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Respiratory viral infections in patients with chronic, obstructive pulmonary disease

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Summary *Objectives*. The purpose of the present study was to apply reverse transcription-PCR (RT-PCR) assays to clinical specimens collected from patients with acute respiratory illness and chronic obstructive pulmonary disease (COPD).

Methods. One hundred and ninety-four samples from two different study cohorts were analysed using RT-PCR assays for picornaviruses, coronaviruses 229E and OC43, influenza A and B viruses, respiratory syncytial virus, parainfluenza types 1–3 viruses, and human metapneumovirus and a PCR assay for adenoviruses. The results were added to results obtained previously using cell culture and serologic methods.

Results. RT-PCR assays identified an additional 35 respiratory virus-associated illnesses not identified previously by cell culture or serology ($n=46$). Picornaviruses and coronaviruses were the most common viral infections identified only by RT-PCR. Overall, 41.8% of the acute respiratory illnesses evaluated were associated with a respiratory virus infection, with picornaviruses, coronaviruses and influenza viruses being the most common infections recognized. No human metapneumovirus infections were identified by RT-PCR assay.

Conclusions. Respiratory viral infections are commonly associated with acute respiratory illness in COPD patients, and the use of RT-PCR assays significantly increases the ability to diagnose these infections.

Introduction

The fourth most common cause of death in the United States is chronic lower respiratory disease.[1,2]

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Patients with chronic obstructive pulmonary disease (COPD) also suffer recurrent exacerbations that increase the morbidity of the disease and contribute to its mortality.[3] Acute exacerbations of COPD are associated with acute infections of the respiratory tract caused by bacteria, viruses, or both bacteria and viruses.[4] The frequency in which a respiratory virus infection has been identified in association with acute worsening of respiratory function in patients with COPD has varied in different studies.[3-9]

We previously performed two studies that evaluated the prevalence of acute respiratory viral infections in patients, including those with COPD.[8,10] Both studies utilized cell culture and serologic methods to identify acute respiratory virus infections. Study 1 examined the frequency of specific respiratory virus infections in persons with acute respiratory tract condition that lead to hospitalization.[10] In study 2, a longitudinal cohort of COPD patients were tested for RTVIs in an ambulatory setting, although some patients were also hospitalized.[8] Respiratory viral infections were identified in 19% of COPD subjects with acute respiratory illness.

Reverse-transcription polymerase chain reaction (RT-PCR) assays can identify respiratory virus infection missed by cell culture.[11] The number of respiratory viral infections identified in asthmatic patients with acute exacerbations of disease increase significantly when RT-PCR assays are used in addition to other diagnostic methods.[12,13] A similar observation has been made for acute exacerbations of COPD.[3] In order to extend our understanding of the prevalence of respiratory viral infection in acute respiratory illnesses in patients with COPD, we used RT-PCR assays to evaluate samples from the previous two prospective studies for evidence of respiratory virus infection.[8,10]

Materials and methods

Study population

Patients with COPD were enrolled in two prospective clinical studies designed to identify the respiratory viruses associated with exacerbations of their lung disease.[8,10] Study 1 evaluated the prevalence of RTVIs among patients hospitalized with an admitting diagnosis of acute respiratory illness or congestive heart failure between July 1991 and June 1995.[10] Patients were identified by review of the admission log. Persons with exacerbations of COPD enrolled in this study were identified using discharge diagnosis codes (ICD9 codes 491.2, 491.21, 492, 493.2, 496). Study 2 was a longitudinal study conducted between September 1991 and May 1995 to evaluate the incidence of RTVIs in a cohort of 62 patients with COPD.[8] The criteria for diagnosis of COPD were as defined by the American Thoracic Society.[14] An exacerbation of COPD was defined as meeting one or more of the criteria from Anthonisen et al.[15] increased dyspnoea, sputum production or sputum purulence. RTVIs in both studies were identified by virus culture of combined nasal wash/throat swab specimens and by serologic assays as previously described.[8,10] For this evaluation, available samples collected between September 1991 and June 1995 were used for analysis. Combined nasal wash/throat swab samples were transported to the laboratory on wet ice, inoculated onto cell culture (usually within 12 h), and then stored at $-80\,^{\circ}C$ prior to the performance of viral RT-PCR assays. Microneutralization assays were used to measure antibody levels to influenza A and B viruses, respiratory syncytial virus, parainfluenza types 1, 2 and 3 viruses, and coronavirus 229E. Hemagglutination inhibition assays also were used to measure influenza A and B virus antibody, and an enzyme-linked immunosorbent assay (ELISA) was used to measure antibody to coronavirus OC43.[8,10]

