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Immunochromatographic test for detection of adenovirus from respiratory samples: Is it a real solution for pediatric emergency department?

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Rapid diagnosis of adenoviral respiratory infections is required in order to decide optimal treatment strategies. The adenovirus antigen immunochromatography Adeno Respiratory Card Letitest (Leti diagnostics, Barcelona, Spain), was evaluated versus the shell-vial culture and multiplex PCR (Clart Pneumovir Version 3.0, Genomica, Madrid, Spain), in nasopharyngeal washes and oropharyngeal swabs specimens from subjects with respiratory tract infections.

Between April 2011 and November 2012, 224 patients were included. The IC Adeno Respiratory Card Letitest was positive for 77.9% (74 of 95) of patients diagnosed at bedside. Using multiplex-PCR as the reference standard, the overall sensitivity was 77.9% and the specificity was 73.6%. Taking shell-viral culture as the reference method, the Adeno Respiratory Card Letitest (Leti diagnostics, Barcelona, Spain) sensitivity and specificity values were 80.0% and 60.9%, respectively. Using RT-PCR (Clart Pneumovir Version 3.0, Genomica, Madrid, Spain) as the reference standard, the viral culture sensitivity was 53.2% and the specificity was 100%.

The Adeno Respiratory Card Letitest (Leti diagnostics, Barcelona, Spain) assay could be a simple and rapid method for antigenic detection of adenovirus in pediatric respiratory samples although it would be necessary to improve the specificity and sensitivity of the test.

1. Introduction

Human adenovirus (HAdV) belong to the *Adenoviridae* family and the *Mastadenovirus* genus. They are structurally icosahedral, non-enveloped, double-stranded, linear DNA viruses. There are 52 different adenovirus serotypes, classified into seven groups (A–G) (Pabbaraju et al., 2011).

More severe disease is observed among children, the elderly and immunocompromised persons. Transmission of HAdV can occur by direct contact, small droplet aerosols, through the water system, and via the fecal-oral route. HAdV can survive for long periods on surfaces in the environment. They are resistant to lipid disinfectants, but are inactivated by heat, formaldehyde and chlorine (Pabbaraju et al., 2011).

HAdV are important causes of respiratory tract infections and are responsible for 5–10% of all lower respiratory tract infections occurring in infants and children. They are most often associated with respiratory tract disease, but can also cause gastrointestinal, ophthalmological, neurological and genitourinary manifestations. Infection with adenoviruses can mimic Kawasaki disease and bacterial infections, leading to unnecessary treatments if it is not properly diagnosed (Peltola et al., 2006).

Most individuals exhibit serological evidence of adenovirus infection by the age of 10 years. Clinical manifestations of adenovirus disease vary with age and the presence of immune dysfunction. Adenovirus is the virus isolated most commonly in young children with febrile respiratory illness. The usual length of illness is 5–7 days, but symptoms may persist for up to 14 days. Many of these infections are difficult to distinguish clinically from other respiratory virus infections and some bacterial infections (Fujimoto et al., 2004; Hara et al., 2010).

Rapid diagnosis of adenoviral respiratory infections is required in order to decide optimal treatment strategies. This necessity has led us to search for rapid diagnostic assays for adenoviruses.

In addition to adenovirus, influenza A, influenza B and influenza C virus, RSV, rhinovirus (RV), parainfluenza viruses types 1–4 (PIV),

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and human metapneumovirus (hMPV) have been implicated frequently in acute lower respiratory tract infection episodes (Del Rosal et al., 2011; Reina et al., 2004).

Diagnostic methods used for viral detection include virus isolation, antigen detection and nucleic acid/detection. Virus isolation in cell culture remains a useful approach for viral disease diagnosis, but the delay in obtaining results represents a drawback. Antigen detection by immunochromatography (IC) tests is an option for laboratories not equipped for molecular testing, but their sensitivity is low (Romero-Gómez et al., 2008). Tests based on nucleic acid amplification are rapid and sensitive, and appear to be the most suitable approach, but their high cost has impeded their widespread adoption as the first-choice diagnostic technique.

The adenovirus antigen immunochromatography test Adeno Respiratory Card Letitest (Leti diagnostics, Barcelona, Spain), versus the shell-vial culture and multiplex PCR (Clart Pneumovir Version 3.0, Genomica, Madrid, Spain), in nasopharyngeal washes and oropharyngeal swabs specimens from subjects with respiratory tract infections. Test takes 10–15 min to perform and relies on a monoclonal antibody that binds to a group-reactive antigen common to 52 known human adenovirus serotypes. The sensitivity, specificity, and convenience of the test were assessed.

