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A.A. Alimbekova, Zh.A. Kalmatayeva

Higher school of public healthcare of the MH RK, Almaty, Kazakhstan

INFLUENCE OF PHYSICAL ACTIVITY ON THE QUALITY OF LIFE OF ELDERLY PEOPLE

Abstract. *Currently in Kazakhstan elderly people comprise more than 7,7 % of population. According to the UN experts' forecasts in the near future increase of elderly population is expected in Kazakhstan by 11 % [1].*

Improvement of medical care to elderly people, comprehensive solution of medical-biological, social, psychological aspects have been identified as one of priorities of State program «Salamatty Kazakhstan» for 2011-2015. One of the key indicators of Program implementation is increase of life expectancy of population by 2015 – to 70 years, and one of the tasks is expansion of the coverage of population by mass sport events and development of sectoral program of physical education and sports development for population.

Aim of the research*: identification of influence of physical activity on quality of life of elderle people.*

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A. A. Alisheva, S.K. Karabalin

Higher school of public healthcare, Almaty, Kazakhstan

ATTITUDE OF STUDENTS TO HEALTH AND ITS INFLUENCE ON PREVENTION OF IODID DEFFICIENCY AND IRON DEFFICIENCY ANEMIA

Abstract. *The article presents significance of health condition and youth's attitude to health as an indicator of social-economic welfare of the country. According to the WHO data, health condition depends largely on the lifestyle, which means that to certain extent on the attitude of a person towards his health. Great role is played by awareness of population on the issues of preserving health, prevention of different pathologies. Problems of iodide and iron deficiency have been always relevant for Kazakhstan. So, according to the data of Kazakh nutrition academy up to 60% of women of reproductive age suffer from iodide deficiency and 40 to 76% - from iron deficiency.*

The aim of the work is assessment of attitude of students to their health, awareness on the issues of iodide deficiency, iron deficiency anemia, as well as collective responsibility for their health. Survey of 422 students of Almaty was done, 400 questionnaires have been statistically reviewed. The questionnaire had 33 questions, characterizing life style of the students, attitude to health, awareness in the issues of iodide and iron deficiency and collective responsibility for heath.

The study has shown that more than half of surveyed students (245 students or 61,25%) do not know that Kazakhstan is part of the iodide deficiency region. 48% (191 people) of the respondents understand the problem of iron deficiency anemia in the country, however 1/3 (124

people - 31%) were undecided, among them 19% females – potential mothers. Furthermore statistically significant differences were identified between male and female. With regards to readiness of the students to collective responsibility, only 17 respondents were informed about it, whereas 312 people stated that health is only responsibility of a person itself.

UDC: 614.2:616-053.2 (574-25)

A.A. Assen[1], G.T. Meschanov[2]

[1]Research center for pediatrics and pediatric surgery, Almaty, Kazakhstan
[2]Higher school for public healthcare, Almaty, Kazakhstan

ANALYSIS OF THE KEY PERFORMANCE INDICATORS OF THE RESEARCH CENTER FOR PEDIATRICS AND PEDIATRIC SURGERY, ALMATY

Abstract. *Among the refraction anomalies the leading place belongs to myopia, the prevalence of which among high school graduates is 20-25%. The main reasons for blindness and hypovision are visual nerve and retina pathology, congenital eye diseases (cataract, glaucoma, development anomalies), premature born babies' retinopathy, uveitis, high degree myopia. In the last years the role of retinopathy among premature babies as a cause of blindness and hypovision [1].*

The aim of the study is to analyse activities of the Research center for pediatrics and pediatric surgery (RCPPS) on provision of highly specialized medical care to newborns with retinopathy and development of practical proposals for organizational improvement. In order to achieve the aim one of the objective was to study the key performance indicators of the RCPPS.

Research materials include key performance indicators of the RCPPS.

Key performance indicators were assessed with the help of comparative analysis of the work in 2012-2013. Both qualitative and quantitative indicators were assessed.

In 2013 the main number of children were hospitalized from Almaty region– 51,23±3,1% and Almaty city – 36,4±3,0% (for laser coagulation of retina for prematures with retinopathy). At the same time the number of children admitted with congenital malformations from Almaty decreased by 1,3 times and comprised 8,7±1,0% of the total number of hospitalized children (in 2012 - 11,7±2,1%). 52,6±3,2% of the children with premature retinopathy secondary to conservative treatment regress of the disease was seen. 47,4±2,7% of the children had laser coagulation of avascular retina zones. Efficiency of the surgical treatment was 92±5,2%.

UDC: 614:658.562 (574-25)

A.A. Assen[1], G.T. Meschanov[2]

[1]Research center for pediatrics and pediatric surgery, Almaty, Kazakhstan
[2]Higher school for public healthcare, Almaty, Kazakhstan

ASSESSING THE SATISFACTION LEVELS AMONG EMPLOYEES WITH WORK CONDITIONS AND PARENTS WITH QUALITY OF MEDICAL CARE IN THE RCPPS, ALMATY

Abstract. *Taking into account high prevalence of the sight organs among children, high level of disabilities in such cases, as well as importance of visual functions for active life of a human being, it becomes clear that proper organization of ophthalmological care to children is highly important [1].*

The aim of the study was analysis of the activities of the RCPPS on provision of highly specialized care for newborns with retinopathy and development of the practical recommendations for improvement of care. In order to achieve the aim one of the objectives was to study satisfaction levels of the employees with work conditions and patients with quality of medical care.

The employees of the Almaty Research center for pediatrics and pediatric surgery gave objective evaluation of the work conditions and conditions of stay for patients' mothers.

According to the satisfaction level assessment it should be noted that out of 37 employees slightly more than half is satisfied with the equipment of the workplace. This is a reality of current situation and the center is no exception to it.

The satisfaction level with the quality of medical care on a 5-point scale was 73,3±8,1%. 97±3,1% of the parents are satisfied with the attention levels from the staff. 90±5,5% of the patients are satisfied with explanations and recommendations of the physicians. 87±6,1% of the parents have positively evaluated the qualification of nurses.