RT-PCR studies

The following RT-PCR or PCR assays (limits of detection) were used to assay the clinical specimens for the following: influenza A and B viruses (1–10 tissue culture 50% infectious dose [$TCID_{50}$]), respiratory syncytial virus (10–50 $TCID_{50}$), parainfluenza virus types 1, 2 and 3 (10–50 $TCID_{50}$), coronavirus OC43 and 229E (1–10 $TCID_{50}$), picornaviruses (<1 $TCID_{50}$), adenoviruses (10–50 $TCID_{50}$), and human metapneumovirus (10–50 $TCID_{50}$) (Table 2). These assays consist of three major steps: (1) nucleic acid extraction; (2) complementary DNA (cDNA) synthesis and amplification (RT-PCR); and (3) identification of virus-specific amplicons by Southern blot hybridization. Nucleic acids were extracted from nasal wash/throat swab samples using the Q1Amp Viral RNA mini kit (Qiagen Inc.; Valencia, CA) according to the manufacturer's instructions. RT-PCR/PCR assays were performed as previously described using primers listed in Table 1.[13,16-20] For detection of human metapneumovirus infection, cDNA was synthesized for 1 h at 43 °C. After an initial 5-minute heat denaturation at 94 °C, 40 cycles of heat denaturation at 92 °C for 15 s, annealing at 55 °C for 30 s, and primer extension at

Table 1 Primers and probes used to identify respiratory viruses in clinical specimens

Virus	Virus gene target	Oligonucleotide name	Oligonucleotide sequence	Reference
Influenza A	Matrix	FAM1	5′-CAG AGA CTT GAA GAT GTC TTT GC-3′	17
		FAM2	5′-GCT CTG TCC ATG TTA TTT GRA T-3′	17
		AH2 (probe)	5′-TCC TGT CAC CTC TGA CTA AGG GGA TTT TG-3′	17
	Matrix	INFA-1[a]	5′-GGA CTG CAG CGT AGA CGC TT-3′	21
		INFA-2[a]	5′-CAT YCT GTT GTA TAT GAG GCC CAT	21
		INFA probe[a]	5′-FAM-CTC AGT TAT TCT GCT GGT GCA CTT GCC A-TAMRA	21
Influenza B	Matrix	B1	5′-GAA AAA TTA CAC TGT TGG TTC GGT G	16
		B2B	5′-AGC GTT CCT AGT TTT ACT TGC ATT GA	16
		HYB365	5′-TTC TAG CTG AGA GAA AAA TGA GAA GAT GT	16
		INFB1[a]	5′-AAA TAC GGT GGA TTA AAT AAA AGC AA	21
		INFB2[a]	5′-CCA GCA ATA GCT CCG AAG AAA	21
		INFB probe[a]	5′-FAM-CAC CCA TAT TGG GCA ATT TCC TAT GGC TAMRA	21
Parainfluenza virus type 1	Matrix	PF526	5′-ATT TCT GGA GAT GTC CCG TAG GAG AAC	19
		PR678	5′-CAC ATC CTT GAG TGA TTA AGT TTG ATG A	19
		P640 (probe)	5′-TAC CTT CAT TAT CAA TTG GTA AGT CAA	19
Parainfluenza virus type 2	Matrix	P2 upstream	5′-CAT GTA CTA TAC TGA TGG TGG	18
		P2 downstream	5′-GTT AGT AAC TTA AAT AGG GTA AC	18
		P2 probe	5′-AAT GGA ACA TGC AAC ATC ACC	18
Parainfluenza virus type 3	Matrix	P3HN1	5′-CTC GAG GTT GTC AGG ATA TAG	40
		P3HN2	5′-CTT TGG GAG TTG AAC ACA GTT	40
		P3 (probe)	5′-CAT CTG TAT TTA GGA GTG CTA G	40

Table 1 (*continued*)

