FACTORS AFFECTING THE SUCCESS OF INFLUENZA LABORATORY DIAGNOSIS

Renáta Kissová[1], Marek Svitok[2], Cyril Klement[1, 3], Lucia Maďarová[1]
[1]Regional Authority of Public Health, Department of Medical Microbiology, Banská Bystrica, Slovak Republic
[2]Faculty of Ecology and Environmental Sciences, Technical University in Zvolen, Zvolen, Slovak Republic
[3]Faculty of Health in Banská Bystrica, Slovak Medical University, Bratislava, Slovak Republic

SUMMARY

Influenza is one of the most common human infectious diseases, and has profound health and economic consequences. The laboratory diagnosis of influenza virus infections plays an important role in the global surveillance of influenza. Therefore, there is a growing demand for highly sensitive and rapid methods for detecting influenza. The performance of particular diagnostic methods is affected by various factors. In this study, we assess the effects of patients' age and time to diagnosis on the probability of detecting influenza using four diagnostic methods (virus isolation, rapid test, RT-PCR and real-time RT-PCR). We examined 3,546 samples from central and eastern Slovakia during the influenza seasons from 2005–2006 to 2010–2011. In general, the probability of influenza detection significantly decreased with the time from onset of illness to sample collection (T1) as well as with patients' age (AGE). On the contrary, time from sample collection to delivery (T2) did not play a role in the probability of influenza detection. As judged by odds ratios, the virus isolation method was most sensitive to T1, followed by the rapid test and RT-PCR methods. For the effect of AGE, the rapid test and virus isolation methods were more sensitive than PCR-based methods. The effects of T1 and AGE were independent of each other. Laboratories which participate in influenza surveillance should use several methods to enable rapid and accurate influenza A and B virus detection.

Key words: influenza laboratory diagnosis, influenza surveillance, influenza viruses isolation, PCR, rapid testing

Address for correspondence: R. Kissová, RÚVZ Banská Bystrica, Cesta k nemocnici 25, 975 56, Banská Bystrica, Slovak Republic. E-mail: renata.kissova@vzbb.sk

INTRODUCTION

Influenza is among the most common human infectious diseases, every year affecting about 10–20% and in periods of pandemic even 40–50% of the world population, and is the cause of death of thousands of people around the world. The most at risk groups are the elderly or people with underlying chronic medical conditions, who have a high risk of developing complications. Influenza also causes huge economic losses by affecting the capacity of infected people to work. Few other infectious diseases exert such an adverse influence on public health and the economy worldwide as influenza (1–5).

Influenza viruses are extremely variable, so the influenza vaccine must change in composition every year according to the influenza strains actually circulating in the population. The recent flu pandemic in 2009 and 2010 also highlighted the necessity for the national and global surveillance of influenza. In Slovakia, influenza surveillance is performed according to the recommendations of the World Health Organization (WHO) and the European Centre for Disease Prevention and Control (ECDC), including the systematic collection of biological samples from sentinel sources. General practitioners and paediatricians from throughout Slovakia collect samples from patients with suspected influenza during the influenza season.

The gold diagnostic standard for diagnosing influenza virus infections is the traditional method of isolating the virus in cell cultures or chicken embryos. It is a technically demanding process, requiring significant financial and time investments. The success of laboratory diagnoses depends on the quality of laboratory equipment, the experience of the laboratory staff, the wide range of diagnostic methods as well as on the cooperation of physicians, epidemiologists and virologists (6–10). Molecular biological methods (RT-PCR and real-time RT-PCR) have become an integral part of the virological surveillance of influenza, as was seen during the recent pandemic of influenza A/H1N1 (11). Influenza viruses can also be detected by "near-patient" rapid tests with a time to obtain result of 15–30 minutes (10, 12–15). These tests were developed for the clinical purposes of causal influenza treatment, facilitating decisions on quarantine and the antibiotic therapy of infected patients as well as helping identify outbreaks within institutions. One disadvantage of these rapid tests, however, is the low sensitivity (12, 14–17).

