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REGULAR ARTICLE

# Polymerase-chain-reaction-based diagnosis of viral pulmonary infections in immunocompromised children

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**Keywords**
Bronchoalveolar lavage, Immunodeficiency, Pneumonia, Polymerase chain reaction, Viral infection

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## ABSTRACT

**Aim:** Viral pneumonia is a serious complication in immunocompromised children. Its aetiology is difficult to identify owing to the limitations of conventional microbiological tests. The aim of this study was to determine whether polymerase chain reaction (PCR) assays for respiratory viruses increase the diagnostic yield of bronchoalveolar lavage (BAL) in immunocompromised children.

**Methods:** BAL samples obtained from immunocompromised children hospitalized with pneumonia were processed for respiratory viruses by viral culture, rapid antigen test and PCR (for CMV, adenovirus, influenza, parainfluenza, herpesvirus, RSV and hMPV).

**Results:** The study group included 42 patients (mean age 7.2 ± 5.1 years) with 50 episodes of clinical pneumonia (50 BAL samples). Forty viral pathogens were identified in 30 episodes (60%). PCR increased the diagnostic rate by fourfold (75% identified by PCR alone, $p < 0.0001$). When viral culture and rapid antigen test were used as the gold standard, PCR was found to have high sensitivity (86–100% when assessed) and specificity (80–96%). The PCR results prompted the initiation of specific antiviral therapy and the avoidance of unnecessary antibiotic treatment in 17 (34%) episodes.

**Conclusion:** PCR-based diagnosis from BAL may increase the rate of pathogen detection in immunocompromised children, decrease the time to diagnosis and spare patients unnecessary antimicrobial treatment.

## INTRODUCTION

Viral pneumonia is a serious infectious complication in immunocompromised children and adults. It is associated with high rates of morbidity, including respiratory and multiorgan failure, increased rates of hospitalization and need for intensive care, and mortality rates of up to 50% (1–4). Diagnosis is often difficult because the clinical and radiological findings lack sensitivity and specificity (4,5); viral cultures are time-consuming and have low sensitivity (1,4,5); and direct antigen assays lack sensitivity and, although they allow for rapid diagnosis, are unavailable for a wide range of viral pathogens (1–7).

In recent years, molecular techniques of gene amplification have been applied for the rapid and sensitive detection of infectious agents. Polymerase chain reaction (PCR) assays for viral respiratory pathogens were shown to have both high sensitivity and specificity (1–9). Studies reported a two- to threefold increase in the diagnostic yield when PCR was added to the standard microbiological investigations (1–4,6,7,10). In different studies, only 20–42% of cases that were virus-positive by PCR were also positive by viral culture (1,4,5); the positive rate for rapid antigen tests was about 40% of viral PCR-positive cases (3,4).

In children, several studies have validated the use of PCR for the detection of viral pathogens, including influenza virus, parainfluenza virus, adenovirus, respiratory syncytial virus

**Abbreviations**
BAL, bronchoalveolar lavage; BMT, bone marrow transplantation; PCR, polymerase chain reaction; RSV, respiratory syncytial virus.

**Key notes**

- In immunocompromised children with pneumonia, viral PCR assays, as compared with standard microbiological assays (viral cultures and rapid antigen tests) from bronchoalveolar lavage, increased the diagnosis yield by fourfold.
- When viral culture and rapid antigen test were used as the gold standard, PCR was found to have high sensitivity and specificity.
- PCR assays also supported the discontinuation of unnecessary antibiotic treatment in 34% of episodes.

 

(RSV), human metapneumovirus (hMPV), rhinovirus, coronavirus and herpesvirus (4,7,9,10). However, data on immunocompromised children in particular are still sparse, especially for PCR tests of bronchoalveolar lavage (BAL) fluid.

The aim of the present study was to determine whether PCR tests for respiratory viruses add to the diagnostic yield of BAL in immunocompromised children with pneumonia.

