PEER-REVIEWED ORIGINAL ARTICLE

Rapid Diagnostic Test vs. Real-Time PCR for Rapid Diagnosis of Infection with Adenovirus and Respiratory Syncytial Virus

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Abbreviation list
ADV: adenovirus; RSV: respiratory syncytial virus; RT-PCR: real-time PCR; DNA: deoxyribonucleic acid; RNA: ribonucleic acid; RDT: rapid diagnostic test

Abstract

A new commercial rapid diagnostic test for 10-min for adenovirus (ADV) and respiratory syncytial virus (RSV) is now available for rapid diagnosis. The sensitivity and the specificity of these rapid diagnostic tests were compared to real-time PCR which is a "golden standard" for detecting ADV and RSV. Here, we assessed the 150 of real-time PCR-positive samples for ADV and 100 for RSV, and 81 (54%) and 63 (63%) of them were positive with rapid diagnostic test, respectively. The sensitivity of the rapid diagnostic test was 54% for ADV and 63.0% for RSV. Accordingly, the samples showing over 30 Ct in real-time PCR were detected by the ADV rapid diagnostic test and over 24 Ct by the RSV rapid diagnostic test. These results indicated that these test kits performed well enough to be used for rapid diagnosis of patients infected with ADV and RSV after 10-min with simple step.

Introduction

Most of the respiratory virus infections in early childhood are confined to the upper respiratory tract, leading to symptoms of the common cold, cough, and hoarseness. Respiratory syncytial virus (RSV) is one of the common respiratory virus which usually causes mild and cold-like symptom, but RSV can be serious, especially for infants and children. RSV is the most common cause of bronchiolitis and pneumonia. Beside RSV, the group of adenoviruses (ADV) typically cause respiratory illnesses, such as common cold, conjunctivitis, croup, bronchitis, or pneumonia[1)]. In children, ADV usually cause respiratory tract infections. Early diagnosis of respiratory pathogen is efficiency during treatment of patients. Today, several methods are available for the diagnosis of respiratory virus detection, all of which are time-consuming or require the use of a well-equipped laboratory. Viral culture had been considered as the gold standard for virus diagnosis, but it takes a turnaround time of 3 to 7 days (1 to 2 days for shell vial culture). Real-time PCR (RT-PCR) is more sensitive and specific but requires more time, technique and skill of technician[2)]. Rapid test can be completed within 10-15 min without special instruments. It rapidly provides helpful information for the diagnosis and for developing a treatment plan for patients with suspected RSV and ADV respiratory diseases. These results can be available during the patient's first examination, at the bedside or in the outpatient clinic.

Differentiation of bacterial and other viral infections from RSV infection is a common clinical problem. With RSV, lower respiratory tract infections in infants and young children, especially those with congenital heart disease or chronic lung disease, are often severe[3,4)]. ADV respiratory infections are usually described as being associated with high mortality rates, infection with adenovirus is usually acquired during childhood. Acute lower respiratory tract infections in children is a major health problem. Human adenoviruses have an important role in children's respiratory tract infections. They are estimated to cause 2-5% of the overall respiratory tract infections and 4-10% of all pneumonias[5)]. Consequently, a simple, sensitive, and rapid diagnostic test for RSV and ADV infections would be invaluable to those caring for children. Rapid confirmation or elimination of RSV would allow a pediatrician to counsel a child's parents about the prognosis and would facilitate prompt and adequate measures to restrict transmission of the virus in a children's ward containing high-risk infants. ADV for rapid test was not popular in Vietnam, it is using real-time PCR or ELISA for testing in the hospitals.

In this study, we compared rapid diagnostic test (RDT) and RT-PCR with regard to sensitivity, specificity, and technical complexity to diagnose RSV and ADV infection among Vietnamese children who had bronchitis or pneumonia.