Choosing the most appropriate diagnostic tool depends on the sensitivity and specificity of the tests, time to obtain results, repeatability, the simplicity of the procedure and costs (18). However, the effectiveness of a particular diagnostic method in the direct diagnostics of influenza is also affected by various factors such as the age of the patient (19–21), early sample collection and rapid transport to the laboratory (8, 22, 23). The aim of our study was to assess the effect of patients' age and time to confirm influenza infection using four diagnostic methods (virus isolation, rapid test, RT-PCR, and real-time RT-PCR).

MATERIALS AND METHODS

Samples were collected during three types of influenza seasons: normal influenza seasons, pandemic influenza season and post-pandemic influenza season.

During four normal influenza seasons (2005–2009), samples of nasopharyngeal swabs and post-mortem material for direct diagnostics of influenza viruses were collected from districts in the Banská Bystrica and Žilina regions (central Slovakia). Clinical samples were taken by sentinel and non-sentinel physicians from patients with symptoms of influenza-like illness.

During the influenza pandemic (2009 and the influenza season 2009–2010), samples were collected from districts in the Banská Bystrica, Žilina, Košice, and Prešov regions (central and eastern Slovakia). This biological material was collected by sentinel and non-sentinel practitioners and also by physicians from various departments (infectology, pneumology, internal medicine, paediatrics). Samples from intensive care units, forensic and pathology workplaces (post-mortem material), and samples from patients diagnosed with Severe Acute Respiratory Infection (SARI) were also examined.

During one post-pandemic season (influenza season 2010–2011), samples were collected from districts in the regions of Banská Bystrica and Žilina (central Slovakia), also including samples from SARI patients and post-mortem material.

Samples were collected from both adult and paediatric patients. Nasopharyngeal swabs were collected and placed into containers with viral transport medium (Medium 199 with 0.5% BSA and antibiotics Penicillin G, Streptomycin and Amphotericin) (24) supplied by the laboratory. Samples were then delivered for analysis to the Regional Authority of Public Health Banská Bystrica, Division of Medical Microbiology, Departments of Medical Virology and Molecular Biology. We monitored the presence of influenza viruses by the virus isolation, rapid test and PCR based methods.

Virus Isolation

Virus isolations were carried out in accordance with standard virological techniques and the WHO protocol (24). The process of virus isolation consists of the capture and multiplication of influenza viruses in MDCK cell cultures (Madin Darby Canine Kidney). The presence of influenza viruses manifests as a cytopathic effect, observed under a light microscope. A haemagglutination test in microplates was also performed to demonstrate the presence of influenza viruses. A haemagglutination test with three kinds of red blood cells (chicken, guinea pig and human type 0) was performed with harvested fluids. In case of a positive haemagglutination, the sample was subjected to further analysis to distinguish between influenza virus A, B or the pandemic influenza virus A/H1N1 using RT-PCR or real-time RT-PCR methods. Inoculated cell cultures were passaged at least three times before a sample was considered negative.

Rapid Test

Directigen EZ Flu A+B is a rapid commercial chromatographic immunoassay test for the direct and qualitative detection of influenza viruses A and B from nasopharyngeal swabs and other materials from symptomatic patients. Viral antigens of influenza A and B can be distinguished from each other using a single processed sample with time to result of 15 min. A positive test result was considered indicative of the presence of influenza A or B virus antigens. A negative test result was considered as probably negative for the presence of influenza virus antigens A and B, respectively.

RT-PCR

We used a conventional qualitative RT-PCR method to detect influenza RNA in samples of biological material. The diagnostic kit Amplisens® RNA PCR TEST Influenza A + B Interlabservice (Ukraine) was used according to the manufacturer's protocol. After amplification, the PCR products were detected by electrophoresis in an agarose gel under UV light. The results of RT-PCR were considered positive or negative depending on the presence or absence, respectively, of an amplification product as detected by electrophoresis.