## PATIENTS AND METHODS

### Patients and setting

This retrospective study was conducted in the paediatric intensive care unit of Schneider Children's Medical Center of Israel, a tertiary care, university-affiliated, 250-bed paediatric facility. All infants and children with primary or secondary immunodeficiency who were hospitalized with pneumonia between January 2007 and June 2009 needing BAL were included in the study. Only episodes in which BAL samples were analysed using both the standard microbiological tests (culture, rapid antigen tests) and PCR-based assays were included in the analysis. The study was approved by the Institutional Review Board of Rabin Medical Center which waived the need for informed consent.

### Data collection

The patients' medical files were reviewed for the following data: age and sex, underlying disease and predisposing factors, duration of hospitalization in the intensive care unit, prior viral infections, presence of fever and respiratory distress, laboratory data (blood cell count, chemistry results), radiological findings, antimicrobial therapy before and after BAL fluid analysis, results of BAL fluid analysis, clinical decisions based on BAL test results and clinical outcome of each episode. The present study was conducted simultaneously with a study of fungal pathogens using PCR and standard tests (fungal culture, calcofluor staining and immunofluorescence staining for *Pneumocystis jirovecii)* in the same cohort (11).

### BAL procedure

BAL was performed in the bronchoscopy suite within the paediatric intensive care unit. Patients were prepared for the procedure with inhalation of adrenaline and lidocaine and sedated with midazolam, atropine and propofol. A fiberoptic bronchoscope was wedged into a subsegmental bronchus of the affected lobe/lobes, and 5–15 mL aliquots of sterile normal saline solution were aspirated. The BAL fluid was transferred for examination.

### Virology

Each sample of BAL fluid was tested for the presence of adenovirus, cytomegalovirus (CMV), parainfluenza virus and herpesvirus, RSV, adenovirus and influenza virus. Real-time PCRs were performed (using the TaqMan probe detection system) for adenovirus, CMV, herpesvirus, and real-time reverse transcriptase PCR assays were performed for influenza virus, RSV and hMPV. Reverse transcriptase PCRs were used for parainfluenza virus. Viral cultures and rapid antigen tests (for RSV) were performed in the Microbiology Department of Rabin Medical Center. The PCR assays were performed in the Central Virology Laboratory of Chaim Sheba Medical Center.

Adenovirus was detected through the amplification of a conserved region in the hexon gene (12). To detect HSV-1 infection, the junction between glycoprotein G/J was amplified. HSV-2 was detected by amplification of glycoprotein G (13). For influenza A virus, specific primers were selected to amplify part of the influenza virus M-protein gene and for influenza B virus, and specific primers were designated to amplify part of the HA gene (14). For the detection of RSV A and B, the N gene of RSV was chosen as the target (15), and for hMPV the L gene was selected (16). Parainfluenza 1, 2 and 3 viruses were detected using multiplex RT-PCR assays that detect and differentiate (17,18).

Viral genomic DNA was extracted from the BAL fluid with a QIAamp DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany), and viral genomic RNA was extracted with a High Pure Viral RNA kit (Roche Diagnostics GmbH, Mannheim, Germany). To test for CMV, DNA was extracted from 200 $\mu$L BAL fluid using the MagNaPure LC Total Nucleic Acid Isolation kit (Roche Diagnostics), according to the manufacturer's instructions. The ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) was used. One primer set was designed from the highly conserved region of glycoprotein B, the major envelope glycoprotein of the *CMV* gene, and a second set was designed from the highly conserved immediate early region (19,20).

### Definitions

Immunosuppression was defined as current treatment of chemotherapy or immunosuppressive therapy, bone marrow transplantation or known primary immune deficiency. Clinical pneumonia was diagnosed when at least two of the following four criteria were met: fever, a new pulmonary infiltrate, worsening of pulmonary infiltrates and dyspnoea. The indication for BAL was clinical pneumonia that did not respond to empirical therapy within 48–72 h. The decision to add PCR testing was based on patient history, findings on physical examination, history of viral infections, radiological findings and presence of specific risk factors. Clinical improvement was defined as resolution of at least two of three diagnostic criteria (fever, respiratory symptoms and pulmonary infiltrates on chest films) within 2 weeks of BAL.

