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## Special Article

# Evaluation of Viruses Associated With Acute Respiratory Infections in Long-Term Care Facilities Using a Novel Method: Wisconsin, 2016–2019



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## A B S T R A C T

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Residents of long-term care facilities (LTCFs) have high morbidity and mortality associated with acute respiratory infections (ARIs). Limited information exists on the virology of ARI in LTCFs, where virological testing is reactive. We report on findings of a surveillance feasibility substudy from a larger prospective trial of introducing rapid influenza diagnostic testing (RIDT) at 10 Wisconsin LTCFs. Any resident with symptoms consistent with ARI had a nasal swab specimen collected for RIDT by staff. Following RIDT, the residual swab was placed into viral transport medium and tested for influenza using Reverse transcription polymerase chain reaction, and for 20 pathogens using a multiplex polymerase chain reaction respiratory pathogen panel. Numbers of viruses in each of 7 categories (influenza A, influenza B, coronaviruses, human metapneumovirus, parainfluenza, respiratory syncytial virus, and rhinovirus/enterovirus) across the 3 years were compared using  $\chi^2$ . Totals of 160, 215, and 122 specimens were collected during 2016–2017, 2017–2018, and 2018–2019, respectively. Respiratory pathogen panel identified viruses in 54.8% of tested specimens. Influenza A (19.2%), influenza B (12.6%), respiratory syncytial virus (15.9%), and human metapneumovirus (20.9%) accounted for 69% of all detections, whereas coronaviruses (17.2%), rhinovirus/enterovirus (10.5%) and parainfluenza (3.8%) were less common. The distribution of viruses varied significantly across the 3 years ( $\chi^2 = 71.663$ ;  $df = 12$ ;  $P < .001$ ). Surveillance in LTCFs using nasal swabs collected for RIDT is highly feasible and yields high virus identification rates. Significant differences in virus composition occurred across the 3 study years. Simple approaches to surveillance may provide a more comprehensive assessment of respiratory viruses in LTCF settings.

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Older individuals are poorly represented in respiratory virus surveillance systems because of mismatch between monitoring sites and venues of care. For example, less than 3% of individuals with influenza-like illnesses in a nationwide surveillance system were aged 65 years or older.<sup>1</sup> This contrasts with the finding that during the 2017 to 2018 influenza outbreak, 1 out of every 217 older Americans, aged 65 years or older, was hospitalized because of influenza infection alone.<sup>2</sup> Similarly, during the 2017 to 2018 influenza season across

Wisconsin, 7514 influenza hospitalizations were reported, with 65% of these admissions occurring in older adults, resulting in 1 of every 178 hospitalized with influenza.<sup>3,4</sup> Of these, 11.4% required intensive care unit admission, and 2.9% required mechanical ventilation.<sup>3</sup> Because of the pervasive lack of testing for influenza in hospitalized older patients,<sup>5</sup> however, these estimates are likely to be significantly lower than the true burden of illness. Moreover, the contribution from other respiratory viruses likely adds significantly to hospitalization and death.

Despite significant illness burden, long-term care facilities (LTCFs), with concentrations of older individuals, are rarely used as surveillance sites. LTCF residents experience this high burden of respiratory infections, often with significant morbidity and mortality, owing to 3 attributes: high transmissibility of many respiratory viruses, close quartering of residents, and high morbidity and mortality in this population.<sup>6</sup> For example, influenza attack rates of 7.2% to 27% are

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common in LTCFs and have been associated with mortality rates of 4.4% to 5.2%.<sup>7–11</sup>

The Rapid Assessment of and Prophylaxis for Influenza in Dwellers of Long-Term Care Facilities (RAPID-LTCF) study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02964871) Identifier: NCT02964871) is a randomized controlled trial with the primary objective to evaluate the potential benefits of using rapid influenza diagnostic tests (RIDTs) in LTCFs. Nursing staff-initiated testing<sup>12</sup> is triggered by the appearance of acute respiratory infection (ARI) symptoms and entails collection of a nasal swab specimen for on-site testing. The premise for this feasibility substudy is that nasal swab specimens, collected for point-of-care RIDT testing, in the intervention arm of the study, can be easily repurposed for respiratory virus surveillance within LTCFs. Such an approach would allow for on-site and near real-time diagnosis with RIDT, with the ability to use the residual specimen for broader surveillance efforts. Accordingly, we examined (1) the rate at which residual specimens are sent for expanded testing; (2) the rate of respiratory virus detection from residual specimens; and (3) whether resident characteristics contribute to the detection rate.