Virus	Virus gene target	Oligonucleotide name	Oligonucleotide sequence	Reference
HCoV OC43	Nucleoprotein	CVP3	5′-IIA AAT TGC TII TCT TGT TCT GGC	13,41
		CVP4	5′-CCA AAA TTC TGA TTA GGG CCT CTC	13,41
		CVPP	5′-AAG CAI AIT GCC AAA IAA GTC AGI CAG AAA ATT TT	13,41
	Nucleoprotein	O1.1[a]	5′-CAT CAG GAG GGA ATG TTG TAC C	42
		O3.1[a]	5′-TAC TGG TCT TTA GCA TGC GGT C	42
		Probe[a]	5′-FAM-CAG CAG TTG ACG CTG GTT GCC ATC TAMRA	This study
HCoV 229E	Nucleoprotein	E7	5′-TCT GCC AAG AGT CTT GCT CG	42
		E9	5′-AGC ATA GCA GCT GTT GAC GG	42
		E8 (probe)	5′-GGA AGT GCA GGT GTT GTG GC	42
		E7[a]	5′-TCT GCC AAG AGT CTT GCT CG	42
		E9[a]	5′-AGC ATA GCA GCT GTT GAC GG	42
		Probe[a]	5′-VIC-TGG CCA CAA CAC CTG CAC TTC C-TAMRA	This study
Picornavirus	5′ Non-coding region	R1	5′-ACG GAC ACC CAA AGT A	13
		R2	5′-AGC ACT TCT GTT TCC C	13
		R4	5′-TCC TCC GGC CCC TGA ATG	13
RSV A and B	Fusion	Forward primer	5′-ATT GGC ATT AAG CCT ACA AAG CA-3′	20
		Rev primer	5′-CTT GAC TTT GCT AAG AGC CAT CT-3′	20
		Probe A	5′-TAG YCC AAA TGG AGC CWG AAA ATT ATA GTA-3′	20
		Probe B	5′-CRG TTA AGA AGG AGC TAA TCC ATT TTA GTA-3′	20
Adenovirus	Hexon	Hex1deg	5′-GCC SCA RTG GKC WTA CAT GCA CAT C-3′	43
		Hex2deg	5′-CAG CAC SCC ICG RAT GTC AAA-3′	43
		AD2 (probe)	5′-GCC ACC GAG ACG TAC TTC AGC CTG-3′	44

(*continued on next page*)

Table 1 (*continued*)

Virus	Virus gene target	Oligonucleotide name	Oligonucleotide sequence	Reference
Human metapneumo-virus	Nucleoprotein	N3F	5′GTC TCT TCA AGG GAT TCA CC-3′	This study
		N479R	5′-ATT ATI GGT GTGTCT GGT GC-3′	This study
		N101F	5′-CAG TGA CAC CYT CAT CAT TG-3′	This study

[a] For real-time PCR assay.

72 °C for 30 s were followed by a final primer extension at 72 °C using the PTC 200 thermal cycler (MJ Research, Inc., Cambridge, MA). Virus-specific PCR products were detected by Southern hybridization using digoxigenin-labeled probes (see Table 1). Human metapneumovirus-specific amplimers were identified following hybridization with a virus-specific digoxigenin-labeled oligoprobe at 50 °C.

Real-time RT-PCR assays also were performed for influenza A and B viruses and coronavirus OC43 and 229E viruses. The influenza virus-specific assays were performed as previously described.[21] For the coronavirus-specific assays, cDNA was generated in a single reaction in the thermal cycler at 43 °C for 1 h. Specimens were assayed in duplicate in a 50 μl reaction containing 25 μl of 2× Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 5 μl of cDNA, 900 nM forward and reverse primers, and 100 nM fluorogenic probes. Amplification and detection were performed using the GeneAmp 5700 sequence detection system (Applied Biosystems) at the following conditions: 10 min at 95 °C for denaturation followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C.

Extraction and reagent positive and negative controls were used in each experiment to assure the validity of the experimental results. Steps to prevent carryover contamination were used routinely such as separation of sample preparation, separate PCR set-up and post-PCR analysis areas, the use of plugged pipette tips, traffic control from 'clean' (pre-PCR) to 'dirty' (post-PCR) areas, and the use of extraction and reagent positive and negative controls. For an assay to be considered valid, negative controls yielded negative results and positive controls yielded positive results.