### Statistical analysis

The diagnostic rates of the standard microbiological methods (viral culture, rapid antigen test) and PCR were calculated as the percentage of viral pathogens identified by each method. The differences in diagnostic rates between the standard methods and PCR were analysed by chi-square test. Culture and rapid antigen test served as the reference (gold standard) for calculating PCR sensitivity and specificity for each viral pathogen. Positive and negative predictive values were not calculated because of the low number

  

of positive findings for each pathogen. Episodes of respiratory illness were divided into two groups: those diagnosed by standard methods (with positive or negative PCR results) and those diagnosed by PCR alone. The groups were compared for percentage of episodes in which clinical improvement occurred out of the total episodes in each group and the percentage of patients who died during the same hospitalization out of the total number of patients in each group. Differences in rates of clinical improvement and mortality between the two groups were analysed by chi-square test. A p value of $\leq 0.05$ was considered significant.

## RESULTS

The study cohort included 42 patients with a total of 50 episodes of clinical pneumonia (50 BAL samples). The patient characteristics are presented in Table 1. In 46 episodes (92%), antibiotic treatment was started prior to BAL because of a suspected bacterial pulmonary infection. In I5 episodes (30%), antiviral therapy (ribavirin, cidofovir or ganciclovir) was also started prior to BAL because of a suspected viral pulmonary infection, based on previous infection (weeks to months before the current pneumonia episode), with CMV and/or adenovirus in most cases. In additional four episodes, patients were receiving antiviral prophylaxis with acyclovir at the time of BAL. In 31 episodes, patients were treated with antifungal therapy (i.e. amphotericin, voriconazole or fluconazole) at the time of BAL examination.

**Table 1** Characteristics of immunocompromised patients with clinical pneumonia

| Patients, no. | 42 |
|---|---|
| BAL examinations (pneumonia episodes), no. | 50 |
| Age, mean ± SD (range) | 7.2 ± 5.1 years (3 months–18 years) |
| Male : female ratio | 23 : 19 |
| Underlying disease, no. (%) | |
| Bone marrow transplantation | 12 (29) |
| Haematological malignancies | 9 (21) |
| Primary immunodeficiency | 7 (17) |
| Solid malignancies treated with chemotherapy | 6 (14) |
| Solid organ transplantation | 4 (9) |
| HIV disease | 1 (2.5) |
| Haemophagocytic lymphohystiocytosis | 1 (2.5) |
| IBD treated with azathioprine | 1 (2.5 |
| Dermatomyositis treated with azathioprine | 1 (2.5) |
| Blood count abnormalities, no. (%) | |
| Neutropenia | 18 (36) |
| Lymphopenia | 32 (64) |
| Prior infections, no. (%) | |
| CMV | 13 (26) |
| RSV | 4 (8) |
| Adenovirus | 2 (6) |
| EBV | 1 (2.5) |
| Clinical characteristics, no. (%) | |
| Fever | 36 (72) |
| New or worsening pulmonary infiltrates | 47 (94) |
| Dyspnoea | 32 (64) |

BAL = bronchoalveolar lavage; IBD = inflammatory bowel disease; PICU = paediatric intensive care unit.

Samples from 20 episodes (40%) were negative for a viral antigen by both standard tests (culture and rapid antigen test) and PCR. In 30/50 episodes (60%), a viral pathogen was identified. In these 30 episodes, 40 viral pathogens were identified (Table 2). Ten of the pathogens (25%) were isolated by standard tests (viral culture, nine pathogens; rapid antigen test, 1 pathogen – RSV), with either negative or positive results on PCR assays. The other 30 pathogens (75%, $p < 0.0001$) were detected by PCR alone. The addition of PCR to the BAL evaluation increased the diagnostic yield by fourfold.

