

NAZIV DISERTACIJE: EVALUACIJA DIREKTNIH METODA DETEKCIJE I ANTIGENSKE KARAKTERIZACIJE INFLUENZA VIRUSA**Autor: Edina Zahirović****Mentor: Prof. dr. Amela Dedeić-Ljubović****Datum odbrane: 07.07.2023.****DOI: <https://doi.org/10.17532/DD.2023.09>****SAŽETAK**

Influenca se definira kao akutna infektivna bolest respiratornog sistema prouzrokovana virusima influence tipa A, B i C. Trenutno, dva podtipa influenza A virusa: A(H1N1)pdm09 i A(H3N2) cirkulišu u ljudskoj populaciji. Postoje brojni dijagnostički postupci (direktni i indirektni), uključujući izolaciju virusa, test amplifikacije nukleinske kiseline, imunohromatografski brzi dijagnostički test, izolacija virusa iz bolesničkog uzorka na ćelijskoj kulturi ili embrionisanim kokošijim jajima, itd. za otkrivanje virusa influence kod ljudi. U našem istraživanju je izvršena evaluacija različitih metoda koje su trenutno dostupne za dijagnosticiranje influence kod ljudi.

Ciljevi ovog istraživanja su utvrditi zastupljenost tipova i podtipova influenza virusa u respiratornim uzorcima u toku dvije uzastopne sezone 2018/19 i 2019/20; ispitati učestalost različitih tipova i podtipova influenza virusa u odnosu na kliničke manifestacije influence (ILI, SARI), dob, spol i geografsku distribuciju; izvršiti izolaciju influenza virusa na ćelijskoj kulturi (MDCK i MDCK-SIAT); izvršiti antigensku karakterizaciju reprezentativnih virusnih izolata; utvrditi filogenetske odnose dokazanih virusa sa virusima različitog geografskog porijekla; izvršiti evaluaciju primjenjenih metoda detekcije i antigenske karakterizacije influenza virusa, te izraditi algoritam detekcije ovog virusa u referalnim laboratorijama.

U studiji su uključeni respiratorni uzorci sa teritorije Federacije BiH. Istraživanje je obavljeno u Odjelu za virusologiju – Klinička mikrobiologija, u Kliničkom centru Univerziteta u Sarajevu. Svi uzorci su analizirani metodom rtRT-PCR. Antigenska karakterizacija i izolacija virusa influence - umnožavanje MDCK ćelijskih kultura i inokulaciju uzoraka pozitivnih na influenza viruse izvršeni su u skladu sa preporukama SZO. Određeni broj uzoraka je testiran brzim testom za kvalitativnu detekciju virusa Influenca A i B. Sekvencirani su reprezentativni uzorci influenza virusa.

Ispitano je ukupno 948 respiratornih uzoraka u dvije sezone influence 2018/19 i 2019/20, od kojih je bilo 35,1% pozitivno na influenza virus. Influenca A virus bio je signifikantno zastupljeniji u odnosu na influenza B virus. Influenca A(H1pdm09) bio je dominantan u odnosu na ostale podtipove virusa. Podtipovi A(H1pdm09), A(H3) i influenza B virus, značajno češće su bili prisutni kod pacijenata sa SARI u odnosu ILI. Ispitivanje učestalosti tipova i podtipova influenza virusa, pokazalo je da ne postoji statistički signifikantna razlika u broju influenza pozitivnih uzoraka u odnosu na dobne grupe. Najveći broj influenza pozitivnih uzoraka dokazan je u Kantonu Sarajevo (261/333). Brzi test je pokazao nisku dijagnostičku senzitivnost od 13,6%. Procenat izoliranih influenza B virusa u MDCK kulturama iznosio je 76.5%, A(H1N1)pdm09 u sezoni 2018/19 78.8%; 2019/20 76.1%, a A(H3)virus u MDCK-SIAT ćelijskim kulturama 62.8% u 2018/19, a 87.9% u 2019/20. Zadovoljavajuće titrove dalo je samo 5.5% izolata A(H1N1)pdm09 podtipa.