Results

There were 96 study subjects with 194 separate illnesses for which samples were available for analysis (Table 2). Participants in study 1 were younger (61.3 vs. 66.7 years, $P=0.005$), more likely to be non-white (71% vs. 21%, $P<0.05$), and less likely to have received influenza vaccination (49% vs. 89%, $P<0.001$) than those in Study 2. The majority of subjects in both studies had a smoking history, but a larger percentage of persons in Study 1 were still smoking at study enrollment (46% vs. 15%, $P<0.01$). Paired sera were available for analysis for fewer illnesses in Study 1 than in Study 2 (38% vs. 91%, $P<0.001$).

Eighty-eight respiratory viral infections were identified in 81 illnesses in the two studies; there were seven illnesses in which dual respiratory infections were identified (Table 3). Fifty (57%) of the 88 infections were identified by culture or serologic methods while the RT-PCR assays identified an additional 38 infections. Overall, picornaviruses (rhinoviruses and enteroviruses) were the most common respiratory virus infections identified and the most frequent virus group identified only by RT-PCR. Twenty-eight of the isolated picornaviruses were further classified to the genus level: 24 were rhinoviruses and 4 were enteroviruses.

Influenza viruses and coronaviruses were the next most commonly identified viruses. Fourteen of the 16 influenza virus infections were caused by influenza A viruses. Influenza virus infection was more common in Study 1 where none of the influenza virus-infected subjects were documented to have received influenza vaccine in the prior year. Twelve of the 16 coronavirus infections identified were caused by OC43-like strains. Only one of the four infections with 229E-like strains occurred in hospitalized subjects, and all four of these infections were identified by RT-PCR.

Parainfluenza virus infections occurred in less than 5% of the illnesses. Two, two and five parainfluenza virus infections were seen with types 1, 2 and 3 strains, respectively. RT-PCR identified two additional type 3 infections and one additional type 1 infection. RSV infections were

Table 2 Study population demographics

	Study 1	Study 2	Both
Studies			
Number of patients	63	33	96
Number of illnesses	77	117	194
Mean (SD) age in years	61.3 (9.6)	66.7 (7.1)	63.1 (9.2)
Gender—# (%) male	35 (56%)	12 (36%)	47 (49%)
Race/ethnicity			
Asian	1	0	1
Black	39	7	46
Hispanic	5	0	5
White	18	26	44
Number (%) with a history of smoking	55 (87%)	26 (79%)	81 (84%)
Number (%) current smokers	29 (46%)	5 (15%)	34 (35%)
Number (%)[a] with history of influenza vaccination in last year	38 (49%)	104 (89%)	142 (73%)
Number (%)[a] hospitalized	77 (100%)	29 (25%)	106 (55%)
Number (%)[a] with serologic studies	29 (38%)	106 (91%)	135 (70%)

[a] Based on number of illnesses.

seen in 3.6% of the subjects. No additional RSV infections were identified by RT-PCR. RT-PCR was the only assay used to identify human metapneumovirus infections, and no infections with this virus were identified.

Fifteen of the respiratory illnesses in study 2 were upper respiratory illnesses only, and all were managed in the outpatient setting. Eighty percent (12/15) of these illnesses were associated with a respiratory viral infection compared to 38% (39/102) of the acute exacerbations of COPD in this study. There were six picornavirus, four coronavirus, two parainfluenza virus, one influenza virus, and one RSV infections (two dual) associated with these common cold illnesses.

Discussion

The application of RT-PCR assays to clinical specimens collected from patients with COPD and acute respiratory illness significantly increased the number of illnesses in which an associated respiratory viral infection was identified. When culture and serologic studies were used for virus identification, 23.4% of the sampled illnesses were associated with a respiratory virus infection. The addition of RT-PCR assays increased the overall percentage of

Table 3 Number of respiratory viral infections identified in Studies 1 and 2

	Study 1		Study 2			Both Studies	
	Hospitalized (n=77)	All (n=117)	Hospitalized (n=29)	Outpatient (n=88)	Culture or serology	RT-PCR only	Total (%) (n=194)[a]
Picornavirus	9	30	3	27	17	22	39 (20.1%)
Influenza A and B virus	12	4	1	3	13	3	16 (8.2%)
RSV	2	5	2	3	7	0	7 (3.6%)
Coronavirus 229E and OC43	6	10	2	8	6	10	16 (8.2%)
Parainfluenza virus types 1–3	3	6	0	6	6	3	9 (4.6%)
Adenovirus	1	0	0	0	1	0	1 (0.5%)
Human metapneumovirus	0	0	0	0	ND	0	0
Any respiratory viral illness	30	51	8	43	46	35	81 (41.8%)

