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# Use of Nasal Samples and Genom Amplification Methods for Detection of Respiratory Viruses in Infants with Acute Lower Respiratory Tract Infection

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**Aim:** The aim of this study was to evaluate the use of genome amplification methods [polymerase chain reaction (PCR) and reverse transcription (RT)-PCR] for detection of common respiratory viruses (RSV: respiratory syncytial virus, PIV3: parainfluenza virus 3, IVA: influenza virus type A, IVB: influenza virus type B and adenovirus) in nasal wash specimens of infants with acute lower respiratory tract infection (ALRI).

**Materials and Methods:** The nasal and serum samples taken from 90 infants with ALRI were analyzed by genome amplification methods and ELISA.

**Results:** In ELISA, specific IgM to only one virus and to multiple viruses was present in 39 (43.3%) and 7 (7.7%) of the serum samples, respectively. Therefore, IgM positivity to at least one virus was detected in 46 (51.1%) of the serum samples. In the genome amplification methods in nasal samples, 62 (68.8%) of these samples were positive for only one virus, whereas 9 (10%) of the samples were found to be positive for multiple viruses. Therefore, a total of 71 (78.8%) samples were accepted as positive with at least one virus.

**Conclusions:** As a result, we recommend use of the genome amplification methods in nasal wash specimens for diagnosis of the respiratory viruses in ALRI infants.

Key Words: Acute lower respiratory tract infection, ALRI, infant, PCR, respiratory viruses

## Akut Alt Solunum Yolu Enfeksiyonlu Bebeklerde Solunum Virüslerinin Belirlenmesi İçin Nazal Örnekler ve Genom Çoğaltma Metotlarının Kullanımı

**Amaç:** Bu çalışmanın amacı, akut alt solunum yolu enfeksiyonlu (ALRI) bebeklerde genel solunum virüslerinin (RSV; solunum sinsitiyal virüs, PIV3; parainfluenza virüs 3, IVA; influenza virüs A ve IVB; influenza virüs B ve adenovirüs) belirlenmesi için nazal yıkantı örneklerinde genom çoğaltma metotlarının (polimeraz zincir tepkimesi; PCR and tersine transkripsiyon-PCR; RT-PCR) kullanımının etkinliğini belirlemektir.

**Yöntem ve Gereç:** ALRI'lı 90 bebekten alınan nazal yıkama ve serum örnekleri genom çoğaltma metodu ve ELISA ile test edildi.

**Bulgular:** Serum örneklerinin 39'unda (%43,3) bir virüse, aynı örneklerin 7'sinde (%7,7) ise birden fazla virüse özgül IgM'lere rastlandı. Bu nedenle, ELISA'da serum örneklerinin 46'sında (%51,1) en az bir virüse karşı IgM pozitifliği saptandı. Nazal örneklerde genom çoğaltma metotlarıyla, bu örneklerin 62'sinde (%68,8) bir virüs, 9'unda (%10) ise birden fazla virüsün varlığı belirlendi. Bu nedenle, örneklerin toplam 71'inin (%78,8) en az bir virüs yönünden pozitif olduğu kabul edildi.

**Sonuç:** Çalışmanın sonuçlarına göre, ALRI'lı bebeklerde solunum yolu virüslerinin belirlemesi için nazal örneklerinde genom çoğaltma metotlarının kullanımını önermekteyiz.

Anahtar Sözcükler: akut alt solunum yolu enfeksiyon, ALRI, bebek, PCR, solunum virüsleri.

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## Introduction

Acute lower respiratory tract infections (ALRIs) are common in all age groups, with an increased incidence rate during fall and winter months. If not diagnosed and treated appropriately, the infections can cause serious health problems including death (1,2).

In general, viruses are the primary cause of LRIs; the bacteria detected in most of the cases are a cause of super infections, which affect the prognosis of the disease. The main respiratory viral agents that cause most of the LRIs are respiratory syncytial virus (RSV), parainfluenza virus 3 (PIV3), human meta-pneumovirus, influenza virus type A (IVA), influenza virus type B (IVB), and adenoviruses (2,3).

