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Comparison of the “ProDect BCS RV CHIP” assay with the combination of shell vial cell culture and immunofluorescence antibody test for the detection of respiratory viruses

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Abstract

In the present study, a multiplex reverse transcriptase polymerase chain reaction combined with a chip hybridization assay (ProDect BCS RV CHIP) was evaluated as an alternative to the combination of immunofluorescent antibody test and shell vial cell culture assay considered as gold standard for the detection of respiratory viruses. Among 100 specimens, 40 were positive using the combination of immunofluorescent antibody test and shell vial cell culture assay in which 9 of them were infected by two different viruses (27 parainfluenza virus type 3, 10 adenovirus, 9 respiratory syncytial virus, 2 influenza type B, and 1 influenza type A). ProDect BCS RV CHIP detected only 10 positive specimens in which one of them was infected by two different viruses (5 respiratory syncytial virus, 3 parainfluenza virus type 3, 2 adenovirus, and 1 influenza virus type B). The sensitivity, specificity, PPV, NPV, and diagnostic accuracy of ProDect BCS RV CHIP were 25.0%, 100%, 100%, 66.6%, and 70.0%, respectively, compared to the combination of shell vial cell culture and immunofluorescent antibody test. As a result, the specificity of ProDect BCS RV CHIP is high, however, the sensitivity (25%) of the assay is not sufficient for routine laboratory use.

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1. Introduction

Infection of human respiratory tract with different viruses causes diseases with similar signs and symptoms. The clinical signs and symptoms of patients infected with specific respiratory viruses cannot be used to differentiate clearly one virus from another. In this respect, rapid and specific identification of the infecting virus provides many advantages to assist the clinician in making therapeutic decisions, to prevent nosocomial infections, and alert the onset of epidemics. Respiratory syncytial virus (RSV), influenza virus types A and B, adenoviruses, and parainfluenza virus types 1, 2, and 3 cause the vast majority of identified viral respiratory infections in children. These viruses are responsible for a spectrum of acute upper and lower respiratory tract disease and are the major causes of croup, bronchiolitis, and pneumonia in infants and young children. However, these viruses may cause significant morbidity and mortality in adults, especially among elderly and immunocompromised individuals. Detecting these viruses will enable to take appropriate measures for the prevention of viral diseases, avoiding unnecessary antibiotic treatment and thus allowing the proper use of antiviral drugs (Corias et al., 2004; Dowell et al., 1996; Henrickson, 2005; Syrmis et al., 2004).

The diagnosis of viral respiratory infections is based on the use of conventional methods such as viral cell culture and indirect immunofluorescent antibody test (IFAT). Although these methods are valuable, they have significant limitations in terms of sensitivity and specificity. Viral cell culture is considered as the “gold standard” for respiratory virus detection, however, the technique is limited due to the prolonged result turnaround time, rapid loss of viability under unsuitable specimen transport and...
storage conditions (Bellau-Pujol et al., 2005; Liolios et al., 2001; Syrmis et al., 2004). IFAT is a more rapid but less sensitive method than viral cell culture and may be affected by specimen quality, virus type, and incorrect interpretation of results by inexperienced personnel. During the past decade, PCR techniques have improved the sensitivity and specificity for the detection of respiratory viruses. Recently, the development of multiplex PCR assays led to a decrease in expenses and short result turnaround time. In addition, molecular amplification methods do not need viable organisms in contrast to virus cell culture method that requires viable organisms. Multiplex PCR is considered as an advanced method compared to conventional PCR because it detects simultaneously more than one virus in a single reaction (Bellau-Pujol et al., 2005; Corias et al., 2005; Liolios et al., 2001; Syrmis et al., 2004).

