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CO-CIRCULATION OF INFLUENZA A AND B VIRUSES AMONG HUMANS IN THE ARAL REGION OF THE REPUBLIC OF KAZAKHSTAN DURING THE 2015–2017 EPIDEMIC SEASONS

Abstract. In 2015-2017, 2105 biosamples (1978 nasopharyngeal swabs and 127 serums) were obtained from patients in polyclinics and infectious diseases hospitals in Aktobe and Kyzylorda regions of the Republic of Kazakhstan.

Using the polymerase chain reaction for 1978 samples collected from humans, the genetic material of the influenza A virus was detected in 10.86% of cases, that of the influenza B virus in 9.15%. While subtyping influenza A virus RNA, A/H1 subtype was identified in 9.76% of samples, A/H3 subtype in 89.30%. The results obtained from the screening of nasopharyngeal swabs in the polymerase chain reaction, as well as serological data in the hemagglutination inhibition reaction and enzyme immunoassay indicate co-circulation of the A/H1N1, A/H3N2 and B influenza viruses in humans in the Aktobe and Kyzylorda regions of the Republic of Kazakhstan during the 2015-2017 epidemic seasons.

In the virological study of nasopharyngeal swabs obtained from humans, 13 hemagglutination agents were isolated on chick embryos, 10 of which were identified in the hemagglutination inhibition and neuraminidase inhibition assays as influenza A/H1N1 viruses, and 3 as influenza B viruses.

The results from virological and serological studies indicate the need for continuous surveillance of the influenza virus circulation among humans in Aktobe and Kyzylorda regions in order to timely predict epidemic outbreaks and carry out preventive measures.

Keywords: circulation, influenza virus, subtype, isolate, hemagglutinin, neuraminidase, chain polymerase reaction, enzyme-linked immunosorbent assay.

Introduction. Among acute respiratory viral infections, influenza has the greatest clinical and epidemiological importance for humans. Each year about 600 million cases of influenza are registered worldwide; at that, 3 million people suffer from serious diseases that lead to a lethal outcome in 250,000 - 500,000 cases [1].

Since 1890, type A viruses periodically, at intervals of 10 to 40 years, cause pandemics resulting from the emergence of radically new variants of influenza viruses, against which there is little or no immunity in the human population, a process called antigenic shift. The last 2009/2010 influenza pandemic was caused by A(H1N1)pdm09 virus, which contains a complex combination of the gene segments of swine, avian and human influenza viruses. This virus completely replaced circulating earlier seasonal viruses A(H1N1) and continues to circulate around the world together with A(H3N2) and type B viruses [2].

Influenza viruses are the most variable among human viruses due to the high mutation rate, rapid replication, the presence of a segmented genome (which facilitates the gene recombination between different influenza viruses), and cases of the introduction of zoonotic A type viruses [3].

The spectrum of epidemic strains of influenza viruses and their characteristics vary depending on the season of the year. Recently, in Kazakhstan, as in many countries around the world, there is a simultaneous circulation of influenza viruses of the A(H1N1), A(H3N2) subtypes and B genus [4-8].

The purpose of this work was to study the peculiarities of the influenza virus circulation in the Aral region of Kazakhstan during the 2011-2017 epidemic seasons.

Research methods. The collection of clinical samples (nasopharyngeal swabs, serums) from patients was carried out in polyclinics and infectious diseases hospitals during the 2015-2017 epidemic periods in the Aktobe and Kyzylorda regions. The samples were stored in liquid nitrogen before initiation of virological studies.

Primary screening of nasopharyngeal swabs in real-time polymerase chain reaction (RT-PCR) was performed on a RotorGen 6000 amplification system (Corbett Research, Australia) with the RIBO-prep, AmpliSens® Influenza virus A/B-FL and AmpliSens® Influenza virus A type-FL kits (produced by the Central Research Institute for Epidemiology of Rospotrebnadzor, Moscow) [9].

Virus isolation was carried out in two systems using traditional methods: on the MDSC culture with the addition of TRNC-trypsin (2 μg/mL) and 9-11 day-old chick embryos (CE). To indicate the virus in the hemagglutination assay (HA), a 0.75% suspension of the chicken and human 0(1) blood group erythrocytes was used.