The pathogen most often detected was adenovirus, in 12 episodes (24%), followed by CMV in nine episodes (18%), RSV in eight episodes (16%), Influenza virus in four episodes (8%, Influenza B in three episodes and A in one episode), parainfluenza in four episodes (8%) and herpesvirus two in three episodes (6%). RSV and Influenza were positive in episodes occurring during the winter and early spring months. None of the episodes was positive for hMPV. When viral culture and rapid antigen test were used as the gold standard, PCR was found to have high sensitivity (86–100% when assessed) and specificity (80–96%).

Of the 30 episodes positive for viral infection, co-infection with other pathogens was observed in 18 (Table 3): bacterial pathogens in 13 episodes (43%, as compared with 28% in viral-negative episodes), other viral pathogens in eight episodes (27%) and fungal pathogens in six episodes (20%, as compared with 28% in viral-negative episodes).

### Treatment

In 18 of the 20 episodes (90%) in which findings for all three diagnostic methods were negative, empiric antiviral therapy was discontinued or withheld (36% of all episodes included in the study). Among the 30 episodes in which a viral pathogen was identified, antiviral therapy was initiated or continued in 17 (57%). Treatment in these cases consisted of ganciclovir for CMV infection (four episodes), foscarnet for CMV infection (four episodes), adenovirus infection (one episode), cidofovir for adenovirus infection (six episodes), ribavirin for RSV infection (one episode) and valganciclovir for CMV infection (one episode). On the basis of the diagnosis of a viral infection, empiric antibiotic treatment was discontinued or withheld in 17 episodes (36%).

### Outcome

Clinical improvement was noted in 28 of the 47 episodes (60%) for which follow-up data were available. The 47 episodes included 18 of the 20 episodes diagnosed by PCR alone. Within this subgroup, clinical improvement was observed in 10 episodes (56%, compared with 43% for episodes diagnosed by standard methods, $p = 0.34$).

Nine patients died during the same hospitalization in which BAL was performed (18% in-hospital mortality).

**Table 2** Viral detection by PCR and standard microbiological tests and PCR sensitivity and specificity

| | Standard test results | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Adenovirus | | CMV | | RSV | | Influenza virus | | Parainfluenza virus | | Herpesvirus | |
| PCR results | Pos | Neg | Pos | Neg | Pos | Neg | Pos | Neg | Pos | Neg | Pos | Neg |
| Pos | 1 | 11* | 6 | 2 | 1† | 7 | 0 | 4‡ | 0 | 4 | 0 | 2 |
| Neg | 0 | 38 | 1 | 41 | 0 | 42 | 0 | 46 | 0 | 46 | 1§ | 47 |
| Sensitivity | 100% | | 86% | | 100% | | –¶ | | –¶ | | –¶ | |
| Specificity | 78% | | 95% | | 86% | | 92% | | –** | | 96% | |

CMV = cytomegalovirus; RSV = respiratory syncytial virus; Pos = positive; Neg = negative.
Values represent number of episodes.
*In two episodes, adenovirus was isolated by PCR but considered clinically nonsignificant.
†RSV was positive by PCR and by rapid antigen test.
‡In one episode, influenza virus was isolated by PCR but considered clinically nonsignificant.
§PCR for herpesvirus was not performed in this episode.
¶PCR sensitivity could not be assessed, as no episode was positive for the same virus by standard and PCR assays.
**PCR sensitivity and specificity could not be assessed, as no conventional assay for parainfluenza was done.

