The effectiveness of common respiratory viruses detection by indirect immunofluorescence test in samples from upper respiratory tract

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Abstract
Study presents a preliminary analysis of the diagnostic effectiveness of the common respiratory viruses detection in the nasal and throat swab samples by the indirect immunofluorescence test. Samples referred for diagnostic test from 15 consecutive patients were examined. All evaluated throat swab samples proved positive (100%), while the rate of respiratory viruses detection in nasal samples was as low as 13.3%. Thus, our preliminary though quite informative data demonstrate considerably higher sensitivity of throat samples in IF-based diagnosis of respiratory viral infection.

Key words: *viral respiratory tract infections, nasal swabs, throat swabs, indirect immunofluorescence.*

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Introduction

Viral respiratory tract infections are the most common contagious diseases responsible for considerable morbidity but also mortality in humans. Consequently, they are accountable for enormous economic burden due to the medical care needs, pharmacological treatment, hospitalizations as well as work absences. It is believed that better monitoring and diagnosis of respiratory viral infections might potentially reduce health-care costs first of all in terms of appropriate/rational treatment and antibiotics consumption but also shortened hospitalization time and absence from work [1].

Clinical diagnosis cannot identify the causative agent and usually is not adequate to differentiate between viral and bacterial infection. Therefore, only laboratory methods provide data essential for introduction of effective clinical procedures and targeted therapy.

Diagnostic methods available for the respiratory virus (RV) detection include nucleic acid amplification tests (NATs), virus isolation in cell cultures, shell vial technique and rapid antigen testing [non-immunofluorescence and immunofluorescence tests (IF)]. However, the most commonly used are IF tests which rapidly, within 60-120 minutes, provide valid results, are cheaper than molecular methods as well as technically simpler [2]. Though overall reported sensitivity of the IF tests in comparison to viral culture is approximately 81%, it very much depends on the virus type [3]. Accordingly, the sensitivity of RV detection by IF method ranges from 84-99% in comparison to cell cultures and is the highest for respiratory syncytial viruses (RSV) [4, 5]. The sensitivity for adenovirus detection by IF method is the lowest between 0-58% [5].

Apart from the virus type, other methodological issues like specimen type, proper sampling technique, as well as its safe transportation to the laboratory are crucial for the rapid and accurate diagnosis of respiratory viral infection [6].

Nasopharyngeal aspirates or nasal wash are the most effective sampling techniques for the detection of respiratory viruses [7-11]. However, in real-life clinical environment reliable sample collection might not be that

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Table 1. The comparative sensitivity of the nasal and throat swabs specimens collected from patients with respiratory tract infections symptoms

Results	Throat swabs	Nasal swabs
Positive	15/15 (100%)	2/15 (13.3%)
Negative	0	11/15 (73.3%)
Nondiagnostic	0	2/15 (13.3%)

easy. Aspirate is a method relatively invasive and uncomfortable for the patient. While nasal and throat swab collection is easier and could be performed everywhere, quality procedures and proper staff training prerequisite.

There are many studies comparing the sensitivity of respiratory viruses detection in respect of sample type (nasopharyngeal aspirates and swabs, nose-throat and nasal swabs) and diagnostic method (viral culture, antigen detection and polymerase chain reaction methods) proving the importance of both factors [7-11].

Our study presents preliminary though quite informative analysis of the clinical usefulness of nasal and throat swabs in detection of seven respiratory viruses by indirect immunofluorescence test.

Material and methods

Study group

Throat and nasal swabs were collected from fifteen consecutive patients developing clinical symptoms of infection (headache, myalgia, throat pain, fever, dry cough, nasal congestion, and runny nose) and from six healthy volunteers free of any symptoms of respiratory tract infection.

Study was performed using the Imagen Respiratory Screen, the qualitative two-step indirect immunofluorescence test. It contains a pool of purified mouse monoclonal antibodies reacting with conserved epitopes of viral proteins, individually specificity for either RSV, influenza A or B virus, parainfluenza virus 1, 2, or 3 or adenovirus. Second, FITC conjugated anti-mouse antibody react with the first one. The assay cannot differentiate between viruses.

