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ASSESSMENT OF THE INFORMATIVENESS OF SEROLOGICAL METHODS FOR THE DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS AND SEROEPIDEMIOLOGICAL DATA ON ITS PREVALENCE

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The aim. *To assess the prevalence of markers of the acute period of infectious mononucleosis (VCA IgM, VCA IgG, EBNA IgG, heterophilic antibodies in the monospot test and EBV DNA by PCR) based on the analysis of the Dila laboratory database in Kyiv during the three-year observation period (2022-2024), to investigate changes in diagnostic approaches in clinical practice, to determine the effectiveness of various laboratory methods in the context of differential diagnosis with SARS-like diseases and to develop optimized recommendations for the rational use of diagnostic resources.*

Materials and methods*. A retrospective analysis of 64,812 laboratory tests performed during 2022–2024 in a single network of Kyiv laboratories was conducted. The analysis included serological tests for IgM antibodies to EBV capsid antigen, IgG to capsid antigen (VCA), IgG to nuclear antigen (EBNA), monospot test and EBV PCR. Statistical research methods.*

Results. *The proportion of positive VCA IgM results remained stable throughout the three years of the study, fluctuating within 15.2-15.8%, which confirms the reliability of this marker of infectious mononucleosis. Serological indicators demonstrated high stability with low coefficients of variation – 4.3% for VCA IgM, 5.2% for VCA IgG and 6.5% for EBNA IgG. In contrast, EBV PCR revealed significant variability (coefficient of variation 118%) with a sharp decrease in positive results from 5.1% to 0.2%. A reorientation of the diagnostic practice of doctors from molecular to serological methods was also revealed - the proportion of serological tests increased from 65.5% to 71.4%, while molecular tests decreased from 34.5% to 28.6%. No seasonal fluctuations in the frequency of acute MI were detected (p=0.153), i.e. infectious mononucleosis was diagnosed equally throughout the year.*

Conclusions. *VCA IgM should be used as the main marker for the diagnosis of the acute phase of MI. VCA IgG and EBNA IgG are important diagnostic elements for determining the stage of the infectious process. The use of PCR should be limited to cases with an atypical course of the disease or in clinically ambiguous situations*

Keywords*: infectious mononucleosis, Epstein-Barr virus, serological diagnostics, VCA IgM, VCA IgG, EBNA IgG, monospot test, EBV PCR*

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1. Introduction

Infectious mononucleosis (IM), caused by Epstein-Barr virus (EBV) or cytomegalovirus, affects almost everyone during their lifetime. More than 90% of the adult population of the world has serological signs of infection, but clinical manifestations develop mainly in adolescents and young people aged 15–24 years. About 60.6 cases per 100,000 population are recorded annually [1], but the real figures may be much higher. In students and military units, the incidence can reach 11–48 cases per 1000 people annually, which makes this problem especially relevant for closed groups [2, 3].

Clinicians are aware of the considerable difficulty in diagnosing infectious mononucleosis. The classic triad - fever, pharyngitis and lymphadenopathy – is not observed in all users, especially early in the disease [4]. The situation is complicated by the fact that the symptoms often resemble ordinary acute respiratory viral infections and only an experienced doctor can suspect IM. At the same time, rapid and accurate diagnosis is critically important and allows to avoid complications of the disease [5].

Modern laboratory diagnostics of IM resembles a complex mosaic of different approaches. Serological methods are still considered the gold standard, as they allow not only to confirm the diagnosis, but also to determine the stage of the infectious process [6]. Antibodies to the capsid antigen VCA IgM appear early and disappear within 4–6 weeks, making them the most specific marker of the acute phase [7]. VCA IgG antibodies are detected in the acute phase and remain lifelong, while EBNA IgG appear 2–4 months after the onset of the disease [8]. This time distribution makes it possible to determine quite accurately when the infection occurred.

However, despite the high specificity of serological methods, the interpretation of their results may be

limited by atypical serological profiles, which can be detected in 28% of patients, which may require additional studies [9]. Atypical serological profiles are defined as results that do not correspond to the classic serological pattern of acute IM (simultaneous presence of VCA IgM and VCA IgG in the absence of EBNA IgG). Such atypical variants include the isolated presence of VCA IgM without concomitant markers, the simultaneous detection of VCA IgM and EBNA IgG (which may indicate reactivation rather than primary infection), weakly positive results at the border of reference values, or serological profiles with the absence of expected markers. The detection of isolated VCA IgM is particularly problematic, which in 52.8% of cases may be false positive or due to cross-reaction with cytomegalovirus [10]. These diagnostic difficulties emphasize the need to develop clear algorithms for the diagnosis of IM, considering possible serological variants.