[a] Seven dual infections involving parainfluenza viruses (N=4), picornaviruses (n=4), coronaviruses (n=4), RSV (n=1), influenza type B virus (n=1), and adenovirus (n=1).

respiratory virus-associated illness to 41.8%. This is similar to the ~40% of patients Seemungal et al.[3] noted to have a respiratory virus infection in association with an acute exacerbation of COPD and somewhat lower than the 56% prevalence rate Rohde et al.[22] noted in COPD patients hospitalized with an acute exacerbation and the 64% reported by Seemungal et al.[9] in a later study. The percentage of dual respiratory infections (7/81, 8.6%) is also similar to rates reported in previous studies.[23]

Picornaviruses and coronaviruses were the most likely viral pathogens to be detected using RT-PCR assays. Picornavirus infections, especially those caused by rhinoviruses, are the most prevalent of respiratory virus infections identified in COPD patients with acute respiratory illnesses in this (44%) and other recent studies (36–58%).[3,22] Many rhinoviruses grow poorly in cell culture and are only detected when RT-PCR assays are used.[3,12,13,24,25] Similarly, coronaviruses are either difficult to grow (229E) or do not grow at all (OC43) using current cell culture methods, so it is not surprising that the number of infections identified increases with the application of RT-PCR techniques. Coronaviruses are associated with 11–20% of viral illnesses in COPD patients when RT-PCR assays are used.[3,25]

Influenza virus remains an important cause of virus infection in COPD patients, especially in the hospitalized patient. Fifteen of the 77 (19%) illnesses in Study 1 were associated with an influenza virus infection compared to only four of 117 (3.4%) in Study 2. Less than half of the patients in Study 1 had received influenza vaccine in the previous year while almost 90% of the patients in Study 2 had accepted influenza vaccine in the same time frame. The use of influenza vaccine is safe, effective and recommended for this population.[26–28] Nevertheless, lack of use of influenza vaccine persists as a modifiable risk factor in patients hospitalized with COPD.[29]

Respiratory syncytial virus is increasingly being recognized as an important viral pathogen in older adults.[30–32] Just under 4% of the illnesses evaluated in the two studies were associated with RSV infection, a number similar to that observed in some studies but lower than that reported in others.[3,22,33] Differences in methods used to identify RSV infection and differences in the sensitivities in the RT-PCR assays likely contribute to the differences observed between studies.[34]

Human metapneumovirus is a newly recognized paramyxovirus that causes lower respiratory disease in children.[35,36] The importance of this pathogen in adults is less well defined. Falsey et al.[37] found up to 10% of subjects admitted with acute cardiopulmonary conditions had serologic evidence of acute human metapneumovirus infection. Prevalence rates were somewhat lower in an ambulatory geriatric population, and there was considerable variability between the two study years. Cases of human metapneumovirus infection also have been observed in elderly patients hospitalized with chronic lower respiratory diseases or immunosuppression.[38,39] In contrast, we failed to identify any illnesses associated with human metapneumovirus infection. Our RT-PCR assay for human metapneumovirus was designed to detect all known groups of human metapneumovirus and has detected these viruses in another study involving hospitalized children (unpublished data), differences in assay performance could explain the results observed. Alternatively, virus circulation during the seasons studied may have been low enough to prevent detection.[37]

A potential weakness of this study is that we did not evaluate the impact of respiratory viruses in our two populations during times of clinical stability. Seemungal et al.[9] reported detection of respiratory viruses other than RSV in 16% of COPD patients during clinical stability and detection of RSV in 23.5%. We have recently completed an evaluation of another cohort of chronic bronchitic patients during periods of clinical stability (unpublished data). The prevalence of RT-PCR positivity (excluding coronavirus 229E and human metapneumovirus) ranged from 0 to 2% for individual agents, with an overall prevalence of 6.4% in 143 encounters. The overall frequency of identification of a respiratory viral infection during illness was similar to that seen in this study. Thus, our experience with the application of RT-PCR assays to cohorts with COPD differs from that described by Seemungal et al.[9]

In summary, the application of RT-PCR assays to clinical specimens collected from patients with COPD during clinical respiratory illness significantly increased the number of respiratory viruses identified. Rhinoviruses, coronaviruses and influenza viruses were the most common respiratory virus infections in this patient population, and no human metapneumovirus infections were found. Respiratory virus infections are an important cause of respiratory illness in COPD patients.

