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Public Hospital-Based Laboratory Experience during an Outbreak of Pandemic Influenza A (H1N1) Virus Infections[▽]

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The experience of a public hospital virology laboratory during a springtime 2009 outbreak of a novel influenza A (H1N1) virus in New York State is described. Influenza virus was isolated from 145 of 613 respiratory swab specimens. Symptoms of fever (temperature, 102.7 ± 0.32°F), cough, upper respiratory infection, myalgia, and headache were reported. Atypical symptoms of nausea/vomiting and diarrhea were also observed. Illness occurred mainly in patients ≤21 years of age (85/145 patients). Only two patients were ≥65 years old. Compared to the results of traditional culture methods, the sensitivities of a rapid chromatographic influenza A and B virus immunoassay and rapid shell vial culture were 70.3% and 98.6%, respectively. A sensitivity of 80% was obtained by testing 50 specimens by a direct fluorescent-antibody (DFA) assay. The observation of adequate numbers of cells on the DFA assay slides suggests that the low sensitivity of the chromatographic immunoassay may result from its intrinsic nature and not from improper specimen collection. A reverse transcription-PCR (RT-PCR) assay of 45 specimens performed off-site yielded 21 novel (H1N1) viruses and 2 seasonal (H3N2) influenza viruses. The mean time interval of 5.69 ± 0.37 days from specimen collection to the availability of RT-PCR results limited the value of this assay for patient care. In laboratories lacking on-site molecular capabilities, shell vial techniques can rapidly (about 1 day) confirm negative results and/or identify false-negative chromatographic immunoassay results. Laboratories lacking culture capabilities may also use the DFA assay to confirm or replace the results obtained by these immunoassays. Increasing testing demands caused shortages in commodities and personnel. Alternative testing strategies and planning are necessary in order to optimize virus detection and ensure appropriate resource allocation.

In March 2009, an outbreak of a novel strain of influenza A (H1N1) virus, first identified in Mexico, spread worldwide, becoming a new pandemic virus (1, 2, 7). New York City and vicinity became an early epicenter of this pandemic when an outbreak, presumably transmitted by students returning from vacation in Mexico, occurred in a Queens County (adjacent to Nassau County) high school (2, 10).

Increased numbers of patient visits to hospital emergency departments (EDs) followed in the wake of this outbreak. Likewise, the heightened need for laboratory testing ultimately caused shortages in personnel and supplies over the following 10 weeks, until the levels of illness subsided.

A number of laboratory assays are available for the diagnosis of influenza (3, 8, 10, 11). Rapid antigen detection assays, including chromatographic immunoassay techniques, are widely utilized, as they are easily adapted to the hospital ED and physician office settings on a 24-h-a-day, 7-day-a-week basis. Testing requires limited technical expertise and little or no equipment, is inexpensive, and rapidly produces results (in about 15 min).

Testing by the direct fluorescent-antibody (DFA) assay can also be used to rapidly identify influenza virus. The DFA assay offers an advantage over chromatographic immunoassays because it assesses specimen adequacy; however, the time to the availability of the results is longer, and special equipment and enhanced technical expertise are required to perform the assay (10, 11).

The combined use of a rapid shell vial culture assay (with A549 and Mv1Lu cells) and a pool of respiratory virus fluorescent antibodies (with a D^3 respiratory virus antibody pool) allows common respiratory pathogens to be identified in 24 to 48 h postinoculation (10). Shell vial culture identifies pathogens more rapidly than traditional tube culture and has roughly the same sensitivity and specificity as traditional tube culture. Many hospital-based laboratories do not perform culture testing on-site, as this assay often requires additional laboratory permits and accreditations, various pieces of equipment and supplies, and a high degree of technical experience.

Hospital laboratories offering molecular techniques for the detection of influenza viruses are even more limited. Molecular-based assays are often not approved by the Food and Drug Administration (FDA) and require extensive in-house validation testing before approval for their use may be obtained (9, 10). These assays may incorporate a multiplex format, which can simultaneously identify numerous viral and bacterial agents (10). The results are rapidly obtained without the need for pathogen viability, and the assays demonstrate superlative sensitivities and specificities (10).