The monospot test (Paul-Bunnell-Davidsohn test) is a rapid diagnostic screening method based on the detection of heterophilic antibodies that are produced in the body during EBV infection and can react with sheep erythrocyte antigens. This test attracts attention for its ease of performance, accessibility, and the ability to obtain a result within a few minutes, which may be especially useful when used in outpatient settings. However, its diagnostic reliability is questionable. The sensitivity of the test ranges from 70% to 92%, and the specificity from 96% to 100%, with sensitivity decreasing more critically in children younger than 4 years of age to 27–76% [11, 12]. Given this and other global data, the US Centers for Disease Control and Prevention does not recommend the use of the monospot test for routine screening due to the high frequency of false-negative/false-positive results, especially in the early stages of the disease [7]. In addition, false monospot test results can be observed in other diseases – HIV, systemic lupus erythematosus, and lymphomas [13].

Molecular genetic methods seem to be an ideal solution – PCR, according to the literature, has a sensitivity and specificity of over 95% [14]. However, practice shows otherwise – positive PCR results are detected in only 39% of patients with confirmed IM [15]. This calls into question the feasibility of widespread use of molecular methods and suggests their possible irrational use. Most experts are inclined to believe that PCR should be left for complex cases, when serological methods may be uninformative [16]. According to our data, PCR results are also affected by the duration of the disease – with its late use, blood test results may be negative.

The economic component of the issue is particularly important in conditions of limited health care resources. Hospitalization and treatment of a child with IM costs an average of $ 970.59 with a median hospital stay of 8 days [17]. Rational use of diagnostic methods for IM in primary diagnosis can significantly reduce these costs by reducing unnecessary examinations and accelerating the diagnosis. An economically justified approach involves the use of a complete blood count with leukoformula and a rapid monospot test, with subsequent use of serological studies as needed [18]. However, given the limitations of the monospot test mentioned earlier, this approach may not be effective enough.

An interesting change has occurred in diagnostic practice in recent years. There has been a trend toward a decrease in the use of molecular genetic methods and an increase in the use of serological tests [19]. These changes may reflect both a reassessment of the clinical relevance of PCR diagnostics for typical cases of IM and a desire for more cost-effective diagnostic strategies for IM.

Given the complexity of IM diagnosis and the variety of available laboratory methods, the development of optimized diagnostic algorithms is an urgent task. Such algorithms should consider clinical effectiveness, economic feasibility and the characteristics of local populations. The need for such an approach is confirmed by the high frequency of differential diagnosis with SARS-like diseases and the need for rational use of diagnostic resources in modern conditions.

The aim of the research. To assess the prevalence of markers of the acute period of infectious mononucleosis (VCA IgM, VCA IgG, EBNA IgG, heterophile antibodies in the monospot test and EBV DNA by PCR) based on the analysis of the Kyiv laboratory database during the three-year observation period (2022–2024), to investigate changes in diagnostic approaches in clinical practice, to determine the effectiveness of various laboratory methods in the context of differential diagnosis with SARS-like diseases, and to develop optimized recommendations for the rational use of diagnostic resources.

2. Materials and methods

A single-center retrospective cohort study with a follow-up period of 3 years was conducted. The conceptual approach of the study was based on the analysis of the assessment of the real incidence of infectious mononucleosis (IM) through the analysis of the acute phase marker (VCA IgM), the study of changes in methodological approaches to laboratory diagnostics in clinical practice, and a comparative assessment of the effectiveness of different diagnostic approaches. Such a design provided the possibility of recognizing epidemiological changes depending on variations in diagnostics, which is important for the correct interpretation of laboratory trends.

