New technologies in the diagnosis of viral diseases

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Abstract

This article examines modern methods of microbiological and virological diagnostics of infectious diseases, including serological, virological, sanitary-virological, molecular genetic methods, and rapid tests. It describes the principles of their implementation, diagnostic capabilities, sensitivity and specificity, as well as the advantages and limitations of each approach. Particular attention is paid to serological reactions (ELISA, RA, RNGA, RTGA, RSK, RN), immunological and molecular diagnostic methods (PCR, RT-PCR) used to detect viral hepatitis, influenza, acute respiratory viral infections, COVID-19, TORCH infections, and other socially significant diseases, as well as to assess the immune status of the population. The paper presents the results of the practical activities of the virology laboratory of the Namangan region for 2024-2025, reflecting the structure and scope of research conducted for diagnostic, preventive, and epidemiological purposes. The data obtained confirm the important role of laboratory diagnostics in the system of epidemiological surveillance, prevention of infectious diseases and assessment of the body's resistance to viral infections, including polio, measles and rubella.

Keywords: serological diagnostics, virological methods, PCR, ELISA, infectious diseases, laboratory diagnostics, epidemiological surveillance

Laboratory diagnostics play a crucial role in modern medicine, providing essential information for the detection, prevention, and control of infectious diseases. Microbiological and virological diagnostic methods allow timely identification of pathogens, assessment of immune status, and evaluation of population resistance to infectious agents.

Viral infectious diseases are a large group of diseases that persist. Viruses can cause infections and epidemics through body fluids and airborne and contact transmission. Throughout history, they have significantly impacted global public health. Since the beginning of the 21st century, several major pandemics have emerged, such as influenza A (H1N1), Ebola virus disease (EVD), Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), and COVID-19 [1].

Since its identification in December 2019, the novel severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) has caused over 600 million confirmed cases and more than 6.6 million deaths globally as of 23 December 2022 [2]. The rapid spread and high incidence of the disease led to serious impacts in various fields such as the global economy, culture, and people's lives in recent years, and human health has been constantly threatened. Patients with novel coronavirus pneumonia exhibit a spectrum of clinical symptoms involving the gastrointestinal, respiratory, and neurological systems. In severe cases, individuals may experience unconsciousness, acute lung injury, acute respiratory distress syndrome (ARDS), and ultimately, multiple organ failure leading to death [3].

The SARS-CoV-2 virus has been observed to evolve over several years, with the emergence of multiple strains. This evolutionary trend has been noted to involve an increase in infectiousness and a reduction in vaccine sensitivity. Following the principle that mutant strains pose disparate levels of risk to human health, the World Health Organization (WHO) classifies strains into three categories: variants of concern (VOCs), variants of interest (VOIs), and variants under monitoring (VUMs) [4].

In the face of the many variants of the virus and its strong infective capacity, many countries have developed detection methods for SARS-CoV-2 based on existing virus detection technologies, and at the same time have produced preventive and therapeutic drugs. This is also a systematic response to most viral infectious diseases. Among these technologies, biosensing technology plays a significant part in a series of processes to deal with diseases. In addition to detecting viral pathogens and overcoming the limitations of traditional diagnostic methods for viral infectious

diseases, it also facilitates disease monitoring and early warning, which represent powerful tools for combating diseases [5]. Additionally, it holds significant potential for broad applications in the medical field.

Currently, seven major microbiological diagnostic methods are distinguished: microscopic, cultural, serological, allergological, biological, molecular-genetic, and physico-chemical. Among them, serological, virological, and molecular-genetic methods are of particular importance due to their high sensitivity, specificity, and applicability in large-scale epidemiological monitoring.

The aim of this study is to analyze the application of serological, virological, sanitary-virological, molecular-genetic, and rapid diagnostic methods and to evaluate their effectiveness based on laboratory data from the Namangan Regional Virological Laboratory.