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A.Zh. Duysenbekova[1], G.T. Meschanov[2]

[1]«OpenClinic» LLP, Almaty, Kazakhstan
[2]Higher school of public healthcare, Almaty, Kazakhstan

HEALTH CONDITIONS OF THE CHILDREN WITH IDENTIFICATION OF THE MOST FREQUENT PATHOLOGY

Key words: *health condition of the children, screening study, morbidity, pathology.*

Abstract. *Health of pediatric population is a part of public health, because comprising of the whole range of signs of individual health and integrating social-economic features, it receives new features and qualities.*

Material for the research included results of the morbidity data studies among pediatric population of the Republic of Kazakhstan in 2005-2010. Information basis was the data of the Ministry of healthcare of the RK (statistical collections for the abovementioned years). Statistical analysis of the time series was used as a mathematical tool.

Leading positions were taken by respiratory diseases (18,4%), diseases of the blood and blood-forming organs (16,7%), diseases of digestive system (13,7%). Then came endocrine system diseases (9,2%), diseases of musculoskeletal system (7,6%) and genitourinary system diseases (5,9%), which combined comprised 71,5% of total morbidity.

Big influence on health of a child is exerted by living conditions, quality and regime of nutrition, psychological and emotional environment in the family, school, availability of modern medical care. The data prove once again that children are considered to be the most sensitive population group to any influence of negative environmental factors.

UDC: 614-378.4

L.T. Imangaliyeva[1], G.T. Meschanov[2]

[1]City student clinics, Almaty, Kazakhstan
[2]Higher school of public healthcare, Almaty, Kazakhstan

HEALTH CONDITION EVALUATION OF THE STUDENTS OF MEDICAL HIGHER EDUCATIONAL INSTITUTIONS IN ALMATY

Abstract. *It is commonly known that health condition of the students directly depends on the inconvinieently organized learning process, decrease of physical activity, incorrect schedule of workload and recreation, as well as living conditions. Lately analysis of the research, devoted to health expertise of the urban student population shows that the number of «healthy» students is decreasing every year. As the results of the studies in this direction have shown, the number of first-year student in this group was on average 43%, but by the last year this indicator decreased to 34%[1].*

Materials of the research included morbidities identified according to the reports on instances of turning for medical care by the students: 356 first-year students and 214 5th year students and the data from the samples 112/y and 025/y. The study timeframe was 2011-2012. The identified data was subject to statistical review with application of the Student's criterion and subdivided into lines according to the groups; comparisons were made between the groups, which was the basis for graphic representations and tables.

Intergroup spread of the signs and collection of claims related to the diseases of the blood and blood-forming organs are similar to the regularities of the spread of the abovementioned diseases; the amount among 1st year students was 190,4±7,33; the established representative indicator among the 5th year students was 100,5±7,32. Signs of the diseases of respiratory system have also shown high prevalence in the group of 1st year students, they were registered 1,3 times more often (181,8±6,45), than the indicator of the 5th year students, approximately 242,8±12,13.

UDC: 616.98:579.852

R.O.Kasymova[1], D.A. Bayizbekovaa[2], K.B. Bekturdiev[3], O.T. Kasymov[2]

[1]Kyrgyz-Russian Slavonic University of the Ministry of Education and Science of the Kyrgyz Republic and the Ministry of Education and Science of the Russian Federation, Bishkek
[2]Scientific and Production Centre for Preventive Medicine of the Ministry of Health of the Kyrgyz Republic, Bishkek
[3]Republican Centre for Quarantine and Especially Dangerous Infections of the Ministry of Health of the Kyrgyz Republic, Bishkek

EPIDEMIOLOGY OF ANTHRAX IN THE KYRGYZ REPUBLIC

Key words: *epidemiology, anthrax, natural nidality*

Abstract: *In the Kyrgyz Republic, which has a centuries-long orientation of economy to livestock breeding, there have formed persistent soil foci of anthrax almost all over the country creating a constant threat of anthrax epidemics. The analysis of official reporting data on anthrax cases between 1960 and 2013 revealed a considerable increase in cases in the last years. Territorial distribution of anthrax cases is uneven in Kyrgyzstan, with 66 to 93% of cases occurring in the southern regions. In the south of the country there exists a persisting poor epizootic situation*

which is related mainly to the presence of a great number of soil foci (665). The location of 270 (40.6%) foci is not established.

UDC: 614.253.83(002)

G. K. Kaussova [1] **, A.I. Nuftieva** [2]

[1] Higher school of public healthcare of the MH RK,

[2] S.D. Asfendiyarov Kazakh national medical university, Almaty, Kazakhstan

TO THE ASSESSMENT OF STATE PROGRAM FOR CARDIAC AND CARDIAC SURGERY CARE DEVELOPMENT IN THE REPUBLIC OF KAZAKHSTAN FOR 2007-2009 ON THE LEVEL OF PRIMARY MEDICAL CARE

Abstract. *Circulatory system diseases (CSD) in the last quarter of a century take the first place among the causes of disability and mortality among the population of the Republic of Kazakhstan (RK), which justifies the need to take effective measures for improvement of cardiac and cardiac surgical care in the republic.*

The Program for cardiac and cardiac surgery care development in the Republic of Kazakhstan for 2007-2009 was developed in accordance with the legislation and is aimed at taking urgent measures on development of cardiac and cardiac surgery care.

The aim of the program is to decrease mortality from the CSD. The main objectives of the Program are: development and improvement of organizational structure of cardiac care and cardiac surgery in the RK; implementation of efficient prevention, early identification, treatment and medical rehabilitation measures for patients and disabled people with CSD; training and professional development of the cardiac surgery specialists [1].

One of the tasks of medicine according to the address of the President of the RK N. Nazarbayev is creation of an efficient medical care systems, based on priority development of socially oriented primary medical care (PMC).