The analysis included all laboratory studies aimed at diagnosing IM, conducted from January 1, 2022 to December 31, 2024 in the conditions of one network of laboratories “Dila” in the city of Kyiv. The inclusion criteria were the availability of serological test results with complete documentation, the absence of technical artifacts, and the performance of studies for diagnostic purposes in patients with clinical symptoms requiring differential diagnosis of infectious mononucleosis. Studies with incomplete results, control studies as part of treatment monitoring, as well as preventive screening studies without clinical indications were excluded. The total number of analysed laboratory studies was 64,812, which provided sufficient statistical power to detect both significant trends and relatively small changes in diagnostic indicators. The analysis included five main diagnostic methods routinely used for diagnosing IM in clinical practice. Serological studies included the determination of IgM antibodies to the Epstein-Barr virus capsid antigen (VCA IgM) as a marker of acute infection, IgG antibodies to the capsid antigen (VCA IgG) to detect past

infection, and IgG antibodies to the EBV nuclear antigen (EBNA IgG) to confirm past infection. The monospot test was performed according to the classical Paul-Bunnell-Davidsohn method to detect heterophilic antibodies characteristic of infectious mononucleosis. Molecular genetic diagnostics was performed by polymerase chain reaction (EBV PCR) for direct detection of Epstein-Barr virus DNA in biological samples. All laboratory studies were performed according to standardized protocols using validated commercial diagnostic systems. Interpretation of results was carried out according to the reference values established by the manufacturers, considering the clinical context and recommendations of professional associations.

Statistical analysis. Statistical analysis was performed using descriptive and analytical statistical methods. Mean values, medians, standard deviations, and 95% confidence intervals were calculated for quantitative variables. Confidence intervals for proportions were calculated using the Wilson method, which provides greater accuracy compared to the binomial method. Temporal changes in the frequency of positive results of diagnostic methods were analysed using appropriate statistical tests to identify significant trends during the study period. To assess seasonal variations, cosinor analysis was used, which allows for the detection of cyclical components in time series and their statistical significance. The reproducibility of diagnostic methods during the study period was assessed by calculating the coefficients of variation for each method. Correlation analysis between different diagnostic approaches was performed to identify relationships and potential complementarity of methods in the diagnostic process. For a comprehensive assessment of different diagnostic methods, an integral indicator was developed that considered three key components: the average frequency of positive results of the method during the study period, the consistency of the results (assessed through the coefficient of variation), and the clinical relevance of the method for different phases of EBV infection. Each component was standardized to a scale from 0 to 1, where 1 corresponded to the optimal value. Clinical relevance was assessed considering the specific purpose of each diagnostic method. Thus, VCA IgM received the highest weight as a marker of acute infection, serological markers of past infection (VCA IgG and EBNA IgG) were evaluated from the position of their informativeness for excluding primary infection, the monospot test was considered as a screening method, and EBV PCR was considered as a method for confirming active viral replication.

We also conducted a detailed analysis of changes in the structure of the use of diagnostic methods during the study period. Both the absolute volumes of studies for each method by year and their relative shares in the overall structure of laboratory studies were calculated. Special attention was paid to the analysis of the ratio of serological and molecular methods as an indicator of changes in diagnostic approaches in clinical practice. To identify statistically significant changes in the methodology of appointments, appropriate criteria for comparing proportions with correction for multiple comparisons were used. Analysis of the dynamics of the volume of studies allowed us to assess not only quantitative changes, but also qualitative transformations in diagnostic practice.

Seasonal variations in IM incidence were analysed by grouping data by calendar seasons – winter (December-February), spring (March-May), summer (June-August) and autumn (September-November). For each season, the frequency of positive VCA IgM results with corresponding confidence intervals was calculated. The statistical significance of seasonal differences was assessed using the nonparametric Kruskal-Wallis test. Additionally, cosinor analysis was performed to identify possible circadian rhythms in incidence with the calculation of amplitude, acrophase and coefficient of determination.

Statistical analysis was performed using Python version 3.8 software with the main libraries for statistical data processing (pandas, numpy, scipy and matplotlib). The level of statistical significance was set at $p<0.05$ for all analyses. In cases of multiple comparisons, an appropriate correction was applied to control for type I error. Results were presented as point estimates with 95% confidence intervals to ensure the possibility of assessing the clinical significance of the differences found.

The study was approved by the bioethics committee of the medical institution (protocol No. 193 dated 03/24/2025). The study was performed in accordance with the principles of the Declaration of Helsinki and the requirements of national legislation on medical research. Only anonymized laboratory data without the possibility of identifying patients were used. The study protocol met ethical standards for retrospective studies using laboratory data.