Real-time RT-PCR

The CDC Real-time RT-PCR (rRT-PCR) protocol was used for the real-time RT-PCR detection of pandemic A/H1N1 influenza virus. This protocol includes a panel of primer pairs and dual-labelled hydrolysis (Taqman®) probes for the in vitro qualitative detection and characterization of pandemic influenza viruses in respiratory specimens. The InfA primer and probe set was designed for the universal detection of type A influenza viruses. The swInfA primer and probe set was designed to specifically detect all swine influenza A viruses. The swH1 primer and probe set was designed to specifically detect swine H1 influenza viruses. Each sample RNA extract was tested by each of the three primer/probe sets described above plus the RNase P gene to serve as an internal positive control for human nucleic acid. At the same time and under the same conditions, the real-time RT-PCR procedure was controlled using positive and negative controls.

The results were evaluated qualitatively, with a sample considered positive when the fluorescence curves increased during the amplification suggesting the presence of target cDNA. The results were validated using positive control samples.

Data Analysis

Influenza detection data obtained by the four different methods (virus isolation, rapid test, RT-PCR, and real-time RT-PCR) were analyzed using Generalized Linear Models (GLM) (25). GLM with binomial error distribution and the logit link function (logistic regression) were employed in order to test the effect of patients' age (AGE), time from the onset of influenza illness to sample collection (T1) and time from the sample collection to delivery of samples to the laboratory (T2) on the probability of influenza virus detection. Second order interaction terms (AGE × T1 and AGE × T2) were also included into the modelling to account for possible multiplicative effects on the probability of detection. Only samples with $T1 \leq 15$ days were included in the analysis, since the remaining 4.7% of observations with T1 longer than 15 days appeared as severe outliers in the models. Initially, full models were fitted to the data, including all explanatory variables

Table 1. *Summary of logistic regression models testing the effects of the time from onset of illness to sample collection (T1), time from sample collection to delivery of samples to the laboratory (T2), and patients' age (AGE) on the probability of detecting influenza virus during three types of influenza seasons. Characteristics of the final models (results of likelihood ratio tests − χ^2 and p, (pseudo) determination coefficient − R^2_{MF}, sample size − n) as well as characteristics of particular parameters (estimates of regression coefficients − b, standard error of estimates − SE, results of likelihood ratio tests − $\chi^2_{(1)}$ and p, relative changes in odds ratios – OR and associated bootstrapped confidence limits − 95% CL) are shown.*

Season	Method	Whole model				Model parameters					
		χ^2	p	R^2_{MF}	n	Variable	b	SE	$\chi^2_{(1)}$	p	OR (95% CL) [%]
Normal influenza seasons	Virus isolation	18.7	< 0.001	0.033	660	T1	−0.269	0.080	15.55	< 0.001	−23.5 (−35.5,−12.1)
						T2	non-significant				
						AGE	−0.014	0.007	3.9	0.049	−1.4 (−2.8,−0.2)
	RT-PCR	30.8	< 0.001	0.051	455	T1	−0.312	0.066	30.8	< 0.001	−26.8 (−35.5,−12.1)
						T2	non-significant				
						AGE	non-significant				
Pandemic influenza season	Rapid test	35.9	< 0.001	0.071	941	T1	−0.183	0.054	14.2	< 0.001	−16.7 (−26.9,−7.5)
						T2	non-significant				
						AGE	−0.030	0.007	19.0	< 0.001	−3.0 (−4.6,−1.5)
	RT-PCR	37.5	< 0.001	0.021	1,563	T1	−0.075	0.019	15.9	< 0.001	−7.2 (−10.8,−3.8)
						T2	non-significant				
						AGE	−0.012	0.003	17.2	< 0.001	−1.2 (−1.8,−0.7)
	real-time RT-PCR	6.5	0.011	0.082	620	T1	non-significant				
						T2	non-significant				
						AGE	−0.012	0.005	6.5	0.011	−1.1 (−2.1,−0.2)
Post-pandemic influenza season	Virus isolation	6.7	0.010	0.079	187	T1	−0.383	0.180	6.7	0.010	−31.8 (−51.0,−16.2)
						T2	non-significant				
						AGE	non-significant				
	RT-PCR	4.9	0.027	0.032	121	T1	−0.145	0.067	4.9	0.027	−13.5 (−26.4,−0.9)
						T2	non-significant				
						AGE	non-significant				
	real-time RT-PCR	non-significant			122	T1	non-significant				
						T2	non-significant				
						AGE	non-significant				