**Table 3** Viral pneumonia episodes with co-infections of viral, bacterial and fungal pathogens

| Episode no. | Viral pathogens | Bacterial pathogens | Fungal pathogens |
|---|---|---|---|
| 3 | Parainfluenza | *PCP* | |
| 4 | CMV | *PCP* | *Candida parapsilosis* |
| 5 | RSV, CMV | | |
| 7 | RSV | *Morganella Morganii* | |
| 12 | Adenovirus, Herpes | *Enterococcus Faecium* | |
| 20 | Adenovirus, Parainfluenza | *Pseudomonas* | *Candida glabrata* |
| 22 | CMV | *Pseudomonas* | |
| 23 | RSV | | *Aspergillus* |
| 28 | CMV, Influenza | *S. pneumoniae, Klebsiella, E. coli* | *Candida albicans* |
| 33 | Adenovirus | *Haemophilus* | |
| 35 | Adenovirus, RSV | | |
| 37 | Adenovirus, RSV | | *Aspergillus* |
| 40 | Adenovirus | *Haemophilus* | |
| 41 | CMV, Herpes, RSV | | |
| 43 | Influenza | *S. peumoniae, Pseudomonas* | *Candida albicans* |
| 45 | RSV | *Enterococcus Faecium* | |
| 49 | Adenovirus | *S. pneumoniae* | |
| 50 | Adenovirus, Herpes, CMV | *S. peumoniae* | |

CMV = cytomegalovirus; PCP = pneumocystis jiroveci; RSV = respiratory syncytial virus.

These included 4 of the 20 patients (and 20 episodes) diagnosed by PCR alone. The in-hospital mortality rate of PCR-based diagnosis group was lower than the mortality rate of the standard methods–diagnosed group (20% and 37%, respectively, p = 0.54).

## DISCUSSION

In the present study, BAL samples from immunocompromised children with clinical pneumonia were tested by PCR assay in addition to standard microbiological methods of viral culture and rapid antigen test. Compared with the standard tests, PCR had high rates of sensitivity (86–100%, when assessed) and specificity (78–96%). The addition of PCR increased the rate of diagnosis of each viral pathogen tested, especially adenovirus, RSV, influenza virus and parainfluenza virus and significantly increased (by fourfold) the diagnostic yield of BAL sampling (30 pathogens identified by PCR alone vs 10 pathogens identified by standard methods). PCR assays also promoted initiation or continuation of specific anti-viral therapy in 17 of the 30 positive episodes. To the best of our knowledge, this is the only published study to compare effectiveness of viral PCR to standard microbiological methods using BAL fluid from immunocompromised children.

Our 60% rate of diagnosis of viral infections with the addition of PCR is consistent with previous findings in immunocompromised patients (38–80%) (1,3,4,6). Gerna and colleagues found that 50% of all respiratory infections in adult lung transplant recipients were positive for

  

respiratory viruses by PCR (3). Bredius and colleagues showed that PCR assay of nasal washes and throat swabs in children after hematopoietic stem cell transplantation yielded a higher rate of diagnosis of viral respiratory illness (caused by influenza virus, parainfluenza virus, adenovirus, RSV, hMPV and rhinovirus) than culture (80% vs 60%) in less time (by a median of 8 days) (4). Accordingly, viral infections appear to play a very significant role in immunocompromised patients.

The information provided by the PCR assays was also important in preventing unnecessary treatment. Antiviral treatment was discontinued or withheld in 18 of the 20 episodes (90%) in which both standard tests and PCR yielded negative findings. Antibiotic therapy was discontinued or withheld in 17 of 30 episodes (57%) in which a viral pathogen was identified. It is noteworthy that bacterial cultures from BAL may have been largely negative because in 46 of the 50 episodes, patients were already treated with antibiotics at the time of BAL. The detection of a viral pathogen by PCR further decreased the likelihood of bacterial infection and supported the avoidance of antibiotic therapy. Antibiotic therapy was discontinued after 7–10 days in most cases. This was the average time between the occurrence of fever and the receiving of PCR results of the BAL (which was done 2–3 days after antibiotic treatment was started in most cases).

Co-infection of viral pathogen with bacterial, viral and fungal pathogens was common in our study (20–43%). The two most common viruses in co-infections were RSV and adenovirus. Co-infection has been reported in other studies of viral PCR assays, especially in children and immunocompromised patients, with rates of up to 50% (2,3,7).