3. Results

During the observation period, 64,812 laboratory tests aimed at diagnosing infectious mononucleosis in patients with a clinical picture requiring differentiation from acute respiratory viral infections were analysed. The overall frequency of positive results for the diagnosis of IM was 37.6%, which reflects the high frequency of prescribing laboratory tests in clinically ambiguous cases. The most indicative for understanding the true frequency of acute infectious mononucleosis was the analysis of VCA IgM as a marker of the acute phase of the disease. This indicator demonstrated stability throughout the entire study period, fluctuating within 15.2–15.8%. Statistical analysis of time series showed no significant changes in the frequency of positive VCA IgM results during the three years of the study ($p=0.695$, which means the absence of a statistically significant trend), which indicates the invariability of the epidemiological situation regarding infectious mononucleosis. Thus, in 2022, out of 3,510 VCA IgM tests performed, 15.5% [14.3; 16.7] were positive, in 2023 out of 6,853 tests - 15.8% [15.0; 16.6], and in 2024 out of 7,266 tests – 15.2% [14.5; 16.0]. This stability of VCA IgM results contrasts sharply with significant changes in the frequency of appointments and results of other diagnostic methods (in particular, a 96% decrease in the use of EBV PCR and an 81% increase in the use of the monospot test). This emphasizes that the observed variations in positive MI results in other methods reflect changes in the diagnostic approaches of clinicians (possibly related to economic factors, accumulation of clinical experience,

or changes in clinical protocols). A detailed analysis can be found in Table 1.

Regarding seasonal features in morbidity, when analysing the frequency of acute IM, we did not find statistically significant fluctuations during the year (p=0.153), which may be due to the overlap of periods of increased incidence of infectious mononucleosis and SARS, which create a constant need for differential diagnosis. Minor fluctuations from 15.3% in autumn to 16.0% in summer have no clinical significance and confirm the need for equal vigilance regarding infectious mononucleosis throughout the year.

Table 1

Stability of the acute IM marker (VCA IgM) during 2022–2024

Year	Number of studies	Positive results	Frequency of positive results (%)	95% CI	Change from previous year
2022	3510	544	15.5	14.3–16.7	–
2023	6853	1,083	15.8	15.0–16.6	+0.3%
2024	7266	1,104	15.2	14.5–16.0	–0.6%

When analysing the use of serological methods of laboratory diagnostics, significant changes were found, reflecting the adaptation of medical practice to economic and methodological factors. Thus, the most significant changes affected molecular genetic diagnostics – the frequency of positive EBV PCR results decreased from 5.1% in 2022 to 0.2% in 2024, which represents a 96% relative decrease. Such changes cannot reflect true epidemiological processes and are probably associated with incorrect interpretation of the clinical picture of the disease. That is, given the high sensitivity of this method, it can be assumed that it was prescribed mainly not according to indications, or to exclude IM in the atypical course of respiratory diseases. At the same time, there was a parallel increase in the use of the monospot test with a frequency of positive test results that increased from 7.0% to 12.7% (+81% relative increase). Serological markers of past infection (VCA IgG and EBNA IgG) showed a moderate decrease in the frequency of positive results but remained at a high level (over 75%), confirming the wide prevalence of EBV infection in the population and the validity of their use to exclude primary infection.

Structural changes in the volume of research demonstrate a clear trend towards a shift from molecular methods of research to serological methods. The share of serological studies increased from 65.5% in 2022 to 71.4% in 2024, while the share of molecular methods in diagnostic appointments of doctors decreased from 34.5% to 28.6%, as shown in Table 2. Such a shift may be associated with economic factors (serological studies are significantly cheaper), difficulties in interpreting PCR results (detection of latent persistence instead of acute infection), and the prevalence of serological diagnostics, which is available in most cities of Ukraine.

