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ADENOVIRUS INFECTION: DIAGNOSIS BASED ON RAPID TESTS

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ABSTRACT - Human adenoviruses (HAdVs) are significant agents that cause serious infections in children and immunocompromised patients. Clinical manifestations such as gastroenteritis, cystitis, hepatitis, keratoconjunctivitis, meningoencephalitis and myocarditis were also related to these viruses. The epidemiological studies of adenovirus infections present limitations due to the relatively high incidence of doubtful and inconclusive results obtained from the currently available tests. Direct immunofluorescence (IFD) and Enzyme Linked ImmunonoSorbent Assay (ELISA) assays are techniques that are not particularly sensitive for the diagnosis of adenovirus infections, compared to cell culture methods and molecular diagnosis. Virus isolation in cell cultures is a sensitive method for adenovirus detection, but this method is costly and time consuming, taking several days to perform the isolation. As some adenovirus serotypes are difficult to culture, to maximize sensitivity, the virus should culture on at least two or three different cell lines. The turn-out of culture results is immediately available for the clinician and is impractical for studies with a large number of clinical samples. Molecular diagnosis is a relatively quick and sensitive tool, enabling direct laboratory diagnosis in the clinical sample. Depending on the DNA region amplified by PCR, it is possible to distinguish between species and serotypes. However, this technique is still expensive and requires a specialized team of technicians to perform it. Therefore, it remains relatively inaccessible. The structuration of the rapid test with better performance is that could detect adenovirus antigens in the sample with greater sensitivity allowing for different opportunities for interaction of the polyclonal antibody with the various epitopes of the hexon.
KEYWORDS: Adenovirus. Rapid test. Diagnosis.

RESUMO - Os adenovírus humanos (HAdVs) são agentes significativos que causam infecções graves em crianças e pacientes imunocomprometidos. Manifestações clínicas como gastroenterite, cistite, hepatite, ceratoconjuntivite, meningoencefalite e miocardite também foram relacionadas a esses vírus. Os estudos epidemiológicos de infecções por adenovírus apresentam limitações devido à incidência relativamente alta de resultados duvidosos e inconclusivos obtidos a partir dos testes atualmente disponíveis. Ensaios de imunofluorescência direta (IFD) e ensaio de imunoadsorção enzimática (ELISA) são técnicas que não são particularmente sensíveis para o diagnóstico de infecções por adenovírus, em comparação com métodos de cultura celular e diagnóstico molecular. O isolamento de vírus em culturas de células é um método sensível para detecção de adenovírus, mas esse método é caro e demorado, levando vários dias para realizar o isolamento. Como alguns sorotipos de adenovírus são difíceis de cultivar, para maximizar a sensibilidade, o vírus deve ser cultivado em pelo menos duas ou três linhas celulares diferentes. A saída dos resultados da cultura fica imediatamente disponível para o clínico e é impraticável para estudos com um grande número de amostras clínicas. O diagnóstico molecular é uma ferramenta relativamente rápida e sensível, permitindo o diagnóstico laboratorial direto na amostra clínica. Dependendo da região do DNA amplificada por PCR, é possível distinguir entre espécies e sorotipos. No entanto, essa técnica ainda é cara e requer uma equipe especializada de técnicos para realizá-la. Portanto, permanece relativamente inacessível. O melhor teste imunocromatográfico a ser feito é aquele que pode

detectar adenovirus na amostra com alta sensibilidade proporcionado pelos diferentes tipos de interação com antígeno hexon.
PALAVRAS-CHAVES: Adenovírus. Teste rápido. Diagnóstico.

1INTRODUCTION

The incidence of adenovirus (HAdV) infections varies widely, between 8 to 50% in children submitted to hematopoietic stem-cell transplants (depending on the diagnostic methods). Mortality varies from 3.2 to 6.0% (AUGUST et al.,1987; PAULINI et al.,2017).