In the present study, a multiplex reverse transcriptase polymerase chain reaction combined with a chip hybridization assay (ProDect BCS RV CHIP) was compared to IFAT and shell vial cell culture where the combination of these methods was considered as the “gold standard”. ProDect BCS RV CHIP is a multiplex reverse transcriptase PCR technique that rapidly detects and differentiates eight different respiratory viruses (Influenza virus type A and B, RSV, adenovirus, parainfluenza virus type 1, 2, and 3, and SARS CoV). This method is based on the detection of the biotin-labelled PCR amplified products obtained from multiplex PCR assays. Those PCR products bind with high specificity to the complementary sequences of the immobilized oligonucleotide probes arrayed on a nitrocellulose membrane. The arrays were analyzed using a reader (BCS AIM Reader) developed by the manufacturer (bcs Biotech S.p.A., Italy).

2. Materials and methods

2.1. Patient specimens

Between February 2005 and November 2005, nasopharyngeal swab specimens were collected from 88 (30 girl, 58 boy) pediatric and 12 (4 female, 8 male) adult patients with acute lower respiratory tract infections (bronchiolitis, bronchopneumonia, pneumonia). For the pediatric and adult patients, the age rates are between 5 days to 16 years (median: 1 year) and 27–89 years (median: 73 years), respectively. Nasopharyngeal swab specimens were transported to laboratory in viral transport medium (Eurotubo collection swab, Rubi, Spain) within few hours after collection and all the specimens were added to 4 ml of medium containing minimal essential medium (MEM) supplemented with penicillin/streptomycin (100 U/ml), gentamycin (10 mg/ml), amphotericin B (0.25 mg/ml). IFAT, shell vial cell culture, and ProDect BCS RV CHIP assay were performed as soon as the specimens arrived at the laboratory and the remaining specimens were stored at −80°C.

2.2. Indirect immunofluorescent antibody test (IFAT)

Specimens were vortexed thoroughly for 60 s and centrifuged at 1000 rpm at 4°C for 10 min. The supernatant was kept for shell vial cell culture. The cell pellet was immediately re-suspended in 500 µl of PBS (phosphate buffered saline; pH 7.4). One hundred microliters of the cell suspension was placed in a cytofunnel and cytocentrifuged at 2000 rpm for 5 min. Then, the slides were air dried, fixed in pre-chilled acetone for 10 min at −20°C and stained with Respiratory Virus Panel IFA kit (Bartels Immunodiagnostic Supplies Inc., Bellevue, Washington, USA) according to the manufacturer’s protocol. Positive specimens, detected using pooled monoclonal antibody, were stained again using fluorescein labeled monoclonal antibodies specific for influenza virus type A and B, RSV, adenovirus, parainfluenza virus type 1, 2, and 3 (Bartels Immunodiagnostic Supplies Inc., Bellevue, Washington, USA). The presence of at least three cells with typical staining was considered as positive. Non-immune mouse antiserum (Bartels Immunodiagnostic Supplies Inc., Bellevue, Washington, USA) was used as the negative control to exclude non-specific fluorescence.