Table 2

Evolution of methodological preferences in the diagnosis of infectious mononucleosis

Year	Serological methods		Molecular methods		Total number of studies
	Number	%	Number	%	
2022	9449	65.5	4972	34.5	14421
2023	17355	69.5	7609	30.5	24964
2024	18153	71.4	7274	28.6	25427

Regarding the comparative assessment of the effectiveness and stability of the studied methods, their comprehensive assessment, carried out using the analysis of coefficients of variation, revealed significant differences in the reliability of the methods during the studied period. The highest stability was demonstrated by serological markers – VCA IgM with a coefficient of variation of 4.3%, VCA IgG (5.2%) and EBNA IgG (6.5%), which confirms their reliability for routine clinical diagnostics. These indicators contrast with the extremely high variability of EBV PCR (coefficient of variation 118%), which indicates certain shortcomings in the use of this method in routine practice – potentially PCR was mainly used for purposes other than its intended purpose.

The integral assessment of the clinical utility of diagnostic methods, which considered the average frequency of positive examination results, the stability of results and clinical relevance, which is presented in more detail in Table 3, demonstrated the predominance of serological methods in diagnostic efficiency. EBNA IgG showed the highest integral score (0.830), which reflects its value for confirming past EBV infection and excluding the acute phase of infection. At the same time, VCA IgG took the next position with a not less significant result (0.787). VCA IgM, despite the highest stability of results, received a moderate integral score (0.660) due to its specificity for diagnosing only the acute phase of infection and the relatively low frequency of positive test results in the general population of subjects. The monospot test and EBV PCR showed the lowest clinical utility rates (0.572 and 0.571, respectively), which calls into question the feasibility of their widespread use for the diagnosis of IM.

Table 3

Comprehensive assessment of the effectiveness of diagnostic methods for IM

Diagnostic method	Coefficient of variation (%)	Average frequency of positive test results	Integral benefit assessment	Rating
VCA IgM	4.3	15.5%	0.660	3
VCA IgG	5.2	79.7%	0.787	2
EBNA IgG	6.5	83.9%	0.830	1
Monospot	28.9	10.0%	0.572	4
EBV PCR	118.1	1.9%	0.571	5

When analysing the correlation relationships between different diagnostic methods, a rather complex system of associations was revealed, reflecting different aspects of the infectious process in MI and confirming the need for a differentiated approach to the choice of diagnostic methods. The strongest negative correlation was observed between EBV PCR and monospot test (r=-0.76), which may reflect different clinical situations in which these methods are used, or technical features of their implementation. Moderate positive correlations between serological markers (VCA IgG and EBNA IgG - r=0.41) confirm their complementarity in the diagnosis of chronic forms of EBV infection. The absence of significant correlations of VCA IgM with other methods emphasizes its unique role as an independent marker of the acute phase of infection and justifies its use as the main criterion for the diagnosis of infectious mononucleosis. More detailed relationships between methods are shown in the correlation matrix (Fig. 1).

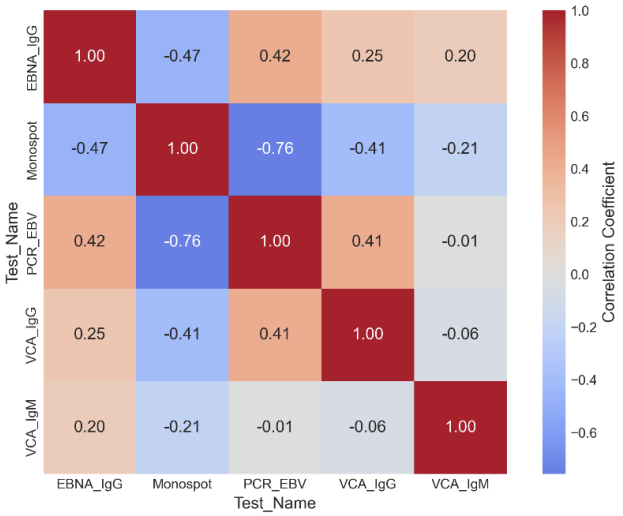

Fig. 1. Correlation matrix of IM research methods

Our analysis revealed several important aspects that require deeper interpretation. The most important of them is the decrease in positive results in EBV PCR, which has no real justification and potentially, as noted earlier, may be associated with the irrational use of this research method. The detected seasonal variations in acute MI have minimal clinical significance due to the low amplitude of fluctuations and the lack of statistical significance, which confirms the need for uniform diagnostic vigilance throughout the year. The stability of VCA IgM as a marker of acute infection against the background of various changes in other methods emphasizes its status as the gold standard for the diagnosis of infectious mononucleosis and justifies its priority use in clinical algorithms for differential diagnosis with SARS-like diseases.