In response to the emerging pandemic, the Centers for Disease Control and Prevention released a reverse transcription-PCR (RT-PCR) molecular assay, on an emergency authorization use basis, designed to definitively identify the novel influenza A (H1N1) virus. The Wadsworth Center laboratory

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of the New York State Department of Health, Albany, NY, was given authority to run this assay in New York State (9).

The present study evaluated the performance of our virology laboratory prior to, during, and following pandemic influenza activity. Data on patient symptoms and demographics were collected. The performance and potential value of nonmolecular diagnostic testing and off-site RT-PCR testing for patient care were evaluated. In addition, the impact of increased testing demands on laboratory resources was also investigated in order to formulate strategies to improve laboratory performance for the 2009–2010 respiratory virus season.

MATERIALS AND METHODS

Specimen collection and handling. Physicians, physician's assistants, and nurses collected nasopharyngeal swab specimens (ideally, one from each nostril) from patients seen at hospital clinics and the emergency department. A testing algorithm was utilized in which patients presenting with temperatures of greater than 100°F and having a respiratory illness, including pneumonia, acute respiratory distress syndrome, and/or symptoms of an upper respiratory infection (URI), were examined for respiratory viruses. Specimens were placed in viral transport medium (Diagnostic Hybrids, Inc. [DHI], Athens, OH), and both a chromatographic immunoassay and viral culture testing were ordered for the specimens. The collection of information on the demographics, underlying medical conditions, and symptoms of the patient was required before specimen testing orders were allowed to be processed. Specimens were immediately delivered for rapid chromatographic immunoassay testing on a 24-h-a-day, 7-day-a-week basis. The results were reported to the emergency department within 1 h of collection. Specimens were then inoculated into a culture within 4 h or were stored at refrigerated temperatures (2 to 8°C) and inoculated within 48 h of collection.

Rapid diagnostic methods. The BD Directigen EZ Flu A+B chromatographic immunoassay (Becton Dickinson and Co., Sparks, MD) was used to rapidly identify influenza virus-positive patients. A volume of 0.3 ml of specimen was tested by using the procedures outlined by the manufacturer.

For 50 patients, a 0.2-ml volume of specimen was also used to make microscope slide preparations with the aid of a Cytospin 3 centrifuge (Thermo, Inc., Pittsburgh, PA). Following centrifugation, the slides were fixed in acetone for 10 min, air dried, and then coded to remove the patient's identity. The preparations were stained with fluorescent monoclonal antibodies to influenza A virus (DHI) and were examined at ×400 magnification with an episcopic microscope equipped with a 460- to 490-nm excitation filter (Nikon Inc., Garden City, NY). The presence of cellular fluorescent activity was noted, and estimates of cell numbers were made.

Culture techniques. Aliquots of the specimens (0.2 ml) were inoculated into tube cultures with rhesus monkey cells, A549 cells (DHI), and MRC-5 cells (Viromed Inc., Minnetonka, MN) by established techniques. The tubes were incubated at 34 to 36°C and were observed by inverted light microscopy at least 3 times per week over a 2-week period. Cultures demonstrating a cytopathic effect were vortexed, and cell spots were prepared on glass microscope slides by using a Cytospin 3 centrifuge (Thermo, Inc.). Following treatment with acetone, the slides were stained with the appropriate fluorescent monoclonal antibodies (DHI) by prescribed techniques to confirm the culture cytopathic effect.

An aliquot(s) of specimen (0.2 ml) was also inoculated into shell vial cultures with A549 and Mv1Lu cells (R-mix; DHI), which were centrifuged at 1,200 × *g* for 30 min. Following replacement of the R-mix maintenance medium (0.5 ml; DHI), the vials were incubated at 34 to 36°C for approximately 24 h. The vials were then fixed in acetone and were stained with a pool of respiratory virus monoclonal antibodies (D^3 DFA assay respiratory virus antibody pool; DHI) which identified the presence of adenoviruses, influenza A and B viruses, parainfluenza types 1 and 2, and respiratory syncytial virus (RSV) in the cells.