Data regarding incidence and mortality from HAdV infections in adults indicates a lower incidence (2-5%) and mortality (0-1%) (LION, 2014). In comparison with the herpes virus and HIV, the persistence of adenoviruses does not seem to be prolonged, being limited to months or at the most some years. Clinical observations have shown that almost 80% of pediatric patients maintain HAdV-C DNA in their nasopharynx tissue and that the average number of HAdV genomes in the amygdala tissue diminishes over time. Adenovirus DNA can persist in a latent state, without significant genic expression or relevant production of infectious viral particles however being able to replicate and cause occasional infections patients (LION, 2014; GAZENMUELLER; HEIM, 2012).

In this work, we reviewed adenovirus infection and your level damage. Furthemore, we showed rapid tests for application. Several types of imunochromatographic tests were studied. Rapid tests with good sensibility and specificity are complex for production.

2 DEVELOPMENT

2.1 HUMAN ADENOVIRUS IN RESPIRATORY INFECTIONS

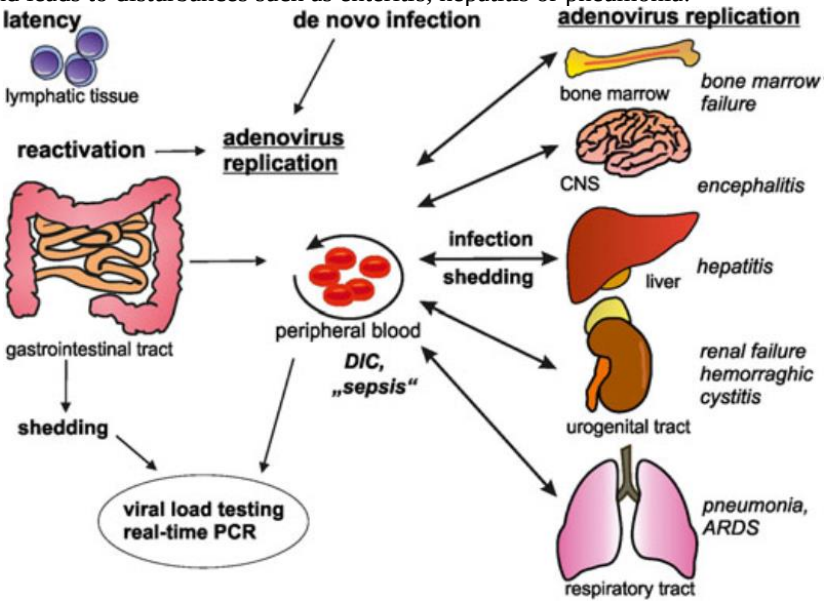
Acute respiratory infections (ARI) are considered one of the most significant causes of morbidity and mortality around the world, being responsible for the death of four million people a year (WORLD HEALTH STATISTICS, 2008). The viruses are the main etiological agents that cause ARI, responsible for 45 to 60% of all respiratory diseases in children. Until the end of April 2015, of the 8,294 samples tested and reported by the World Health Organization of the United States (WHO/NREVSS), 542 (6.5%) were positive for influenza (TEMPORADA ..., 2018). In the state of São Paulo, among the epidemiological weeks (EW), 5,278 cases of hospitalized with Severe Acute Respiratory Syndrome (SARS), with 729 (13.8%) evolving to death. Of the SARS cases, 648 (12.3%) were confirmed for the influenza virus, with 124 (17.0%) deaths (YAMAMOTO et al., 2014).

The pathogenesis of the disease disseminated by HAdV is associated with the replication of the virus in various affected organs (for example, intestine, liver, pancreas, adrenal glands, lungs, bone marrow and kidneys) (Figure 1). This has been demonstrated through the quantification of HAdV DNA in autopsy samples of organs from patients with disseminated infection. A lymphocyte count below 300 cells/uL was associated with higher mortality. C and B HAdV species cause acute, disseminated disease in immunocompromised patients (LION, 2014; GAZENMUELLER et al., 2012).