New qualitative level of healthcare can be reached by significant changes in the technology of services provision, primarily on the level of PMC. In order to increase availability, efficiency, quality and development of PMC a comprehensive improvement is planned, including development and implementation of rational forms and methods of PMC provision on the basis of general practice.

The article analyses implementation of the program for cardiac and cardiac surgery care development on example of Almaty. Review of cardio-vascular morbidity was done among the aged population; results of screening and preventive check-ups. The following was identified: growth of the CSD, low quality of medical examinations and rehabilitation of cardiac patients. It is necessary to strengthen interest and participation of population with medical organizations for the purpose of improving health by means of awareness raising.

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S.M. Kisselyov

Zaporizhia medical university, Zaporizhia, Ukraine

MARKERS OF PARIETAL THROMB FORMATION AMONG PATIENTS WITH Q-WAVE HEART ATTACH WITH ACUTE ANEURYSM OF LEFT VENTRICLE

Abstract. *In the developed countries myocardial infarction (MI) is one of the leading causes for persistent occupational disability and mortality of the patients. Regardless of the recent successes in treatment of Q-wave AMI due to fundamental and clinical studies and prevention of complications, the frequency of left ventricle aneurysm and parietal thromb occurrence is still high and reaches about 30%. Detailed study of the mechanisms of aneurysm and thromb formation in the left ventricular cavity will improve prevention and treatment of such dangerous complications of the Q-wave AMI.*

Aim*: to identify the main markers for formation of parietal thromb in patients with Q-wave MI with left ventricle aneurysm.*

Patients and methods. *Serumal C-protein level, von Willebrandt factor, expression of the vascular adhesion molecules sVCAM-1, aggregation properties of the platelets were studied among 110 patients (average age – 63,2±5,7 years) with acute Q-wave MI and aneurism and parietal thromb. The first group included 72 patients with post-MI aneurysm of LV, the second – 38 patients with post-MI aneurysm and parietal thromb of LV.*

UDC: 614.2:616.9(574.13-25)

K.E. Kozbagarov[1], G.T. Meschanov[2]

[1]Regional clinical infectious diseases hospital, Aktobe, Kazakhstan
[2]Higher school of public healthcare, Almaty, Kazakhstan

ANALYSIS OF THE KEY PERFORMANCE INDICATORS OF THE REGIONAL CLINICAL INFECTIOUS DISEASES HOSPITAL OF AKTOBE SITY

Key words: *average stay, mortality, cytomegalovirus infection.*

Abstract. *Lately due to changes in social-economic conditions the situation with infectious morbidity aggravated significantly not only in our republic, but in other CIS countries as well, namely in the Russian Federation (RF), especially in large cities and towns [1,2]. Diphteria, influenza epidemics were serious problems for healthcare, taking leading positions according to morbidity [3, 4].*

The aim of the research was analysis of activity of the regional clinical infectious diseases hospital (RCIDH) of Aktobe and development of practical recommendations for improvement. In order to achieve the aim one of the objectives was to evaluate the levels and dynamics of key performance indicators of the RCIDH of Aktobe.

Materials for research were performance indicators of the hospital.

Key performance indicators were assessed with the help of comparative analysis of the work in 2011-2013. Both qualitative and quantitative indicators were identified.

Decrease of the average stay was noted in the dynamics, which is related to the fact that patients do not want to have a lengthy stay in the in-patient care and in accordance with the new treatments protocols of the MH RK. Irregular increase and decrease of mortality among children and adults is seen in the period from 2011 to 2013, which can be related to hospitalizations in different seasons of the year and the level of medical care provision in different parts of PMC and emergency medical care.

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M.M. Pisareva, A.B. Komissarov, Z.V. Buzitskaya, M.A. Stukova, M.P. Grudinin

Federal State Budget-Supported Institution Influenza Research Institute of Russia's Ministry of Health

GENE DIAGNOSIS OF INFLUENZA

Abstract. *The epidemic spread of influenza, airborne route of transmission, severity of illness and related complications cause the need for rapid and effective diagnosis, which further defines the timely antiviral therapy.*

The epidemic situation of human influenza in the world and in Russia in the last decade was determined by the circulation of antigenically similar variants of influenza A (H1N1), A (H3N2) and B. The complication of the epidemic situation in this period is due to the infection of people with the avian influenza A viruses (H5N1, H7N7, H7N3, H9N2, H7N9), influenza A(H1N2) virus which is the result of reassortment of co-circulating human influenza viruses A/H1N1 and A/H3N2 [6, 17] and, finally, the emergence of the swine-origin triple-reassortant influenza A virus (H1N1pdm09) in the human population in 2009.

Diagnosis of influenza viruses to date includes several methods that allow direct detection of influenza viruses.

The culture method which is used in virology laboratories for the isolation and typing of virus strains has a high specificity and sensitivity, but requires a significant amount of time prior to the result, depends to a large extent on the quality of cell cultures and can be used only in virology laboratories with appropriate levels of biological safety.

Currently, the **method of direct immunofluorescence** (antigen detection of the pathogen in the biological material) is widely used in the laboratory practice. The shortcomings of the method are low specificity and sensitivity, and subjective evaluation of test results. In addition, high requirements are imposed in this method for storage of the clinical material (no more than 24 hours from the time of collection).

In 1983, the American scientist Cary Mullis invented the polymerase chain reaction (PCR), for which he was awarded the Nobel Prize [12]. Currently, the PCR diagnosis is perhaps the most accurate and sensitive method for the diagnosis of infectious diseases. The PCR is a method of molecular diagnosis, which has become a "gold standard" test for a number of infections, which have been proven over time and thoroughly tested clinically.

The method of PCR analysis explicitly indicates the presence of a specific fragment of the influenza virus RNA in the material collected from the patient.

This method does not have all above-mentioned shortcomings, has the highest

specificity and sensitivity and allows obtaining reliable results in a short time.

