broad range of pathogens (e.g., adenovirus) can have a significant impact on patient care. Additionally, in an outpatient setting, the ability to interrogate multiple pathogens to triage patients may be appealing. For repeat test orders, both assays saw an increase in the proportion of repeat testing within ICUs. This may be attributed to patient severity and the need for ruling out respiratory infections versus other pathologies. Ordering of the RP assay within the computerized physician order entry was not available outside of ICU and transplant locations, which may also contribute to this difference.

Our study suggests that in our institution opportunities exist for improving test utilization for both assays. We identified a number of contexts in which such opportunities may occur: the ordering of both a Flu and RP assay on a single patient, repeat testing with either the Flu or RP assay, and the ordering of Flu assays outside of Flu season.

Despite the redundancy of the Flu and RP assays to detect influenza A/B, many patients were tested with both assays. In patients who received both assays, only 1.4% had discrepant results for influenza A and B, suggesting limited benefit of this practice. This is consistent with a previous study by Wahrenbrock et al. (29), in which they compared the Xpert Flu/RSV XC with the RP assay, demonstrating a large amount of agreement between the 2 assays on the same nasopharyngeal swab specimens. There are clinical scenarios in which detection for other pathogens (e.g., adenovirus in immunocompromised patients) would still be clinically relevant, and therefore testing with a broad array may be appropriate. Repeat testing with both the Flu and RP assays may be due to a lack of understanding about the redundancy between the 2 assays, a broad differential diagnosis, or a perception that the analytical performance of the assays differs.

In patients who were tested with the Flu assay multiple times, there were only 4.4% instances of repeat tests providing new information (a new positive or negative finding), while 9.2% of instances repeat testing with the RP assay resulted in discrepant information. It is unclear whether this new information affected the management of the patient, nonetheless, the rates of discrepant results for both assays are low. Riley et al. (30) demonstrated that a passive clinical decision support tool could prevent some unnecessary testing. Reprioritization of EMR laboratory orders is another technique that laboratories might employ to assist in the reduction of repeat testing (31).

For the RP assay, the added variable of specimen type may have contributed to repeat testing, as there were many instances in which clinicians submitted multiple specimen types on the same patient. Our results suggest that limited additional information is gained by testing different specimens on the same patient. The elimination of repeat testing of different specimens may help eliminate the need for invasive procedures (e.g., a bronchoalveolar lavage).

A significant proportion of repeat testing was performed on the same specimen type (264/1035, 25.5%). Sometimes this was performed with relatively narrow time interval (fewer than 8 hours) between initial and repeat testing, suggesting that the repeat testing may have been due to communication gaps between different ordering providers.
Finally, there was a surprising number of Flu assays ordered outside of the local Flu season. Between the months of June and October, 1423 Flu assays (14.0% of total) were ordered, with only 6 positive results (<0.1%) returning as positive. This suggests limited clinical utility of the Flu assay outside of the Flu season, and another area for potential improvement of use.

Based on the findings of our study, we propose a generalizable algorithm to assist clinicians in evaluating adult patients with respiratory symptoms, as well as assist with infectious disease prevention (Fig. 3). If a patient presents with respiratory symptoms, the clinician would first evaluate the patient’s immune status. If a patient is immunocompromised, there is an increased risk of severe respiratory infection. Thus, there is greater impetus to determine the causative agent to see if antiviral therapies (e.g., for influenza) need to be initiated, or whether broad-spectrum antibiotics (many with their own side-effects) can be discontinued (32). For immunocompetent patients, the algorithm assesses whether it is Flu season. During Flu season, a targeted influenza assay would be an appropriate first-line approach to triage the patient given the increased pre test probability. If the Flu assay is positive, no further testing related to the respiratory symptoms is recommended. If the Flu assay is negative, a larger, syndromic panel could be pursued, if necessary, for management. In all scenarios, repeat testing is discouraged. Not only does the proposed algorithm assist for recognition and treatment of patients with respiratory symptoms, but it can also be leveraged for infection control within a healthcare setting. One should also consider that testing should only be performed if it will affect patient care, which may be variable depending on the patient setting.

There are myriad advantages to eliminating potentially unnecessary repeat testing, including

![Fig. 3. A proposed algorithm for testing of patients who present with respiratory symptoms.](image-url)
patient comfort, decreased potential for false results, and financial advantages. From a financial standpoint, if all repeat testing were eliminated during the course of this study (a total of 293 Flu assays and 1257 RP assays), this would equate to a savings of approximately $268,680 per year based on midpoint pricing in the Centers for Medicare & Medicaid Services Clinical Laboratory Fee Schedules (33). If repeat testing on the same specimen type was eliminated, this would result in savings of approximately $140,000 per year.

There are several limitations to our study. We did not evaluate the impact of testing on patient management or clinical outcomes. Previous studies have examined the effect of NAAT testing on patient management, noting that NAAT testing did result in a decrease in antibiotic use among patients positive for influenza, but not for patients with other respiratory pathogens (34). Rappo and colleagues (3) also demonstrated that the use of the FilmArray multiplexed assay resulted in a statistically significant decrease in time to diagnosis of causative respiratory agent, chance of admission, length of hospital stay, and use of radiographic studies. The ResPOC study is an ongoing trial examining how the RP assay with nasopharyngeal and throat swabs will affect patient management (35).

Another limitation of our study is that nearly all the patients examined were adults. Pediatric patients have a different distribution of respiratory illnesses, and the clinical decisions related to their testing are significantly more complex. This study was also performed at an urban, tertiary academic medical center, where many patients are critically ill or immunocompromised. This limits the generalizability of our study to smaller community hospitals or clinics. Finally, the algorithm we propose assumes that the laboratory of interest has the capabilities to perform both a targeted influenza assay and a multiplexed respiratory pathogen panel. Many smaller medical centers do not have the staffing needs or expertise to perform these.

Despite these limitations, our study has several strengths. For one, this is one of the most comprehensive studies to date to examine repeat NAAT testing in the context of respiratory pathogens. Our results emphasize the similar performance of both the Flu and RP assays for the detection of Influenza A and B. The large sample size of our study has also enabled us to examine the positivity rates between many different specimen types on the same patient, which no other study to date has accomplished. Furthermore, this study has highlighted that repeat testing for respiratory pathogens does not necessarily result in new, clinically actionable information for clinicians.

In conclusion, repeat testing for respiratory pathogens via both the Flu and RP assays are common, yet, in most instances does not result in any additional, clinically actionable information. Our findings suggest an opportunity for both laboratorians and health care providers to evaluate ordering practices for respiratory pathogen testing at their institutions, and potentially implement ordering algorithms and clinical decision support to optimize their clinical yields and test utilization.

SUPPLEMENTAL MATERIAL

Supplemental material is available at The Journal of Applied Laboratory Medicine online.

Nonstandard Abbreviations: Flu assay, Xpert Flu Assay for influenza A/B; RP assay, the Biofire 2.0; Flu, Influenza FilmArray respiratory panel assay; BAL, bronchoalveolar lavage; NP, nasopharyngeal swab.
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