Human adenoviruses represent 5 to 15% of the virus isolates from children younger than 2 with ARI (YAMAMOTO et al., 2014). Children with respiratory disease caused by an adenovirus present a wide variety of clinical forms including pharyngitis, inflammation of the tonsils, pharyngo-conjunctival fever, bronchiolitis and pneumonia. Pneumonia in children has frequently

been associated with serotypes 3 and 7 (species B) and occasionally, the disease is fatal (WATANABE et al., 2013). Respiratory infections caused by adenoviruses also affect adolescents and adults. HAdV infection epidemics have been reported since the 1950s in military recruits in North America (LION, 2014). These epidemics began to be controlled with the introduction of vaccines for serotypes 4 and 7 in the 1970s. However, in 1996, vaccination was discontinued and new ARI outbreaks in recruits were observed (LION, 2014). To monitor the effects of the interruption of vaccination, agents monitored ARI cases in military quarantine camps. During the winters of 1997 and 1998, adenovirus infections were responsible for more than 90% of the ARIs, with the serotypes 3, 4, 7 and 21 being isolated. Recently, Yamamoto and collaborators (2014) showed high levels of mortality from adenovirus 7h in hospitalized children in India. In Brazil, the main adenovirus serotypes related to ARI cases are those of the species C (1, 2, 5 and less frequently 6) and of species B, (serotypes 3 and 7) (WATANABE et al., 2013) . The studies by our research group of the Federal University of Sao Paulo demonstrated that the capacity for detection of the adenovirus by immunofluorescence is limited (PUERARI et al., 2015). Additionally, the adenovirus was detected in 13.2% of the 643 samples collected between 2001 and 2010 (WATANABE et al., 2013). Of these, nearly 30% were transplant patients; a high rate of prevalence for this virus was observed in immunocompromised patients (FIGURE 1) (WATANABE et al., 2013; PUERARI et al., 2015).

Figure 1: Pathogenesis of the infection disseminated in immunocompromised patients. The source of human adenovirus (HAdV) can be a new infection or latent reactivation of the lymphatic tissue, for example, of the intestine or of the upper respiratory tract. After local respiration, HAdV flows through the peripheral blood to other organs, such as the liver, bone marrow, central nervous system, urogenital organs or the respiratory tract. Replication of HAdV affects the tissues and leads to disturbances such as enteritis, hepatitis or pneumonia.



Source: LION (2014).

2. 2 RAPID TESTS FOR ADENOVIRUS DETECTION

Rocholl and collaborators (2004) reported adenovirus infections in 7.5% of the samples of children by direct immunoflorescence. The average age of patients in this study was 23 months (ROCHOLL et al., 2004). This is consistent with previous studies that reported relatively few cases in older children (7-8%). In a recent study conducted by Carraro and collaborators, it was found that the investigation of the adenovirus as an etiological agent for acute respiratory infection in 412 adults did not find any case through investigation by direct immunoflorescence (WATANABE, et al., 2013, PUERARI et al., 2015). Detection was only possible through the double amplification of the viral genome (nested-PCR) technique. This result demonstrates the need for greater sensitivity for detection of the presence of the virus in the samples from adult patients. Some authors have already suggested that methods for antigenic detection of adenovirus in respiratory tract samples can present sensitivity like the cell cultures, but much less than PCR (WATANABE, et al., 2013, PUERARI et al., 2015). The fact that IFD provides inconclusive results is still a question to be resolved. A plausible explanation may be the lithic capacity of adenoviruses to cause significant devastation in the mucous cells, hampering the specific detection of the hexon (intracellular) by monoclonal antibodies. In other words, the type of cytopathic effect caused by the adenovirus limits its detection. On the other hand, the proof that monoclonal antibodies can react against cytoplasmic epitopes of human cells could explain the elevated level of background noise that emerges when evaluating the samples. Another line of thought, in the case of immunochromatographic testing, is that the combination of a monoclonal antibody in the test line of the nitrocellulose membrane together with the polyclonal antibody as detection and/or vice versa, can provide a promising format for greater sensitivity and specificity for diagnosis.

