Microbiologic Methods in the Diagnostics of Upper Respiratory Tract Pathogens

J. Kompanikova, A. Zumdick, M. Neuschlova, V. Sadlonova, and E. Novakova

Abstract

Upper respiratory tract infection (URI) is a nonspecific term used to describe acute infections involving the nose, paranasal sinuses, pharynx, and larynx above the vocal cords. The aim of this study was to provide a summary of the most common pathogens of URI and to compare advantages and disadvantages of traditional and new rapid microbiological tests used to identify them. Blood samples were simultaneously examined by the enzyme-linked immunosorbent assay (ELISA) and by the FilmArray Respiratory Panel for eight different pathogens in a total of 15 tests performed in nasopharyngeal swabs. The ELISA method is unable to identify the pathologic agent until the host's immune system elicits a response. The method is readily available in many laboratories at a low cost, which puts less strain on economic resources. The FilmArray® Panel, on the other hand, is more expensive, but it is fast and exact in the identification of a broad spectrum etiologic agents. Nonetheless, since most repiratory tract infections are viral in origin and there is no treatment available, the diagnosis provided by the FilmArray Panel does not provide any additional clinical benefit and thus should be used only whenever necessary on the individual basis.

J. Kompanikova, A. Zumdick, M. Neuschlova,
V. Sadlonova, and E. Novakova (✉)
Department of Microbiology and Immunology, Jessenius
Faculty of Medicine in Martin, Comenius University in
Bratislava, Mala Hora 4B, 036 01 Martin, Slovakia
e-mail: novakova@jfmed.uniba.sk

1   Introduction

Upper respiratory tract infections (URIs) involve the moist surface of the eyes and eyelids, the nasolacrimal ducts, the middle ear, paranasal sinuses, mastoid air cells, and the main

respiratory passage of the nose and throat as far as the epiglottis and vocal cords. Acute URIs include the common cold, pharyngitis, epiglottitis, and laryngotracheitis (Nester et al. 1995). A variety of viruses, bacteria, fungi, and parasites can infect the respiratory tract. Transmission of organisms occurs by aerosol droplet or direct hand-to-hand contact with infected secretions, with subsequent passage to the nares or eyes. Most URIs are of viral etiology (Dasaraju and Liu 1996). Epiglottitis and laryngotracheitis are exceptions with severe cases likely caused by *Haemophilus influenzae* type B. Bacterial pharyngitis is often caused by *Streptococcus pyogenes*. Bacterial and viral upper respiratory infections produce highly variable clinical symptoms that cannot be used to identify the etiologic agent. Proper treatment depends on the correct identification of a pathogen involved as antibiotics provide little or no benefit with viral infections (Nester et al. 1995).

aureus are involved with the upper respiratory system (Murray et al. 2005). The most common URI of bacterial origin is pharyngitis caused by Group A beta-hemolytic streptococci, with *Streptococcous pyogenes* as a main representative, which accounts for 5–10% of pharyngitides (Poole and Portugal 2005). *Streptococcus pneumoniae* and *Haemophilus influenzae* are the most important bacterial pathogens in otitis media and bacterial conjunctivitis. Less commonly, *Mycoplasma pneumoniae, Streptococcous pyogenes,* and *Staphylococcus aureus* are the causative agents in otitis media. A study carried out in the Czech Republic in 2004/05 in 16 different cities among healthy children aged 3–6 years show that the overall carriage of pathogens was 62.8%, with *Streptococcus pneumoniae* 38.1%, *Haemophilus influenzae* 24.9%, *Moraxella catarrhalis* 22.1%, and *Staphylococcus aureus* 16% being the most prevalent (Zemlickova et al. 2006).