The infectious activity of isolates was determined according to the conventional method [10], and their titer was expressed in lg $EID_{50/0.2ml}$ and lg $TCD_{50/0.2ml}$.

Identification of isolates was carried out in the hemagglutination inhibition (HI) and neuraminidase inhibition (NAI) assays with polyclonal diagnostic serum kits according to WHO recommendation [11, 12].

The level of specific antibodies against influenza viruses in serum was determined in HI assay and enzyme-linked immunosorbent assay (ELISA). HI assay was carried out according to the WHO recommendation using both reference viruses A/California/04/09 (H1N1), A/Solomon Islands/03/06 (H1N1), A/USA/1976/31 (H1N1), A/Aichi/2/68 (H3N2), A/Panama/2007/99 (H3N2), B/Florida/04/06, and commercial diagnosticums produced by the FSBI Research Institute of Influenza (St. Petersburg). The test systems intended for influenza viruses of A (H1N1), A (H3N2) subtypes and type B produced by EPDP LLC (Enterprise for the Production of Diagnostic Preparations, St. Petersburg) were used in ELISA.

Results and discussion. The materials were collected during the 2015-2017 epidemic seasons in the medical institutions located in the Aktobe and Kyzylorda regions. In total, 1978 upper respiratory tract swabs and 127 serums were taken from the patients.

More than 90% of the samples were collected from patients diagnosed with acute respiratory viral infection. The greatest number of nasopharyngeal swabs (1291) was obtained from children under 14 years of age (65.27%).

Table 1 shows the characteristics of the collected material and results of the primary RT-PCR based screening of nasopharyngeal swabs.

As can be seen from Table 1, while studying 293 samples collected in 2015, the genetic material of the influenza virus was detected in 40 samples (13.6% of the total number of samples). Influenza A virus RNA was detected in 37 samples (12.6%), that of influenza B virus in 3 samples (1.0%). Subtyping made it possible to detect A/H1N1 virus RNA in 8 swabs (2.7% of cases), A/H3N2 virus RNA in 27 samples (9.2%).

Of 112 samples taken from patients in 2016, the genetic material of the influenza virus was detected in RT-PCR in 18 samples (16.1% of the total number of samples). Influenza A virus RNA was detected in 16 samples (14.3%), that of influenza B virus in two samples (1.8%). Subtyping made it possible to detect A/H1N1 virus RNA in 13 swabs (11.6% of cases), A/H3N2 virus RNA in 3 samples (2.7%).

When examining 1573 biosamples obtained in 2017, the genetic material of the influenza virus was detected in 338 samples (21.5% of the total number of samples). Influenza A virus RNA was detected in 162 biosamples (10.3%), that of influenza B virus in 176 samples (11.2%). Subtyping of PCR-positive samples for influenza A virus revealed the presence of the genetic material of A/H3N2 virus in all 162 samples; it was not possible to detect A/H1N1 virus RNA.

Table 1 – Characterization and RT-PCR based screening of clinical samples collected from humans in 2015-2017

Year	Sampling site	Number of nasopharyn-geal swabs	Number of PCR-positive samples				Number of serums
			for influenza A virus	for viruses of subtypes:		for influenza B virus	
				A/H1N1	A/H3N2		
2015	Aktobe region	39	8	5	1	2	25
	Kyzylorda region	254	29	3	26	1	23
Total:		293	37	8	27	3	48
2016	Aktobe region	17	4	3	2	0	22
	Kyzylorda region	95	12	10	1	2	-
Total:		112	16	13	3	2	22
2017	Aktobe region	768	137	0	137	96	25
	Kyzylorda region	805	25	0	25	80	32
Total:		1573	162	0	162	176	57
Total for 3 years		1978	215	21	192	181	127

Therefore, the primary RT-PCR based screening of nasopharyngeal swabs showed that influenza A and B viruses co-circulated among humans in the Aktobe and Kyzylorda regions in 2015-2017. At the same time, the influenza A/H3N2 virus, which prevailed in 2015 and gave the place to the A/H1N1 virus in 2016, manifested itself again in 2017.

As a result of primary infection and subsequent passages on CE and MDCK cultures, 13 hemagglutinating agents were isolated from PCR-positive samples with titers on CE from 1:32 to 1:1024 and on MDCK culture from 1:4 to 1:32.

Identification of 2015-2017 isolates was carried out in HI and NAI assays. The results of determining the hemagglutinin subtype in the isolates are given in table 2.