Though the advances in research achieved over recent decades have resulted in a variety of lab-techniques that make an important contribution to diagnosing diseases generally and infectious diseases in particular, the diagnosis of infections caused by adenoviruses has advanced little. This fact is due to two principal reasons. Until recently, due to dealing with a relatively neglected disease, little commercial return could be expected on investment in research into adenoviruses in addition to the biological complexity of the infection itself. On the other hand, the precise diagnosis of a case of diarrhea, acute respiratory syndrome or highly contagious conjunctivitis as with adenoviruses is important when seeking to avoid the propagation of the disease. The monitoring of this virus is of fundamental importance in institutions such as kindergarten, hospitals, clinics and schools. Additionally, in the case of immunocompromised patients, adenovirus infections are a serious concern. The best diagnosis method for any infectious disease is one with high sensitivity and specificity, quick for realization and presenting a result that does not require personnel with specialized training and that is cheap. Viral isolation via cell cultures requires the presence of viable disease and a specific culture medium for rapid transport to the laboratory. Despite taking longer to get the result in comparison with other methods and the cost for its realization, the cell culture remains the gold standard for diagnosis since the isolation of the infectious agent is definitive. This maybe the source of the second reason for the relative neglect of the diagnosis of the disease, which is its need for highly specialized personnel when employing the currently available techniques. However, the need for a ready response makes point-of-care tests (POCT) a good option for application in clinics and hospital emergency rooms. The rapid tests (also known as dipsticks) involve fast technology, sensitive in relation to the traditional antigenic-antibody tests in solid phase (development at room temperature) and easy to use by the health care agent (ROCHA et al., 2012). Monoclonal antibodies against hexon have been produced and evaluated in various countries with variable sensitivity and specificity. Udeh, Scheneider and Ohsfeldt (2008) found

that approximately 400 million dollars/year of resources destined to health care could be economized with a good diagnosis for conjunctivitis adenovirus in the United States. Additionally, 1.1 million cases of inappropriate antibiotics use could be avoided. In the case of immunocompromised patients, the direct rapid test (research of viral proteins) has an advantage over indirect ELISA given that patients with this condition do not present sufficiently elevated antibody levels for detection (LION, 2014). Levent and collaborators (2009) evaluated an immunochromatographic test for adenovirus (SAS adeno test) and observed 55% sensitivity and 98.9% specificity for the samples of children with flu syndrome. Romero-Gomez and collaborators (2014) analyzed the Adeno respiratory card letitest (Leti diagnositics Barcelona, Spain) in 224 samples of children younger than 15 treated at the emergency ward. When compared with PCR, the rapid test showed 78% sensitivity and 73% specificity. Additionally, researchers speculated that the low specificity of the test indicated that it could be used to make clinical decisions during the period of greater replicative activity of the adenovirus. The crossed reaction of the test in comparison with the other respiratory viruses was considerable.

Sambursky and collaborators (2013) tested 128 samples of conjunctival scrapings to evaluate the sensitivity and specificity of the Adenoplus test in comparison with cellular culture, direct immunoflorescence and PCR. In comparison with the cellular culture and IFD, the Adenoplus test presented 90% sensitivity and 96% specificity. In comparison with only PCR, the Adenoplusr showed 85% sensitivity and 98% specificity. Additionally, it also showed that the detection limit was around 6 ng/mL. The level of coloration of the line is proportional to the quantity of antigen present (SAMBURSKI, et al., 2013). The lower detection limit corresponds to the greater intensity of the positive result. Hara and collaborators (2010) conducted a prospective study between August 2005 and July 2008 in a pediatric clinic and analyzed the Caillia Adeno Test (Tauns, Numazu, Japan). In total, 587 throat swab samples from children were tested. Sensitivity and specificity were 89.2% and 98%, respectively (HARA et al., 2010). The sensitivity of the test in samples from patients with exudative tonsillitis and pharyngoconjunctival fever was greater than in patients with pharyngitis or fever. The high sensitivity seems to reflect high levels of viral load in the throat swab of patients with two symptoms. Patients with pharyngoconjunctival fever presented greater disease severity, possibly presenting concomitantly conjunctivitis, pharyngitis and exudative tonsillitis.