and two-way interactions. To acquire minimal adequate models, the full models were simplified following backward stepwise deletion (26). Starting from the higher-order terms, significance was tested using likelihood-ratio χ^2 tests to compare the change in model deviance after deleting each term. Separate models were built for each combination of detection method and type of influenza season. Diagnostic plots of residuals were employed to check the performance of the final models. In order to facilitate interpretation of the results, relative changes in odds ratios (OR) were calculated and associated 95% confidence limits (CL) were obtained using 1000 non-parametric bootstrap re-samples (27). The fit of the final models was assessed using McFadden's (pseudo) determination coefficient (R2MF) (28). All analyses were performed in the R language (29).

RESULTS

Altogether, 3,546 samples were examined during all influenza seasons. Among the 1,056 samples examined by the virus isolation method, 48 influenza A viruses and 78 influenza B viruses were identified (11.9% of positive samples). The rapid test and PCR methods were used mostly during the pandemic (2009–10) and in the post-pandemic seasons. Out of 1,454 samples examined by the rapid test, 98 (6.7%) were identified as positive for influenza A. No sample was identified as positive for influenza B by this method. Out of 3,067 samples examined by PCR methods, 821 influenza A viruses and 94 influenza B viruses were identified (29.8% of positive samples). The real-time RT-PCR method was mainly employed for subtyping pandemic influenza A/H1N1 in influenza A-positive samples identified by the RT-PCR method. Among 1,035 samples tested by real-time RT-PCR, 579 (55.9%) samples were identified as positive for the presence of pandemic influenza A/H1N1 virus.

The effects of patients' age (AGE), and times to sample collection (T1) and delivery (T2) on the probability of influenza virus detection was examined using logistic regression. Final regression models for each combination of method and type of influenza season are given in Table 1. Time from sample collection to delivery (T2) did not play a role in the detection of influenza. In contrast, T1 and AGE showed significant relationships with the probability of detecting influenza for each combination of method and season, with the exception of real-time RT-PCR during the post-pandemic season. Generally, the probability of detecting influenza decreased with time from the onset of illness to sample collection as well as with patients' age (Fig. 1). Depending on the method and season, the odds of detecting influenza decreased, in a range from 7.5 to 31.8%, with each day from the onset of illness to sample collection. As judged by odds ratios, the strength of the effect of T1 on particular methods was, in descending order, virus isolation, RT-PCR, rapid test and real-time RT-PCR. The significant effect of patients' age was found only during the normal and post-pandemic seasons. The odds detecting influenza decreased, in a range from 1.1 to 3.0%, with every year of patient's age. Again, the rapid test and virus isolation were more sensitive to patients' age than PCR methods. The effects of T1 and AGE were independent of each other since the interaction terms were non-significant. A common feature of all models was the low explanatory power, i.e. explained deviance ranged from 2.1 to 8.2% as expressed by R2MF.