## Methods

### Location

LTCFs throughout Wisconsin were contacted through email by a study coordinator to gauge their interest in participating in a research study about the effectiveness of early detection of respiratory infections. Interested sites were paired based on bed-size, region, and ownership. Twenty sites were then randomized either to control (standardized, site-specific influenza surveillance) or intervention group (RIDT use). Data and respiratory specimens were collected from residents of 10 LTCFs widely scattered across the state of Wisconsin assigned to the intervention group. Together, the 10 sites had a maximum bed capacity of 838 with individual facilities ranging from 30 to 149 beds.

### Timeframe

Specimen collection occurred over 3 consecutive influenza seasons. After initiation of the study protocol in December 2016, LTCFs could test patients at any time of the year, but sampling was prioritized during 17-week study periods for 2016–2017, 2017–2018, and 2018–2019, based on statewide surveillance<sup>1</sup> of influenza in other settings. Sampling concluded on June 8, 2019.

### Participant Identification

Nursing staffs were instructed to collect specimens from any LTCF resident with a newly observed ARI (characterized by any 2 of the following symptoms: cough, nasal congestion, rhinorrhea/runny nose, sore throat, or fever). Fever was determined by each facility's nursing protocol based on Wisconsin Department of Health Services guidelines. Specimens were to be collected as early in the ARI as possible. No exclusion criteria were used. Specimens were collected for on-site RIDT.

### Data and Specimen Collection and Handling

Per protocol, nursing staff received brief, annual on-site training during early autumn on participant identification, obtaining a nasal swab specimen, and performing the RIDT. Training included hands-on instruction of the RIDT and a 1-page "how to" guide. Nursing staff could contact study personnel with questions, if needed. Data pertaining to date of collection, patient age and sex, number of days from symptom onset to collection, illness severity (3 levels— mild,

moderate, or severe) as determined by nursing staff, and the presence or absence of nine symptoms (fever, chills, cough, sore throat, runny nose, nasal congestion, headache, malaise, and myalgia) were collected on a paper requisition data form. Mild illnesses were considered to be self-limiting; moderate illnesses were those for which medical consultation was or could be considered; severe illness required medical attention or transfer to an acute care facility.

A nasal swab specimen was collected from the anterior nostril of each identified participant using a Sterile Foam Tipped Applicator (Puritan, Guilford, ME) for near real-time testing with a RIDT. After the specimen was tested using RIDT, the nasal swab was then placed into a 3.0 mL MicroTest M4RT (Remel, Lenexa, KS) Transport viral transport medium tube, sealed into a small biohazard bag, and maintained at 2°C to 8°C. Specimens, along with the requisition data form, were placed in a Styrofoam container with an ice pack to maintain temperature at 4°C to 8°C and transferred to the Wisconsin State Laboratory of Hygiene (WSLH) by courier within 48 hours of collection.

LTCFs were instructed to continue following the State of Wisconsin Department of Health Services guidance for reporting, prevention, and control of ARIs.<sup>13</sup> In Wisconsin, LTCFs are required by State Statute to report single cases of notifiable diseases to their local health department electronically through the Wisconsin Electronic Disease Surveillance System, by mail or fax, or by other means within 72 hours upon recognition of a case or suspected case. The State of Wisconsin Department of Health Services guidance outlines both laboratory testing protocols and antiviral treatment and prophylaxis to be considered during influenza outbreaks. LTCFs enrolled in this study were strongly encouraged to follow the State of Wisconsin Department of Health Services guidance.