In Brazil, Barbosa and collaborators (2007) found the use of RPS Adenodetector (Rapid Pathogen Screening, Inc, 101 Phillips Park Drive, South Williamsport, PA, 17702) as a diagnostic method for patients with a clinical condition of adenoviral conjunctivitis. 11 patients were evaluated and the RPS adenodetector showed 100% sensitivity and 67% specificity adopting the viral culture as the gold-standard exam for diagnosis.

Uchio and collaborators (1997) evaluated the SAS Adeno test, however, for conjunctivitis samples from patients with acute conjunctivitis, keratoconjunctivitis or pharyngoconjunctival fever. 130 conjunctival swab samples were analyzed (95 samples positive from the PCRT technique and 35 negative from PCR), respectively. Additionally, the researchers found that the rapid test can detect adenovirus antigen up to 13 days after the start of symptoms. They also affirmed that the test is useful for diagnosis of adenovirus of all serotypes, that is, the comparison of hexon protein of the A, B, C, D and F species showed discrete hypervariable regions between the 250 residues. Around 2 to 38 residues presented variability, showing that more than 99% of the serotype-specific residues are common to all serotypes (UCHIO et al., 1997). Kaneko and collaborators (2008) evaluated the possible presence of adenovirus in hospitalized patients (post-ocular surgery) asymptomatic for conjunctivitis. This sample occurred after the case of a patient hospitalized with acute, serious conjunctivitis (KANEKO et al., 2008). A sample coming from the

ophthalmologist proved positive after immunochromatographic testing, isolation in culture, nested-PCR and Real-Time PCR. Additionally, other patients showed an elevated number of copies/mL in the quantitative PCR. An almost imperceptible infection may be responsible for the transmission. The pathway by which the adenovirus is disseminated may be undetermined (KANEKO et al., 2008). Apparently, the fomites spread by the environment may have been the infection pathway. In this sense, immunochromatographic testing could be used to determine the presence of the virus in surgical instruments, surfaces and other nursing areas of a hospital. However, it is known that large quantities of antigen are necessary for detection (KANEKO et al., 2008).

In recent years, other viral agents with the capacity to cause disease in the central nervous system were isolated. The adenovirus is implicated in the genesis of infections such as meningoencephalitis, encephalitis and acute flaccid paralysis (FERREIRA et al 2014). In Brazil, Ferreira and collaborators isolated adenovirus in 68% (13) of 19 samples from patients with paralysis (negative for enterovirus and poliovirus). In Finland, during monitoring of patients with paralysis, four HAdV serotypes originating from samples negative for poliovirus and enterovirus were detected (HOVI; STENVIK, 2000). In China, adenovirus was detected in 5.7% of the samples isolated from cases of paralysis (BINGJUN et al., 2008). During the period from 1997 to 2002, cases of paralysis that had occurred in Brazil, Peru and Bolivia were researched, isolating the HAdV-B from fecal specimens of suspected cases of paralysis (AZEVEDO et al., 2009).

Adenovirus infections are described in 3 to 47% of haematopoietic stem cell transplant patients. Clinical syndromes reported include pneumonia, colitis, hepatitis, hemorrhagic cystitis, tubulo-interstitial nephritis and encephalitis. The disease is frequently disseminated and the mortality rate for symptomatic patients is around 26% (DAWOD et al., 2014).