The specificity of the PCR diagnosis reaches 100%. During the analysis, the RNA fragment, which is specific to a certain type or subtype of influenza virus, is isolated in the tested material. This RNA fragment is unique and cannot be found in any known gene sequence in the world (specific to a given pathogen).

The PCR method has the highest sensitivity compared with other known diagnostic methods. Detection of influenza virus RNA is theoretically possible even if the collected biological material contains only a single virus particle. When compared with other immunological and microbiological diagnosis methods, the PCR sensitivity is 10-100 genome copies in a sample, while the sensitivity by other methods is lower - 10^3-10^5 copies of a pathogen-specific agent.

Various, virtually any material, including the material that cannot be used in tests by other methods, can be used in PCR-tests. Samples taken from the upper respiratory tract are recommended for gene diagnosis of influenza. The following samples should be collected: from nasal openings (nasal swab), nasopharynx (nasopharyngeal swab), nasopharyngeal aspirate, throat or bronchial aspirates. It is not yet known which type of samples gives the best product for diagnosis. Proper precautions should be taken when collecting samples because the collecting person can be exposed to respiratory secretions of patients. In addition, the lung biopsy or autopsy lung or bronchial tissue can be used. The same sample of biological material can be examined for the presence of agents of a number of diseases, unlike bacteriological methods, in which different methods of cultivation are used for different agents.

The processing method, which is common for all materials tested, and the ability to automate the PCR allow diagnosing several pathogens for 4-5 hours, while **culture testing methods** require from a few days to a few weeks.

The high sensitivity of the PCR method allows diagnosing the infection not only in samples collected from patients during the acute stage of illness, but also during about 3 weeks after the onset of symptoms. In addition, unlike bacteriological and virological methods for detecting pathogens, the requirements for storage and transportation of the material from a patient to a laboratory are significantly lower.

Currently, according to the WHO recommendation, the molecular diagnosis is the preferred method of detection of influenza viruses. One method of detecting RNA of influenza A and B viruses is the RT-PCR in real time (rRT-PCR). This method allows determining the type of influenza virus and the affiliation of influenza A virus to subtypes H1, H3, H1pdm09 and others. The main feature of this method is a relatively quantitative analysis of storage of products during PCR, automatic registration and interpretation of the results. The real-time PCR uses fluorescently labeled oligonucleotide probes to detect cDNA during its amplification.

Real-time RT-PCR is used due to the simplicity of its performance and reliability. Real-time RT-PCR allows performing a complete test of the sample during 3 hours and is theoretically capable of detecting even one molecule of DNA or RNA in the sample. This technology does not require the electrophoresis stage, which enables to reduce the requirements for PCR laboratories.

One of the constraints of this method is the availability of the expensive sophisticated equipment - a device for real-time PCR, and qualified personnel.

Testing Algorithms

A general approach to the detection of the viral genome by RT-PCR is to address a particular objective of typing or subtyping of influenza viruses. The testing algorithm determines which gene sequence should be chosen as a target for RT-PCR, and whether to perform a parallel or subsequent testing in the RT-PCR-M, nucleoprotein (NP) and

non-structural (NS) genes and the genes of hemagglutinin and neuraminidase.

Since the influenza virus genome is a single-stranded RNA, the synthesis of complementary DNA (cDNA) should primarily use the reverse transcriptase (RT). RNA genome amplification (RT-PCR) in real time requires a pair of oligonucleotide primers and a probe labeled with a fluorescent dye. The presence of a PCR mixture of such pairs of primers and probes, which have a known sequence of HA, for example, influenza A virus of subtype H1N1, will lead to the amplification of cDNA of only one subtype. Further test of DNA generated by using primers for certain subtypes is possible by molecular genetic methods such as sequencing.

Currently, the following test systems and reagent kits are used in Russia for gene diagnosis of influenza viruses:

1. Test systems AmpliSens developed in Federal State Institution Central Research Institute of Epidemiology, Rospotrebnadzor (Russian agency for protection of consumers' rights), Moscow.

- **"AmpliSens® Influenza virus A H5N1-FL"** (set of reagents for the detection of RNA of influenza A virus (Influenza virus and identification of the subtype H5N1 in the clinical material using the polymerase chain reaction (PCR) with hybridization-fluorescence detection);

- "**AmpliSens ® Influenza virus A-type-FL**" (set of reagents for typing (identification of subtypes H1N1 and H3N2) influenza A viruses (Influenza virus A) using the polymerase chain reaction (PCR) with hybridization-fluorescence detection);

- "**AmpliSens ® Influenza virus A/H1-swine-FL**" (set of reagents for the identification of swine flu A/H1 using the polymerase chain reaction (PCR) with hybridization-fluorescence detection);

- "**AmpliSens ® Influenza virus A/B-FL**" (set of reagents for the detection of RNA of influenza A viruses (Influenza virus A) and influenza (Influenza virus B) in the clinical material using the polymerase chain reaction (PCR) with hybridization-fluorescence detection), there is also a set with detection of amplification products using the electrophoresis method.

All these test systems include reagent kits for a two-step RT-PCR, *i.e.*, reverse transcription reaction and PCR are performed separately. The control of reverse transcription and PCR are performed by adding a heterologous RNA sample before the test, followed by amplification of the RNA fragment with the results for other fluorescent dyes (different from that used for labeling of specific viral cDNA).

2. The test systems developed by the DNA Technology, Moscow

- a set of reagents for detection of RNA of influenza A virus subtype H5N1 ("avian flu") (**Influenza A virus subtype H5N1**) by the polymerase chain reaction with reverse transcription (RT-PCR)

- a set of reagents for detection of RNA of pandemic influenza A (H1N1) viruses, similar to the strain A/California/4/2009 ("swine flu") by polymerase chain reaction with reverse transcription **PAN H1N1**

- a set of reagents for detection of influenza A virus RNA by polymerase chain reaction with reverse transcription **Influenza A virus**

3. Kits of reagents for RNA isolation (RNeasy Mini Kit, Qiagen, Germany) and RT-PCR (SuperScript III One-Step RT-PCR System, Invitrogen, USA) recommended by CDC (Centers for Disease Control and Prevention, USA), specific primers and probes developed by CDC experts and recommended by WHO [3].