Rapid and accurate diagnosis of viral respiratory infections is very important for proper selection of therapeutic options, for controlling the infection, and for accurate collection of epidemic data. Cell cultures, the traditional method used to detect most viruses, are accepted as a gold standard method in diagnosis of respiratory viruses. However, the method requires a period of up to two weeks to obtain the results, which limits the use of this method for diagnosis (4,5). In addition, chance of viral isolation in cell culture is possible only when samples are sent to the diagnostic laboratory under appropriate conditions and under optimal processing conditions. Consequently, all these factors limit the clinical use of cell cultures for identification of viruses. The other traditional method, which is based on detection of increase of IgG titers in serum samples during acute and convalescent periods, requires two weeks for results as well. The sensitivity of detection of IgM and viral antigens by enzyme linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA) during the acute period of infections is low (4-7). The polymerase chain reaction (PCR) is a highly sensitive and specific method giving rapid results. This method may detect inactive viruses, which facilitates archiving samples for retrospective studies. Hence, PCR has been accepted as an efficient method in diagnosis of viral respiratory infections (3,4,6,7).

Collection of samples from the infected region of an infant with ALRI is a painful method, and may cause a number of health risks. Medical equipment and professional medical staff are needed for this procedure. Due to the pathogenesis of respiratory viruses, use of nasal samples may be considered as an alternative approach (8,9).

The aim of this study was to evaluate the use of genome amplification methods [PCR or reverse transcription-PCR (RT-PCR)] for detection of common respiratory viruses (RSV, PIV3, IVA, IVB, and adenovirus) in nasal wash specimens of infants with ALRI. The results of the methods were compared with the specific IgM responses to the viruses detected by ELISA.

## Materials and Methods

**Samples:** All samples were collected from 90 infants with ALRI, aged between 6 and 16 (mean 10) months old. These infants were admitted to Firat Medical Center, Department of Pediatrics, with a suspicion of acute respiratory infection during the winter months of 2003-2004. After physical examination and clinical investigation, they were accepted as patients with ALRI. History of presenting illness of all infants consisted of general symptoms such as fatigue, anorexia, flu-like symptoms, eye redness, hoarseness, coughing, fever, restlessness, wheezing and difficulty in breathing. According to information taken from the parents, symptoms had started 4-6 days before the hospital visit and none of the infants had a history of vaccinization against respiratory viruses. Nasal wash specimens were collected in 2 ml of viral transport medium (Eagle minimal essential medium). Upon arrival in the laboratory, each sample was vortexed for 30 s and was centrifuged at 5,000 rpm for 30 min at 4ºC. The supernatants were transferred into vials. In addition, blood samples were taken from these infants. The supernatants and serum samples were stored at –80ºC until analyzed.

**ELISA:** Detections of the specific IgM and IgG antibodies to the test viruses from the serum samples were performed using commercial ELISA kits by following the procedures recommended by the manufacturers (R-Biopharm AG, Darmstadt-Germany and Genzyme Virotech GmbH, Rüsselsheim-Germany).

**Isolation of DNA and RNA:** DNA and RNA were isolated from the nasal samples using a commercial extraction kit by following the procedures recommended by the manufacturer (Wizard Genomic DNA Purification System, Promega Corp., Madison, WI and EZ-RNA Total RNA Isolation Kit, Biological Industries Corp., Beit Haemek-Israel). The resulting DNA and RNA pellets were dissolved in 50 µl $dH_2O$.

**Genome amplification methods:** RT-PCR for RSV (9), PIV3 (10), IVA and IVB (11) and PCR analysis for adenovirus (12) were carried out from the genome of nasal samples as described earlier. Primers used in this study and the expected sizes of the target sequences amplified with these primers are described in Table 1. Although the original study (11) for IVA and IVB was performed with nested RT-PCR using four different primers, this study was carried out with RT-PCR using two different primers. Ten microliters of each reaction product was run on a 2% agarose gel and the products were visualized by ethidium bromide staining.