1.1 Etiology of Upper Respiratory Tract Infections

The URIs are in 69% of viral origin (Mäkelä et al. 1998). Orthomyxoviruses (influenza A and B), paramyxoviruses (parainfluenza and respiratory syncytial viruses), coronaviruses, adenoviruses, and enteroviruses (coxsackie and ECHO viruses) cause common cold. However, most colds are caused by more than 89 types of rhinoviruses (Musher 2003; Cooper et al. 2001). More than 40 strains of adenoviruses cause pharyngitis resembling a strep throat. Rhinoviruses are unresponsive to antibiotics and other medications that control bacterial infections. Antibiotic treatment of adenovirus infections is of no value and sometimes can even be harmful, because it supresses normal bacterial flora and enables the resistant opportunistic pathogens to grow in an uncontrolled way (Nester et al. 1995). Different bacteria, including *Chlamydia pneumoniae, Mycoplasma pneumoniae, Sreptococcus pneumoniae, Bordetella pertussis, Haemophilus influenzae,* and *Staphylococcus*

1.2 Diagnostic Methods of Upper Respiratory Tract Infections

The diagnosis of URIs is based on a review of symptoms, physical examination, and laboratory tests. Direct identification of bacterial pathogens is based on routine laboratory tests, including growing bacteria in cultures, detection of bacterial metabolic activity, single enzyme tests (catalase, oxidase, urease, or coagulase tests), and molecular methods (Balentine and Siamak 2015; Harvey et al. 2007). Complement fixation test, direct agglutination technique, latex agglutination and enzyme linked immunosorbent assay (ELISA) are traditional serological methods used to detect antibodies in the patient's serum. Traditional diagnostic methods for viral pathogens include growth of the virus in a cell culture, observation of virus particles by electron microscopy, and detection of viral nucleic acid or virus-specific antibodies in the blood (Meneghetti 2016).

1.3 FilmArray Respiratory Panel

The FilmArray Panel is a multiplexed nucleic acid test intended for the simultaneous qualitative detection and identification of multiple respiratory pathogen nucleic acids in nasopharyngeal swabs. This new platform combines automated sample preparation, nucleic acid extraction, and polymerase chain reaction (PCR)-based detection from a single unprocessed sample in 1 h (BioFire Diagnostics; Salt Lake City, UT). This method allows for identification of 21 different respiratory pathogens from a nasopharyngeal swab, 18 of viral etiology and three of bacterial origin (Idaho Technology 2007).

In the present study we seek to determine the individual advantages and disadvantages of different diagnostic tools for pathogens underlying the URIs, as well as under which circumstances a specific method would have an advantage over another one.

2 Methods

2.1 Study Design

The study was approved by the Ethics Commitee of Jessenius Faculty of Medicine in Martin, Slovakia. Results of FilmArray® Respiratory Panel and ELISA tests performed in the Department of Clinical Microbiology of Martin University Hospital were evaluated and compared for the functionalities, advantages, and disadvantages of these methods. We focused on a total number of FilmArray Panel tests, the number of positive results, and the spectrum of pathogens detected in connection with the clinical diagnosis.

2.2 Detection of Pathogens by FilmArray Respiratory Panel

Nasopharyngeal swabs were examined using the FilmArray® Respiratory Panel developed by IDAHO Technology (BioFire Diagnostics; Salt Lake City, UT) for the presence of 21 pathogens (adenovirus, bocavirus, coronavirus HKU1, coronavirus NL63, coronavirus 229E, coronavirus OC43, human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza A/H1, influenza A/H3, influenza A/H1-2009, influenza B, parainfluenza 1–4, respiratory syncytial virus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*). To assess the diagnostic efficacy of this new method, a venous blood sample was draw from two patients and it was concurrently examined in the same laboratory for the presence of eight pathogens (adenovirus, influenza A, influenza B, parainfluenza virus 1, respiratory syncytial virus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*) in 15 tests with the hitherto commonly used ELISA method. Additionally, a hemagglutination inhibition test for influenza A and B was performed in a patient in the Department of Virology of the Regional Institute of Public Health in Banska Bystrica, Slovakia.

3 Results

The FilmArray Panel was performed 15 times to identify URI pathogens in hospitalized patients with acute respiratory infections in January 2016. Positive results were obtained in 8 samples. Four samples turned out positive for human rhinovirus/enterovirus. The other detected pathogens were parainfluenza virus 3, human metapneumovirus, coronavirus OC43, influenza B, and adenovirus. Two different pathogens were identified in one patient (human rhinovirus/enterovirus and adenovirus). Positive results of laboratory tests are summarized in Table 1. These results corresponded with a number of respiratory infections, as diagnosed before hand, such as URIs, pneumonia, acute inflammation of nasopharynx, acute bronchitis, hypothermia unrelated to external temperature, and undefined fever.