### Laboratory Procedures

RIDT was completed by each site's nursing staff using the Sofia (Quidel, San Diego, CA) influenza A + B fluorescent immunoassay for initial assessment of nasal specimens.<sup>14</sup> All specimens, regardless of RIDT result, were tested at WSLH for influenza A and B viruses using the in vitro diagnostic Food and Drug Administration-approved Centers for Disease Control and Prevention human influenza virus real-time Reverse transcription polymerase chain reaction (RT-PCR) diagnostic panel (Cat.# FluIVD03).<sup>15</sup> In addition, all specimens were tested at the WSLH for the presence of 14 noninfluenza respiratory viruses and 2 atypical bacterial pathogens using a multiplexed PCR respiratory pathogen panel (RPP: NxTAG Respiratory Pathogen Panel; Luminex, Madison, WI).<sup>16</sup> Viral targets included influenza A (FluA), influenza B (FluB), rhinovirus/enterovirus (R/E), adenovirus (AD), parainfluenza virus (PIV: 1, 2, 3, 4), coronavirus (CoV: HKU1, NL63, 229E, OC43) respiratory syncytial virus (RSV: A, B), human metapneumovirus (hMPV), and human bocavirus (BoV). Bacterial targets include *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. Most specimens were tested upon receipt at the WSLH; specimens not tested immediately were frozen at –70°C until testing was performed. We tracked time from collection until receipt at WSLH. All results from influenza PCR and multiplex RPP were faxed, within 2 hours of completion, to the originating LTCF following usual WSLH procedures.

### Data Analysis

For this study, we compiled descriptive statistics on the rate of virus detection by year and the percentage of specimens positive for Flu A, Flu B, R/E, AD, PIV, CoV, RSV, and hMPV. Categorical variables were compared using the  $\chi^2$  test. For all analyses, a *P* value of < .05 was considered statistically significant.

## Ethics/Institutional Review Board

This study was approved by the University of Wisconsin Health Sciences Institutional Review Board, and was in compliance with Health Insurance Portability and Accountability Act and all other federally mandated human subjects regulations.

## Results

Respiratory specimens were collected from 497 LTCF residents during the 3-year study. Characteristics of the sample are provided in Table 1. The mean age of participants was 83.7 (median 86.0) years with 70.3% of the sample being female (Figure 1A). Specimens tended to be collected early in the illness, occurring an average of 1.97 (median 2.0) days after the onset of symptoms (Figure 1B). Depending on facility, 81%–99% of residents received seasonal influenza vaccination. Numbers of specimens collected varied by year (Table 1). During the first 2 years, 9 of 10 sites contributed specimens, with 8 sites contributing specimens in year 3. The percentage of specimens for which RIDT was performed that were shipped to WSLH for RT-PCR and RPP—a proxy measure of protocol compliance—experienced a nonsignificant decline following the first year ( $\chi^2 = 5.09$ ;  $P = .079$ ) and was 86.3% overall. Time from specimen collection to testing at the WSLH averaged 3.03 (median 2.0) days. The rate of virus detection was 54.8% overall, with higher rates during 2016–2017 and 2017–2018 than in 2018–2019. This decline, however, was not significant ( $\chi^2 = 5.70$ ;  $P = .058$ ).

The rate of virus detection from specimens was not related to the age of the resident ( $\chi^2 = 0.917$ ;  $P = .821$ ) across 4 age groups (Figure 2A), nor was it dependent on the time between symptom onset and specimen collection ( $\chi^2 = 1.557$ ;  $P = .821$ ; Figure 2B).

A wide variety of viruses was detected from the LTCF residents. Influenza viruses were most frequently detected from LTCF residents (31.8%), followed by hMPV (20.9%). Together, FluA, FluB, hMPV, and RSV comprised 69% of all detections (Figure 3), and CoV was frequently (17.2%) detected. R/E and PIV were detected at lower levels (Table 2). Across years, there were significant differences in the composition of viruses detected in LTCF residents ( $\chi^2 = 71.663$ ;  $df = 12$ ;  $P < .001$ ). For example, hMPV varied between 0% (2016–2017) and 37.8% (2018–2019) of annual detections.

## Discussion

Surveillance of influenza and other respiratory viruses in LTCFs using nasal swab specimens, initially collected for RIDT, is highly feasible and potentially valuable as shown by our work here. A simple process allowing nursing staff-initiated testing was well-accepted, resulted in wide sampling across the demographic spectrum, and resulted in a reasonable virus identification rate of 55% using influenza RT-PCR and a RPP.<sup>12</sup> This identification rate is in close agreement with other studies designed to assess the epidemiology of respiratory viruses in LTCFs.<sup>17,18</sup> Moreover, the specimens were collected early in the course of illness when diagnostic rates should be at their optimum. We were unable to detect declines in the rate of virus detection with increasing age or with increasing time from symptom onset. Accordingly, using “low threshold” criteria of ARI symptoms to trigger sampling and testing in the absence of a known influenza outbreak (eg, new onset ARI symptoms), as opposed to established criteria (eg, “When an influenza outbreak has been identified in a long-term care facility or hospital, influenza testing should be done for any resident/patient with one or more acute respiratory symptoms, with or without fever...”),<sup>19</sup> holds the potential for early detection and characterization of a number of circulating respiratory viruses.