During analyses, $dH_2O$ was used as a negative control. For sensitivity of PCR, RSV standard B2 strain was 10-fold diluted from $10^{6.7}$ to $10^{0.7}$ tissue culture infectious $dose_{50}$ ($TCID_{50}$) and then tested by PCR. Furthermore, for

Table 1. Primers used for genome amplification analysis (PCR and RT-PCR) of the nasal samples taken from infants with acute lower respiratory tract infection.

| Viruses | Sequences of the primers | | Amplification products |
|---|---|---|---|
| IVA | AMPA | 5'-ccgtcaggccccctcaaagc-3' | 640 bp |
| | AMPDII | 5'-gaccagcactggagctagga-3' | |
| IBV | BMPA | 5'-tgtcgctgtttggagacaca-3' | 430 bp |
| | BMPDII | 5'-agttttacttgcattgaata-3' | |
| Adenovirus | A2H/4R | 5'atgacttttgaggtggatcccatgga-3' | 134 bp |
| | A2H/1 | 5'-gcggagaagggcgtgcgcaggta-3' | |
| RSV | RSV1 | 5'-ttaaccagcaaagtgttaaga 3' | 243 bp |
| | RSV2 | 5'-tttgttataggcatatcattg 3' | |
| PIV3 | P1 | 5'-acgcaatccaactctactcata-3' | 880 bp |
| | PR | 5'-ctttgggagttgaacacagtt-3' | |

RSV: Respiratory syncytial virus. PIV3: Parainfluenza virus 3. IVA: Influenza virus type A. IVB: Influenza virus type B. bp: Base pair.

assurance of accuracy of the PCR results, the positive clinical samples were double checked by a second DNA extraction and PCR analysis.

The relationship between the serological test results for IgM and the genome amplification methods was analyzed using chi-square test (13).

## Results

In this study, findings of clinical, serological and genome amplification method of 90 infants with ALRI were evaluated. All of the infants had general symptoms such as coughing, high fever, nasal discharge, and hyperemic oropharynx and tonsils. In addition to these symptoms, vomiting, axillary lymphadenopathy and conjunctivitis were observed in some of the infants. Sixty-four (71.1%) of the infants had bronchiolitis, and 26 (18.9%) had bronchopneumonia. IgM positivity to at least one virus was detected in 46 (51.1%) of serum samples taken from these infants. Specific IgM response to only one virus and to multiple viruses was found in 39 (43.3%) and 7 (7.7%) of these serum samples, respectively. According to specific IgM presence, most of the samples were found to be positive for IVB (Table 2). IgG seropositivity to the tested viruses was detected in 26 (28.8%) of the serum samples (Table 2). Sixteen (61.5%) of the IgG seropositives were detected from the sera taken from infants younger than 9 months old.

When RNA extraction and RT-PCR method were carried out with standard RSV strain, the lowest detection limit of these methods was determined as approximately 10 virus particles for each reaction.

RT-PCR and PCR results carried out from nasal wash specimens are summarized in Table 2. Eight (8.8%), 32 (35.5%), 26 (28.8%), 9 (10%) and 5 (5.5%) of the samples were positive for IVA virus, IVB virus, RSV, PIV3, and adenovirus, respectively. While 62 (68.8%) of the samples were infected with only one virus, 9 (10%) of the samples were detected as infected with multiple viruses. Therefore, a total of 71 (78.8%) samples were accepted as infected with at least one virus (Table 2).

When the serological test results for IgM were compared to the genome amplification methods, the genome amplification methods were found to be significantly higher in positivity ($P<0.01$). Specificity of the serological test was 100%, and sensitivity was 64.7%.