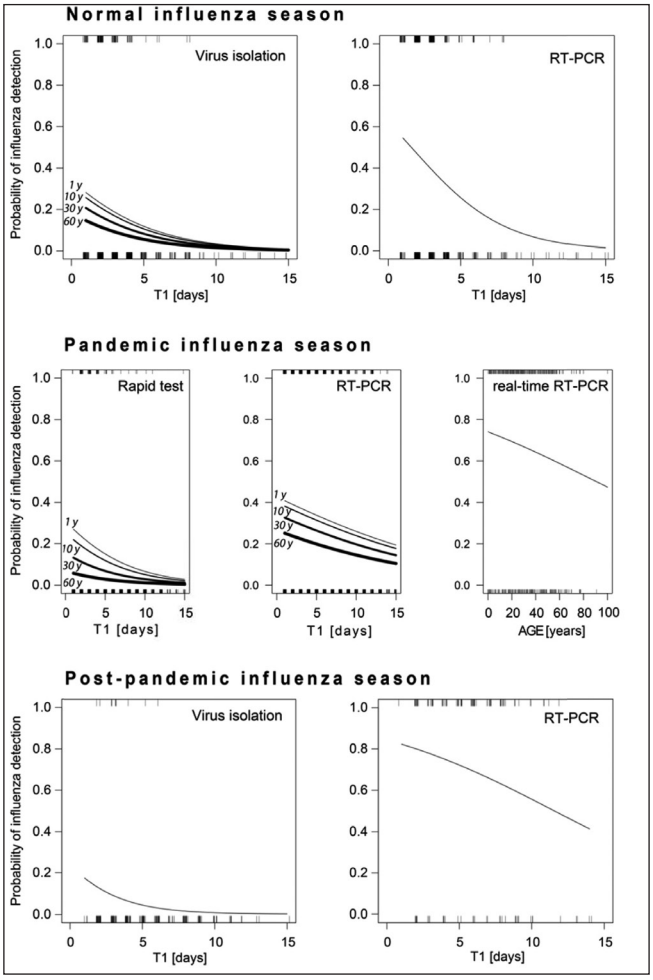

Fig. 1. *Final logistic regression models showing relationships between the time from onset of illness to sample collection (T1), patients' age (AGE) and the probability of detecting influenza virus based on four different methods during three types of influenza seasons. In order to enhance the clarity of the results, models with two parameters (T1+AGE) are shown in the same plots while separate curves of different thickness are shown for 1, 10, 30 and 60 year-old patients.*

DISCUSSION

Over six years, we detected influenza viruses using four standard methods: RT-PCR for influenza A and B (from 2005), real-time RT-PCR for pandemic influenza A/H1N1 (from 2009), the rapid test for influenza A and B (from 2007), and virus isolation in MDCK cells (all years with the exception of the pandemic influenza period).

An exact comparison of the sensitivity of these various methods cannot be conclusively performed on the basis of our results because we did not apply all methods on the same samples. However, some reasonable inferences can be made from the patterns in our findings. Among the methods compared here, PCR-based methods appear to be the most sensitive for the detection of influenza viruses, as expected. This is true for both RT-PCR and especially for real-time RT-PCR, which was used to identify and subtype pandemic influenza A/H1N1 during the pandemic and the post-pandemic periods. Real-time RT-PCR was used mainly to test samples positive by RT-PCR. A few samples tested by real-time RT-PCR consisted of material from patients

with severe respiratory symptoms, since there was not enough time to perform RT-PCR.

The rapid test method (Directigen EZ Flu A + B) appears to be least sensitive, although it was used to test samples from patients with SARI, which have a higher probability of being positive. A negative test result does not eliminate the possibility of an influenza infection, so it is necessary to evaluate the patient's clinical symptoms with regard to the epidemiological situation. Results of this test need to be confirmed by virus isolation and/or PCR methods (10, 15–17, 30).

Time from onset of illness to sample collection (T1) showed a significant effect on the detection of influenza using all but one method (real-time RT-PCR). However, it should be again emphasised that real-time RT-PCR was used mainly for subtyping and more precise identification of influenza-A positive samples detected by RT-PCR.

Virus isolation (MDCK) appeared to be the most sensitive method to changes in T1. The odds of detecting influenza decreased by 23.5% (normal seasons) or even by 31.8% (post-pandemic season) with every successive day from the onset of illness. The yield of positive samples can be enhanced when swabs are taken early (24–48 hours) after the disease onset. This reflects the pattern of virus shedding (8). Samples which can be detected as positive must contain a sufficient number of viable viral particles capable of replication (6, 8, 10, 23, 24), making it difficult or even impossible to isolate influenza viruses from samples taken at later stages of the disease.

The rapid test method also showed high sensitivity to T1. For a one-day change in T1 there was a 16.7% decrease in the odds of detecting influenza. This could be explained by the generally lower sensitivity of this test (10, 12, 15, 31, 32). Samples which can be detected as positive must contain a relatively large number of virus particles (not necessarily viable) (12, 17, 24, 30, 32). Also, the manufacturer of the test (Directigen) recommends only using swabs obtained within 48 hours after the onset of clinical symptoms. This limitation has been confirmed by several studies (9, 10, 14, 22, 33).

PCR methods are able to identify viruses in samples with a lower concentration of viral particles and which do not need to be viable. For this reason PCR is a more sensitive technique than the other methods used. This increases the possibility of detecting influenza viruses also in samples taken in the later stages of the disease (34–38).