2.3. Shell vial cell culture assay

For each patient, four shell vials containing human laryngeal carcinoma (Hep-2) cells (National Public Health Institute, Helsinki, Finland), four shell vials containing Madin Darby canine kidney (MDCK) cells (National Public Health Institute, Helsinki, Finland), and two shell vials containing African green monkey kidney (Vero) cells line (German Collection of Microorganisms and Cell Cultures, DSMZ, Germany) were prepared and each vial was inoculated with 0.2 ml specimen supernatant for the recovery of the following five respiratory viruses: adenovirus, RSV (Hep-2), influenza virus type A and B (MDCK), and parainfluenza virus (Vero). Thus, two vials are prepared for each virus to be detected. Next, the vials were centrifuged at 3000 × g for 30 min at 25°C, incubated at 37°C for 1 h, and supernatants were aspirated from each vial. Subsequently, 1 ml isolation medium containing Eagles MEM supplemented with 2% FCS and antibiotics (BiochromAG, Leonorenstr, Berlin, Germany) was added to the vials containing Hep-2 and Vero cells and 1 ml serum free medium containing 2 µg/ml trypsin-TPCK (Applichem, USA) was added to the vials containing MDCK cells. Then, the vials were incubated in moist chamber at 37°C in a 5% CO2 atmosphere for 48 h as described (Wiedbrauk and Johnston, 1993). Coverslips that are inside the first set of vials were removed carefully and fixed in pre-chilled acetone for 10 min at −20°C and stained with a fluorescein isothiocyanate labelled monoclonal antibody specific for RSV, parainfluenza virus, adenovirus, influenza virus type A and B (Light Diagnostics, Chemicon International, USA) according to the manufacturer’s protocol. The coverslips were examined for typical fluorescent inclusion bodies and was considered positive if one or more fluorescing inclusions bodies were present. If the specimens were negative for any type of virus in shell vials containing MDCK, Vero, and Hep-2 cells, then the second set of shell vials were incubated up to 5 days, coverslips were removed, fixed with pre-chilled acetone and stained with fluorescein isothiocyanate labelled monoclonal antibody as described previously.
After the inoculation of specimens, it takes approximately 5 days to detect parainfluenza virus in shell vial cell culture. Thus, the specimens were stored at −80 °C in the meantime and the parainfluenza virus positive specimens were thawed. 0.2 ml specimen was incubated in three different shell vials containing Vero cells as described previously and tested for the presence of parainfluenza virus types using fluorescent isothiocyanate labelled monoclonal antibodies specific for parainfluenza virus type 1, 2 and 3 (Light Diagnostics, Chemicon International, USA).

2.4. Extraction of viral nucleic acids

Viral nucleic acids were extracted from 0.2 ml of specimen which is in viral transport medium using the High Pure Viral Nucleic Acid kit according to the manufacturer’s protocol. Purified nucleic acid was eluted from the column using 50 μl elution buffer provided by the kit (Roche Applied Science, Germany).

2.5. ProDect BCS RV CHIP method

ProDect BCS RV CHIP method is a multiplex reverse transcriptase polymerase chain reaction followed by a chip hybridization assay (bc Biotech, Italy).

2.5.1. Multiplex reverse transcriptase PCR

Multiplex reverse transcriptase PCR (RT-PCR) was performed according to the manufacturer’s protocol. Two distinct 0.2 ml DNase/RNase-free PCR tubes labelled as tube 1 and tube 2 for each sample. Two microliters of RT-PCR mix 1 and mix 2 were added to tube 1 and tube 2, respectively. Then, 4 μl of purified nucleic acid and 4 μl Ready-To-Go enzyme mix (RT-PCR beads, Amersham, Biosciences, UK) were added to each tube. The tubes were incubated at 42 °C for 45 min in a thermal cycler (Eppendorf, Germany) to synthesize the cDNA. Then, PCR amplification reaction was prepared by adding mix RM solution (The mix RM solution contains biotin labelled primers designed from highly conserved genetic sequences of the eight respiratory viruses and targets the hemagglutinin neuraminidase gene of parainfluenza virus type 1, 2, and 3, the non-structural gene 2 of RSV, the hemagglutinin gene of adenovirus, and BNI-1 fragment of SARS) and DNA polymerase to each tube. The PCR amplification reactions were performed in an thermal cycler (Eppendorf, Germany) using the following calculated protocol: 5 min initial denaturation step at 95 °C, followed by 35 cycles of 20 s at 95 °C, 20 s at 50 °C, and 40 s at 72 °C, and an extension of 10 min at 72 °C.