Specimens were collected by trained nurse, using a sterile polyester collection swab (Equimed) which were liquefied by few drops of sterile PBS (phosphate-buffered saline). Afterwards specimens were transported to the laboratory within one hour.

Further specimens were transferred into 2 ml of sterile PBS, vortexed and centrifuged at room temperature (15-30°C) for 5 minutes at 3000 rpm. After discard the supernant, obtained pellet was resuspended in 200-500 µl PBS maintaining a high cell density. 25 µl aliquots were spotted on a teflon coated slides, air dried and then fixed with cold acetone.

For each specimen two wells of fixed cell preparation were required. To one well of the test slide we added 20 µl of Screening Reagent and to the other well we added 20 µl Negative Control Reagent. Next, slides were incubated in moist chamber for 30 minutes at 37°C. After incubations all slides were washed off excess reagent with PBS in an agitating bath, three times for 1 minutes. Then we added 20 µl of FITC reagent to each specimen well, we incubated and washed the slides one more time under the same condition as previously. After drying the slides we added one drop of Mounting Fluid to the centre of each well and placed the coverslip.

The examination of entire wells area containing the stained specimen was performed using an epifluorescence microscope immediately after staining.

A positive diagnosis was made when one or more cells in the fixed stained specimen, demonstrated typical fluorescence pattern (apple-green cytoplasmic and/or nuclear fluorescence). A negative diagnosis was made when fixed, stained specimens do not exhibit fluorescence with the screening reagent.

Results

The comparative sensitivity of the nasal and throat swabs specimens for detection of respiratory viruses, collected from patients with typical symptoms of respiratory tract infection is shown in Table 1. While all evaluated throat swab samples proved positive (100%), the rate of respiratory viruses detection from nasal swabs was significantly lower. Only two samples were positive (13.3%) and eleven (73.3%) negative. As much as two (13.3%) nasal samples were inadequate for testing due to the low cell density. All samples collected from healthy volunteers, free of any respiratory symptoms, were negative.

Discussion

Present study was designed to compare the real-life sensitivity of respiratory viruses identification by qualitative indirect immunofluorescence test in nasal or throat swabs specimens. In the group of consecutive 15 patients throat swabs samples proved much more effective than nasal specimens. Surprisingly, only 2 of 15 nasal samples (13.3%) shown positive in contrast to all samples (15/15) obtained from throat swab.

There are several factors that could be held responsible for throat samples superiority shown in our data. Since study group is rather small and consist of consecutive patients at least some samples could represent similar causative agent characterized by significantly higher viral titer in the throat than in the nose, as it was shown for influenza virus A/H5N1 [12]. Hence, the superiority of throat samples could follow.

Certain technical issues that might have influenced the study outcome should be considered as well. Proper specimen collection is crucial for the accurate test result and reliable diagnosis of respiratory viral infection. Appropriate sampling must maximize the collection of respiratory epithelial cells, as the test sensitivity strictly depends on the number of infected cells examined [6]. It has been demonstrated that nasal swabs are characterized by the lower cell density then throat swabs. Also, significantly higher mucus content in nasal samples could result in considerably lower test sensitivity due to the nonspecific staining [13].

The fact that as much as 2 out of 15 nasal samples (13.3%) were nondiagnostic due to the lack of tissue material emphasizes the importance of continuous medical staff training. Proper specimen collection – insufficiently vigorous (lack of cellular material), sample contamination – critically affects final test outcome. Drexler *et al.* clearly demonstrated that reliability of respiratory viruses diagnostic tremendously depends on the medical staff training in the collection of biological material. Sensitivity and specificity of testing in samples collected by trained personnel versus untrained operators differed significantly showing respectively 60% vs. 34.6% and 96.6% vs. 89.7% [14].

Present study suggest that throat swabs seem more suitable for detection of respiratory viral infection by indirect immunofluorescence method. Meanwhile, nasal swabs as the only source of the biological material for virus detection might not be adequate.

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