Similar to other studies,<sup>17,20</sup> influenza viruses were commonly encountered in LTCF residents and accounted for 32% of detections over the 3-year study period. Human metapneumovirus, CoV, and RSV were also common. Together, 4 previously determined significant pathogens for older adults (FluA, FluB, hMPV, and RSV)<sup>21,22</sup> comprised nearly 70% of all virus detections. Co-detections, with residents having more than 1 virus, were uncommon and occurred in 4 residents (1.7%). Two of the co-detections included FluA (H3N2) and CoV.

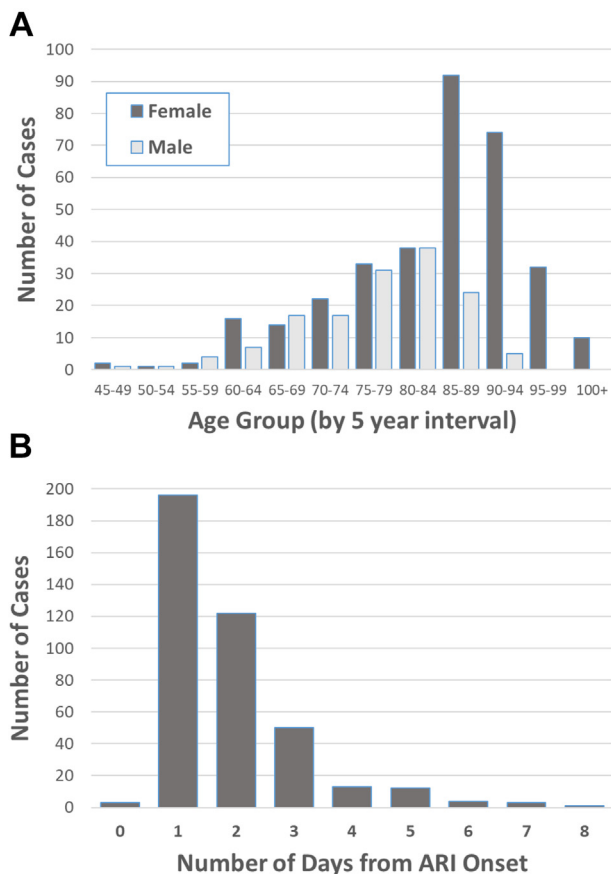
Significant differences in the constellation of viruses detected occurred across the study time period. The relative prevalence of viruses varied widely. For example, influenza viruses (FluA and FluB) comprised between 8.8% and 41.7% of all detections depending on year; hMPV, absent in 2016–2017, was the most commonly identified virus (37.7%) in 2018–2019. Accordingly, we demonstrate great variability of numbers and types of respiratory viruses across years in LTCFs.

A number of limitations were encountered in this substudy. First, this assessment is part of a larger study evaluating the potential

**Table 1**  
Characteristics of Long-Term Care Facility Residents With Acute Respiratory Infections (ARIs) From Whom Specimens Were Collected and Numbers of Specimens Collected During the 3 Consecutive Influenza Seasons (2016/2017–2018/2019)