2. Materials and methods

Over a nineteen months period, nasopharyngeal washes and oropharyngeal swabs specimens were obtained from all patients with acute lower respiratory tract infection episodes admitted to the Paediatric Emergency Department of La Paz Hospital, a tertiary care hospital in Madrid, Spain. The results of the IC Adeno Respiratory Card Letitest were compared with those obtained by multiplex RT-PCR and viral culture. The applicability of the assay for routine adenovirus testing is discussed.

2.1. Patients and microbiological specimens

Two hundred and twenty four nasopharyngeal washes and oropharyngeal swabs specimens were collected from children of less than 15 years old with respiratory tract infections, from April 2011 to November 2012, who were attended in the pediatric emergency department.

Briefly, one tube of an aspiration trap was connected to a vacuum source and the other to a suction catheter of appropriate size. Then, 2–3 ml of sterile physiological saline was instilled into one nostril, and the nasopharyngeal washes was collected. The same procedure was done in the other nostril, with the same aspiration trap. For the oropharyngeal swabs specimens, a swab from the posterior pharynx was placed in viral transport medium (Copan, Madrid, Spain) and both nasopharyngeal washes and oropharyngeal swabs specimens were rapidly transported to the laboratory and kept at 4 °C for a maximum of 24 h. Then, IC Adeno Respiratory Card Letitest and culture by shell vial tested an aliquot of each nasopharyngeal washes. The remaining nasopharyngeal washes portion and the oropharyngeal swabs specimens were frozen at −80 °C until they were tested by molecular methods.

2.2. Virus isolation

For the shell-vials culture, two vials of HEp-2 and A-549 cells lines were inoculated with 200 μl of nasopharyngeal aspirate (Vircell, Granada, Spain). The vials were centrifuged at 700 × *g* at room temperature followed by incubation at 37 °C for 1 h for adsorption. The inoculum was discarded and 1 ml of maintenance medium (minimal essential medium without fetal bovine serum) was added. The vial was incubated for 48 h at 37 °C in a CO_2 incubator. The coverslip was removed, fixed in cold acetone for 30 min and stained by an indirect immunofluorescence assay (IFA) using a monoclonal antibody to Adenoviruses (Vircell. Granada, Spain) and viewed at 200× and 400× magnification with a fluorescence microscope.

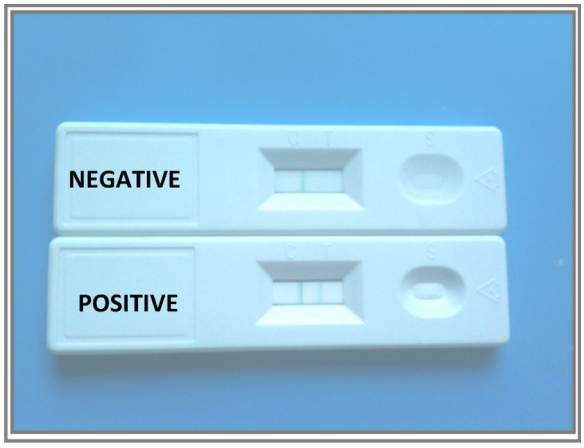

Fig. 1. The results are interpreted as follows: two colored lines on the test stick are positive for adenovirus antigen; one colored line is negative for adenovirus antigen. (For interpretation of the references to color in text, the reader is referred to the web version of the article.)

2.3. Immunochromatography test

Adenovirus antigen IC test was performed according to the manufacturer's instructions. 250 μL of nasopharyngeal aspirates specimens were mixed with 250 μL or 8 droplets of the buffer to achieve a sample dilution ratio of 1/2. Stir thoroughly to homogenize the solution. Immerse the sensitized strip in the direction indicated by the arrow during 15 min. The test is based on the use of a homogeneous immunochromatographic system. The results are interpreted as follows: two colored lines on the test stick are positive for adenovirus antigen; one colored line is negative for adenovirus antigen (Fig. 1).

2.4. Molecular testing

Viral nucleic acids were extracted from clinical samples using the NucliSens easyMAG system (bioMérieux, Marcy l'Etoile, France), according to the manufacturer's protocol. Finally, the nucleic acids were eluted in 70 μl of the elution buffer included in the kit and stored at −80 °C until analysis by multiplex PCR analysis.

The PCR method used, was capable of detecting and characterizing the presence of the 19 most frequent types of human viruses causing respiratory infections: Influenza A (H1N1, H2N3 and H1N1-2009), influenza B, influenza C, parainfluenza 1, parainfluenza 2, parainfluenza 3, parainfluenza 4a, parainfluenza 4b, RSV-A, RSV-B, rhinovirus, adenovirus, enterovirus, bocavirus, coronavirus, metapneumovirus A and metapneumovirus B.