Table 2 – Identification of hemagglutinin subtypes for the 2015-2017 influenza virus isolates in HI assay

Isolate	Titer of immune serum antihemagglutinin					
	A/USA/1976/31 (H1N1)	A/Solomon Islands/03/06 (H1N1)	A/California/04/09 (H1N1)pdm	A/Aichi/2/68 (H3N2)	A/Panama/2007/99 (H3N2)	B/Florida/04/06
	1280*	**640**	**640**	**640**	**640**	**640**
Aktobe/02/15	160	160	160	<20	<20	<20
Aktobe /03/15	80	40	40	<20	<20	<20
Aktobe /06/15	80	20	20	<20	<20	<20
Aktobe /18/15	80	20	20	<20	<20	<20
Aktobe /20/15	320	160	160	<20	<20	<20
Kyzylorda/83/15	160	160	160	<20	<20	<20
Kyzylorda /176/16	40	80	40	<20	<20	<20
Kyzylorda /177/16	160	40	20	<20	<20	<20
Kyzylorda /178/16	80	20	20	<20	<20	<20
Kyzylorda/185/16	320	160	160	<20	<20	<20
Kyzylorda /21/17	<20	<20	<20	<20	<20	80
Kyzylorda/28/17	<20	<20	<20	<20	<20	160
Aktobe /73/17	<20	<20	<20	<20	<20	80

*Homologous antibody titers for reference serums are presented; homologous antibody titer for reference serums against A/USA/1976/31 (H1N1) strain was of 1:1280, for the remaining ones of 1:640.

As can be seen from table 2, the hemagglutinating activity of the Aktobe/02/15, Aktobe/03/15, Aktobe/06/15, Aktobe/18/15, Aktobe/20/15, Kyzylorda/83/15, Kyzylorda /176/16, Kyzylorda /177/16, Kyzylorda /178/16, and Kyzylorda/185/16 isolates from 1/32 to 1/4 of the homologous titers was suppressed by immune serums against the A/USA/1976/31 (H1N1), A/Solomon Islands 03/06 and A/California /04/09 (H1N1) pdm viruses. This allowed attributing HAA to the influenza A virus with the H1 hemagglutinin subtype.

The hemagglutinating activity of three isolates (Kyzylorda /21/17, Kyzylorda/ 28/17 and Aktobe /73/17) from 1/8 to 1/4 of the homologous titer was suppressed by immune serums against the influenza B/Florida/04/06 virus. Serums against influenza A/USA/1976/31 (H1N1), A/SolomonIslands/03/06 (H1N1), A/California /04/09 (H1N1) pdm, and A/Aichi/2/68 (H3N2) viruses gave the negative results, which made it possible to classify the 2017 isolates as influenza type B virus.

The results from subtype identification of the second surface glycoprotein for influenza A virus isolates in NAI assay are presented in table 3.

Table 3 – Identification of neuraminidase subtype for the 2015-2016 influenza virus isolates in NAI assay

Isolate	Antibody titer against neuraminidase subtypes	
	N1	*N2*
A/Aktobe/02/15	100	<20
A/Aktobe /03/15	100	<20
A/Aktobe /06/15	100	<20
A/Aktobe /18/15	100	<20
A/Aktobe /20/15	100	<20
A/Kyzylorda/83/15	100	<20
A/Kyzylorda/176/16	100	<20
A/Kyzylorda/177/16	100	<20
A/Kyzylorda/178/16	100	<20
A/Kyzylorda/185/16	100	<20
Note. The reciprocals of antineuraminidase antibody titers are presented.		

It can be seen from Table 3 that the neuraminidase activity of all isolates in titers of 1:100 was suppressed by the immune polyclonal serum against the A/H1N1 virus.

Therefore, according to the results of HI and NAI assays, the 2015-2016 isolates were attributed to influenza A viruses with A/H1N1 antigenic formula, and the 2017 isolates to influenza type B virus.

To evaluate the 2015-2017 seroepidemiological situation of influenza in the Aral region, 127 serums were examined in HI assay and ELISA. The results of HI assay are shown in figure 1.