On the contrary, time from sample collection to delivery (T2) did not play a role in the probability of influenza detection in our study. Undoubtedly, proper collection and transport of samples increases the probability of a successful result (6, 24, 30). In general, cool holding temperatures (not freezing), short transport time (optimally 1–2 days, max. 3–4 days after sampling), and high virus concentrations are important for diagnosis. However, influenza viruses can be successfully detected in the samples transported at ambient temperatures and analysed several days after the sampling (8, 23).

Age showed significant relationships with the probability of detecting influenza for each combination of method and season, with the exception of real-time RT-PCR during the post-pandemic season. These results again reflect the limits of particular diagnostic methods to detect the number of viral particles in a sample. The probability of detecting influenza decreased with patients' age. Generally, the highest morbidity is in preschool and school-age children (1, 3, 39). Moreover, virus shedding is significantly higher and lasts for a longer period in children than in adults (21). Possible explanation is immature immune system of children and low previous exposition to influenza virus, which implicates a lower level of immunity against influenza (1, 20, 21, 39).

The generally low fit of the models (explained deviance < 10%) could be caused by various external factors that affect the outcome of these tests and that were not included in our models. This includes mainly the technique of sample collection, and the prevalence or incidence of influenza in the population, according to the strict clinical case definition of influenza and influenza–like illness by physicians (8). The immunological status of the patient and previous use of antivirals are also factors that may affect the success of influenza laboratory diagnosis (1, 19, 21, 39).

Laboratory results based on virus isolation are usually not available in less than 3–5 days, sometimes up to 10 days (especially in the case of negative results) (7, 11, 40, 41). The time to result of the rapid test is 15 minutes, but there is a high probability of false negative results (10, 15, 30, 32). The advantages of PCR methods are the short time to results (4–5 hours) and high sensitivity of the tests (17, 34, 36, 38). However, the high cost and necessity of special laboratory equipment and trained staff are disadvantages (34, 36, 37). Virus isolation is the only method by which is possible to obtain viable flu virus strains. This can be especially useful since isolates can be subtyped, antigenic and genetic characterized, used for the production of vaccines or for monitoring of antiviral drugs sensitivity (2, 16, 24, 30, 41). This method is also essential for the diagnosis of new and unexpected infections (7, 18, 37, 40). However, virus isolation methods are very sensitive to the time of sample collection and its quality (40, 42, 43).

CONCLUSION

The introduction of molecular-biological methods (RT-PCR and real-time RT-PCR) and rapid screening tests in diagnostic practice has significantly accelerated and improved the diagnosis of influenza. Rapid detection is important not only for the treatment of individual patients, but also for public health and for defining and managing outbreaks of influenza in the population. However, viral isolation in cell cultures still remains an integral and irreplaceable part of influenza surveillance (43). Rapid and accurate diagnosis of influenza allows the early initiation of antiviral therapy and prophylaxis, limiting antibiotic therapy and other tests, and allows the implementation of appropriate infection control strategies for individuals and public health issues (10, 30, 42). Laboratories which participate in influenza surveillance should use several methods to enable rapid and accurate influenza virus detection, and to provide a useful tool for public health institutions in the management and surveillance of influenza and influenza-like illness (1, 8, 10, 30, 37).

Acknowledgement

We are grateful for language correction by David Hardekopf.