2.5.2. ProDect BCS RV CHIP hybridization

The biotinylated amplified PCR product was hybridized to a set of eight immobilized oligonucleotide probes which were bound covalently to the nitrocellulose membrane on the chip, using manufacturer’s protocol. Briefly, amplified PCR product from tube 1 and tube 2 as well as 480 μl BCS Hyb buffer (peroxidase-labelled probe solution) were mixed in the same tube. The tube was then incubated for 5 min at 100 °C and immediately chilled on ice for 2 min. Next, the mixture was transferred onto the chips provided by the manufacturer and incubated at 45 °C for 1 h in an incubator (BCS mini oven, Italy). After the PCR product was hybridized to the oligonucleotide probe on the chip, each chip was washed five times with 500 μl wash buffer provided by the kit, incubated with a Strept-AP (streptavidin–alkaline phosphatase) diluted to 1:1000 with blocking reagent for 30 min at room temperature and washed with 500 μl wash buffer for 1 min. Next, the chips were inverted and tapped onto paper towel 10 times to remove residual liquid. To visualize the reaction on the chips, 490 μl detection buffer mixed with 10 μl NBT/BCIP was added onto each chip and incubated in the dark for 10 min at room temperature. The chips were then washed with distilled water and air-dried.

After hybridization and colorimetric development, only the specific probes for the pathogen present in the PCR product bind to oligonucleotide probe and probe-target hybrids forms blue-purple precipitants on the chip. The developed pattern on the chip was detected by a reader provided by the manufacturer (BCS Reader, bc Biotech, Italy). The different viruses that were present in the specimens could be distinguished by their different hybridization patterns on the chip (Fig. 1).

2.6. CAPvir method

CAPvir method is a multiplex RT-PCR followed a nitrocellulose strip hybridization assay (Genome Identification Diagnostic GmbH, Gen ID, Germany). In this method, 10 specimens were randomly selected out of 24 frozen specimens that were positive for parainfluenza virus type 3 using shell vial cell culture and negative with ProDect BCS RV CHIP. The viral nucleic acids of the selected thawed specimens were extracted as previously described and used in CAPvir method.

2.6.1. Multiplex reverse transcriptase PCR

Multiplex RT-PCR was performed according to the manufacturer’s protocol. For each specimen three groups of tubes (each containing three 0.2 ml DNase/RNase free PCR tubes) were prepared. Three types of reaction mixes were prepared for each group of tube. Each tube in the first group of tubes contains reaction mix 1 prepared with the following reagents: 30 μl CAPvir Flu solution (contains biotin labelled primers designed for non-structural protein 1 of influenza virus type A and B), 10 μl 5 × RT-buffer, 5 μl 125 mM Mn(OAc)2, 20 units RNase inhibitor, 5 units Tth-DNA polymerase (Roche, Germany). Each tube in the first group of three tubes that contain reaction mix 1 was gently mixed with 3 μl extracted viral nucleic acid, 3 μl RNase free distilled H2O (negative control), and 3 μl β-2 microglobulin (amplification control; extracted RNA from HeP-2 cells), respectively. Each tube in the second group of tubes contains reaction mix 2 prepared with the following reagents: 30 μl CAPvir PIV solution (contains biotin labelled primers designed for hemagglutinin gene of parainfluenza virus type 1, nucleocapsid protein of parainfluenza virus type 2 and 3), 10 μl 5 × RT-buffer, 5 μl 25 mM Mn(OAc)2, 20 units RNase inhibitor, 5 units Tth-DNA polymerase. Each tube in the second group of three tubes that contain reaction mix 2 was mixed gently
Fig. 1. ProDect BCS RV CHIP probe design. This chip model is a representative of a ProDect BCS RV CHIP, which is probed with a specimen that has respiratory viruses (parainfluenza virus type 1, 2, and 3, influenza virus type A and B, adenovirus, respiratory syncytial virus, and SARS Co-V). The chip has five control spots. Control spots, located at the corners of the chip, are hybridization controls that show the proper handling of the reagents used during the assay. The control spot, located in the centre of the chip, is the amplification control that is the target of the internal control and shows that the amplification reaction occurred. In addition, the chip has an amplification control which is designed to identify the disturbing effect of inhibitory substances inside the sample that hampers the amplification reaction.