Izolati influenza A(H1N1)pdm09 su, prema sekvenci HA gena, pripadali 6B genetičkoj grupi. Većina izolata A(H3N2) podtip bila je je grupisana u podklasteru 3C.2a2. Sva 3 izolata influenza B (Victoria) pripadala su 1A genetičkoj grupi. Metode molekularne detekcije virusa imaju veću specifičnost od metoda izolacije virusa u kulturi. Zbog veoma niske senzitivnosti RIDT u poređenju sa rtRT-PCR-om, ovaj test nije preporučen za predloženi algoritam.

DISSERTATION TITLE:**Author:** Edina Zahirović**Mentor:** Prof. dr. Amela Dedeić-Ljubović**Date of defence:** 07. July 2023.**DOI:** <https://doi.org/10.17532/DD.2023.09>**SUMMARY**

Influenza is defined as an acute infectious disease of the respiratory system caused by influenza viruses of type A, B and C. Currently, two subtypes of influenza A virus are circulating in the human population: A(H1pdm09) and A(H3). There are numerous diagnostic procedures (direct and indirect), including virus isolation, nucleic acid amplification test, immunochromatographic rapid diagnostic test, virus isolation from a patient sample on cell culture or embryonated chicken eggs, etc. for the detection of influenza viruses in humans. In our research, an evaluation of the various methods currently available for diagnosing influenza in humans was performed.

The aims of this research are to determine the prevalence of types and subtypes of influenza virus in respiratory samples during two continuously seasons 2018/19 and 2019/20; to examine the frequency of different types and subtypes of influenza virus, in relation to clinical manifestations of influenza (ILI, SARI), age, gender and geographic distribution, implement isolate influenza virus on cell culture (MDCK and MDCK-SIAT 1); carry out antigenic characterization of representative virus isolates; determine the phylogenetic relationships of confirmed viruses with viruses of different geographical origins; evaluate the applied methods of detection and antigenic characterization of influenza viruses, and create an algorithm for the detection of this virus in referral laboratories.

Respiratory samples from the territory of the Federation of Bosnia and Herzegovina were included in the study. The research was carried out in the Department of Virology - Clinical microbiology, at the Clinical Center of the University of Sarajevo. All samples were analyzed by the rtRT-PCR method. Antigenic characterization and isolation of influenza viruses - multiplication of MDCK cell cultures and inoculation of samples positive for influenza viruses were performed in accordance with WHO recommendations. A certain number of samples were tested with a rapid test for the qualitative detection of Influenza A and B viruses. Representative samples of influenza viruses were sequenced.

A total of 948 respiratory samples were tested in the two influenza seasons 2018/19 and 2019/20, of which 35.1% were positive for the influenza virus. Influenza A virus was significantly more prevalent than influenza B virus. Influenza A(H1pdm09) was dominant compared to other virus subtypes. Subtypes A(H1pdm09), A(H3) and influenza B virus were significantly more often present in patients with SARI than ILI. Examination of the frequency of influenza virus types and subtypes showed that there is no statistically significant difference in the number of influenza positive samples in relation to age groups. The largest number of influenza positive samples was proven in Sarajevo Canton (261/333). The rapid test showed a low diagnostic sensitivity of 13.6%. The percentage of isolated influenza B viruses in MDCK cultures was 76.5%, A(H1pdm09) in the 2018/19 season 78.8%; 2019/20 76.1%, and A(H3) virus in MDCK-SIAT cell cultures 62.8% in 2018/19, and 87.9% in 2019/20. Satisfactory titers were given by only 5.5% of isolates of the A(H1pdm09) subtype.

Influenza A(H1N1)pdm09 isolates, according to the HA gene sequence, belonged to the 6B genetic group. Most of the A(H3N2) subtype isolates were grouped in subcluster 3C.2a2. All 3 isolates of influenza B (Victoria) belonged to the 1A genetic group. Methods of molecular detection of viruses are more specific than methods of virus isolation in culture. Due to the very low sensitivity of RIDT compared to rtRT-PCR, this test is not recommended for the proposed algorithm.