A possible pitfall in relying on PCR assays is the risk of false positives. As healthy subjects may carry viral particles in their respiratory secretions (mostly due to a previous infection), viral PCR assays may sometimes yield positive results even in the absence of respiratory illness. (4) Thavagnanam and colleagues tested non-bronchoscopic BAL samples from healthy and asthmatic children by PCR assays for 13 common viral pathogens. The rate of positive results in the children without a respiratory illness was 35% (21). However, this problem may be less relevant in immunocompromised patients. In one study of adult lung transplant recipients, the false-positive rate of PCR assays for viral pathogens was only 4% in the patients with no respiratory symptoms compared with 55% in the patients with a respiratory tract infection. When BAL samples taken after the respiratory illness were evaluated for the same viral pathogens by PCR, all were negative. These findings suggest that respiratory viruses do not cause latent infections (1).

In the present study, we could not accurately evaluate the false-positive rate because the PCR results were analysed against standard tests which are known to have low sensitivity. Nevertheless, although the findings were not statistically significant, patients in whom treatment was directed solely by the (positive) PCR results had higher rates of clinical improvement and lower mortality. We could not have expected this finding had some of the PCR results been falsely positive, supporting the assumption that the false-positive rate of PCR is low.

Another limitation is the potential risks of BAL. Bronchoscopy is an invasive procedure which carries several risks, especially in immunocompromised patients with respiratory disease, among them are bleeding and respiratory failure. In our study, bronchoscopy was done in the paediatric intensive care unit under sedation (not general anaesthesia) and no complications were observed. There were also no cases of mortality in the 10 days following the procedure. However, the benefit of BAL should be considered against the potential risk of the procedure in every patient.

Other limitations of the study are the retrospective, single-centre design of the study, the relatively small number of episodes evaluated and the heterogeneity of the patients (e.g. primary immunodeficiency, bone marrow transplantation patients). In addition, some PCR assays were not available to us, including coronavirus and rhinovirus, which are increasingly acknowledged to be significant pathogens in patients with respiratory tract infections, especially immunocompromised patients. It is possible that the diagnostic yield would have been even higher with these additional examinations.

In conclusion, viral PCR assays increased the diagnostic yield of BAL in immunocompromised children with pneumonia, prompted the initiation of specific antiviral treatment and spared patients unnecessary treatment.

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## CONFLICTS OF INTEREST AND SOURCE OF FUNDING

None declared.

## References

1. Garbino J, Gerbase MW, Wunderli C, Deffernez C, Thomas Y, Rochat T, et al. Lower respiratory viral illnesses. Improved diagnosis by molecular methods and clinical impact. *Am L Respir Crit Care Med* 2004; 170: 1197–203.
2. Bassinger AL, Einsele H, Hamprecht K, Schumacher U, Kandolf R, Loeffler J, et al. Infectious pulmonary complications after stem cell transplantation or chemotherapy: diagnostic yield of bronchoalveolar lavage. *Diagn Microbiol Infect Dis* 2005; 52: 275–80.
3. Gerna G, Vitulo P, Rovida F, Lilleri D, Pellegrini C, Oqqionni T, et al. Impact of human metapneumovirus and human cytomegalovirus versus other respiratory viruses on the lower respiratory tract infections of lung transplant recipients. *J Med Virol* 2006; 78: 408–16.
4. Bredius RG, Templeton KE, Scheltingta SA, Claas EC, Kroes AC, Vossen JM. Prospective study of respiratory viral infections in pediatric hemopoietic stem cell transplantation patients. *Pediatr Infect Dis J* 2004; 23: 518–22.
5. Garbino J, Soccal PM, Aubert JD, Rochat T, Meylan P, Thomas Y, et al. Respiratory viruses in bronchoalveolar lavage: a hospital based cohort study in adults. *Thorax* 2009; 64: 399–404.

