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Major article

Respiratory virus identification by interval polymerase chain reaction testing in the southeastern United States

Diane C. Halstead PhD [a,*], Eydie R. Garcia BS [b], Sharon Wright BS [b], Kimberly McKean BS [b], Page McKitrick BS [b]

[a] Jacksonville Pathology Consultants, PA, at Baptist Health, Jacksonville, FL
[b] Infectious Disease Laboratory Service, Baptist Medical Center, Jacksonville, FL

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Background: This study was designed to determine if testing the first ~40 nasal washings (interval) each month for 1 year, could be used as an epidemiologic tool for seasonality and prevalence of respiratory viruses such as human metapneumovirus in an adult and pediatric population in the southeastern United States.

Materials and Methods: Results of interval polymerase chain reaction (PCR) testing of 469 specimens for 8 viruses were compared with our current procedures using PCR, culture, or respiratory synctial virus antigen for all 7435 specimens (routine).

Results: One hundred thirty-six viruses out of 469 specimens (29.0%) and 1,495 viruses out of 7,435 specimens (20.1%) were identified by interval and routine testing, respectively. Seasonal detection varied among viruses and to some degree between interval and routine testing. A higher percent of positives and dual infections were detected by interval testing of pediatric specimens, likely due to the use of PCR for viruses commonly seen in this population. Human metapneumovirus was detected in both pediatric and adult specimens between January and August.

Conclusions: Interval testing can be used to provide a snapshot of prevalence and seasonality of respiratory viruses, although as currently designed they may not be sensitive enough to identify the beginning of a specific virus season. Exclusive use of interval PCR testing identified several dual infections, including human metapneumovirus, throughout most of the year in Florida. A rapid turnaround time to results translates into improved infection control and improved patient care.

Respiratory viral infections can lead to significant morbidity and mortality even in light of the availability of vaccines and antivirals for prevention or treatment of some of these infections. Many of these infections present with similar signs and symptoms that make it difficult if not impossible to clinically differentiate 1 virus infection from another.[1-3] Although virus isolation has during the past decades been the mainstay for diagnosing respiratory viral infections, polymerase chain reaction (PCR) assays have now been introduced for both traditional respiratory viruses and emerging viruses like human metapneumovirus (hMPV), which was first identified in 2001.[4] These assays are more sensitive with improved turnaround time to results compared with culture,[3,5,6] allowing better understanding of respiratory virus epidemiology and improved infection control. In clinical practice however, a specific virus is often not identified due to lack of availability of sensitive assays or resources to perform these tests. Therefore, physicians must rely on community surveillance programs, if available, to provide information on the viruses that are circulating in their community.

Those laboratories that have viral testing capabilities are encouraged to perform routine respiratory virus surveillance testing and share this information with the medical community within and outside of their facility. In this way current information would be available to alert the community to circulating viruses and the need for immunization or prophylaxis, and alert the institution of infection control practices when appropriate. To provide this service, the Baptist Health Infectious Disease Laboratory Service in northeast Florida performs weekly surveillance and

* Address correspondence to Diane C. Halstead, PhD, Infectious Disease Laboratory Service, Jacksonville Pathology Consultants, PA, Baptist Health, Main 3 N, 800 Prudential Dr, Jacksonville, FL 32207.
E-mail address: diane.halstead@bmcjax.com (D.C. Halstead).
Conflicts of interest: None to report.

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reporting to IMS; the Florida Department of Health Emergency Notification System; and the Jacksonville, FL, medical community via e-mail and postings on physician and employee Web sites.

The study presented here was designed to determine if the results from the first ~40 nasal washing specimens received each month (interval testing by PCR) compared with results of all specimens received each month for virus identification (routine testing by culture, PCR, or respiratory syncytial virus [RSV] antigen detection), could be used as an epidemiologic tool to determine the prevalence of respiratory viral activity in the community. We also compared the presence of respiratory virus activity using interval and routine testing in our pediatric and adult populations. We were particularly interested in the prevalence and seasonality of hMPV in our region because we were not routinely testing for hMPV and there were no published data on the seasonality of this virus in the southeastern United States. We therefore evaluated the use of a molecular real time (RT)-PCR assay to identify this virus in northeastern Florida during the study period (October 2010 through September 2011). Finally, we compared the overall sensitivity of using RT-PCR exclusively used for interval testing versus our routine testing procedure.

MATERIALS AND METHODS

Our approach to identify respiratory viruses (routine testing) of 7,435 (4,484 pediatric and 2,951 adult) nasal washing specimens included the use of several methods. Clinicians could order molecular multiplex RT-PCR for influenza A and B and RSV, a comprehensive viral culture used to detect 7 of the most common respiratory viruses with the exception of hMPV, or a rapid antigen test specifically for RSV used exclusively for patients seen in the emergency center at Wolfson Children's Hospital. For comparison each month, the first 40 nasal washings received for a total of 469 specimens (297 pediatric and 172 adult) were tested by RT-PCR for 8 respiratory viruses (interval testing).