Materials and methods

Specimens: The study materials consisted of 250 nasal wash from children were diagnosed with bronchiolitis and pneumonia at Vietnam National Children's Hospital. 150 samples of children positive ADV and 100 samples positive with RSV were confirmed by real-time RT-PCR. Their average age was 12.07 ± 9.74 months (range 2 to 55 months) with ADV and 3.73 ± 3.42 months (range 0.5 to 19 months) with RSV.

Preparation of lateral flow immunoassay (LFIA) strips

Gold nanoparticles (AuNPs) of 60 nm diameter were prepared according to the citrate reduction of HAuCl4. Anti-ADV hexon monoclonal antibody (anti-ADV Mab, Tanaka Kikinzoku Kogyo K.K., Tokyo) or anti-RSV fusion protein monoclonal antibody (anti-RSV Mab, Tanaka Kikinzoku Kogyo K.K., Tokyo) was immobilized on the prepared AuNPs. The antibody-AuNPs conjugates were centrifuged at 12,000 rpm for 10 min at 4°C

to wash with 5mM PBS (pH7.4) containing 1% BSA. The conjugation pad was prepared by spraying the antibody-AuNPs conjugates, prepared as described above, and then drying it under vacuum for 2 hours at room temperature. The counterpart of anti-ADV MAb or anti-RSV MAb was sprayed onto nitrocellulose membrane (Merck) as a test line using Bio-Dot dispensing machine. Similarly, anti-mouse IgG polyclonal antibody (Nippon Bio-test Laboratories Inc, Tokyo) was sprayed onto the membrane as a control line. After spraying, the membranes were dried at 37°C for 1 hour. Then, the nitrocellulose membranes were placed on a plastic backing, and a conjugation pad and an absorption pad were applied at the upper part of the membranes. Furthermore, the sample pad was applied at the upper part of the conjugation pad. Subsequently, the membranes were cut into 5 × 77 mm strips and they were stored in sealed plastic laminated aluminum bags with a desiccant agent at room temperature until use.

LFIA procedure

To collect nasal or pharynges swab clinical samples, the sterile swabs were inserted into the nostril or throat of subjects being tested. The swabs were immersed and swirled in extraction buffer, consisted of non-ionic detergent, casein and PBS (pH7.4). Then, the swabs were removed from extraction buffer, and the extraction buffer tubes were fitted with filter nozzles. Three drops (approximately 120µL) of the prepared sample, passed through the filter nozzle, were dispensed on sample pad. The results of the LFIA were observed at 10 minutes by eye.

Real-time RT-PCR

Total nucleic acid extraction was performed on a MagNA Pure LC 2.0 (Roche Diagnostics, Vietnam). Specimens were extracted using a Total nucleic acid extraction kit (Roche Diagnostics, Germany) and the Total NA variable-elution-volume protocol with a sample input of 200µl and an elution volume of 100µl. Amplification was then 25µl reaction volumes including 5µl template in an ABI 7500 real-time PCR system (Thermos Fisher's Scientific) [2].

Results

The RDT test is a sandwich immunoassay that uses a paper membrane with a monoclonal antibody in the liquid phase and two polyclonal antibodies in the solid phase. The signal antibody segment is adjacent to the round well of the sample aliquot. Briefly, the 10-min, one-step procedure is as follows. The result in ADV and RSV showed that strong positive in RDT occurred with Ct value <18 in RT-PCR, color line of samples clear same or stronger than positive control.

The total number of 100 RSV samples detected by RT-PCT when using RDT was 63 samples (63%), including 50 strong positive samples and 13 weak positive but wasn't detected in 37 positive samples which was confirmed by RT-PCR during one-step around 15.76±0.28 min (Figure 1). Besides, among 150 ADV samples which were positive by RT-PCR when using RDT, there were 81 positive samples (54%) including 56 samples strong positive and 25 samples weak positive, 69 samples positive with DNA but negative with RDT during 10.51±0.26 min (Figure 1). We demonstrated that the sensitivity of RDT was 73% of RSV, higher than ADV with 68.5%. RT-PCR is now the reference diagnosis method for RSV and ADV because of sensitivity and specificity but RDT testing for RSV and ADV detection is potentially faster than RT-PCR, although it is less sensitive and requires considerable technical skill. RSV infection is usually higher than ADV and RDT developed with RSV longer than ADV, so may be the sensitivity of RSV higher than ADV.