## Discussion

Respiratory viruses are a common cause of respiratory infection epidemics for all age groups. These viruses are highly contagious and may cause infections of both upper and lower respiratory tracts (2). LRIs in particular may cause serious complications in infants. In different studies, RSV, PIV3 and IVB viruses were reported as the most common causes of LRIs (1-3). In the current study,

Table 2. Results of genome amplification methods (PCR and RT-PCR) and ELISA carried out in infants with acute lower respiratory tract infection (n: 90).

| Viruses | Genome positive n (%) | IgM positive n (%) | IgG positive n (%) |
|---|---|---|---|
| IVA | 8 (8.9) | 4 (4.4) | 2 (2.2) |
| IVB | 24 (26.6) | 17 (18.8) | 10 (11.1) |
| RSV | 20 (22.2) | 12 (13.3) | 4 (4.4) |
| PIV3 | 6 (6.6) | 4 (4.4) | 4 (4.4) |
| Adenovirus | 4 (4.4) | 2 (2.2) | - |
| IVB + RSV | 5 (5.5) | 4 (4.4) | 6 (6.6) |
| IVB + PIV3 | 3 (3.3) | 3 (3.3) | - |
| Adenovirus + RSV | 1 (1.1) | - | - |
| Total | 71 (78.8) | 46 (51.1) | 26 (28.8) |

RSV: Respiratory syncytial virus. PIV3: Parainfluenza virus 3. IVA: Influenza virus type A. IVB: Influenza virus type B.

IVB and RSV were detected as the responsible agents in most of the infants. Incidence rates of respiratory viruses differ from season to season. Generally, influenza and RSV viruses cause epidemics during winter months, while PIV3 typically may be isolated in spring epidemics (14-16). The higher level of IVB and RSV positivity detected in this study may be due to collecting nasal samples during the winter period.

For the diagnosis of most of the microbial infections, collection of samples from tissues and organs with the most specific symptoms are recommended. However, in some cases, collection of samples from the primarily affected organ or tissue may be impossible because of the serious complications that may result from the procedures. For example, it is painful and risky to collect lower respiratory tract tissue samples from infants with ALRI. Generally, respiratory viruses initiate the infection from the upper respiratory tract epithelium. After replication period from the epithelium, these viruses are spread to the lower respiratory tract by upper respiratory tract secretions, and start different courses of the lower respiratory tract infections (5,17). Because of the pathogenesis of these infections, nasal samples may be used for diagnosis from different clinical forms of viral ALRI (5,8,17,18). However, in some studies, the use of lower respiratory tract samples was found more sensitive than nasal samples in ALRI cases (6,16,19). For using nasal samples, the phase of infection may be an important factor affecting the sensitivity of the methods used. All ALRI cases evaluated in our study were in the acute phase of the infection. One week after the initial symptoms, it may be impossible to detect the viruses in the nasal mucosa because of the possible transportation of the agent to the lower respiratory tract. For this reason, we think that use of nasal samples in the late phases of the disease may cause false-negative results in the diagnostic tests.

Maternal antibodies may protect newborns against respiratory viruses until 6-9 months of age. However, sometimes maternal antibodies and acquired immunity may fail to protect infants from viral respiratory infections. One of the most important causes of this failure is inadequate antibody level in infants when compared with the antibody level synthesized from children. These low antibody titers in infants may also lead to false negativity in serological tests (1). This may explain the lower sensitivity of serological tests found in the present study. In addition, the lower sensitivity of serological tests may be due to lack of IgM at the time of collection of the sera. In a previous study, although RSV presence was determined by molecular techniques, RSV-specific IgM was positive in only 22.7% of the serum samples taken in the acute period and in 68.2% of the serum samples taken two weeks after the beginning of the disease (3). We did not have a chance to compare IgM positivity in acute and convalescent serum samples, since we could not collect serum samples for the second time. In addition, due to the

unavailability of second serum samples, we did not perform detection of an increase in IgG titers in serum samples during acute and convalescent periods. In this study, presence of IgG was detected by ELISA only in serum samples taken during the acute period of the illness. According to the ELISA results, we think that the IgG detected in infants younger than 9 months old is maternal antibodies. IgG seropositivity detected in another five infants older than 9 months may point to previous infections with these viruses.

In conclusion, we recommend use of the genome amplification methods in nasal samples for detection of the respiratory viruses in ALRI infants. Use of nasal samples in infants is the safe approach, and genome amplification methods in nasal sample are more sensitive than the conventional serological method. The technique also provides results faster than with cell culture technique. Therefore, use of genome amplification methods and nasal samples would improve patient management and infection control.