The distinguishing feature of the method is that the stages of reverse transcription and subsequent PCR occur in one reaction mixture. The analysis of storage of polymerase chain reaction products is performed using only one fluorescent dye. RNA isolated from a sample of a patient is added to various reaction mixtures for RT-PCR with real time detection, which contain specific primers and probes for identifying:

- RNA of influenza A virus,
- RNA of influenza B virus,
- RNA of influenza virus H1N1,

- RNA of influenza virus H3N2,
- RNA of influenza virus H1N1pdm09 (swine),
- RNA of influenza virus H5N1a, H5N1b (Asian and European lines)
- RNA of gene that encodes human RNP (ribonucleoprotein synthesized in all human cells) to control collection of the material from a patient and perform RT-PCR.

It should also be noted that AmpliSens test systems and CDC reagent kits differ in sensitivity of reactions when typing influenza viruses A and B and subtyping of influenza A virus: if test systems developed in the Central Research Institute of Epidemiology are more sensitive in detecting RNA, which are specific for subtypes of influenza A, CDC reagent kits are more sensitive in detecting RNA, which is characteristic for influenza A and B viruses. All the above kits of reagents and their target genes are shown in Table 1.

Reagent kit	Target gene	Number of fluorescent dyes/detection channels	Control of material collection/PCR
1. AmpliSens® Influenzavirus A H5N1-FL	M of influenza A virus, HA, NA (H5, N1) Heterologous RNA	2	Heterologous RNA is added to the sample from the patient no/yes
AmpliSens® Influenza virus A-тип-FL	HA, NA (H1, H3, N1, N2) Heterologous RNA	3	Heterologous RNA is added to the sample from the patient no/yes
AmpliSens® Influenza virus A/H1-swine-FL	HA (H1v swine) Heterologous RNA	2	Heterologous RNA is added to the sample from the patient no/yes
AmpliSens® Influenza virus A/B-FL	M of influenza A virus, NS influenza B virus Heterologous RNA	3	Heterologous RNA is added to the sample from the patient no/yes
2. Influenza A virus subtype H5N1, RNA-technology	HA, NA (H5, N1) Heterologous RNA	2	Heterologous RNA is added to the sample from the patient no/yes
ПАН H1N1, RNA-technology	NA (N1 swine) Heterologous RNA	2	Heterologous RNA is added to the sample from the patient no/yes
Influenza A virus, RNA-technology	M of influenza A virus, Heterologous RNA	2	Heterologous RNA is added to the sample from the patient no/yes
3. Primers and probes for detection of RNA of influenza A virus, CDC	M of influenza A virus	1	-
Primers and probes for detection of RNA of influenza B, CDC	NS of influenza B virus	1	-
Primers and probes for detection of RNA of influenza A H1N1 virus, CDC	HA (H1) of influenza A virus	1	-
Primers and probes for detection of RNA of influenza A H3N2 CDC	HA (H3) of influenza A virus	1	-
Primers and probes for detection of RNA of influenza A H1N1v	HA (H1v swine) of influenza A virus	1	-

(swine), CDC			
Primers and probes for detection of RNA of influenza A H1N1v (swine), CDC	NP of influenza A/H1N1v (swine) virus	1	-
Primers and probes for detection of RNA of influenza A H5N1 a, b, CDC	HA (H5) of influenza A virus	1	-
Primers and probes for detection of RNA gene that encodes human RNP	Human RNP	1	yes /yes

Table 1 *– kits of reagents and their target genes*

Currently, WHO developed the algorithm of gene diagnosis of influenza A and B viruses and other infections that cause acute respiratory illnesses. This algorithm and interpretation of PCR results are shown in Fig. 1 [18].