4. Discussion of research results

The American Academy of Family Physicians published updated guidelines for the diagnosis and treatment of IM in 2023 [20]. According to these guidelines, the initial evaluation of acute IM should include a complete blood count with differential (to detect >40% lymphocytes and >10% atypical lymphocytes) and a rapid heterophile antibody test (optionally a monospot). It is emphasized that the heterophile antibody test has a sensitivity of about 87% and a specificity of 91% but may give false-negative results in children younger than five years and in adults during the first week of illness. The guidelines also indicate that elevated liver enzymes are considered an additional criterion for the diagnosis of IM if the heterophile antibody test is negative.

In contrast, a British study by Naughton and colleagues, published in 2021 [21], proposed a standardized diagnostic algorithm for IM, which involves a stepwise approach, where first the clinical assessment of the classic triad of symptoms (fever, pharyngitis, lymphadenopathy) is performed, followed by laboratory confirmation through complete blood count and serological testing. The authors emphasized the importance of early, accurate, and informative laboratory testing for diagnosis, appropriate treatment, and patient management, especially in cases of atypical disease.

The US Centers for Disease Control (CDC) also updated its recommendations for laboratory diagnosis of EBV infection in 2021. The authors cautioned clinicians against using the monospot test for routine screening of IM due to the high rate of false-negative and false-positive results [7]. However, as an alternative, the CDC recommends the use of serological markers as the gold standard for diagnosis, especially in children under five years of age, where approximately 40% of cases do not develop heterophile antibodies after primary EBV infection.

According to our data, Ukrainian doctors most often use serological diagnostic methods. In this case, monospot is in second place. Thus, the stability of VCA IgM indicators during a three-year observation period is of particular interest, especially when compared with the results of a large-scale study by Chinese scientists Shi et al. [22]. Their work involving 616 children with suspected IM also demonstrated high stability of serological methods during different stages of the disease – the sensitivity of VCA IgG reached 98.27% with a specificity of 91.13%. Interestingly, similar reproducibility of statisti-

cal indicators of serological markers is found in most countries of the world, regardless of geographical region and population characteristics. At the same time, the data we obtained on EBV PCR raise serious questions. The drop in positive results from 5.1% to 0.2% is difficult to explain solely by epidemiological factors; they may be due to low rates of occurrence, including potentially insufficient assessment of patients' clinical data and, as a result, the irrational use of additional research methods. Regarding the method for determining heterophile antibodies, Wang and colleagues in 2021, studying the monospot test in the population, obtained a specificity of the test of 90.6% - which is significantly lower than previously published data [23]. Given this, the authors emphasized in the article the need to confirm positive results of the monospot test by serology. However, despite certain limitations and shortcomings of the monospot, our doctors have now begun to use it for IM screening more often – during the observation period in our sample, the frequency of use of the method increased from 7.0% to 12.7%.

Regarding the parameters that we calculated during the study, the coefficients of variation reveal an interesting picture. Serological markers demonstrate quite good stability - VCA IgM varies by only 4.3%, VCA IgG by 5.2%, EBNA IgG by 6.5%. In contrast, EBV PCR shows a coefficient of variation of 118%, which practically makes it impossible to use it as a reliable diagnostic tool for IM. De Paschale and Clerici in 2012 [6] warned about the shortcomings of serological diagnostics of EBV infection related to the interpretation of the results, about atypical serological profiles in 28% of patients. However, even these "problematic" serological methods turn out to be much more stable than molecular diagnostics.

The lack of seasonal variation in the incidence of acute MI was not surprising. Crawford and Ando in their epidemiological review noted that infectious mononucleosis does not show seasonal variations in incidence [24]. Our variation from 15.3% in the fall to 16.0% in the summer is so small as to be of no clinical significance.

Structural changes in the use of diagnostic methods reflect broader trends in laboratory diagnosis of infectious diseases. The increase in the proportion of serological tests from 65.5% to 71.4% is consistent with the findings of a meta-analysis by Liu and colleagues [25] on the diagnostic value of EBV DNA and EBV-specific antibodies. The authors emphasized the advantages of serological methods in terms of availability and cost, whereas molecular methods, despite potentially higher specificity, require sophisticated equipment and specially trained personnel.