6. Murali S, Langston AA, Nolte FS, Banks G. Detection of respiratory viruses with a multiplex polymerase chain reaction assay (MultiCode-PLx respiratory virus panel) in patients with hematologic malignancies. *Leuk Lymphoma* 2009; 50: 619–24.
7. Rovida F, Percivalle E, Zavattoni M, Torsellini M, Sarasini A, Campanini G, et al. Monoclonal antibodies versus reverse transcription-PCR for detection of respiratory viruses in a patient population with respiratory tract infections admitted to hospital. *J Med Virol* 2005; 75: 336–47.
8. Camps Serra M, Cervera C, Pumarola T, Moreno A, Perello R, Torres A, et al. Virological diagnosis in community-acquired pneumonia in immunocompromised patients. *Eur Respir J* 2008; 31: 618–24.
9. Christensen MS, Nielsen LP, Hasle H. Few but severe infections in children with cancer: a prospective RT-PCR and PCR-based 12-month study. *Pediatr Blood Cancer* 2005; 45: 945–51.
10. Weinberg GA, Erdman DD, Edwards KM, Hall CB, Walker FJ, Griffin MR, et al. Superiority of reverse-transcription polymerase chain reaction to conventional viral culture in the diagnosis of acute respiratory tract infections in children. *J Infect Dis* 2004; 189: 706–10.
11. Kadmon G, Nahum E, Sprecher H, Stein J, Levy I, Schiller O, et al. Polymerase-chain-reaction-based diagnosis of invasive fungal pulmonary infections in immunocompromised children. *Pediatr Pulmonol* 2012; 47: 994–1000.
12. Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol* 2003; 70: 228–39.
13. Filen F, Strand A, Allard A, Blomberg J, Herrmann B. Duplex real-time polymerase chain reaction assay for detection and quantification of HSV type 1 and HSV type 2 in genital and coetaneous lesions. *Sex Transm Dis* 2004; 31: 331–6.
14. Hindiyeh M, Levy V, Azar R, Versano N, Regev L, Shalev Y, et al. Evaluation of a multiplex real-time reverse transcriptase PCR assay for detection and differentiation of influenza viruses A and B during the 2001-2002 influenza season in Israel. *J Clin Microbiol* 2005; 43: 589–95.
15. Hu A, Colella M, Tam JS, Rappaport R, Cheng SM. Simultaneous detection, subgrouping, and quantitation of respiratory syncytial virus A and B by real-time PCR. *J Clin Microbiol* 2003; 41: 149–54.
16. Regev L, Hindiyeh M, Shulman LM, Barak A, Levy V, Azar R, et al. Characterization of human metapneumovirus infections in Israel. *J Clin Microbiol* 2003; 44: 1484–9.
17. Aguilar JC, Pérez-Breña MP, García ML, Cruz N, Erdman DD, Echevarría JE. Detection and identification of human parainfluenza viruses 1, 2, 3, and 4 in clinical samples of pediatric patients by multiplex reverse transcription-PCR. *J Clin Microbiol* 2000; 38: 1191–5. Erratum in: *J Clin Microbiol* 2000; 38:2805. PMID: 10699020
18. Choi EH, Lee HJ, Kim SJ, Eun BW, Kim NH, Lee JA, et al. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000-2005. *Clin Infect Dis* 2006; 43: 585–92.
19. Tanaka N, Kimura H, lida K, Saito Y, Tsuge I, Yoshimi A, et al. Quantitative analysis of cytomegalovirus load using a real-time PCR assay. *J Med Virol* 2000; 60: 455–62.
20. Boeckh M, Huang M, Ferrenberg J, Stevens-Ayers T, Stansland L, Nichols WG, et al. Optimization of quantitative detection of cytomegalovirus DNA in plasma by real-time PCR. *J Clin Microbiol* 2004; 42: 1142–8.
21. Thavagnanam S, Christie SN, Doherty GM, Coyle PV, Shields MD, Heaney LG. Respiratory viral infection in lower airways of asymptomatic children. *Acta Pediatr* 2010; 99: 394–8.


  