For comparison, specimens obtained from two patients were tested simultaneously with

Table 1 Positive results of filmarray respiratory panel – January 2016

Patient	Result	Clinical diagnosis
1	Human rhinovirus/enterovirus	Acute URI
2	Human rhinovirus/enterovirus and adenovirus	Acute URI
3	Human rhinovirus/enterovirus	Acute URI
4	Parainfluenza virus 3	Acute bronchitis
5	Coronavirus OC43	Hypothermia
6	Human metapneumovirus	Pneumonia
7	Human rhinovirus/enterovirus	Undefined fever
8	Influenza B	Acute nasopharyngitis

URI upper respiratory tract infection

Table 2 Results of ELISA tests performed in two patients.

	Patient 1		Patient 2		
Pathogen	Date	Result	Date	Result	Costs
Adenovirus	15.01.2013	Negative	31.01.2013	Negative	IgM = 1.81 €; IgA = 2.07 €
Influenza A	13.01.2013	Negative	31.01.2013	Negative	IgM = 1.91 €; IgA = 1.91 €
Influenza B	13.01.2013	Negative	31.01.2013	Negative	IgM = 1.91 €
Parainfluenza 1	13.01.2013	Negative	31.01.2013	**Positive**	IgM = 2.08 €
Respiratory syncytial virus	12.02.2013	Negative	12.02.2013	Negative	IgM = 1.81 €; IgA = 2.07 €
Bordetella pertussis	13.01.2013	Negative	31.01.2013	Negative	IgM = 1.99 €; IgG = 1.99 €; IgA = 1.99 €
Chlamydophila pneumoniae	18.01.2013	Negative	31.01.2013	Negative	IgG = 1.33 €; IgA = 1.33 €
Mycoplasma pneumoniae	17.01.2013	Negative	31.01.2013	Negative	IgG = 1.33 €; IgA = 1.33 €
Entire testing time	**28 days**		**29 days**		**Total cost – 165 € either patient**

traditional ELISA and FilmArray Panel methods. A venous blood sample and a nasopharyngeal swab were taken from both patients for either method, respectively. In one of these patients, all results were negative and no etiologic agent could be identied by the ELISA method. In total, 28 days were was required to obtain the full panel of results (Table 2; Patient 1). In addition, culture of the specimen obtained from a nasopharyngeal swab from the same patient also was negative for any pathogens. However, nasal swab specimen, examined by the FilmArray Panel, yielded a positive result for human rhinovirus or human enterovirus (Table 3; Patient 1), which was negative when with ELISA.

ELISA performed in a second patient showed an elevation of IgG against parainfluenza virus 1 (IgG positive – 1.53, cut off – 0.709, index – 2.1). This result, however, is inconclusive

regarding an acute current infection as it rather confirms a previous encounter with the virus. The result regarding influenza A infection was also negative (IgM – 0.355, cut off – 0.832, index – 0.4; and IgG – 0.083, cut off – 0.381, index – 0.2). The testing time for all eight pathogens amounted to 22 days (Table 2; Patient 2). The examination with the FilmArray Panel in this patient identified the etiologic agent as influenza A/H1-2009 (Table 3; Patient 2). The identification had to be confirmed with a serum hemagglutination inhibition test (HIT), which was done at the Department of Virology of the Regional Institute of Public Health, in Banská Bystrica, Slovakia. The HIT was performed twice 2 weeks apart to assess a possible change in antibody titers. While in the first sample influenza A and B titers were negative, there was a significant rise in the antibody titer against

Table 3 Results of filmarray panel performed in two patients

Pathogen	Date	Patient 1	Patient 2
Adenovirus	15.01.2013	Negative	Negative
Bocavirus	15.01.2013	Negative	Negative
Coronavirus HKU1	15.01.2013	Negative	Negative.
Coronavirus NL63	15.01.2013	Negative	Negative
Coronavirus 229E	15.01.2013	Negative	Negative
Coronavirus OC43	15.01.2013	Negative	Negative
Human metapneumovirus	15.01.2013	Negative	Negative.
Human rhinovirus /enterovirus	15.01.2013	**Positive**	Negative
Influenza A	15.01.2013	Negative	Negative
Influenza A/H1	15.01.2013	Negative	Negative
Influenza A/H3	15.01.2013	Negative	Negative
Influenza A/H1–2009	15.01.2013	Negative	**Positive**
Influenza B	15.01.2013	Negative	Negative
Parainfluenza virus 1, 2, 3, 4	15.01.2013	Negative	Negative
Respiratory syncytial virus	15.01.2013	Negative	Negative
Bordetella pertussis	15.01.2013	Negative	Negative
Chlamydophila pneumoniae	15.01.2013	Negative	Negative
Mycoplasma pneumoniae	15.01.2013	Negative	Negative