As can be seen from figure 1, in the 2015 epidemic season, antihemagglutinins to the influenza A/H3N2 virus were detected in human serums in 60.4% (29 samples), in 10.5% of cases (5 samples) the serums were found to be seropositive against the influenza A/H1N1 virus. The serums were positive against influenza B virus in 6.3% of cases (3 samples); antihemagglutinins simultaneously to influenza A/H1N1 and A/H3N2 viruses were detected in 4.2% (2 samples) and to influenza A/H3N2 and B viruses in 2.1% (1 sample). Antibody titers were of 1:80-1:320.

In 2016, antihemagglutinins to the influenza A/H1N1 virus were detected in human serums in 40.9% of cases (10 samples), 18.2% of cases (4 samples) were found to be seropositive against the influenza A/H3N2 virus. In 9.1% of cases (2 serums) antihemagglutinins to influenza B virus were detected.

In 2017, antihemagglutinins to the serotype A/H3N2 virus were detected in 47.4% of cases (27 samples), to influenza type B virus in 5.3% (3 samples); antibodies simultaneously to influenza A (H1N1 + H3N2) viruses were detected in 19.3% of cases (11 samples). Antibody titers were of 1:80-1:320.

Figure 2 presents the results of a serological study of 127 serums in ELISA.

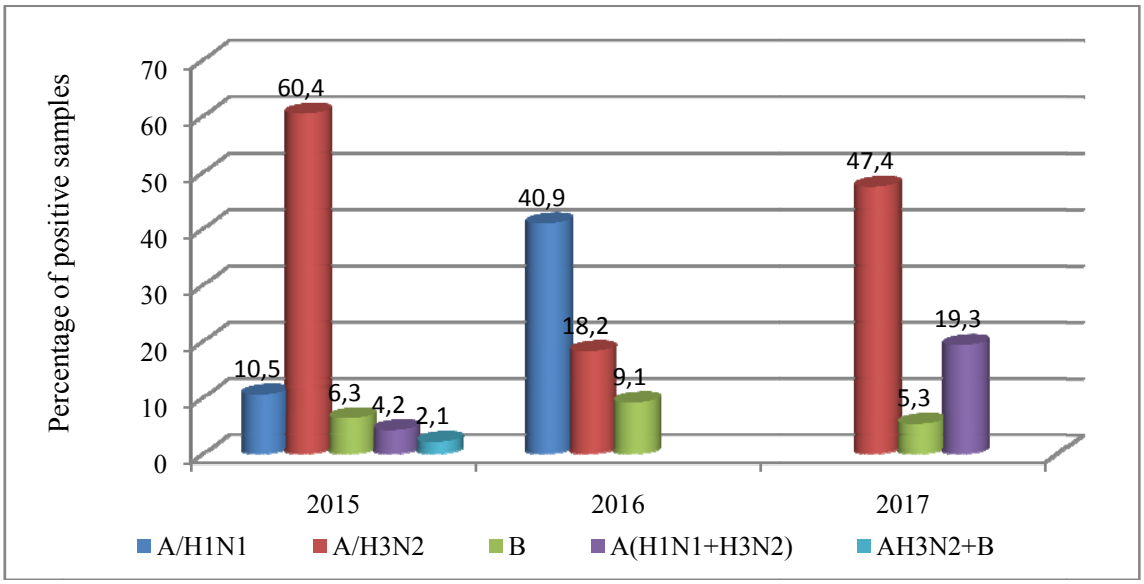

Figure 1 – Detection of specific antibodies against influenza viruses in serums in HI assay