with 3 µl extracted viral nucleic acid, 3 µl RNase free distilled H2O and 3 µl β-2 microglobulin, respectively. Each tube in the third group of tubes contains reaction mix 3 prepared with the following reagents: 30 µl CAPvir RSV/ADV solution (contains biotin labelled primers designed for hexon protein of adenovirus, polymerase gene of RSV), 10 µl 5× RT-buffer, 5 µl 25 mM Mn(OAc)2, 20 units RNase inhibitor, 5 units Tth-DNA polymerase. Each tube in the third group of three tubes that contain reaction mix 3 was mixed gently with 3 µl extracted viral nucleic acid, 3 µl RNase free distilled H2O and 3 µl β-2 microglobulin, respectively. The PCR amplification reactions were performed in a thermal cycler (Eppendorf, Germany) using the following calculated protocol: 1 cycle 40 min at 58 °C and 4 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 45 s at 65 °C, and 45 s at 70 °C, and a final extension of 8 min at 72 °C.

2.6.2. CAPvir nitrocellulose strip hybridization

The biotinylated amplified PCR product was hybridized to a set of seven immobilized oligonucleotide probes which were bound covalently to the nitrocellulose strip using manufacturer’s protocol. Briefly, 20 µl amplified PCR product was collected from each tube that contains viral nucleic acid was mixed in a well of an incubation tray. Sixty microliters of denaturing agent was added in to each well, gently mixed and incubated for 5 min at room temperature. One microliter of pre-warmed hybridization buffer was added to each well and strips were transferred to the wells that contain the mixture. The trays that contain the strips were incubated for 30 min at 47 °C in the shaking water bath. The strips were washed twice with Rinse I solution for one min on a horizontal shaker at room temperature. Each strip was incubated with 1 ml conjugate diluted to 1:100 with conjugate buffer for 30 min on a horizontal shaker at room temperature, washed three times 1 ml Rinse II solution for 1 min at room temperature. To visualize the reaction on the strips 1 ml substrate solution was added on to the each strip and incubated in the dark for 10 min at room temperature. The reaction was stopped by washing the strips twice with 1 ml distilled water. Strips were transferred on to absorbent paper, air dried in the dark. For the negative and amplification controls, 20 µl sample, collected from each groups’ corresponding negative and amplification control tubes, were mixed in two different wells of an incubation tray. Sixty microliters of denaturing agent was added in to each well and CAPvir nitrocellulose strip hybridization method was performed as described above. Therefore, at the end of the assay, three strips that contain viral nucleic acid, negative and amplification control strips have been prepared for each specimen. The bands on the strips were analyzed using the supplied template provided by manufacturer (Fig. 2).

2.7. Statistical analysis

Sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy were calculated using standard methods (Dawson-Saunders and Trapp, 1994).

3. Results

3.1. Viruses detected in pediatric and adult patients

As shown in Table 1, in the group of 88 pediatric patients, 34 (38.6%) of them were positive for respiratory viruses and in
Fig. 2. Interpretation of the results of CAPvir method. The strips presented in this figure are nitrocellulose strip templates that were used to analyse the results of CAPvir method. The strip has conjugate control band that appears when efficient conjugate binding occurred. Amplification control zone on the strip has a probe for mRNA coding sequence of β-2 microglobulin which serves as a marker for human mRNA and develops when there is an appropriate RNA extraction and RT-PCR. (A) Representative of a strip probed with negative control. Only the conjugate band appears. (B) Representative of a strip probed with amplification control. The conjugate and amplification control bands appear. (C) Representative of a strip probed with a specimen that have parainfluenza virus type 1, 2, and 3, influenza virus type A and B, adenovirus, and respiratory syncytial virus. Conjugate band, amplification band and the bands specific for viruses appear. The specificity control zone the strip develops when the washing temperature is to low and shows unspecific hybridizations.