2.5. Statistics

Comparisons between assays were done using the McNemar nonparametric test for paired proportions. A specimen was considered to be a true positive when it was positive by culture or by molecular tests. Agreement between assays was measured using the kappa statistic. We used the following nomenclature to describe the relative strength of agreement associated with kappa statistics: 0 = poor; 0–0.2 = slight; 0.21–0.4 = fair; 0.41–0.6 = moderate; 0.61–0.8 = substantial; and 0.81–1 = almost perfect (Landis and Koch, 1977). All reported *p*-values are two-sided, and $p < 0.05$ was considered to be significant.

Table 1
Comparison between IARL and viral culture.

IC Adeno Respiratory Letitest	Viral culture		Total
	Positive	Negative	
Positive	40	68	108
Negative	10	106	116
Total	50	174	224

Table 2
Comparison between IARL and RT-PCR.

IC Adeno Respiratory Letitest	RT-PCR		Total
	Positive	Negative	
Positive	74	34	108
Negative	21	95	116
Total	95	129	224

3. Results

A total of 224 patients were studied. The average age of the patients was 32.4 months with a range of 3.4 months to 9.4 years.

The majority of patients presented fever 87.0% and respiratory symptoms 64.8%, but only 10.5% of children requiring hospitalization. A chest radiograph was performed for 44.8% of the patients. Abnormalities were diagnosed in 25.5% of patients, including perihilar infiltrates in the 10.6% and a focal infiltrate consistent with pneumonia in 19.2%. Other symptoms reported were vomiting (28.6%), and diarrhea (12.4%). However, clinical signs, such as conjunctivitis and rash, commonly associated with adenovirus infection were relatively infrequent in our patient population. Conjunctivitis was present in 23.8% of patients, and rash was noted in the 9.5% of patients (Rocholl et al., 2004).

All specimens were assayed by culture, multiplex PCR and IC Adeno Respiratory Card Letitest. A specimen was considered to be a true positive when it was positive by culture or by molecular tests. Comparing the results between the viral culture and IC Adeno Respiratory Card Letitest, 146 patients (106 negative and 40 positive) had the same results, 68 patients were shown to be positive on IC only and ten patients were shown to be positive on viral culture only (Table 1). McNemar χ^2-test showed $p < 0.0001$, suggesting that these two methods had extremely statistically significant difference on the test results. In addition, consistency test showed $k = 0.290$ ($p < 0.001$), suggesting that IC Adeno Respiratory Letitest had strength of agreement to be "fair" with the virus culture in detecting Adenovirus.

Comparing the results between the PCR and that IC Adeno Respiratory Card Letitest, 169 patients (95 negative and 74 positive) had the same results, 34 patients were shown to be positive on that IC Adeno Respiratory Letitest only and 21patients were shown to be positive on PCR only (Table 2). McNemar χ^2-test showed $p = 0.1056$, suggesting that two methods has no statistically significant difference on the test results. Consistency test showed $k = 0.506$ ($p < 0.001$), suggesting that IC Adeno Respiratory Card Letitest has moderate consistency with PCR in identifying adenovirus disease.

Using PCR as the reference standard, the overall sensitivity was 77.9% and the specificity was 73.6%. The negative predictive values and the positive predictive values were for PCR 81.9% and 68.5%, respectively. Taking viral culture as the reference, the IC Adeno Respiratory Card Letitest sensitivity and specificity values were 80.0% and 60.9% and the negative predictive values and positive predictive values were 91.4% and 37.1%, respectively (Table 3)

Clart Pneumovir identified 95 patients with adenovirus, 31 with bocavirus, 19 with influenza virus A (17 H3N2 and 2 H1N1), 19 with enterovirus, 20 with rhinovirus, 16 with respiratory syncytial virus (7 RSV-A and 9 RSV-B), 6 with parainfluenza virus type 3, 3 with human metapneumovirus, 3 with parainfluenza virus type 1, and 1 with parainfluenza virus type 2.

The presence of viral coinfection was 44.2% (42/95): three cases of co-infection with adenovirus/rhinovirus, four with adenovirus/bocavirus, three with adenovirus/influenza A (H3N2), four with adenovirus/respiratory syncytial virus, seven with adenovirus/enterovirus, two with adenovirus/parainfluenza virus type 1, two with adenovirus/parainfluenza virus type 2, three with Adenovirus/parainfluenza virus type 3 and three with adenovirus/metapneumovirus.

4. Discussion

Viral infections, which represent more than 90% of acute infectious diseases in children, are considered the most important cause of lower respiratory tract illness commonly observed during wintertime (Van Woensel et al., 2003). Despite its association with severe respiratory infections, the use of specific diagnostic laboratory tests for viral agents in the case of acute respiratory infections is limited due to financial and technical problems, or due to the fact that viruses are underestimated as agents of respiratory infections requiring hospitalization.

The current available methods for the diagnosis of adenovirus infection include: viral culture, nucleic acid detection, serological tests and antigen detection (Hara et al., 2010; Levent et al., 2009).