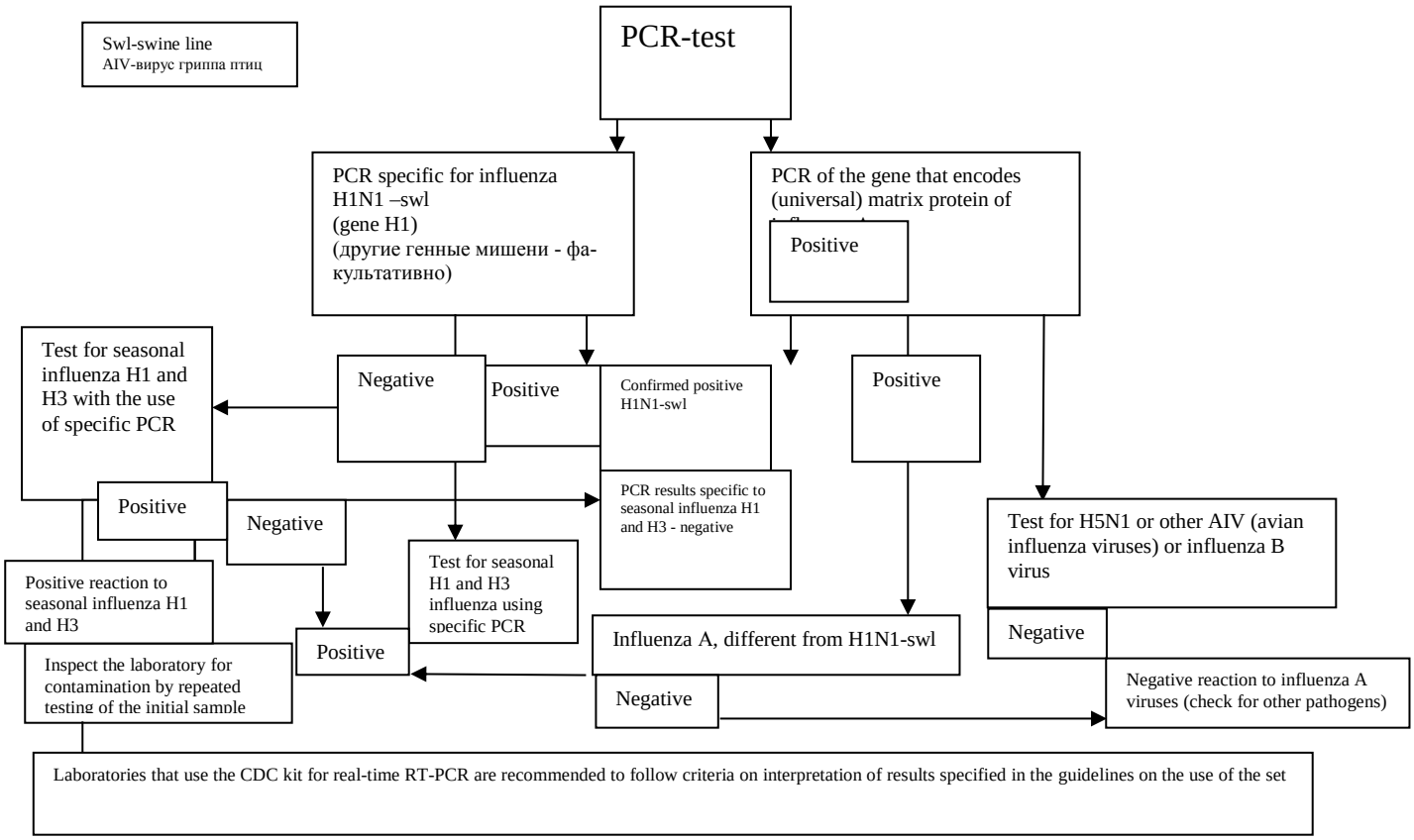

Figure 1 *– Algorithm of PCR-testing and interpretation of results*

The gene diagnosis of influenza viruses and infectious agents that cause influenza-like illnesses can decode disease outbreaks and monitor the circulation of viruses in the human population. However, the PCR diagnosis does not enable to identify molecular genetic characteristics of circulating viruses, and, consequently, their antigenic characteristics. One of the methods to supervise circulating strains of influenza virus in relation to their genetic structure and emergence of resistance mutations to antiviral drugs is to determine the nu-

cleotide sequence (sequencing) of the genome segments that are responsible for such mutations. This monitoring helps to determine the strain composition of vaccines for the following season, and choose drugs that are effective for treatment of influenza.

Major drugs that are currently used for treatment of influenza virus are adamantanes and neuraminidase inhibitors.

Rimantadine, a classic anti-influenza adamantane drug, blocks ion channels of cell membranes and inclusion of the virus into the host cell, and inhibits the release of the viral genome. The widespread use of rimantadine resulted in emergence of resistant influenza virus variants, which was the consequence of mutations in the virus genome segment that encodes M2 protein. Tests of more than 60,000 strains of H3N2 influenza A virus isolated in different countries showed an increase in resistance to inhibitors of M2 channels from 0.4% in 1995 to 12.3% in 2004 [2] and 70% in 2007 (data of the Influenza Research Institute).

Neuraminidase plays an important role in virus replication. Although the genetic structure of influenza viruses and neuraminidase are constantly changing, the amino acid sequence of the active site of the enzyme remains constant, which makes it an ideal target for antiviral therapy. The study of a three-dimensional structure of neuraminidase became the basis for development of inhibitors of this enzyme [13]. Two drugs of this group are widely used now - oseltamivir (Tamiflu) and zanamivir (Relenza). When using neuraminidase inhibitors for treatment of influenza, resistant variants of influenza virus A are also formed. The most well-known mutation that causes influenza virus resistance to oseltamivir occurs in NA gene and leads to the substitution of histidine for tyrosine at position 275 of NA (N1). Such substitutions were widely reported for the first time in 2007 in circulating strains of influenza virus A/H1N1. In 2008, the amount of H1N1viruse, which are resistant to Tamiflu and carry the H275Y substitution in the NA, reached 90% of circulating influenza A viruses in many European countries [16].

Currently, two fundamentally different sequencing methods: automatic enzymatic Sanger sequencing and pyrosequencing, and the test of restriction fragment length polymorphism (RFLP) are used to oversee the emergence of resistance mutations to antiviral drugs and the reconstruction of the evolution of viruses [7].

Determination of the DNA sequence became possible in the 1970s. In 1977, two sequencing methods were proposed: method of chemical degradation [11], which is rarely used today, and the enzymatic Sanger method [15].

Sanger Method

The principle of the method is based on the enzymatic synthesis of the complementary strand of DNA on the unknown single-stranded matrix with probabilistic breakage (termination) in each position of the sequence.

DNA-matrix, primer, DNA polymerase, dNTPs and chain extension terminators (ddNTPs labeled dideoxynuceloside triphosphate) are added to the reaction mixture. ddNTP does not have a 3'OH group, that is why further chain elongation becomes impossible when such nucleotide is added. The ratio of dNTPs and ddNTPs is chosen in such a way that they should compete with each other for incorporation to the growing DNA chain. Since the number of molecules of the DNA-matrix in the reaction mixture greatly exceeds the length of a sequence, the mixture contains the fragments of all lengths that are terminated as complementary to ddNTPs matrix. Radiolabeled ddNTPs, which are indistinguishable from each other, were used in the classic method of Sanger. Therefore, the sequencing of one sequence required four sequencing reactions, each of which had only one type of ddNTP. The sequencing reaction just as PCR includes stages of denaturation, primer annealing and chain elongation. The analysis of sequencing reaction products was performed by electrophoresis in polyacrylamide gels of high resolution that enabled to distinguish the DNA molecules which differed in length by one nucleotide. Fragments were detected by autoradiography.