Table 1
Single and dual virus infections detected in children and adult patients

| Groups            | Viruses                          | Pediatric | Adult |
|-------------------|----------------------------------|-----------|-------|
| Single virus infections | Parainfluenza virus type 3       | 16        | 2     |
|                   | Influenza virus type B           | 1         | –     |
|                   | Adenovirus                       | 5         | 2     |
|                   | RSV                              | 5         | –     |
| Dual virus infections | Parainfluenza virus type 3 + RSV | 4         | –     |
|                   | Parainfluenza virus type 3 + adenovirus | 1         | 2     |
|                   | Parainfluenza virus type 3 + influenza virus type A | 1         | –     |
|                   | Parainfluenza virus type 3 + influenza virus type B | 1         | –     |
| Total             |                                  | 34        | 6     |

In the group of 12 adult patients, 6 (50%) patients were positive for respiratory viruses. Parainfluenza virus type 1 and 2 was not detected during the study. Thus, 49 respiratory viruses were isolated and identified in 40 specimens obtained from 100 patients (Table 2).

3.2. Comparison of the methods used to detect viruses

In the present study, IFAT, shell vial cell culture, and ProDect BCS RV CHIP method were performed in 100 specimens. CAPvir method was carried out on 10 specimens that were randomly selected out of 24 frozen specimens that were positive for parainfluenza virus type 3 using shell vial cell culture and negative with ProDect BCS RV CHIP.

In the 40 respiratory virus positive specimens, IFAT detected respiratory viruses in 24 specimens in which five were dual virus infections and 19 were single virus infections. Therefore, 29 respiratory viruses were detected in 24 specimens using IFAT. Shell vial cell culture detected respiratory viruses in 40 specimens in

Table 2
Number of respiratory viruses detected by each method

| The results of the methods | The amount of the viruses |
|----------------------------|--------------------------|
| Shell vial cell culture    | IFAT | ProDect BCS RV CHIP | Parainfluenza virus type 3 | Adenovirus | RSV | Influenza virus type A | Influenza virus type B | Total |
| +                          | +    | +                    | 3                         | 2           | 1   | –                      | –             | 6     |
| +                          | +    | –                    | 13                        | 1           | 4   | –                      | –             | 19    |
| +                          | –    | +                    | –                         | –           | –   | –                      | 4             | 1     |
| +                          | +    | +                    | –                         | –           | –   | 4                      | –             | 4     |
| +                          | –    | –                    | 11                        | 7           | –   | 1                      | –             | 19    |
| Total                      | 27   | 10                   | 9                         | 1           | 2   | 49                     |               | 49    |
which five were dual virus infections. Thus, 45 viruses were detected in 40 specimens using shell vial cell culture. The last assay ProDect BCS RV CHIP detected respiratory viruses in 10 specimens in which one of them was dually infected. Eleven respiratory viruses were detected in 10 specimens by ProDect BCS RV CHIP method. The combination of IFAT and shell vial cell culture which is considered as the “gold standard” in the present study detected respiratory viruses in 40 specimens in which 9 (22.5%) were dual virus infections and 31 were single virus infections. In these 40 specimens, 49 respiratory viruses were detected using the combination of IFAT and shell vial cell culture. As a result, shell vial cell culture, IFAT and ProDect BCS RV CHIP detected 92% (45/49), 59.2% (29/49), and 22.4% (11/49) of the respiratory viruses in the specimens, respectively. In addition, ProDect BCS RV CHIP detected only one dual infection compared to the combination of IFAT and shell vial cell culture which detected nine dual infections (Tables 2 and 3).

The sensitivity of ProDect BCS RV CHIP in detecting RSV, adenovirus and parainfluenza virus type 3 were 55.5%, 20%, and 11%, respectively, and the specificity was 100% for these viruses. As influenza virus type A and B were detected only in a small group of specimens (n = 3) in this study, the ProDect BCS RV CHIP results were not compared with shell vial cell culture and IFAT. The overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of ProDect BCS RV CHIP were 25.0%, 100%, 100%, 66.6%, and 70%, respectively, compared to the combination of IFAT and shell vial cell culture.

Ten specimens which were parainfluenza virus type 3 positive with shell vial cell culture and negative with ProDect BCS RV CHIP assay were also positive for parainfluenza virus type 3 using CAPVir method. Inhibitory substances were not detected in any of the specimens by the ProDect BCS RV CHIP and CAPvir methods.