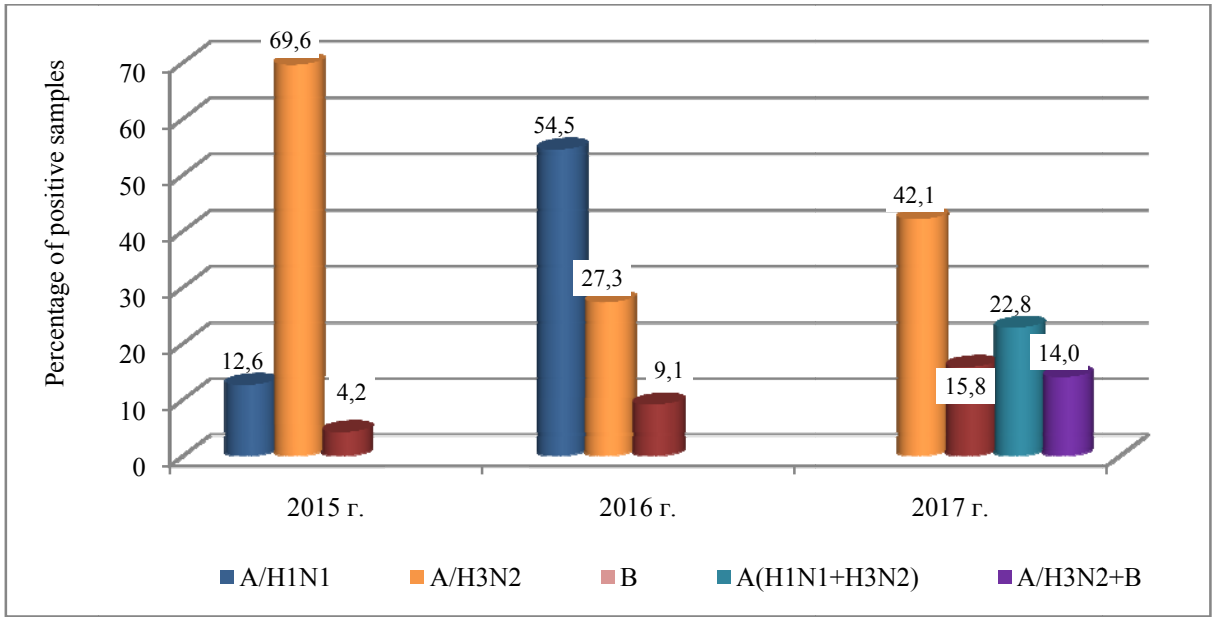

Figure 2 – Identification of antibodies against influenza viruses in serums in ELISA

As can be seen from figure 2, in the 2015 epidemic season, antibodies against the influenza A/H3N2 virus were detected in 69.6% of cases (28 samples), influenza A/H1N1 virus in 12.6% (6 samples), and influenza B virus in 4.2% (2 serums).

While studying 22 serums obtained in 2016, antibodies against the influenza A/H1N1 virus were detected in 54.5% of cases (12 samples), influenza A/H3N2 virus in 27.3% (6 samples), and influenza B virus in 9,1% (2 serums).

In the 2017 epidemic season, antibodies against the influenza A/H3N2 virus were detected in the vast majority of serums (42.1% - 24 samples), influenza B virus in 15.8% of the serums (9 samples); antibodies simultaneously against A (H1N1+H3N2) viruses were detected in 22.8% of cases (13 serums), A/H3N2 and B viruses in 14.0% (8 samples).

Therefore, the results from serological studies of serums in ELISA and HI assay indicate co-circulation of influenza A/H1N1, A/H3N2, type B viruses and mixed influenza infection in the Aktobe and Kyzylorda regions during the 2015-2017 epidemic seasons. A distinctive feature of the 2017 epidemic season is the high content of antibodies against the A/H3N2 and B virus.

According to the literature data, recently there has been a simultaneous circulation of strains representing various evolutionary lines of influenza A and B viruses [4, 13-16]. At that the antigenic composition of the viral population varies depending on the epidemic seasons [17-19]. Subtypes H1N1 and H3N2 of influenza A viruses are widespread among humans. Influenza B virus infection proceeds easier as compared with type A, produces small outbreaks and rare mutations [20].

The unique antigenic variability of influenza viruses, which allows them to overcome interspecies barriers, leads to the emergence of viruses with new biological properties that are capable of wide epidemic spread. [21] In connection with this the most important areas of the fight against influenza include the surveillance of the infection spread, timely pathogen diagnostics, and disease prevention.

Conclusions. In the initial screening of nasopharyngeal swabs and serological studies of serums collected in the 2015 -2017 epidemic season from the patients in Aktobe and Kyzylorda regions, co-circulation of influenza A/H3N2, A/H1N1 and B viruses was established in RT-PCR, HI assay, and ELISA.

As a result of virological studies, ten isolates of influenza A/H1N1 and three isolates of influenza B viruses were obtained from clinical samples, which confirmed the circulation of influenza viruses in the region.