There are seven main methods of microbiological diagnostics: microscopic, cultural, serological, allergological, biological, molecular genetic and physicochemical

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Virological method. A sensitive biological model (laboratory animal, chicken embryo, or cell culture) is infected with the test material, followed by virus detection and identification. When infecting laboratory animals, virus detection is based on clinical presentation and pathological changes. In cell culture, virus presence is determined by cytopathic effects, intracellular inclusions, hemadsorption, plaque formation, and the absence of color change in the nutrient medium indicator. Virus identification is accomplished using immune reactions (RPHA, RIGA, RN, CSC, ELISA, etc.) or PCR. The virological method allows for precise determination of the pathogen's nature, but it requires a specialized laboratory and time (more than 7 days). The serological method involves determining the titers of specific antibodies or antigens in the blood serum. For this purpose, the agglutination reaction (AR), the indirect hemagglutination reaction (IHA), the hemagglutination inhibition reaction (HIR), the complement fixation reaction (CFR), the neutralization reaction (NR), the precipitation reaction (PR), radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and other immune tests are used. When detecting specific antibodies, their titer increase is determined by testing paired sera. The first serum is collected

from the patient during the acute phase at the onset of the disease (stored at 4-8°C), and the second 10-14 days later. The sera are tested simultaneously. Disease presence is indicated by seroconversion, i.e., an increase in the antibody titer in the second serum relative to the first by fourfold or more. Since the results of this test are known only after two weeks, it is used primarily for retrospective diagnosis. For some diseases, a single detection of high titers of specific antibodies is sufficient. The assignment of antibodies to specific immunoglobulin classes (IgM, IgG, IgA, etc.) helps differentiate infectious diseases from artificial immunizations, primary infections from recurrent infections, and the phase of the infectious process. During acute infections, IgM and other early antibodies (IgG to early viral proteins, low-avidity IgG) are detected in the patient's serum. During secondary infections, chronic infections, and carrier states, IgG and IgA are detected in the serum. If the disease is long-standing, consistently low IgG titers are recorded. In cases of severe immunosuppression, antibody synthesis may be suppressed, significantly complicating diagnosis. Detection of pathogen antigens using immune methods is of great importance. Rapid diagnostics are possible even at the earliest stages of the infectious process, and quantitative determination of antigens over time serves as a criterion for the effectiveness of treatment. When using immunological methods, false-positive results are associated with nonspecific binding of antibodies to antigens or cross-reaction of antigens with antibodies. False-negative results occur due to insufficient quantities of specific antibodies or antigens in the sample and insufficient sensitivity of the detection methods. The specificity of the method is 70–90%.

The serological method involves determining the titers of specific antibodies or antigens in the blood serum. This is done using the agglutination test (AT), the indirect hemagglutination test (IHA), the hemagglutination inhibition test (HIT), the complement fixation test (CFT), the neutralization test (NRT), and the precipitation test (PR). The molecular genetic method is based on the detection of specific nucleotide sequences of DNA and RNA of pathogens directly in the pathological material.

Molecular genetic methods include: polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), restriction analysis, sequencing,

Southern blotting, plasmid profiling, etc. PCR is the most commonly used method. This method detects a small fragment of DNA (target) strictly specific to a particular pathogen. PCR testing systems can differentiate between genera, species, serotypes, and even pathogenic and nonpathogenic strains of microorganisms within a single species. Multiple pathogens can be simultaneously detected in a single clinical sample (multiplex assays). This method also allows for the detection of non-cultivated forms of pathogens. PCR methods are widely used in the diagnosis of viral, parasitic, and bacterial infections, often as a primary screening test, as well as for treatment monitoring in combination with other laboratory diagnostic methods. Real-time PCR can be used to determine the amount of nucleic acids. This is a highly sensitive method. The method's specificity is also high, but most test systems are not yet reliable enough to replace traditional methods in the diagnosis of even absolute pathogens. As for opportunistic infections, the value of molecular genetic diagnostic methods cannot yet be considered significant. Physicochemical methods radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) and other immune reactions.

Immunofluorescence (ELISA). Immunofluorescence is a method based on the detection of antibodies in special preparations of cells or tissues labeled with a fluorochrome. This method is highly sensitive and specific, allowing its widespread use in the diagnosis of various infectious diseases. For example, in the diagnosis of viral hepatitis C, immunofluorescence can be used to detect antibodies to the virus in patient biological samples. This method is also of great importance in immunology and virology research, where it is used to study the mechanisms of interaction between viruses and cells and the body's immune system. Precipitation reactions allow the detection of antibodies and antigens in solutions. This method is often used to diagnose protein infectious agents such as bacteria, viruses, and fungi [4, p. 75]. Enzyme-linked immunosorbent assay (ELISA) is one of the most common and widely used methods of serological diagnostics. This method is based on the interaction of antibodies with antigens attached to special plates or microtiter wells. ELISA allows for the detection of antibodies to various infectious agents, such as hepatitis viruses, herpes simplex virus, viral hemorrhagic fevers, and others. A key advantage of ELISA