4. Discussion

One of the most widely used methods for the diagnosis of viral respiratory infections is the isolation of viruses in shell vial cell culture and by immunofluorescence antibody tests (Halstead et al., 1990; Henrickson, 2005). Even though these methods are effective and often complementary, they have some disadvantages. Cell culture techniques are considered traditionally as the “gold standard” during routine diagnosis of viral respiratory agents, however, these methods are laborious and time consuming. Although immunofluorescence antibody tests are easy to perform, antigen detection is less sensitive and specific (Bellau-Pujol et al., 2005; Landry and Ferguson, 2000). Currently, the traditional cell culture and IFAT techniques for the diagnosis and identification of viral respiratory agents are being replaced gradually by molecular techniques such as PCR to increase the diagnostic accuracy of the routine assays and decrease the turnaround time of the result (Corias et al., 2005; Henrickson, 2005; Syrmis et al., 2004). The short result turnover time is very important during a viral outbreak or in the case of a severely infected patient for initiating antiviral therapy on time (Adcock et al., 1997; Corias et al., 2004; Woo et al., 1997). Thus, the present study aimed to compare a multiplex reverse transcriptase polymerase chain reaction followed by a chip hybridization assay that has 4.5 h result turnaround time to the combination of shell vial cell culture and IFAT that have totally 5 days turnaround time for the result.

RSV and influenza viruses are the major viral agents that cause respiratory illness in pediatric and adult patients, respectively (Henrickson, 2005; Weigl et al., 2000). In this study, parainfluenza virus type 3 and adenovirus/parainfluenza virus type 3 were the major viral agents detected in 23 pediatric and 6 adult patient specimens, respectively. Parainfluenza virus type 3 was frequently isolated from the pediatric patients during the research because some of the specimens used in this study were collected during a parainfluenza virus type 3 outbreak which was reported in Izmir municipality, Turkey between December 2004 and March 2005 (Gülen et al., 2005).

In the present study, the combination of shell vial cell culture and IFAT detected nine dual infections compared to ProDect BCS RV CHIP, which detected only one dual infection. In two studies published previously, a large numbers of dual infection were detected with multiplex RT-PCR compared to the traditional diagnostic assays (Bellau-Pujol et al., 2005; Corias et al., 2004). Thus, the ProDect BCS RV CHIP was considered as not suitable for detecting dual infections. However, the shell vial cell culture used in this study could not detect the RSV in the four cases that have dual infection caused by RSV and parainfluenza virus type 3 but IFAT detected the RSV in these specimens. As reported in previous studies, the reason for this discrepancy might be due to the rapid loss of the viability of RSV under unsuitable transport and/or storage conditions (Bellau-Pujol et al., 2005; Liolios et al., 2001; Syrmis et al., 2004).
The previous studies that compare multiplex RT-PCR to traditional respiratory virus diagnostic assays such as shell vial cell culture, IFAT and/or individual RT-PCR reported often that multiplex PCR is more sensitive and specific in detecting all respiratory viruses (Bellau-Pujol et al., 2005; Corias et al., 2005; Liolios et al., 2001; Syrmis et al., 2004). In these studies, Liolios et al. (2001) used nucleocapsid and hemagglutinin-neuraminidase gene regions for RSV and parainfluenza virus type 3, respectively. Bellau-Pujol et al. (2005) used nucleocapsid and hemagglutinin-neuraminidase gene regions for RSV and parainfluenza virus type 3, respectively. Syrmis et al. (2004) used nucleocapsid, hexon protein, and L protein for parainfluenza virus type 3, adenovirus and RSV, respectively, Corias et al. (2005) used fusion protein, hexon protein, and hemagglutinin gene regions for RSV, adenovirus, and parainfluenza virus type 3, respectively. However, in two other studies, Gröndahl et al. (1999) and Puppe et al. (2004) reported that multiplex RT-PCR was less sensitive in detecting only RSV and parainfluenza virus type 3 among all tested pathogens compared to traditional diagnostic assays. In these studies, F1 subunit of fusion glycoprotein gene, 5’ non-coding region of the fusion protein, hexon gene regions were used for detecting RSV, parainfluenza virus type 3, and adenovirus, respectively (Gröndahl et al., 1999; Puppe et al., 2004). Similar to these two studies, the ProDect BCS RV CHIP was also less sensitive in detecting RSV, adenovirus, and parainfluenza virus type 3 compared to shell vial cell culture and IFAT. The ProDect BCS RV CHIP used non-structural gene 2, hemagglutinin, and hemagglutinin-neuraminidase gene regions for the detection of RSV, adenovirus, and parainfluenza virus type 3, respectively. ProDect BCS RV CHIPs gene regions for the detection of RSV and adenovirus were used for the first time in literature. Therefore, the reason for the low sensitivity obtained while detecting RSV and adenovirus by ProDect BCS RV CHIP might be due to the selection of primer gene regions different than the studies described previously that have high sensitivity for these viruses.