The correlation structure between diagnostic methods deserves special attention. The strong negative correlation between EBV PCR and monospot test (r=-0.76) may indicate that these methods are used in fundamentally different clinical situations. Shi et al. [22] showed that the EBV-DNA test is superior to the VCA-IgG avidity test in children younger than 6 years, especially up to 3 years of age. That is, potentially in cases of diagnosis of IM in children under 6 years of age, PCR has better results. However, based on the obtained data on PCR variability in our study, it can be argued that although PCR has its place in the diagnosis of IM (children), high variability indicates that this method is used irrationally in many cases where serological methods would be more reliable and cost-effective.

The results of the integrated assessment of the clinical utility of the methods are not surprising. EBNA IgG (0.830) and VCA IgG (0.787) top the ranking, which is consistent with the meta-analysis by Liu and colleagues [26]. Their study showed that EBV-DNA, VCA-IgA, EBNA1-IgA and Rta-IgG have high accuracy in the early diagnosis of EBV-associated diseases, with the combined serological markers demonstrating the highest diagnostic efficiency compared to the individual tests.

Study limitations. Regarding the limitations of our study, the retrospective nature of the study and the focus on data from a single laboratory network limit the possibility of extrapolating the results. The lack of clinical data does not allow us to assess the impact of changes in diagnostic approaches on the quality of medical care.

Prospects for further research. Future prospective studies that include clinical outcomes and cost-effectiveness of different diagnostic strategies will help to better understand the optimal approaches to diagnosing infectious mononucleosis.

Practical relevance. The practical implications and utility of this study are clear. The stability of VCA IgM confirms its status as a primary diagnostic method for MI, while the high variability of EBV PCR requires a review of the indications for its use.

5. Conclusions

1. The analysis of 64,812 laboratory tests conducted during 2022–2024 demonstrated the stability of the true incidence of infectious mononucleosis at 15.2–15.8% according to the VCA IgM marker, which indicates the immutability of the epidemiological situation. At the same time, despite significant changes in the choice of diagnostic methods for IM (in particular, a decrease in the use of EBV PCR by 96% and an increase in the use of the monospot test by 81%), the real frequency of acute infectious mononucleosis remained constant, which confirms the stability of the VCA IgM marker as a reliable indicator of the epidemiological situation.

2. Serological markers of EBV infection demonstrated the highest stability during the studied period with coefficients of variation of VCA IgM - 4.3%, VCA IgG - 5.2%, EBNA IgG - 6.5%, which confirms their status as the main diagnostic tools in IM.

3. EBV PCR showed extremely high variability of results (coefficient of variation 118%) and a sharp decrease in positive test results from 5.1% to 0.2%, indicating systemic shortcomings in the use of this method in the routine diagnosis of infectious mononucleosis.

4. A clear trend towards reorientation of clinical practice from molecular to serological diagnostic methods has been identified - the share of serological studies has increased from 65.5% to 71.4%, while molecular methods have decreased from 34.5% to 28.6%, reflecting adaptation to economic and methodological factors.

5. An integrated assessment of clinical benefit demonstrated the predominance of serological markers of infection (EBNA IgG (0.830) and VCA IgG (0.787)), which justifies their priority use to exclude primary EBV infection in differential diagnostic algorithms.

6. The analysis did not reveal statistically significant seasonal fluctuations in the frequency of acute infectious mononucleosis (p=0.153), which confirms the need for uniform diagnostic vigilance throughout the year.

7. The increase in the frequency of positive monospot testing results from 7.0% to 12.7% against the background of its limited diagnostic value (integral score 0.572) requires a review of the indications for its use and mandatory confirmation of positive results by serological methods.

Thus, based on the results obtained, it is recommended to use VCA IgM as the main marker of the acute phase of infectious mononucleosis, complementary use of VCA IgG and EBNA IgG to determine the stage of the infectious process, and limit the use of EBV PCR exclusively to cases with an atypical clinical course or in immunocompromised patients.

Conflict of interests

The authors declare that they have no conflict of interest regarding this study, including financial, personal, authorship, or other, that could influence the study and its results presented in this article.

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Data availability

Data will be provided upon reasonable request.

Using artificial intelligence technologies

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

Authors' contributions

Nataliia Dziubenko: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Visualization; **Olga Golubovska:** Conceptualization, Methodology, Data Curation, Writing – review & editing, Supervision, Project administration.

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