Table 4 Hemagglutination inhibition test in Patient 2

Pathogen	First sample 21.01.2013	Second sample 07.02.2013	Costs
Influenza A	Negative	Positive titer – 1:160	Influenza A = 2 € x 2 = 4 €
Influenza B	Negative	Negative titer	Influenza B = 2 € x 2 = 4 €
			Total cost = 8 €

influenza A in the second sample. The HIT assessment took 17 days in all. These results are shown in Table 4.

Health insurance gave 600 points for each of the 15 tests, which makes a total 9000 points, plus 150 points for sample culture from upper respiratory tract and 320 points for a culture form lower respiratory tract. Each insurance point has a value of 0.0066 €. The laboratory therefore received 59.40 € for running the ELISA and additionally 3.10 € for the cultures, which sums up to a total of 62.50 € or 224% of the real material costs. On the other hand, health insurance gave 2500 points for each of the 21 pathogen targets in the FilmArray Panel, which makes a total of 52,500 points and comes to 346.50 € or 210% of the real material costs amounting to 165.00 € per sample. The insurance reimbursed 11.70 € for influenza A and B testing each. Each type of influenza was tested twice giving the cost

of 46.80 € in total. The actual laboratory costs for all HIT tests were 8.00 €, which is about 6 times less than the insurance reimbursement.

4 Discussion

Laboratory testing is generally not recommended in the evaluation of upper respiratory infections. Tests for specific pathogens are helpful when therapy depends on the results. Targeted therapy is not available for most viruses that cause URI. Therefore, viral testing is rarely indicated for uncomplicated URIs in the outpatient setting. However, confirmation of a viral condition such as influenza may reduce inappropriate use of antibiotics (Balentine and Siamak 2015). Considering the benfits for patient, the speed to identify the etiologic agent clearly favors the FilmArray System. This method readily identified the

etiologic agent in both patients in whom it was applied in the present study, whereas the HIT identified the virus only in the repeat sample of the second patient. However, identification of the etiologic agent did not have any benefit for the first patient with mild symptomatology of coryza because no targeted treatment for human rhinovirus/enterovirus infection was needed. The symptomatology in the second patient was severe and the speed of pathogen identification is crucial for the commencement of appropriate treatment, especially in suspected cases of influenza where early administration of neuraminidase inhibitors significantly reduces mortality rates. In such cases, accurately targeted therapy has an enormous benefit for the patient. The laboratory costs to run one examination with different methods showed that the FilmArray multiplex PCR respiratory panel is more expensive than the ELISA, HIT, and the cultivation. One examination with the FilmArray panel brought a 181.50 € *per* patient profit for the laboratory, whereas the profit from running ELISA together with cultivation was 34.62 €, and that from HIT was 38.80 € *per* patient. Therefore, FilmArray respiratory panel is best in terms of profit margin for the laboratory and least favorable for health insurance.

5   Conclusions

Serologic diagnostic methods, such as ELISA and HIT, cannot identify the pathologic agent until the host's immune system elicits a response. However, advantages of those methods are that they are readily available in many laboratories and are least pricey for health insurance. The disadvantage is that the spectrum of pathogens detected is small. A clinical benefit of the FilmArray respiratory panel is that it is quick and exact and may identify a broader spectrum of possible pathogens. Since most URIs are viral in origin and there is no treatment available, the diagnosis provided by the FilmArray panel is not always necessary and the method should be used

on an individual basis when clinically justified. From the economic standpoint, FilmArray respiratory panel is the most profitable for the laboratory. In critically ill patients, a spectrum of diagnostic methods should be used to obtain diagnosis as fast as possible. In patients with minor respiratory tract infections, a more rational approach should be undertaken since the speed and accuracy of diagnosis are less crucial. The decision to choose a specific diagnostic method rests with the medical caregiving staff.

Conflict of Interest The authors declare no conflicts of interest in relation to this article.

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