Routine testing was performed within 24 hours of specimen receipt and included the use of R-Mix A549/Mv1Lu shell vials for comprehensive viral cultures (Diagnostic Hybrids, Athens, OH), NOW RSV antigen test (Binax Inc, Scarborough, MA) for specimens collected in our pediatric emergency center, and Prodesse ProFlu+ Influenza A and B, RSV multiplex RT-PCR (Gen-Probe/Hologic, San Diego, CA). Interval testing not only included results from the routine Prodesse ProFlu+ but also ProAdeno+, ProParaflu+, and ProhMPV+ (Gen-Probe/Hologic, San Diego, CA). The molecular assays were modified and verified using a Universal Internal Control for RNA and DNA (Gen-Probe/Hologic, San Diego, CA) and nasal washings in M4RT VTM (Remel Inc, Lenexa, KS). Otherwise the tests were performed according to manufacturer's instructions. Specimens were extracted using NucliSENS easyMAG (bioMerieux, l'Etoile, France) before molecular testing. If RT-PCR was ordered for influenza A and B and RSV, the remaining extracted eluates were stored at −70°C and batch tested (interval testing) for adenoviruses; hMPV; and parainfluenza viruses 1, 2, and 3. The study was in compliance with investigational review board requirements.

RESULTS

During the 12-month period, a higher percent of viruses per number of specimens were identified by interval testing using RT-PCR (Fig 1). A total of 136 viruses in 469 specimens (29.0%) were identified by interval testing: 25 viruses in 172 adult patients (14.5%) and 111 viruses in 297 pediatric patients (37.4%). A total of 1,495 viruses out of 7,435 specimens (20.1%) were identified in routine testing: 578 viruses in 2,951 specimens from adult patients (19.6%) and 917 viruses in 4,484 speciments from pediatric patients

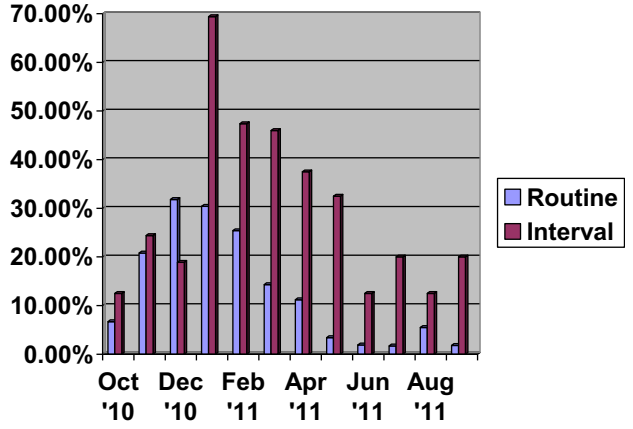

Fig 1. Percent of positive specimens tested each month by routine and interval testing (October 2010-September 2011).

Table 1
Peak month of virus detection by interval versus routine testing

	Interval		Routine	
	Initial detection	Peak detection	Initial detection	Peak detection
Influenza A	November	January	October	January
Influenza B	January	February	November	February
Respiratory syncytial virus	Throughout year	January	Throughout year	January
Adenovirus	Throughout year	April	Throughout year	December/ March
Parainfluenza 1 -3	Throughout year	May	Throughout year	March
Human metapneumovirus	January-August	May	ND	ND

ND, not done.

(20.5%). None of the samples included in routine testing nor any of the adult specimens included in interval testing were positive for >1 virus. In contrast, 11 pediatric samples in interval testing were positive for 2 viruses; 9 out of 11 were positive for adenovirus and another virus. Table 1 compares the seasonality of each virus along with the peak month of detection. Although there was discordance in initial influenza A and B activity detected by interval and routine detection, low activity was observed by routine testing until November for influenza A and January for influenza B. The remaining viruses were detected throughout most of the year by both methods of testing. In pediatric specimens, hMPV activity was identified from January through August in the pediatric population. When comparing results from pediatric and adult specimens, the highest percent of positive pediatric population specimens were for adenovirus and RSV by interval testing and RSV and influenza A by routine testing, whereas for adults, influenza A was the predominating virus identified overall (see Table 2 and Fig 2). Even though 60% of specimens submitted for routine testing were from our pediatric population, the percent positive pediatric and adult specimens were almost the same. In contrast, although the same percent of pediatric specimens were included during interval testing, a significantly higher percent were positive compared with routine testing.

DISCUSSION

Respiratory virus infections are recognized as a significant cause of morbidity and mortality, and a source of economic strain on the national health care budget. Viruses have overlapping clinical