Figure 2 shows that comparison of the result of RDT to Ct value of RT-PCR. From the clinical specimens, Ct value as high equivalent with the low viral load and Ct values are less equivalent with the high viral load. With Ct value >30 of RT-PCR was not detected by RDT of ADV. In RSV, the relatively poor sensitivity in this group resulted from a high proportion of samples with low viral load (Ct value >24). RT-PCR runs 45 cycles with samples have Ct value >38 it is negative. In our result, RDT with RSV and ADV, required 10-15 min but RT-PCR required 5 hours including extract nucleic acid and amplification reaction.

Discussion

In this study, the sensitivity and specificity of RDT for ADV were 68.5% and 100% and those for RSV were 73% and 100%. However, samples with 18< Ct value <25 in RT-PCR have not clear color line as positive control may be the concentration and sensitivity of RDT of samples lower Nasal wash for testing may be some samples which have a more viscosity so that it also affects the capillary of the samples, whereas the cellular release in the sample might blocked by the viscosity makes the surface antigen more lower sensitive than the nasal swab. Nasal washing may provide effective alternatives to nasal swab in RT-PCR. We demonstrated that the sample condition might be important in achieving absolute specificity. RDT kits for RSV and ADV antigen have been widely used in Japan. These kits are reported to have moderate sensitivity compared with RT-PCR. Rapid detection of RSV, ADV infection in children and infants can be critical for effective patient management by focusing appropriate drug treatment, reducing unnecessary use of antibiotics, and preventing nosocomial spread. Diagnosis of RSV, ADV infection can be made by observation of clinical signs and symptoms, characteristic chest radiographs, rapid antigen detection, viral culture, or RT-PCR of clinical specimens[6]. Thus, RDT was accepted results of screen test of sensitivity of kit.

Conclusions

Rapid test could be more rapid around 10 min and easier test compared with RT-PCR. Detection of RSV and ADV antigen by this simple and rapid method will serve physicians as a useful tool for early diagnosis and prevention of adenoviral conjunctivitis. It is convenience for outpatients when they will be examined in the hospital.

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Table 1. Comparison of ADV and RSV by RDT method and real-time RT-PCR

	RT-PCR	RDT			Rapid test RT-PCR
	Positive	Negative	Positive	Weak Positive	Negative Control
ADV	150 (100%)	69 (46%)	56 (54%)	25 (25%)	10
RSV	100 (100%)	37 (37%)	50 (63%)	13 (13%)	10