The first Sanger sequencing of the genomic fragment of influenza A virus was conducted in 1979 [1].

The method of Sanger has undergone a number of modifications that enabled to automate it, significantly increasing its productivity. For example, fluorescent labels, which are different for different ddNTP terminators, are used instead of a radioactive label. This allows performing the sequencing reaction in one tube. The electrophoresis of the reaction products and fluorescence detection are performed in an automatic analyzer (sequencer). The majority of modern sequencers perform the electrophoretic separation in the capillaries; however, there are devices in which the electrophoretic unit is represented as an electrophoretic gel or a mini-gel. Fluorophores and lasers used for fluorescence excitation vary in devices from different manufacturers, which makes the reagent sets and sequencers of different firms incompatible. The characteristics of a number of modern sequencers are shown in Table 2. The sequence data are transmitted to a computer and after processing they are represented as sequenograms (see Fig. 2.)

Sanger sequencing is the reference method for detecting mutations of influenza A virus to adamantanes and neuraminidase inhibitors.

1. Isolation of RNA of influenza A virus
2. RT-PCR fragment of 6 segment (NA) and / or 7 (M) of influenza A virus
3. Electrophoresis of PCR products by agarose gel followed by the isolation of target DNA fragments
4. Sequencing reaction
5. Purification of the labeled products of the sequencing reaction
6. Electrophoresis of products of the reaction in a sequencer
7. Analysis of sequenograms

The sequencing method is characterized by such parameters as the length of read (LOR), mean quality value (QV) and the sequencing depth. Sequencing depth means the number of reads of each positions in the sequence. The length of read is not important in overseeing the emergence of resistance mutations to antiviral drugs, since we are only interested in individual codons in the sequence. The quality of reading these codons should be QV $\geq$ 30, the depth of sequencing $\geq$ 2.

The whole process from the isolation of viral RNA until sequences can be performed during 1 day, but usually it takes 2-3 days. The duration of sequencing a fragment of 600-700 nucleotides is 2-3 hours on average. The capacity depends on the instrument used (the quantity of samples simultaneously analyzed) and the selected sequencing depth (how many times we want to sequence the same sample).

Manufacturer	Sequenator	Form of Electrophoresis	Type of Laser	Number of Detected Dyes – Name of Dyes	Number of Samples per cycle	Number of Nucleotides/Time
Amersham Pharmacia Biotech www.apbiotech.com	ALFexpress MegaBACE 500 MegaBACE 1000 MegaBACE 4000	Gel 48 capillaries 96 capillaries 96 capillaries	HeNe-laser (632.5 nm) Ar-laser (488 nm) -//- -//-	1 -Cy5 4 - DYEnamic™ET 4 - -//- 4 - -//-	10 48 96 96	600/6 hours 650/2 hours 650/2 hours 650/2 hours
Applied Biosystems www.appliedbiosystems.com	ABI 373 ABI 377 ABI 310 ABI 3100 ABI 3700	Gel Gel 1 capillary 16 capillaries 96 capillaries	40mW Ar-laser (488-514 nm) 40mW ar-laser (488-514 nm) 10mW Ar-laser (488-514 nm) 25mW Ar-laser (488-514	4 – TAMRA, ROX, R110, R6G 4 – dTAMRA, dROX, dR110, dR6G or BigDye 4 - -//- 4- -//- 4- -//-	16-24 18-96 1 16 96	450/9 hours 700-900/9 hours 450/1.5 hour 650/3 hours 650/3 hours

			nm) 40mW Ar-laser (488-514nm)			
Beckman Coulter www.beckman.com	CEQ 2000	8 capillaries	Two solid-phase semiconductor lasers (650 nm and 750 nm)	4 – D2, D3, D4, Cy™	8	500/2 hours
LI-COR www.licor.com	IR[2]	gel	Two solid-phase semiconductor lasers (680 nm and 780 nm)	2 – IRDye700, IRDye800	16-32	2000/12
Visible Genetics www.visgen.com	MicroGene Blaster MicroGene Clipper MicroGene Tower	Minigel Minigel Minigel	HeNe-laser (632.5 nm) Solid-phase semiconductor laser (640 nm) Solid-phase semiconductor laser (640 nm)	1 – Cy5.5 2 – Cy5, Cy5.5 2 - -//-	4 8 8	300/30 min 450/30 min 700/3 hours

Table 2 *– Technical specifications of certain modern sequencers*

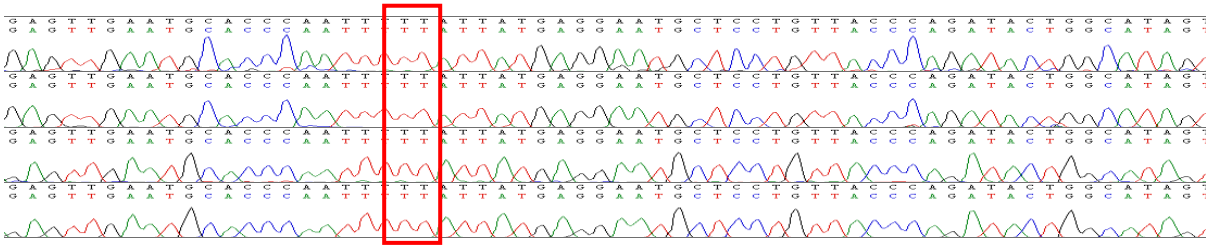

Figure 2 *– Fragment of segment 6 (NA) sequenograms of influenza virus A/St.Petersburg /66/09 (H1N1) that carry mutation of resistance to oseltamivir H275Y (in codon 275 CAT > TAT). Depth of read 4.*

Pyrosequencing

The pyrosequencing method was proposed in 1988 by E. Hyman [8]. Detection of pyrophosphate that is formed during the merger of dNTPs to the DNA chain underlies the method. Added dNTPs are sequentially added to the matrix. Inclusion of the next nucleotide and, consequently, the release of pyrophosphate depends on the sequence of the DNA-matrix. The sulfurylase in the presence of adenosine phosphosultate transforms into ATP. ATP triggers the oxidation of luciferin by the luciferase accompanied by luminescence, which is recorded by a special camera. Unincorporated dNTPs in the growing chain are split by the apyrase. The following dNTP goes next.