Conflict of Interests

None declared

REFERENCES

1. Beran J, Havlík J. Influenza: clinical picture, prevention and treatment. 2nd ed. Praha: Maxdorf; 2005. (In Czech.)
2. Fukuda K, Levandowski RA, Bridges CB, Cox NJ. Inactivated influenza vaccines. In: Plotkin SA, Orenstein WA, editors. Vaccines. 4th ed. Philadelphia: Elsevier; 2004. p. 339-70.
3. Stephenson I, Zambon M. The epidemiology of influenza. Occup Med (Lond). 2002 Aug;52(5):241-7.
4. Monto AS. Epidemiology and virology of influenza illness. Am J Manag Care. 2000 Mar;6(5 Suppl):S255-64.
5. Nicholson KG, Wood JM, Zambon M. Influenza. Lancet. 2003 Nov 22;362(9397):1733-45.
6. WHO Regional Office for Europe guidance for influenza surveillance in humans [Internet]. Copenhagen: WHO Regional Office for Europe; 2009 [cited 2009 Sept 1]. Available from: http://influenzatraining.org/static/toolkit/WHO_GIPCCare_CDRom_V3Master_June_2013/resource/U02_doc5.pdf.
7. World Health Organization. Recommendations and laboratory procedures for detection of avian influenza A(H5N1) virus in specimens from suspected human cases [Internet]. Geneva: WHO; 2007 [cited 2008 July 7]. Available from: http://www.who.int/influenza/resources/documents/RecAIlabtestsAug07.pdf.
8. Leitmeyer K, Buchholz U, Kramer M, Schweiger B. Enhancing the predictive value of throat swabs in virological influenza surveillance. Euro Surveill. 2002 Dec;7(12):180-3.
9. Smit M, Beynon KA, Murdoch DR, Jennings LC. Comparison of the NOW Influenza A & B, NOW Flu A, NOW Flu B, and Directigen Flu A+B assays, and immunofluorescence with viral culture for the detection of influenza A and B viruses. Diagn Microbiol Infect Dis. 2007 Jan;57(1):67-70.
10. World Health Organization. WHO recommendations on the use of rapid testing for influenza diagnosis [Internet]. Geneva: WHO; 2005 [cited 2008 July 21]. Available from: http://www.who.int/influenza/resources/documents/RapidTestInfluenza_WebVersion.pdf?ua=1.
11. Mad'arová L, Feiková S, Kissová R, Klement C, Blaškovičová H, Takáč B, et al. Polymerase chain reaction (PCR) - an efficient tool for diagnosis of influenza and acute respiratory illnesses during influenza season. Cent Eur J Public Health. 2008 Jun;16(2):59-64.
12. Wunderli W, Thomas Y, Müller DA, Dick M, Kaiser L. Rapid antigen testing for the surveillance of influenza epidemics. Clin Microbiol Infect. 2003 Apr;9(4):295-300.
13. Kristufkova Z, Blaskovicova H. Influenza and influenza-like acute respiratory illnesses in the Slovak Republic in the 1999-2000 season. Bratisl Lek Listy. 2000:101(11):603-10.
14. Ruest A, Michaud S, Deslandes S, Frost EH. Comparison of the Directigen Flu A+B test, the QuickVue influenza test, and clinical case definition to viral culture and reverse transcription-PCR for rapid diagnosis of influenza virus infection. J Clin Microbiol. 2003 Aug;41(8):3487-93.
15. Pachucki CT. Rapid tests for influenza. Curr Infect Dis Rep. 2005 May;7(3):187-92.
16. Center for Disease Control and Prevention. Influenza symptoms and the role of laboratory diagnostics [Internet]. Atlanta: CDC [cited 2008 July 7]. Available from: http://www.cdc.gov/flu/professionals/diagnosis/labrolesprocedures.htm.
17. Newton DW, Treanor JJ, Menegus MA. Clinical and laboratory diagnosis of influenza virus infections. Am J Manag Care. 2000 Mar;6(5 Suppl):S265-75.
18. Carman WF, Wallace LA, Walker J, McIntyre S, Noone A, Christie P, et al. Rapid virological surveillance of community influenza infection in general practice. BMJ. 2000 Sep 23;321(7263):736-7.
19. Surveillance of Influenza. Acta Hyg Epidemiol Microbiol. 2006;(6):52-6. (In Czech.)
20. Grijalva CG, Poehling KA, Edwards KM, Weinberg GA, Staat MA, Iwane MK, et al. Accuracy and interpretation of rapid influenza tests in children. Pediatrics. 2007 Jan;119(1):e6-11.