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2015-2017 ЖЖ. ТҰМАУ ІНДЕТІ АРАЛЫҒЫНДАҒЫ ҚАЗАҚСТАН РЕСПУБЛИКАСЫ АРАЛ МАҢАЙЫ ТҰРҒЫНДАР АРАСЫНДАҒЫ А ЖӘНЕ В ТҰМАУ ВИРУСТАРЫНЫҢ АЙНАЛЫМЫ

Аннотация. 2015-2017 жж. аралығында Ақтөбе және Қызылорда облыстарындағы инфекциялық емханаларымен поликлиникаларындағы сырқат адамдардан 2105 биосынамалар алынды. (1978 танау-мұрын жағындысы және 127 қан сарысуы).

Полимеразды тізбекті реакциясында адамдардан жиналған 1978 үлгіден А тұмау вирусының генетикалық материалы 10,86% жағдайында анықталды, В тұмау вирусы – 9,15%. А тұмау вирусын субтиптеу кезінде А/Н1 тұмау вирусы – 9,76% сынамасында анықталса, А/Н3 – 89,3% құрады.

Мұрын-танау жағындысын полимеразды тізбекті реакциясында скрининг жүргізу және қан сарысуын гемагглютинация тежеу рекциясымен иммуноферментті талдаудағы зерттеу нәтижелері, Ақтөбе және Қызылорда облыстарындағы адамдар арасында 2015-2017 жж. А/Н1N1, А/Н3N2 және В тұмау вирустары айналымда жүргендігін көрсетеді.

Адамдардан жиналған мұрын-танау жағындыларын вирусологиялық зерттеу нәтижесінде, тауық эмбриондарында 13 гемагглютининдеуші агент бөлініп алынды. Нейраминидаз белсенділігін тежеу реакциясы және гемагглютинация тежеу реакциясында 10 А/Н1N1 тұмау вирусы, 3 В тұмау вирусы болып анықталды.

Вирусологиялық және серологиялық зерттеулердің нәтижелері Ақтөбе және Қызылорда облыстарындағы адамдар арасындағы тұмау індетін алдын-ала болжау және профилактикалық іс-шараларды жүргізу үшін, тұмау айналымын үздіксіз қадағалау қажеттілігін көрсетеді.

Түйін сөздер: айналым, тұмау вирусы, типасты, изолят, гемагглютинин, нейраминидаза, полимеразды тізбекті реакция, иммунноферметті талдау.

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СОЦИРКУЛЯЦИЯ ВИРУСОВ ГРИППА А И В СРЕДИ ЛЮДЕЙ В АРАЛЬСКОМ РЕГИОНЕ РЕСПУБЛИКИ КАЗАХСТАН В ЭПИДЕМИЧЕСКИЕ СЕЗОНЫ 2015-2017 ГГ.

Аннотация. В 2015-2017 гг. в Актюбинской и Кызылординской областях РК от больных людей в поликлиниках и инфекционных больницах получено 2105 биопроб (1978 носоглоточных смыва и 127 сывороток крови).

В полимеразной цепной реакции в 1978 образцах, собранных от людей, генетический материал вируса гриппа А был обнаружен в 10,86% случаев, вируса гриппа В – в 9,15%. При субтипировании РНК вируса гриппа А подтип A/H1 идентифицирован в 9,76% проб, A/H3 – в 89,30%.

Результаты, полученные при скрининге носоглоточных смывов в полимеразной цепной реакции, также как и данные серологических исследований в реакции торможения гемагглютинации и иммуноферментном анализе, указывают на социркуляцию вирусов гриппа A/H1N1, A/H3N2 и В у людей в Актюбинской и Кызылординской областях РК в эпидемические сезоны 2015-2017 гг.

При вирусологическом исследовании носоглоточных смывов, полученных от людей, на куриных эмбрионах выделено 13 гемагглютинирующих агентов, 10 из которых идентифицированы в реакции торможения гемагглютинации и реакции ингибиции нейраминидазной активности как вирусы гриппа A/H1N1, три – как вирусы гриппа В.

Результаты вирусологических и серологических исследований свидетельствуют о необходимости проведения постоянного надзора за циркуляцией возбудителей гриппа среди людей в Актюбинской и Кызылординской областях с целью своевременного прогнозирования эпидемических вспышек и проведения профилактических мероприятий.

Ключевые слова: циркуляция, вирус гриппа, подтип, изолят, гемагглютинин, нейраминидаза, цепная полимеразная реакция, иммунноферметный анализ.

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