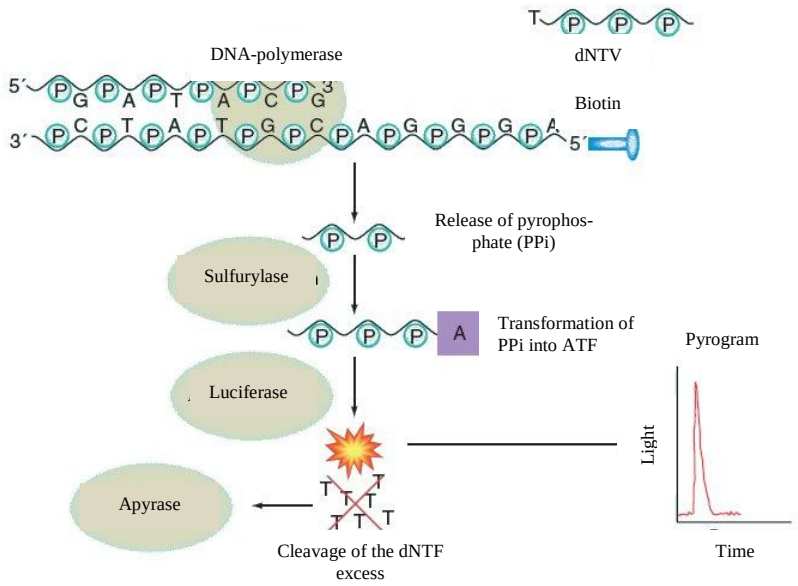

Figure 3 *– Principle of sequencing (from [4])*

Sample preparation consists of the following steps:

1. Isolation of RNA of influenza A or B virus.
2. Pyro-RT-PCR. A particular feature is that the reverse primer is biotinylated.
3. Cleaning of the biotinylated amplicon, immobilization of amplicon on streptavidin-coated particles and DNA denaturation.
4. Pyrosequencing.
5. Analysis of the pyrograms.

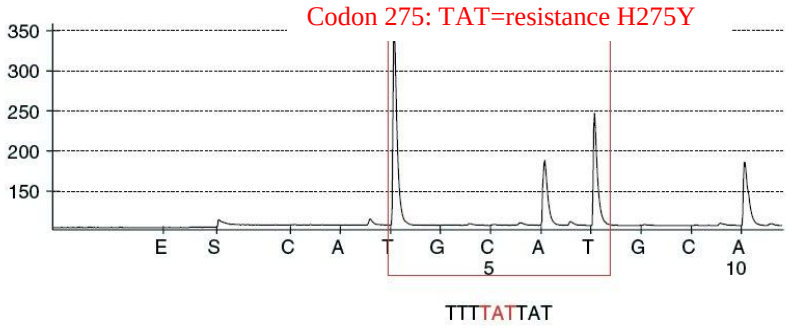

Figure 4 *– Fragment of segment 6 (NA) pyrograms of influenza virus A/New Hampshire/02/2008 (H1N1) (From [4]).*

Pyrosequencing has been used to monitor resistance mutations of influenza viruses A and B to antivirals since 2005 [2]. The method has the productivity that greatly exceeds automated Sanger sequencing. It enables to analyze 96 samples in 1.5-2 hours. Another important advantage of pyrosequencing is the ability to perform quantity evaluation of the content of a nucleotide in a specific position of the sequence. Minor sequences are quantitatively detected if their share is greater than or equal to 10%. Currently, the WHO recommends 2 protocols to identify drug resistance mutations [9, 10].

PCR with mass spectrometry detection

There are many modifications and variations of PCR. PCR with electrophoretic detection and real-time PCR are the most widely used as the method for detection of the PCR-product. Only one target can be detected by electrophoresis. Real-time PCR enables to look for up to five targets in a tube owing to different fluorophores. A ma-

jor limitation is the difficulty in selecting primers and probes for multiplex reactions, and a limited set of detection channels. It was suggested to use mass spectrometry for the analysis of amplification products (Mass-Tag-PCR).

Primers labeled with a mass spectrometer tag (w-tag) are used for amplification. After purification, labeled PCR products are UV irradiated, which leads to cleavage of the mass-tag, then mass tags are detected by the mass spectrometer. The Mass-Tag PCR method allows detecting up to 30 targets in a single tube [5]. The Mass-Tag PCR is one of the fastest ways to simultaneously check the analyzed clinical data for 30 pathogens.

A commercial platform for PCR with mass spectrometric detection - PlexID (Abbott) has been developed [14]. An automated complex PLEX-ID developed on the basis of the ideas of David Acker has been performed using the time-of-flight (TOF) high-precision mass spectrometry, electro-spray ionized (ESI) PCR amplicons followed by a computerized calculation of the masses of corresponding PCR amplicons and their nucleotide composition, and comparison of the results with specially formed databases of nucleotide sequences of microorganisms used for automatic identification.

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O.T. Umarov, Zh.A. Kalmatayeva

Higher school of public healthcare, Almaty, Kazakhstan

FACTORS, INFLUENCING THE CONSUMER BEHAVIOR ON THE MEDICATIONS MARKET

Abstract. *It is known that rational consumption of medications improves the quality of life of population. So the study of the factors, influencing choice of consumers in the medications markets is one of the relevant problems in modern healthcare.*

The aim of the research was study of the consumer behavior and influence of several factors on it.

With the help of questionnaire 397 respondents were surveyed.

Method of correlation analysis identified the link between consumers behavior on pharmaceutical market with such factors as education, age and income, as well as marital status and presence of chronic pathology.