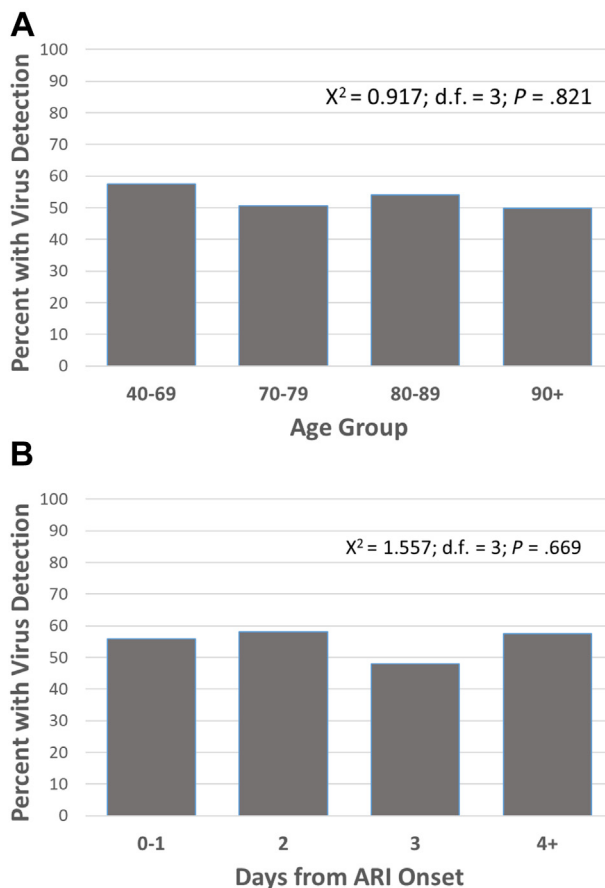
| Resident Characteristics  | N                                  | Range   | Mean (SD)  | Median (IQR)     |
|---|------------------------------------|---|--|------------------|
| Age in y (missing)  | 489 (8)                            | 48–104  | 83.7 (9.9)   | 86.0 (78.0–91.0) |
| 40–69   | 48                                 |   |  |                  |
| 70–79   | 91                                 |   |  |                  |
| 80–89   | 202                                |   |  |                  |
| ≥90   | 148                                |   |  |                  |
| Sex (missing)   | 489 (8)                            |   |  |                  |
| Female (%)  | 344 (70.3)                         |   |  |                  |
| Male (%)  | 145 (29.7)                         |   |  |                  |
| Time in d from illness onset to specimen collection (missing)         | 470 (27)                           | 0–8   | 1.97 (1.26)  | 2.0 (1.0–2.0)    |
| 0–1   | 212                                |   |  |                  |
| 2   | 147                                |   |  |                  |
| 3   | 67                                 |   |  |                  |
| 4+  | 44                                 |   |  |                  |
| Time in d from specimen collection to testing at WSLH (missing)       | 429 (68)                           | 0–37  | 3.03 (3.68)  | 2.0 (1.0–4.0)    |
| Percent of residents receiving seasonal influenza vaccination by site | 10 sites                           | 81–99   | 91.8%  |                  |
| Specimens Collected   | Number of with Specimens Collected | Number with RT-PCT/RPP (% of Samples w/PCR Results) | Number with Virus Detection (% of PCR Results w/Detection) |                  |
| 2016/2017   | 160                                | 146 (91.3)  | 83 (56.8)  |                  |
| 2017/2018   | 215                                | 182 (84.7)  | 107 (58.8)   |                  |
| 2018/2019   | 122                                | 101 (82.8)  | 45 (44.6)  |                  |
| Total   | 497                                | 429 (86.3)  | 235 (54.8)   |                  |

\*IQR, interquartile range



**Fig. 1.** (A) Age and sex distribution of LTCF residents from whom nasal swab specimens were obtained for rapid influenza diagnostic testing and further virological assessment. (B) Frequency of specimen collection based on time in days from symptom onset.

benefit of RIDT use in LTCF for early identification of influenza. The subsequent handling and transfer of nasal swabs was a secondary outcome, as RAPID-LTCF was not designed to be an epidemiologic assessment of respiratory viruses in such locations. Nevertheless, as the 10 study sites were randomly selected from a pool of 20 sites and had a wide geographic distribution, we do have a generalizable sampling frame. Second, we did not evaluate compliance of individual sites. In each of the first 2 years, personnel at 9 sites collected specimens; this dropped to 8 sites in the third year. The third year, however, was marked by a very late influenza season and manifested lower levels of respiratory virus activity across Wisconsin as evidenced by a 14% decline in ARI cases for patients ≥65 years of age recorded in electronic health record monitoring of Wisconsin surveillance clinics.<sup>1</sup> Moreover, because of early autumn training and staff turnover, late season testing may be vulnerable to attrition in trained nursing staff. Third, other factors may have created interference with specimen transport. The WSLH changed couriers following the first year; the decline in the number of specimens that were transferred to WSLH for RT-PCR and RPP in the second 2 years was attributable, in part, to courier issues. Fourth, the RPP used in this study had a limited number of pathogens. Accordingly, the rate of virus identification may have been limited by the array of pathogens. The overall rate of virus detection, however, was similar to that found in our other surveillance systems using the same laboratory testing<sup>1</sup> as well as in studies designed primarily to assess the epidemiology of respiratory viruses in LTCF settings.<sup>17,18</sup> Fifth, we were unable to couple virus identification with clinical outcomes. Consequently, we do not know if there was selection bias in nurse identification of residents for testing. Finally,