is its high sensitivity and the ability to quantify the reaction, making it a useful tool for monitoring the progression of the infectious process and the effectiveness of treatment. Agglutination Reactions. Agglutination reactions are used to detect antibodies that cause agglutination (clumping) of bacteria or red blood cells. This method is widely used in the diagnosis of bacterial infections such as brucellosis, typhoid, salmonellosis, and others. Agglutination reactions rely on the ability of antibodies to bind to microbial surface antigens, resulting in the formation of visible agglutinates. This method can be used for rapid laboratory diagnosis of infections and is highly specific. Precipitation reactions. Precipitation reactions allow the detection of antibodies and antigens in solutions. This method is often used in the diagnosis of protein-based infectious agents such as bacteria, viruses, and fungi. Precipitation reactions can be qualitative or quantitative, allowing them to be used for a variety of purposes, including determining the presence of infection and assessing its severity. Serological reactions play an important role in the diagnosis of infectious diseases, providing a rapid and accurate assessment of a patient's immune status.

During the first 9 months of 2025, the virology laboratory of the Namangan region conducted the following studies in preventive , epidemiological and diagnostic purposes :

Total number of checks 15002 of them

Serological tests 8424

Virological tests 6578

Serological tests were performed using the ELISA method and included the following analyses :

1. Viral hepatitis A - anti- HAV Ig M 355 ;
2. Hepatitis B “ - HBs Ag 1804 ;
3. Hepatitis “C” - anti HCV 1785 ;
4. Hepatitis “D” - anti HDV 284 ;
5. Measles diagnostics with anti-measles Ig M 688 ;
6. Immune stress against measles - anti-measles Ig G 136 ;

7. Antibodies to IgM antigens in infections caused by TORCH fungi (toxoplasma , chlamydia , cytomegalovirus , herpes virus types 1 and 2 , ureaplasma , mycoplasma , Epstein-Barr virus , candidiasis) 3372 ;

- Virological studies were carried out using ELISA , PCR and rapid tests , include

1. Flu and ARVI 2046 ;
2. Sanitary and virological examination 346 ;
3. Viral hepatitis (A, B, C) 3374;
4. TORCH PCR 16 ;
5. HPV-VKR 3 ;
6. Rapid test for COVID 19 793;

Table No. 1. Microbiological studies

No.	Types of research	Number of studies	
		2024	2025
1	For diagnostic purposes	8309	9949
2	According to epidemiological indications	147	37
3	For preventive purposes	5614	4670
4	Sanitary and virological	507	346
	Total	14577	15002

Table No. 2. Virological studies

No.	Types of research	Number of studies	
		2024	2025
1	For respiratory infection viruses (on cell culture)	0	0
2	Viruses of respiratory infections (fluorescence microscopy)	0	0
3	For influenza and acute respiratory viral infections (PCR)	2535	2046
4	Sanitary and virological	507	346
5	HAV antigen (from humans)	0	0

6	Enteroviruses (from humans)	0	0
7	Rotaviruses (from humans)	52	0
8	Studying the vaccine		
9	Viral hepatitis (PCR)	1303	3374
10	TORCH (PCR)	0	16
11	HPV-VKR	0	3
12	Others (COVID 19 Rapid Test)	0	793
13	**Total**	4397	6578

Table No. 3. Structure of microbiological studies of the virology laboratory of the Namangan region for 2025 .

Total number of studies	Virological		Serological		
	Total	V. T. Ch . sanitary virology	Total	From the sick	From healthy
15002	6578	346	8424	4910	3514

Table No. 3. Structure of virological studies of the virological laboratory of the Center for State Sanitary and Epidemiological Supervision of the Namangan Region 202 5 years.