Molecular testing. The Wadsworth Center laboratory at the New York State Department of Health was given clearance by the FDA to perform an RT-PCR assay to definitively identify the novel influenza H1N1 virus. Seasonal influenza A (H3N2 and H1N1) virus strains could also be identified. A total of 45 patient nasopharyngeal swab specimens were forwarded to the Wadsworth Center laboratory. The specimens submitted included those from both immunoassay-positive and immunoassay-negative patients, which were sent on the basis of a triage process implemented by the Nassau County Department of Health, which used the patient history, physician interview, medical conditions (i.e., pregnancy), etc. Estimates of the time (in days) from specimen collection to receipt at the Wadsworth Center laboratory and to final reporting of the results were collected and evaluated with respect to the time of reporting of the culture result. Statistical analysis was done by using a computer-generated descriptive analysis program (Excel; Microsoft, Inc., Redmond, WA).

Potential laboratory shortages. A list of the potential and actual laboratory supply shortages, along with possible remedies, was evaluated.

TABLE 1. Respiratory viruses isolated in tube culture during weeks ending 28 March through 18 July 2009[a]

End day of wk in 2009	No. of specimens[b]					
	Total	Culture positive[c]				
		INF A	INF B	PIV	ADENO	RSV
28 March	17	0	0	0	1	1
4 April	25	0	0	1	0	0
11 April	18	0	0	0	0	0
18 April	31	1	0	1	0	0
25 April	12	0	1	0	0	0
2 May	20	8	0	0	0	0
9 May	23	1	0	6	1	0
16 May	44	4	0	1	0	0
23 May	33	10	0	5	0	0
30 May	46	14	0	5	0	0
6 June	66	32	0	1	0	0
13 June	67	26	0	0	1	0
20 June	73	31	0	1	2	0
27 June	83	12	0	1	1	0
4 July	47	5	0	0	0	0
11 July	34	1	0	0	1	0
18 July	24	0	0	0	0	0
Total	613	145	1	22	7	2

[a] Confirmation of suspected viral isolates was done by fluorescent monoclonal antibody techniques. The viruses isolated included influenza A virus (INF A), influenza B virus (INF B), parainfluenza virus types 1 to 3 (PIV), adenoviruses (ADENO), and respiratory syncytial virus (RSV). Specimens from a total of 52 newborns routinely screened for respiratory viruses while they were in the neonatal intensive care unit were included in the population tested.

[b] Respiratory specimens included throat and nasopharyngeal swab, bronchoalveolar lavage, and autopsy tissue specimens.

[c] Inoculated in tube cultures with rhesus monkey, A549, and MRC-5 cells for 14 days at 34 to 36°C.

RESULTS

A total of 177 respiratory viruses were isolated in tube culture from 613 specimens tested from 22 March through 18 July 2009 (Table 1). Influenza A viruses accounted for 145 of these isolates. Other viruses included parainfluenza virus (22 isolates), adenovirus (7 isolates), respiratory syncytial virus (2 isolates), and influenza B virus (1 isolate). The peak influenza A virus activity occurred during the weeks ending on 30 May 2009 through 20 June 2009.

Both the number of respiratory specimens tested and the number of viruses isolated far exceeded what is normally seen during that time of year. By comparison, a total of 156 specimens tested during the same time period in 2007 and 146 specimens tested during the same time period in 2008 only yielded five and seven viral isolates, respectively.

A variety of symptoms were identified in the 145 patients harboring influenza A virus. The most prevalent symptom was fever (temperature, 102.7 ± 0.32°F), which occurred over a mean duration of 2.9 ± 0.61 days prior to examination. Other reported symptoms included cough (n = 134 patients), URI (i.e., rhinorrhea, congestion, and sore throat; n = 127), nausea/vomiting (n = 59), myalgia (n = 55), headache (n = 52),

TABLE 2. Age distribution of patients culture positive for respiratory viruses between 22 March and 18 July 2009[a]