Latent or persistent adenovirus could explain the clinical state in immunosuppressed patients, which could reactivate a latent virus during the period of immunosuppression. However, infections in patients submitted to renal transplant more frequently stem from exogenous viruses apparently originating in the donated organ (LION, 2014). Another explanation could be the capacity for a stable connection of the gene sequence E1A and E1B of the adenovirus to the host chromosome (SIQUEIRA-SILVA et al., 2009). Watanabe and collaborators evaluated samples from haematopoietic stem cell transplant patients and found HAdV in 30% of them. The infection was detected throughout the year studied, with greater occurrence observed using immunoflorescence from March to April 2008, possibly due to an outbreak that occurred in the ward. In the case of renal transplants, HAdV was found in 6% of a total of 643 samples (WATANABE et al., 2013).

Lee and collaborators isolated HAdV-41 from a patient with respiratory disease without diarrhea. Given this, the question of whether a supposed change in pathogenesis of the disease could arise from this condition emerged. In any case, the diverse serotypes that could have tropism by different tissues reaffirms the need for an effective diagnostic detection tool with coverage for all species (A-G) of the adenovirus (FERONE et al., 2014; LEVENT et al., 2009).

Paulini and collaborators (2017) have standardized a test in which the combination of monoclonal antibodies in the test line (capture) (together with sucrose at 3%) with polyclonal antibodies of rabbits conjugated to the gold (detection) provided better sensitivity and specificity. With the antibody (concentration 1.2 mg/mL) together with the sucrose (5%) and trehalose (2.5%), success was achieved for detection of adenoviruses 2, 3, 5 and 41.

The structuration of the rapid test with better performance reported by Paulini and collaborators was with the monoclonal antibody as capture and the polyclonal antibody from rabbit as detection (conjugated with colloidal gold nanoparticles), as already seen in other studies (ROCHA et al., 2012). This led to the hypothesis that the assay could detect adenovirus antigens

in the sample with greater sensitivity allowing for different opportunities for interaction of the polyclonal antibody with the various epitopes of the hexon. Following this, the immune-complex formed could be captured with high specificity by the fixed monoclonal antibody in the solid phase (PAULINI et al.,2017).

3 CONCLUSIONS

This study aimed to describe immunochromatographic tests with positive results. Once standardized in more precisely, rapid tests assays will be available for the accurate and rapid diagnosis of respiratory infections in the emergency room. Their use will provide benefits when there is suspicion of outbreaks, allowing the physician to prescribe immediate and appropriate treatment for the disease.

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REFERENCES

AUGUST, M.J.; WARFORD, A.L. Evaluation of a comercial monoclonal antibody for detection of adenovirus antigen. **J. Clin. Microbiol**, v.25, n.11, p.2233-5, 1987.

AZEVEDO J.P.et al. Characterization of species B adenoviruses isolated from fecal specimens taken from poliomyelitis-suspected cases. **J. Clin Virol.** v.31, n.4, p. 248-252, 2004.

BARBOSA JUNIOR, J.B. et al. Diagnóstico de conjuntivite adenoviral pelo RPS Adenodetector. **Arq Bras Oftalmol.** v.70, n.3, p. 441-444, 2007.

BINGJUN, T. et al. Molecular typing and epidemiology of nonpolio enteroviruses isolated from Yunnan province the people's Republic of China. **J Med Virol**. v.80, n.4, p.670-679, 2008.

DAWOD, U.S.et al. Disseminated adenovirus infection in kidney transplant recipient. **Nephrology**, 19, Suppl, p.110–13, 2014.

FERONE E.A. et al. Clinical and epidemiological aspects related to the detection of adenovírus or respiratory syncytial virus in infants hospitalized for acute lower respiratory tract infection. **J Pediatr**, v.90, p.42-49, 2014.

FERREIRA, J.A., et al. Detecção e caracterização de adenovírus humanoproveniente de casos de paralisia flácida aguda, na Região Norte do Brasil. **Rev Pan-Amaz Saude**, v.5, n.3, p.47-54. 2014.

GANZENMUELLER, T.; HEIM, A. Adenoviral load diagnostics by quantitativepolymerase chain reaction: techniques and application. **Rev. Med. Virol**, v.22, p.194–208, 2012.