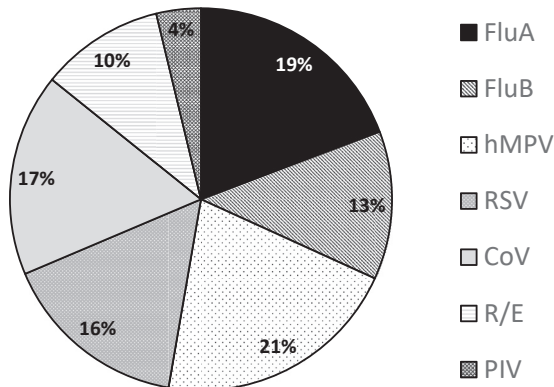


**Fig. 2.** (A) Percent of specimens from which viruses were detected by RT-PCR or RPP based on age group of LTCF residents. (B) Percent of specimens from which viruses were detected by RT-PCR or RPP based on time in days between symptom onset and collection of nasal swab specimen.

our study sites were distributed statewide, but were limited to less than 3% of Wisconsin's LTCFs.

**Conclusions and Implications**

Simple approaches to surveillance may provide a more comprehensive assessment of respiratory viruses in LTCF settings. The use of nasal swab specimens, obtained for on-site rapid influenza testing, was associated with a relatively high rate of respiratory virus identification by subsequent influenza RT-PCR and RPP. Detection of



**Fig. 3.** Composition of respiratory viruses detected over the entire 3-year study period. Wisconsin LTCFs; December 2016 to June 2019.

**Table 2**  
Viruses Identified From LCTF Residents During the 3 Consecutive Influenza Seasons (2016/2017, 2017/2018, 2018/2019)

| Year      | Virus Detected |           |           |           |         |           |           | Total |
|-----------|----------------|-----------|-----------|-----------|---------|-----------|-----------|-------|
|           | CoV            | Flu A     | Flu B     | hMPV      | PIV     | R/E       | RSV       |       |
| 2016–2017 | 18             | 16        | 19        | 0         | 5       | 10        | 16        | 84*   |
| 2017–2018 | 14             | 26        | 11        | 33        | 0       | 5         | 21        | 110†  |
| 2018–2019 | 9              | 4         | 0         | 17        | 4       | 10        | 1         | 45    |
| Total     | 41 (17.2)      | 46 (19.2) | 30 (12.6) | 50 (20.9) | 9 (3.8) | 25 (10.5) | 38 (15.9) | 239   |

Viruses were assigned to the following groups: coronavirus (CoV including 229E, HKU1, OC43, NL63), influenza A (FluA including H3N2, H1N1), influenza B (FluB), human metapneumovirus (hMPV), parainfluenza viruses (PIV including 1, 2, 3, 4), rhinovirus/enterovirus (R/E), and respiratory syncytial virus (RSA including RSV, RSVB).

\*Includes 1 co-detection (PIV 1-RSV B)

†Includes 3 co-detections (CoV NL63-FluA[H3]; CoV OC43-FluA[H3]; hMPV-R/E)

respiratory viruses was not significantly affected by resident age or time from symptom onset (up through 4 days). This approach demonstrated a wide variety of viruses and significant variability from year to year. Despite this variability, however, 4 viruses with high potential for morbidity and mortality in LCTF settings were found in nearly 70% of ARI cases. On-site testing of nasal swab specimens—collected on-site from residents with ARI by nursing staff—with additional virological testing off-site may not only provide a useful alternative for both respiratory virus diagnostic and surveillance efforts, but may ultimately provide useful information for infection control efforts and justification for prophylactic treatment of LCTF resident populations. This approach is consistent with and enhances existing guidelines that call for collection of specimens early in illness, appropriate testing as soon as possible, and reporting of results to public health and the originating facility.<sup>1</sup>

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