Age group (yr)	Influenza A virus				No. of patients positive			
	No. of patients positive	No. of patients negative	% positive	95% CI[b]	Influenza B virus	Parainfluenza	Adenovirus	RSV
	145	434			1	22	7	2
<1	10	95	18.8	4.4	0	8	3	1
1–3	11	65	18.8	5.5	1	3	4	1
4–12	26	38	50.6	10.5	0	3	0	0
13–21	38	40	56.8	10.9	0	1	0	0
22–31	21	61	34.4	7.4	0	1	0	0
32–41	18	34	37.3	10.3	0	2	0	0
42–51	9	36	23.5	8	0	2	0	0
52–64	10	37	21.7	7.5	0	0	0	0
>64	2	28	6.7	4.9	0	2	0	0

[a] Virus identity was confirmed by fluorescent microscopy with the appropriate antibody reagents (DHI). The specimens were inoculated into tube cultures with rhesus monkey, A549, and MRC-5 cells, incubated at 33 to 35°C, and examined for a cytopathic effect over a 14-day period.
[b] CI, confidence interval.

bronchitis ($n = 45$), diarrhea ($n = 39$), and pleurisy ($n = 23$). A majority of patients ($n = 98$) reported either exposure to a person known to be influenza A virus positive or recent travel outside the United States.

The age distribution of the patients harboring respiratory viruses is found in Table 2. A majority of influenza A virus infections (38/145) occurred in those 13 to 21 years of age, whereas only 2 patients were older than 64 years of age. Influenza A virus infection was significantly greater in the 13- to 21-year-old age group than in those in the age groups under 1 year, 42 to 51 years, 52 to 64 years, and 65 years and older ($P < 0.05$). Over the previous two influenza seasons, a majority of infected patients were found to be over 64 years of age.

Parainfluenza virus infection was observed across all age groups. In the previous five respiratory virus seasons, parainfluenza virus was exclusively isolated from children 5 years old or younger. Adenovirus and RSV were isolated from pediatric patients 3 years of age or younger.

By using traditional tube culture as the "gold standard," we were able to evaluate other rapid diagnostic assays for influenza A virus utilized in the laboratory (Table 3). Rapid shell vial culture (R-mix) was able to generically identify the presence of common respiratory viruses by approximately 1 day postinoculation. The sensitivity and specificity were 98.6% and 100%, respectively, for those patients infected with influenza A virus. The R-mix shell vials demonstrated 100% sensitivity and 100% specificity for the other respiratory viruses.

TABLE 3. Evaluation of a chromatographic immunoassay, R-mix shell vial culture, and tube culture methods for identification and isolation of influenza A virus in respiratory specimens

Item	EZ Flu[a]	Shell vial[b]	Tube culture[c]
No. of specimens positive	102	143	145
% specimens positive ± 95% CI[d]	16.6 ± 6.6	21.3 ± 6.1	23.6 ± 6.1
No. of specimens negative	511	470	468
Sensitivity (%)	70.3	98.6	100
Specificity (%)	100	100	100

[a] EZ Flu, BD Directigen EZ Flu A+B assay.
[b] Aliquots (0.2 ml) of respiratory specimens was inoculated into shell vials with A549 and Mv1Lu cells (R-mix; DHI). The vials were spun at 1,200 × *g* for 30 min, incubated at 33 to 35°C, harvested at 20 to 26 h, fixed in acetone, and stained with D^3 DFA assay respiratory virus antibody pool (DHI).
[c] Tube cultures with rhesus monkey cells, A549 cells (DHI), and MRC-5 cells (Viromed, Inc.) were inoculated with aliquots (0.2 ml) of respiratory swab specimens, incubated at 33 to 35°C, and observed for a cytopathic effect for 14 days. Positive results were confirmed by fluorescent microscopy with the appropriate staining reagents (DHI).
[d] CI, confidence level, found to be statistically nonsignificant.

Reporting of the rapid chromatographic immunoassay result for influenza A and B viruses occurred within 1 h of specimen collection. The immunoassay failed to detect influenza A virus in 43 of 145 patients, yielding a sensitivity of 70.3%. The specificity was found to be 100%.