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References

1. Hoyert DL, Arias E, Smith BL, Murphy SL, Kochanek KD. Deaths: final data for 1999. *Nat Vital Stat Rep* 2001;**49**: 1–113.
2. Arias E, Anderson RN, Kung HC, Murphy SL, Kochanek KD. Deaths: final data for 2001. *Natl Vital Stat Rep* 2003;**52**: 1–115.
3. Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;**157**:1418–22.
4. Fagon JY, Chastre J. Severe exacerbations of COPD patients: the role of pulmonary infections. *Semin Respir Infect* 1996; **11**:109–18.
5. Smith CB, Golden C, Klauber MR, Kanner R, Renzetti A. Interactions between viruses and bacteria in patients with chronic bronchitis. *J Infect Dis* 1976;**134**:552–61.
6. Wiselka MJ, Kent J, Cookson JB, Nicholson KG. Impact of respiratory virus infection in patients with chronic chest disease. *Epidemiol Infect* 1993;**111**:337–46.
7. Greenberg SB, Atmar RL. Chronic airway disease: the infection connection. *Trans Am Clin Climatol Assoc* 1999; **110**:38–48.
8. Greenberg SB, Allen M, Wilson J, Atmar RL. Respiratory viral infections in adults with and without chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000;**162**: 167–73.
9. Seemungal T, Harper-Owen R, Bhowmik A, et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;**164**:1618–23.
10. Glezen WP, Greenberg SB, Atmar RL, Piedra PA, Couch RB. Impact of respiratory virus infections on persons with chronic underlying conditions. *JAMA* 2000;**283**:499–505.
11. Wedzicha JA. Role of viruses in exacerbations of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2004;**1**: 115–20 doi:10.1513/pats.2306030.
12. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ* 1995;**310**:1225–9.
13. Atmar RL, Guy E, Guntupalli KK, et al. Respiratory tract viral infections in inner-city asthmatic adults. *Arch Intern Med* 1998;**158**:2453–9.
14. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am Rev Respir Dis* 1987;**136**:225–44.
15. Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med* 1987;**106**:196–204.
16. Atmar RL, Baxter BD. Typing and subtyping clinical isolates of influenza virus using reverse transcription-polymerase chain reaction. *Clin Diagn Virol* 1996;**7**:77–84.
17. Atmar RL, Baxter BD, Dominguez EA, Taber LH. Comparison of reverse transcription-PCR with tissue culture and other rapid diagnostic assays for detection of type A influenza virus. *J Clin Microbiol* 1996;**34**:2604–6.
18. Bandi V, Jakubowycz M, Kinyon C, et al. Infectious exacerbations of chronic obstructive pulmonary disease associated with respiratory viruses and non-typeable *Haemophilus influenzae*. *FEMS Immunol Med Microbiol* 2003;**37**: 69–75.
19. Fan J, Henrickson KJ. Rapid diagnosis of human parainfluenza virus type 1 infection by quantitative reverse transcription-PCR-enzyme hybridization assay. *J Clin Microbiol* 1996;**34**:1914–7.
20. van Milaan AJ, Sprenger MJ, Rothbarth PH, Brandenburg AH, Masurel N, Claas EC. Detection of respiratory syncytial virus by RNA-polymerase chain reaction and differentiation of subgroups with oligonucleotide probes. *J Med Virol* 1994;**44**: 80–7.
21. van Elden LJ, Nijhuis M, Schipper P, Schuurman R, van Loon AM. Simultaneous detection of influenza viruses A and B using real-time quantitative PCR. *J Clin Microbiol* 2001;**39**: 196–200 doi:10.1128/JCM.39.1.196-200.2001.
22. Rohde G, Wiethege A, Borg I, et al. Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalisation: a case–control study. *Thorax* 2003;**58**:37–42.
23. Drews AL, Atmar RL, Glezen WP, Baxter BD, Piedra PA, Greenberg SB. Dual respiratory virus infections. *Clin Infect Dis* 1997;**25**:1421–9.
24. Johnston SL, Sanderson G, Pattemore PK, et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol* 1993;**31**:111–7.
25. Falsey AR, Walsh EE, Hayden FG. Rhinovirus and coronavirus infection-associated hospitalizations among older adults. *J Infect Dis* 2002;**185**:1338–41.
26. Centers for Disease Control and Prevention. Prevention and control of influenza: recommendation of the American Committee on Immunization Practices, (ACIP). *MMWR* 2003;**52**:1–34.
27. Poole PJ, Chacko E, Wood-Baker RWB, Cates CJ. Influenza vaccine for patients with chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* 2003;**1**. Accessed: 4-30-2004.
28. Tata LJ, West J, Harrison T, Farrington P, Smith C, Hubbard R. Does influenza vaccination increase consultations, corticosteroid prescriptions, or exacerbations in subjects with asthma or chronic obstructive pulmonary disease? *Thorax* 2003;**58**:835–9.
29. Garcia-Aymerich J, Barreiro E, Farrero E, Marrades RM, Morera J, Anto JM. Patients hospitalized for COPD have a high prevalence of modifiable risk factors for exacerbation (EFRAM study). *Eur Respir J* 2000;**16**:1037–42.
30. Walsh EE, Peterson DR, Falsey AR. Risk factors for severe respiratory syncytial virus infection in elderly persons. *J Infect Dis* 2004;**189**:233–8.
31. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003;**289**:179–86.
32. Griffin MR, Coffey CS, Neuzil KM, Mitchel Jr EF, Wright PF, Edwards KM. Winter viruses: influenza- and respiratory syncytial virus-related morbidity in chronic lung disease. *Arch Intern Med* 2002;**162**:1229–36.
33. Walsh EE, Falsey AR, Hennessey PA. Respiratory syncytial and other virus infections in persons with chronic cardiopulmonary disease. *Am J Respir Crit Care Med* 1999;**160**: 791–5.
34. Walsh EE, Falsey AR, Swinburne IA, Formica MA. Reverse transcription polymerase chain reaction (RT-PCR) for diagnosis of respiratory syncytial virus infection in adults: use of a single-tube 'hanging droplet' nested PCR. *J Med Virol* 2001;**63**:259–63.

35. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001;**7**:719–24 doi:10.1038/89098.
36. Hamelin ME, Abed Y, Boivin G. Human metapneumovirus: a new player among respiratory viruses. *Clin Infect Dis* 2004; **38**:983–90 doi:10.1086/382536.
37. Falsey AR, Erdman D, Anderson LJ, Walsh EE. Human metapneumovirus infections in young and elderly adults. *J Infect Dis* 2003;**187**:785–90.
38. Boivin G, Abed Y, Pelletier G, et al. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. *J Infect Dis* 2002; **186**:1330–4.
39. van den Hoogen BG, van Doornum GJ, Fockens JC, et al. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. *J Infect Dis* 2003; **188**:1571–7.
40. Karron RA, Froehlich JL, Bobo L, Belshe RB, Yolken RH. Rapid detection of parainfluenza virus type 3 RNA in respiratory specimens: use of reverse transcription-PCR-enzyme immunoassay. *J Clin Microbiol* 1994;**32**:484–8.
41. Murray RS, Cai GY, Hoel K, Zhang JY, Soike KF, Cabirac GF. Coronavirus infects and causes demyelination in primate central nervous system. *Virology* 1992;**188**:274–84.
42. Arbour N, Day R, Newcombe J, Talbot PJ. Neuroinvasion by human respiratory coronaviruses. *J Virol* 2000;**74**:8913–21.
43. Allard A, Albinsson B, Wadell G. Rapid typing of human adenoviruses by a general PCR combined with restriction endonuclease analysis. *J Clin Microbiol* 2001;**39**:498–505 doi:10.1128/JCM.39.2.498-505.2001.
44. Allard A, Albinsson B, Wadell G. Detection of adenoviruses in stools from healthy persons and patients with diarrhea by two-step polymerase chain reaction. *J Med Virol* 1992;**37**: 149–57.