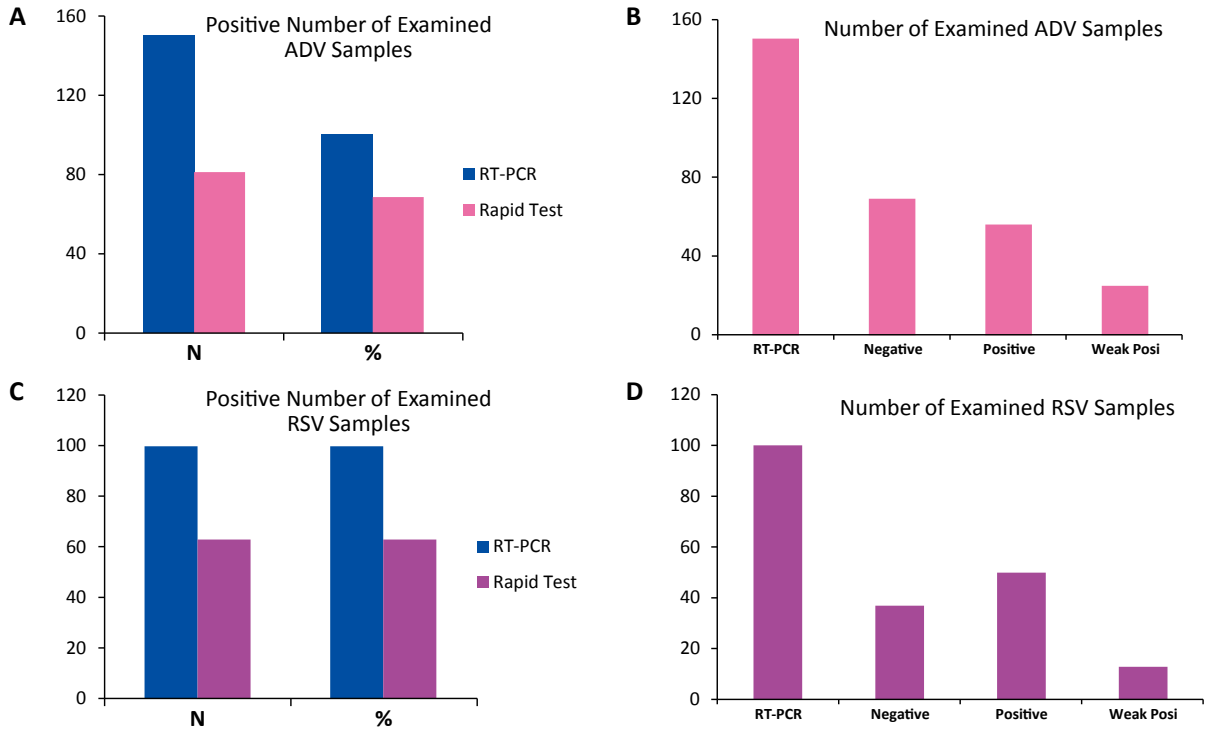

Figure 1. Number of examined ADV and RSV samples
A: Number of examined for 150 DNA-positive of ADV and the percent of ADV samples positive by RT-PCR and RDT. B: The number of ADV samples negative, positive and weak positive by RDT compare with positive samples by RT-PCR. C: Number of examined for 100 RNA –positive of RSV samples with RDT and the percent of RSV samples positive by RT-PCR and RDT. D: The number of RSV negative, positive and weak positive by RDT compare with positive samples by RT-PCR.

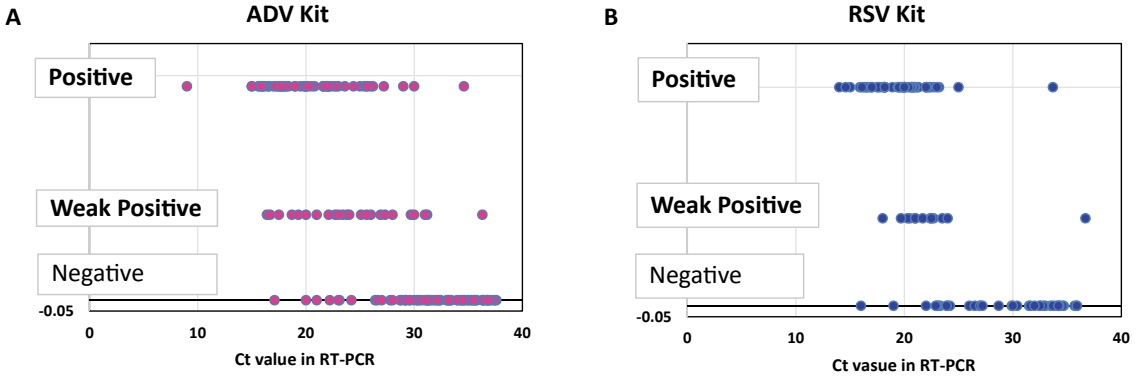

Figure 2. The comparison of Ct value in RT-PCR with sensitivity of RDT
A: The samples were Ct value of RT-PCR >30 that sample negative by RDT of ADV. B: The samples were Ct value of RT-PCT >24 that samples negative by RDT of RSV.

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