For 50 patients, the DFA assay was added to the testing regimen. A total of 15 patients were positive for influenza A virus by the tube culture assay (Table 4). Compared to the results of the tube culture assay, sensitivities of 66.7, 100, and 80% were obtained by the chromatographic immunoassay, shell vial culture, and DFA assay methods, respectively. The specificities of all assays were 100%.

Estimates of cell numbers on the DFA assay slides yielded 39 cases in which more than 100 cells were observed and only 3 cases in which less than 10 cells were found. Ample cell numbers (>100 cells) were available in those specimens for which the chromatographic immunoassay or the DFA assay yielded false-negative results. Differentiation between squamous and columnar epithelial cells was not done in the present study.

Of 45 specimens forwarded to the Wadsworth Center laboratory for testing by RT-PCR, 21 were found to be positive for the novel influenza A (H1N1) virus and 2 were found to be positive for seasonal influenza A (H3N2) virus. The specimens containing seasonal influenza A (H3N2) virus were collected in early May of 2009, whereas specimens positive for the novel influenza A (H1N1) virus strain were collected from mid-May into July 2009. The culture results were identical to the findings of RT-PCR for these 45 specimens. In addition, in three patient specimens, tube culture isolated 3 adenoviruses that were not identified by RT-PCR. The chromatographic immunoassay yielded seven false-negative results (70% sensitivity) compared with the results of RT-PCR.

The mean time intervals from specimen collection to reporting of the results were found to be 5.12 ± 42 days for culture and 5.69 ± 0.37 for RT-PCR, demonstrating statistically nonsignificant differences ($P = 0.33$). Further analysis of the RT-

TABLE 4. Evaluation of assay performance and nasopharyngeal swab specimen adequacy with 50 respiratory specimens examined for influenza A virus

Item	Result by the following assay[a]:				Result for the following no. of cells/slide[b]:		
	EZ Flu	R-mix	Tube	DFA assay	<10	10–100	>100
No. negative	40	35	35	38			
No. positive	10	15	15	12			
% positive ± 95% CI[c]	20 ± 22	30 ± 20	30 ± 20	12 ± 21			
Sensitivity (%)	66.7	100	100	80			
Specificity (%)	100	100	100	100			
Total no. of specimens					3	8	39
No. of specimens with true-positive results					0	3	12
No. of specimens with true- negative results					3	5	27
No. of specimens with false-negative results by RIA[d]					0	0	5
No. of specimens with false-negative results by DFA assay					0	0	3

[a] EZ Flu, BD Directigen EZ Flu A+B assay; R-mix, shell vial cultures with A549 and Mv1Lu cells (DHI) inoculated with 0.2 ml specimen harvested at 18 to 28 h and stained with a respiratory virus monoclonal antibody pool (D^3 DFA assay respiratory virus antibody pool; DHI); Tube, tube cultures with rhesus monkey cells, A549 cells (DHI), and MRC-5 cells (Viromed, Inc.) inoculated with 0.2 ml of specimen, incubated at 34 to 36°C, and examined for 14 days; DFA assay, microscope slides were prepared with a Cytospin 3 centrifuge (Thermo, Inc.) and by using 0.2 ml specimen per slide, and the slides were stained with influenza A fluorescent monoclonal antibodies (DHI).

[b] Microscope slides were examined with a Nikon episcopic fluorescent microscope (Nikon, Inc.) at ×100 to ×400 magnification.

[c] CI, confidence interval, found to be statistically nonsignificant.

[d] RIA, rapid immunoassay.

PCR procedures demonstrated a mean time interval of 1.62 ± 0.21 days from specimen receipt at the Wadsworth Center laboratory to reporting of the results. In contrast, it took approximately 4.1 days to evaluate the need for molecular testing and for specimen processing and transport to the laboratory to occur.

A number of potential shortages resulted from the increased testing demands placed on the laboratory. We were able to substitute culture maintenance medium, which regularly undergoes monthly quality control testing, for viral transport medium. Aliquots of maintenance medium were placed in sterile conical centrifuge tubes, packaged along with sterile swabs, and labeled with the maintenance medium lot number and an expiration date set before the next scheduled quality control testing. The shortage of transporting supplies was handled by obtaining suitable boxes and preparing the necessary shipping labels.