HARA, M.et al. Rapid diagnosis of adenovirus respiratory tract infections using the chromatografic Immunoassay test in the pediatric outpatient setting. **The Pediatric Infectious Disease Journal**, v.29, n3, 2010.

HOVI, T.; STENVIK, M. Surveillance of patients with acuteflaccid paralysis in Finland: report of a pilot study. **Bull World Health Organ**. v.78, n.3, p.298-304, 2000.

KANEKO, H.et al. The Possibility of Human Adenovirus Detection From the Conjunctiva in Asymptomatic Cases During Nosocomial Infection. **Cornea**. v. 27, n.5, 2008.

LEVENT, F. et al. Performance of a new immunochromatographic assay for detection of adenoviruses in children. **J. Clin. Virol.** v.44, p.173–175, 2009.

LION, T. Adenovirus infections in immunocompetent and immunocompromised patients. **Clin Microbiol Rev**, v.27, p.441, 2014.

PUERARI, D. et al. Aplicação de teste molecular para detecção de adenovírus em pacientes pediátricos distintos. **Rev. Paul. Ped**., v.33, p.136-141, 2015.

ROCHA, L.B. et al. Interaction between Shiga toxin and monoclonal antibodies: binding characteristics and in vitro neutralizing abilities. **Toxins,** v.4, n. p.729–747, 2012.

ROCHOLL, C. et al. Adenoviral Infections in Children: The Impact of Rapid Diagnosis. **Pediatrics,** v.113, n.1, 2004.

ROMERO-GOMEZ, M.P., et al. Immunochromatographic test fordetection of adenovírus from respiratory samples: is it a real solution for pediatric emergency department? **J. Virol. Methods**. v.195, p.236-239, 2014.

SAMBURSKY, R.et al. Sensitivity and specificity of the adenoplus test for diagnosing adenoviral conjunctivitis. **JAMA Ophthalmol**., v.131, n.1, p.17-22, 2013.

SIQUEIRA-SILVA J, HARSI CM. Infection kinetics of human adenovirus serotype 41 in HEK 293 cells. **Mem. Inst. Oswaldo Cruz**, Rio de Janeiro, v.104, p.736-744, 2009.

PAULINI, I.J. et al. Development of a prototype immunochromatographic test for rapid diagnosis of respiratory adenovirus infection. **Braz J Infect Dis.** v.21, n.5, p. 500-506, 2017. https://doi.org/10.1016/j.bjid.2017.03.023

TEMPORADA de influenza 2018-2019. **Informe semanal de vigilancia de la influenza en los EE. UU**. Disponível em: https://espanol.cdc.gov/enes/flu/weekly/index.htm>. Acesso.

UCHIO, M.D. et al. Rapid diagnosis of adenoviral conjunctivitison conjunctival swabs by 10-minute immunochromatography. **Ophthalmology,** v.104, p.1294–1299, 1997.

UDEH, B.L.; SCHENEIDER, J.E.; OHSFELDT, R.L. Cost effectiveness of a point-of-care test for adenoviral conjunctivitis. **Am. J. Med. Sci.**, v. 336, p.254-264, 2008.

WATANABE, A. et al. Human adenovirus detection among immunocompetent and immunocompromised patients presenting acute respiratory infection. **Rev. Soc. Brasileira Med. Tropical**. v.46, p.161-165, 2013.

WORLD HEALTH STATISTICS. WHO Library Cataloguing-in-Publication Data. Disponível em: < https://www.who.int/gho/publications/world_health_statistics/EN_WHS08_Full.pdf?ua=1> Acesso em: 10 fev. 2018.

YAMAMOTO, D. et al. Impact of Human Adenovirus Serotype 7 in Hospitalized Children with Severe Fatal Pneumonia in the Philippines. **Japanese Journal of Infectious Diseases**. v. 67, n.2, p. 105-110, 2014.

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