Puppe et al. (2004) reported low sensitivity (23%) for parainfluenza virus type 3 using 5’ non-coding region of the fusion protein gene region in a multiplex RT-PCR ELISA method compared to traditional diagnostic assays. The previous studies that detected parainfluenza virus type 3 with high sensitivity used mainly hemagglutinin-neuraminidase or nucleocapsid gene regions during multiplex RT-PCR (Bellau-Pujol et al., 2005; Corias et al., 2005; Liolios et al., 2001; Syrmis et al., 2004). ProDect BCS RV CHIP also used hemagglutinin-neuraminidase gene region for the detection of parainfluenza virus type 3, however, it was less sensitive compared to the combination of shell vial cell culture and IFAT. In order to investigate the technical problems such as sampling error, loss of RNA during extraction or degradation of RNA that might cause ProDect BCS RV CHIPS low sensitivity in detecting of parainfluenza virus type 3, CAPVir assay was applied to randomly selected 10 specimens which were parainfluenza virus type 3 positive with shell vial cell culture and negative with ProDect BCS RV CHIP assay. As a result, CAPVir detected all of the parainfluenza virus type 3 positive specimens that were not detected with the ProDect BCS RV CHIP, which eliminated the presence of technical problems described above. Another parameter that can cause the decrease in sensitivity might be the selection of primer gene regions different than the recommended ones. However, the CAPVir and ProDect BCS RV CHIP use the recommended nucleocapsid and hemagglutinin-neuraminidase gene regions, respectively, for the detection of parainfluenza virus type 3. Another parameter that is important during the application of multiplex RT-PCR is the large number of primer sets combined in a single reaction that can hamper the sensitivity of the assay (Syrmis et al., 2004). Interestingly, the studies that reported high sensitivity in detecting respiratory viruses by the multiplex RT-PCR and the ProDect BCS RV CHIP used a high number of primer sets combined in a single reaction except the multiplex RT-PCR developed and CAPVir which used low number of primer in three sets of primer mixes (both methods mixed primers of parainfluenza virus types in a tube) that contain low number of prime sets (Bellau-Pujol et al., 2005; Corias et al., 2005; Liolios et al., 2001; Syrmis et al., 2004). Inadequate optimization of large number of primer sets in a single reaction might be causing the low sensitivity of the ProDect BCS RV CHIP in detecting parainfluenza virus type 3. Although the ProDect BCS RV CHIP has decreased sensitivity during routine laboratory use as described above, it has high PPV and specificity because of the absence of cross-reactions with other viruses and false positive results.

Overall, the ProDect BCS RV CHIP has high specificity but the design of primer gene regions and the reactions that have large number of primer sets still needs to be optimized for routine laboratory use as a result of the ProDect BCS RV CHIPs low sensitivity compared to the combination of shell vial cell culture and IFAT which is considered as the “gold standard” in the present study.

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