Increased personnel needs arose. Nighttime staffing of the microbiology laboratory was utilized during "off hours" in the Virology Laboratory to accommodate rapid immunoassay testing on a 24-h-a-day, 7-day-a-week schedule. In addition, approximately 12% increased technical time (12 to 15 h of overtime per week) was needed to process and package the specimens, transport the materials for outside testing, etc.

DISCUSSION

Nassau University Medical Center (NUMC) is a 530-bed, tertiary-care public hospital which, together with a 589-bed skilled nursing facility and a network of seven community health centers, is part of the Nassau County, NY, Health Care Corporation. Annually, NUMC treats more than 75,000 patients in the ED and more than 200,000 patients in its outpatient clinics. The corporation provides a health care safety net for poor and uninsured individuals in Nassau County and vicinity, regardless of their ability to pay for such services.

Most public hospital emergency departments are often used by poor and uninsured individuals for primary health care. In the recent novel influenza A (H1N1) virus epidemic, emergency departments became overwhelmed by a surge of anxious patients who presented with an influenza-like illness with various degrees of severity. The increased diagnostic testing needs also placed demands on laboratory resources during a time when the typical winter respiratory illness season was thought to have ended. This report summarizes the experiences of a public hospital viral diagnostic laboratory during an outbreak of pandemic influenza that occurred in New York State from April through July 2009. The experiences and recommendations presented herein will, it is hoped, prepare the small hospital laboratory for the upcoming influenza season.

Early reports indicated that infection with influenza A (H1N1) virus caused the self-limiting symptoms common in influenza illnesses, including fever, cough, and URI (1, 2, 4). Common risk factors, including pregnancy and chronic underlying conditions (pulmonary, cardiovascular, neurological, and immunological conditions), which can exacerbate influenza illness were also reported (5, 6). In our study population, the symptoms reported with the illness included fever (mean temperature, 102.7 ± 0.32°F), cough (92% of patients), URI (88%), myalgia (37%), and headache (36%). Atypical symptoms of diarrhea and nausea/vomiting reported in previous investigations of pandemic viral infection (7) were also observed in 27% and 40% of our patients, respectively.

Important distinctions between seasonal influenza and the present 2009 influenza A (H1N1) virus pandemic exist. Advanced age did not appear to be a risk factor associated with pandemic influenza A (H1N1) virus infection, as evidenced by early reports citing the occurrence of a majority of infections in adults 30 to 44 years of age and a relative lack of illness among elderly individuals (1, 2, 7). In the present investigation, a majority of cases of illness occurred in those 21 years old and younger (85/145 cases), whereas only 2 cases of illness were identified in those older than 64 years of age. These results

suggest that individuals in younger age groups have a greater susceptibility to infection, that differences in social networks which delay transmission to older people exist, or that elderly people possess some level of cross-reactivity against the novel influenza A (H1N1) virus strain (7). Extreme obesity, not recognized in seasonal influenza, has also surfaced as a possible independent risk factor for severe complications in pandemic virus infection (6).

The poor performance of the chromatographic immunoassay readily became apparent in early investigations of pandemic influenza illness (3, 10). In a study with a large patient volume comparing rapid and culture methods with a molecular multiplex respiratory viral assay (Luminex xTAG), Ginocchio et al. (10) reported a combined sensitivity of 17.8% for the Binax NOW Influenza A+B (Inverness, Scarborough, ME) and the 3M Rapid Detection Flu A+B (3M Medical Diagnostics, St. Paul, MN) antigen detection assays. A CDC study comparing three immunoassay products produced similar findings, with overall sensitivities ranging from 40% to 69% for the Binax NOW Influenza A+B, Directigen EZ Flu A+B, and QuickVue Influenza A+B (Quidel Corp, San Diego, CA) products.