Lateral flow immunochromatographic assays have been developed for use in the rapid laboratory diagnosis of viruses such as influenza A and B, RSV, rotavirus, adenovirus, and bacteria such as group A streptococcal infections. They have recognizable utility in the emergency department and outpatient settings because of its easy use, the rapid turnaround time and the availability of a result within 30 min. A rapid positive result enables a quick diagnosis, targeted treatment options, reduction or elimination of inappropriate antibiotic therapies, opportunity for cohorting seriously ill patients for infection control purposes, and the potential for reduced laboratory test usage and hospitalization costs. Factors involved in the selection of a rapid diagnostic assay include statistical performance characteristics of the assay such as sensitivity, specificity, simple use and interpretation, background clarity, and cost per test result. The statistical performance characteristics of a rapid assay are influenced by the patient population being tested, the prevalence of disease in the community, and the type of specimen submitted for analysis.

Differentiation of bacterial from viral infections is a common clinical problem. Children infected with adenovirus are often

Table 3
Results of IARL, referred to viral culture and RT-PCR.

Test	IC Adeno Respiratory performance (%) against:			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Culture ($n = 224$)	80.0 (65.9–89.5)	60.9 (53.2–68.1)	37.0 (28.1–46.9)	91.4 (84.3–95.6)
RT-PCR ($n = 224$)	77.89 (68.0–85.5)	73.64 (65.0–80.8)	68.52 (58.8–76.9)	81.9 (73.4–88.2)

PPV: positive predictive value; NPV: negative predictive value; n: number of samples tested; values in parentheses are 95 confidence intervals.

characterizing by high-grade, prolonged fever and high levels of acute-phase reactants. These findings are also consistent with those for bacterial infections (Putto et al., 1986). Consequently, a simple, sensitive, and rapid diagnostic test for adenovirus infections would allow the pediatrician to counsel child's parents about the prognosis and give specific advice to limit further transmission of the virus. Furthermore, it would eliminate unnecessary antibiotic use to treat possible streptococcal or other bacterial infections (Hara et al., 2010; Levent et al., 2009).

The IC Adeno Respiratory Card Letitest assay is easy to perform and read does not require additional instrumentation and can be completed within 20 min.

The IC Adeno Respiratory Card Letitest was moderately specific 73.6% (identifying 95 of 129 specimens) and moderately sensitive 77.9% (identifying 74 of 95 specimens) compared to multiplex PCR. Taking viral culture as reference, the IC Adeno Respiratory Card Letitest was low specific 60.9% and moderately sensitive 80.0%. For evaluating the agreement between the IC Adeno Respiratory Card Letitest and two confirmative methods, percent agreement and kappa statistic were calculated and showed moderate agreements (percept agreement, 75.5% for RT-PCR and 65.2% for viral culture; kappa, 0.506 for RT-PCR and 0.290 for viral culture).

The moderately specificity of this test method indicates that it could be relied on in making clinical decisions during periods of peak Adenovirus activity (identifying 95 of 129 specimens) for IC Adeno Respiratory Card Letitest compared with the results of RT-PCR. The results show lower sensitivity than the reported earlier by Tsuguto Fujimoto et al. (9.1%) (Enomoto et al., 2010) but higher than reported by Tsutsumi et al. (72.6%) (Tsutsumi et al., 1999). Studies comparing RT-PCR to culture have also shown a distinct advantage for sensitivity by RT-PCR (Fujimoto et al., 2004).

The IC Adeno Respiratory Card Letitest has a 22.1% false-negative rate compared to multiplex PCR, careful interpretation is required in cases where IC Adeno Respiratory Card Letitest negative results are obtained and adenovirus infection is suspected. One cause of false-negative results by IC Adeno Respiratory Card Letitest may be attributable to the small amount of viral particles in the sample. In thirty-four samples, we detected adenovirus by IC Adeno Respiratory Card Letitest, which were subsequently multiplex PCR negative (false positives). These discrepancies may be explained at least in part by cross-reaction with other viruses (eleven cases the sample was positive for bocavirus, five influenza B-virus, four influenza A-virus, four enterovirus, three RSV-A, two rhinovirus, two metapneumovirus, one influenza C-virus and one parainfluenzavirus type 3). Thus, the IC Adeno Respiratory Card Letitest has moderately sensitivity and specificity compared with those of multiplex PCR and viral culture for detecting the Adenovirus. Samples with negative rapid assay results should have additional testing for adenovirus performed by either viral culture or PCR. The IC Adeno Respiratory Card Letitest was also easy to perform, and provided results in 15 min, a time frame that is useful to decide optimal treatment strategies, when the test is positive.

In conclusion, this study provides preliminary data on issues that need to be addressed when considering routine clinical application of such Adeno Respiratory Card Letitest diagnostic assays in the pediatric emergency department.

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Competing interests

None declared.

Ethical approval

Not required.

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