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**References**

1. Crowe JE Jr, Williams JV. Immunology of viral respiratory tract infection in infancy. Paediatr Respir Rev 2003; 4: 112-119.
2. Mackie PL. The classification of viruses infecting the respiratory tract. Paediatr Respir Rev 2003; 4: 84-90.
3. Baumeister EG, Hunicken DS, Savy VL. RSV molecular characterization and specific antibody response in young children with acute lower respiratory infection. J Clin Virol 2003; 27: 44-51.
4. Mohtasham L, Auais A, Piedimonte G. Advances in viral respiratory infections: new experimental models. Drug Discovery Today: Disease Models 2004; 1: 303-309.
5. Wat D. The common cold: a review of the literature. Eur J Intern Med 2004; 15: 79-88.
6. Simpson JL, Moric I, Wark PA, Johnston SL, Gibson PG. Use of induced sputum for the diagnosis of influenza and infections in asthma: a comparison of diagnostic techniques. Clin Virol 2003; 26: 339-346.
7. Smith AB, Mock V, Melear R, Colarusso P, Willis DE. Rapid detection of influenza A and B viruses in clinical specimens by Light Cycler real time RT-PCR. J Clin Virol 2003; 28: 51-81.
8. Heikkinen T, Marttila J, Salmi AA, Ruuskanen O. Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. J Clin Microbiol 2002; 40: 4337-4339.
9. Henkel JH, Aberle SW, Kundi M, Popow-Kraupp T. Improved detection of respiratory syncytial virus in nasal aspirates by seminested RT-PCR. J Med Virol 1997; 53: 366-371.
10. Swierkosz EM, Erdman DD, Bonnot T, Schneiderheinze C, Waner JL. Isolation and characterization of a naturally occurring parainfluenza 3 virus variant. J Clin Microbiol 1995; 33: 1839-1841.
11. Zhang W, Evans DH. PCR detection and differentiation of influenza virus A, B, and C. In: Persing DH, Smith TF, Tenover FC, White TJ, editors. Diagnostic molecular microbiology: principles and applications. Mayo Foundation; 1993. pp. 374-82.
12. McDonough M, Kew O, Hierholzer J. PCR detection of human adenoviruses. In: Persing DH, Smith TF, Tenover FC, White TJ, editors. Diagnostic molecular microbiology: principles and applications. Mayo Foundation; 1993. pp. 389-93.
13. SPSS for Windows, Release; 2000; 11.5.
14. Welliver RC. Review of epidemiology and clinical risk factors for severe respiratory syncytial virus (RSV) infection. Pediatr 2003; 143: 112-117.
15. Shaw MW, Xu X, Li Y, Normand S, Ueki RT, Kunimoto GY et al. Reappearance and global spread of variants of influenza B/Victoria/2/87 lineage viruses in the 2000-2001 and 2001-2002 seasons. Virology 2002; 303: 1-8.
16. Guney C, Kubar A, Yapar M, Besirbellioglu AB, Doganci L. An outbreak of respiratory infection due to respiratory syncytial virus subgroup B in Ankara, Turkey. Jpn J Infect Dis 2004; 57: 178-180.
17. Stensballe LG, Trautner S, Kofoed PE, Nante E, Hedegaard K, Jensen IP et al. Comparison of nasopharyngeal aspirate and nasal swab specimens for detection of respiratory syncytial virus in different settings in a developing country. Trop Med Int Health 2002; 7: 317-321.
18. Englund JA, Piedra PA, Jewell A, Patel K, Baxter BB, Whimbey E. Rapid diagnosis of respiratory syncytial virus infections in immunocompromised adults. Clin Microbiol 1996; 34: 1649-1653.
19. Covalciuc KA, Webb KH, Carlson CA. Comparison of four clinical specimen types for detection of influenza A and B viruses by optical immunoassay (FLU OIA test) and cell culture methods. J Clin Microbiol 1999; 37: 3971-3974.