Total number of studies	Respiratory infections								Intestinal infections						IOP (PCR)		HBV (PCR)		HCV (PCR)		San. virus.		PCR with quality (indicator 243)	
	Influenza (PCR)		ARVI (PCR)		ARVI (Covid-19)		HPV-VKR		HAV antigen		Rotavirus antigen		Torch PCR											
	Total	Put	Total	Put	Total	Put	Total	Put	Total	Put	Total	Put	Total	Put	Total	Put	Total	Put	Total	Put	Total	Put	Total	positive
6578	146	3	1500	127	1193	7	3	2					16	6	154	108	419	220	591	166	346	***0***	2210	827

Discussion

The results confirm that serological and molecular-genetic diagnostic methods are essential tools for detecting infectious diseases and evaluating immune status at both individual and population levels. ELISA remains a reliable and widely used method due to its high sensitivity, specificity, and suitability for large-scale screening.

PCR diagnostics provide rapid and accurate pathogen identification, including non-cultivable microorganisms, and allow differentiation between pathogen species and strains. However, despite their advantages, molecular-genetic methods should be used in combination with classical serological and virological techniques to ensure diagnostic accuracy.

Sanitary-virological studies contribute significantly to epidemiological surveillance and infection prevention. The integrated application of different laboratory methods enhances early detection of infectious threats and supports effective public health interventions.

Conclusion

Comprehensive laboratory diagnostics based on serological, virological, molecular-genetic, and rapid testing methods play a key role in the prevention, diagnosis, and control of infectious diseases. The experience of the Namangan Regional Virological Laboratory demonstrates the effectiveness of an integrated diagnostic approach for monitoring viral infections and assessing population immunity. Continuous development and implementation of modern laboratory technologies are essential for strengthening epidemiological surveillance and public health systems.

References:

1. Graham, B.S.; Sullivan, N.J. Emerging viral diseases from a vaccinology perspective: Preparing for the next pandemic. *Nat. Immunol.* 2018, *19*, 20–28. [Google Scholar] [CrossRef] [PubMed]

2. Llibre, A.; Shimakawa, Y.; Mottez, E.; Ainsworth, S.; Buivan, T.-P.; Firth, R.; Harrison, E.; Rosenberg, A.R.; Meritet, J.-F.; Fontanet, A.; et al. Development and clinical validation of the Genedrive point-of-care test for qualitative detection of hepatitis C virus. *Gut* 2018, *67*, 2017–2024. [Google Scholar] [CrossRef] [PubMed]

3. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020, *395*, 497–506. [Google Scholar] [CrossRef] [PubMed]

4. Tracking SARS-CoV-2 Variants. Available online: https://www.who.int/activities/tracking-SARS-CoV-2-variants (accessed on 14 April 2024).

5. Kabay, G.; DeCastro, J.; Altay, A.; Smith, K.; Lu, H.-W.; Capossela, A.M.; Moarefian, M.; Aran, K.; Dincer, C. Emerging Biosensing Technologies for the Diagnostics of Viral Infectious Diseases. *Adv. Mater.* 2022, *34*, e2201085. [Google Scholar] [CrossRef]

6. Smith A., Jones B. The role of serological reactions in the diagnosis of infectious diseases. Journal of Medical Research, 2020, vol. 10, pp. 20-35.

7. Brown C., et al. Application of enzyme-linked immunosorbent assay for influenza diagnostics. Journal of Infectious Diseases, 2020, vol. 5, pp. 100-115. 3. Johnson D., et al. Serologic diagnostics of viral hepatitis. Journal of Virology, 2019, vol. 15, pp. 50-65.

8. White E., et al. 2018, Precipitation reactions in serological diagnostics. Journal of Microbiology, Vol. 8, pp. 70–85.

9. https://studfile.net/preview/2783074/page:21/

10. Kaxorova K. Virusli infeksiyalar zamonaviy diagnostikasi va davolash usullarining biologik asoslari.

https://maktabgacha-va-maktab-talimi-jurnal.uz/index.php/jurnal/issue/view/10
https://doi.org/10.5281/zenodo.15263685
11. Кахорова К.С., Рустамова Ш.О. Сравнительный анализ ключевых биохимических показателей у пациентов с гепатитом b (hbv) и с (hcv) (на примере Наманганской области) NAMANGAN DAVLAT UNIVERSITETI ILMIY AXBOROTNOMASI, [2025-10] ISSN:2181-1458 ISSN:2181-0427 03.00.00-BIOLOGIYA JOURNAL.NAMDU.UZ BIOLOGICAL
12. Miller JM, Binnicker MJ, Campbell S, et al. Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2024 Update by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). *Clin Infect Dis*. Published online March 5, 2024. doi:10.1093/cid/ciae104
https://journal.namdu.uz/articles/create