The present study found that the Directigen EZ Flu A+B immunoassay had a sensitivity of approximately 70%. The increased sensitivity of the assay in our study most likely results from the use of culture instead of PCR as the standard for assay evaluation. The RT-PCR assays used in other evaluations are considered more sensitive than culture and, consequently, would produce lower sensitivities.

It is possible that the reduced sensitivity of the immunoassay could result from inadequate swab specimen collection. Nasopharyngeal aspirates or washes can collect more cells than swabs. Nevertheless, swab specimens are considered easier and safer to obtain, especially in light of the potential for aspiration pneumonia resulting from increased bacterial colonization and the reduced gag reflex of elderly individuals (the usual risk group for complicated influenza virus infections) (11). For a subgroup of 50 patients, testing by the DFA assay was added to the study regimen to ascertain if the low sensitivity of the immunoassay was a result of poor specimen collection. Our results suggest that ample numbers of cells were available for testing (>100 in cases in which immunoassay and the DFA assay produced false-negative results) and that the low sensitivity of the immunoassay may be a product of the intrinsic properties of the assay itself.

Shell vial culture demonstrated sensitivities of 98.6% in the present study and 88.9% in an earlier investigation (10). The availability of results in approximately 1 day postinoculation and the ability to generically identify seven respiratory viruses make this assay attractive in facilities lacking on-site RT-PCR capabilities. Negative shell vial assay results can substantiate the negative findings obtained by immunoassay. Alternatively, a positive shell vial assay result can be further confirmed by using individual viral fluorescent antibodies to specifically rule in the presence of influenza virus and other respiratory pathogens.

Shell vial culture has the versatility to provide positive results for other respiratory viruses, a property lacking in the CDC molecular assay authorized for emergency use. There is certainly a positive psychological benefit to the rapid identification of the cause of illness in patients. Rapid viral identification can also maximize resource utilization in the hospital setting.

In laboratories that lack culture or molecular diagnostic capabilities, the use of the DFA assay as a replacement or an adjunct to immunoassays may also improve the sensitivity of virus detection. A recent study demonstrated that DFA and PCR assays had similar performance characteristics for the identification of pandemic influenza virus (12). The pitfalls of testing by the DFA assay rather than the rapid immunoassay include equipment needs, the need to use a larger specimen volume, and the greater specimen processing time; performance of the DFA assay also requires greater technical skill. These factors may preclude the use of the DFA assay in hospitals with large test volumes.

The time necessary to obtain off-site RT-PCR assay results (5.69 ± 0.37 days from the time of specimen collection to the time of the availability of results) offered little clinical benefit to patient management. Further analysis indicated that a majority of the time was spent in assessing the need to send the specimen for RT-PCR and the actual transport process (mean, 4.1 days). In contrast, it took only 1.6 days from the time that the specimen was received at Wadsworth Laboratory to the time of reporting of the results. Improvements in specimen triage and transport have evolved, and these should result in shorter times to the reporting of the results. Ultimately, timely RT-PCR results may be achieved by altering the role of the state health department laboratory from being a testing site to a training and validation site for laboratories that are familiar with FDA-approved molecular techniques but that lack experience in formulating and validating "home-brewed" assays.

Regardless of the diagnostic mode used, the potential for laboratory supply shortages and problems are increased at times of high demand. A proactive strategy for obtaining or substituting assay materials, supplies, and disposables should be in place well before the start of the flu season. These replacements may include substituting culture maintenance medium (which routinely undergoes quality control testing) for viral transport medium and obtaining and validating a stock of frozen cultures (obtainable from DHI) for use in times of testing surges. Validation of the capabilities of staff from other laboratories to assist with a 24-h-a-day, 7-day-a-week testing regimen and assignment of blocks of time when staff are available to work overtime can be invaluable to accommodating personnel needs. If the plans of a laboratory are to perform tests off-site, personnel must be trained and certified to transport potentially dangerous goods. Having a number of people certified and responsible for acquiring the forms, labels, and transport materials needed may reduce shipping times. The success of the hospital-based laboratory in providing quality patient care during the influenza season will depend on laboratory preparedness and cooperation from the entire hospital staff.

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