21. Brankston G, Gitterman L, Hirji Z, Lemieux C, Gardam M. Transmission of influenza A in human beings. Lancet Infect Dis. 2007 Apr;7(4):257-65.
22. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics. J Infect Dis. 2006 Nov 1;194 Suppl 2:S98-110.
23. Johnson FB. Transport of viral specimens. Clin Microbiol Rev. 1990 Apr;3(2):120-31.
24. World Health Organization. WHO Manual on animal influenza diagnosis and surveillance [Internet]. Geneva: WHO; 2002 [cited 2013 March 5]. Available from: http://www.who.int/csr/resources/publications/influenza/en/whocdscsrncs20025rev.pdf.
25. McCullagh P, Nelder JA. Generalized linear models. 2nd ed. London: Chapman and Hall; 1989.
26. Crawley MJ. The R book. Chichester: John Wiley & Sons; 2007.
27. Tibshirani R, Knight K. Model search by bootstrap "bumping". J Comput Graph Stat. 1999;8(4):671-86.
28. McFadden D. Conditional logit analysis of qualitative choice behavior. In: Zarembka P, editor. Frontiers in econometrics. New York: Academic Press; 1974. p. 105-42.
29. R Development Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2010.
30. Dwyer DE, Smith DW, Catton MG, Barr IG. Laboratory diagnosis of human seasonal and pandemic influenza virus infection. Med J Aust. 2006 Nov 20;185(10 Suppl):S48-53.
31. Cazacu AC, Chung SE, Greer J, Demmler GJ. Comparison of the Directigen Flu A+B membrane enzyme immunoassay with viral culture for rapid detection of influenza A and B viruses in respiratory specimens. J Clin Microbiol. 2004 Aug;42(8):3707-10.
32. Drinka PJ. Experience with the rapid Directigen test for influenza. J Am Med Dir Assoc. 2006 Jan;7(1):37-9.
33. Fleming DM. Influenza diagnosis and treatment: a view from clinical practice. Philos Trans R Soc Lond B Biol Sci. 2001 Dec 29;356(1416):1933-43.
34. Ellis JS, Zambon MC. Molecular diagnosis of influenza. Rev Med Virol. 2002 Nov-Dec;12(6):375-89.
35. Stone B, Burrows J, Schepetiuk S, Higgins G, Hampson A, Shaw R, et al. Rapid detection and simultaneous subtype differentiation of influenza A viruses by real time PCR. J Virol Methods. 2004 May;117(2):103-12.
36. Read SJ, Burnett D, Fink CG. Molecular techniques for clinical diagnostic virology. J Clin Pathol. 2000 Jul;53(7):502-6.
37. Pérez-Ruiz M, Yeste R, Ruiz-Pérez MJ, Ruiz-Bravo A, de la Rosa-Fraile M, Navarro-Marí JM. Testing of diagnostic methods for detection of influenza virus for optimal performance in the context of an influenza surveillance network. J Clin Microbiol. 2007 Sep;45(9):3109-10.
38. Fouchier RAM, Bestebroer TM, Herfst S, Van Der Kemp L, Rimmelzwaan GF, Osterhaus ADME. Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the matrix gene. J Clin Microbiol. 2000 Nov;38(11):4096-101.
39. Collignon PJ, Carnie JA. Infection control and pandemic influenza. Med J Aust. 2006 Nov 20;185(10 Suppl):S54-7.
40. U.S. Department of Health and Human Services. HHS Pandemic Influenza Plan [Internet]. Washington, D.C.: U.S. Department of Health and Human Services; 2005. Supplement 2, Appendix 1, Influenza diagnostic assays; [cited 2008 July 7]; p. S2-12-7. Available from: http://www.flu.gov/planning-preparedness/federal/hhspandemicinfluenzaplan.pdf.
41. Meguro H, Bryant JD, Torrence AE, Wright PF. Canine kidney cell line for isolation of respiratory viruses. J Clin Microbiol. 1979 Feb;9(2):175-9.
42. Zitterkopf NL, Leekha S, Espy MJ, Wood CM, Sampathkumar P, Smith TF. Relevance of influenza a virus detection by PCR, shell vial assay, and tube cell culture to rapid reporting procedures. J Clin Microbiol. 2006 Sep;44(9):3366-7.
43. Ogilvie M. Molecular techniques should not now replace cell culture in diagnostic virology laboratories. Rev Med Virol. 2001 Nov-Dec;11(6):351-4.

Received March 28, 2013
Accepted in revised form February 28, 2014