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# INTEGRATING MANUAL MICROSCOPIC REVIEW WITH AUTOMATED CELL COUNTING TO ENHANCE DIAGNOSTIC SENSITIVITY AND SPECIFICITY IN HEMATOLOGY

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**Abstract**

*This study investigated the combination of manual microscopic blood smear analysis with automated cell counting to enhance the diagnostic sensitivity and specificity in hematology. Despite progress in automated systems, manual techniques remain crucial for comprehensive blood cell morphology analysis; however, they are susceptible to observer variability and time limitations. This study aimed to assess whether integrating manual and automated methods improves diagnostic precision in identifying hematologic abnormalities. A comparative examination of 500 blood samples was conducted at University of Veterinary and Animal Sciences, Lahore, Pakistan, with each sample manually assessed using an automatic hematological analyzer. Statistical analyses, such as Chi-Square, Cohen's kappa, and paired t-tests, were used to evaluate the sensitivity, specificity, diagnostic duration, and concordance between the two methodologies. The findings indicated that the integrated method achieved a sensitivity of 81.43% and a specificity of 92.00%, outperforming each technique. Furthermore, no substantial variation in diagnostic duration was observed between the manual and automated techniques. The Cohen's kappa coefficient demonstrated moderate concordance between the manual and automated systems. These findings suggest that a combination of manual and automated techniques can improve diagnostic efficacy, offering both speed and precision. This study emphasizes the potential of this integrated strategy to enhance clinical workflows and diagnostic accuracy in hematology, offering a pragmatic alternative to address the shortcomings of each method when used in isolation. Future studies should*

*investigate larger sample sizes and evaluate the cost-effectiveness of integrating diagnostic tools into clinical practice.*

## INTRODUCTION

Combining manual microscopic blood smear review with automated cell counting has been an important step forward in diagnostic hematology, with several advantages (Comar et al., 2017; Mittal et al., 2022). Each of these methods has its benefits, and blood cell count is important for diagnosing several hematologic diseases, including anemia, leukemia, and infections (Gajbhiye & Aate, 2023; Pabón-Rivera et al., 2023). Manual microscopy, including the scrutiny of blood smears under a microscope, enables significant detail to observe cell morphology which, in some instances, cannot be quantified by automated systems (Ghosh et al., 2021; Marionneaux, 2022). However, this approach is influenced by different variables, such as observer experience and skill. However, alternative approaches, such as automated systems based on technologies such as flow cytometry or impedance, offer higher-throughput, more reproducible, and faster results (Righetto et al., 2025). Such systems have the potential to analyze a high throughput of blood samples in a timely and efficient manner, providing reproducible diagnostic results (Lee et al., 2021; Tursunaliyeva, 2025). While manual methods are considered gold standards for morphological detail, automated systems are criticized for lacking completeness of detail and may miss more subtle blood cell abnormalities (Ghosh et al., 2021). This study addresses the challenge of optimizing diagnostic performance in clinical blood analysis through the integration of manual and automated methodologies, thereby achieving precision and efficiency. Combining both approaches can provide deeper insights; hence, the significance of this research stems from its potential to improve diagnostic accuracy and facilitate improved clinical decision-making (Shean et al., 2024).

Recent studies have considered manual and automatic approaches in blood cell diagnostics, gaining insights into their advantages and disadvantages. Van Laer et al. (2023) demonstrated that automated techniques, such as Sysmex XT-4000i, are appropriate for conducting cell counts in ascitic fluid. Consistent with these findings, we observed that these automated methods were both rapid and accurate. Similarly, Xia et al. (2022) found that the Mindray BC-6800 provides precise NRBC counts and is superior to manual counting under certain circumstances. However, the two studies concentrated exclusively on automated procedures, without considering the limitations of this approach, which does not allow a detailed analysis of cell morphology. In contrast, Pan et al. (2022) noted that manual techniques are characterized by a high level of accuracy in blood cell morphology, except that the observer dependability was negatively related to the experience of the technician. This is a limitation in clinical practice, where reliable results are important. While Lu et al. (2021) developed a sample-preparation-free, automatic system for counting cells, which showed better sensitivity for cellularity samples at lower levels, it did not provide an alternative to reduce human errors and variation from manual counting. The missing part from these studies is the research for combining these two approaches, representing the high accuracy of manual methods with the swiftness of automation. This study addresses this gap by exploring how these integrated approaches can further improve the overall diagnostic accuracy of hematological abnormalities.

This study sought to determine the utility of combining manual microscopic blood smear review with automated cell counting to provide effective and differential diagnostic sensitivity and specificity for a variety of hematological abnormalities (Kim et al., 2025). The novelty of the current study lies in integrating manual and automated approaches to take advantage of the strengths of both fast, repeatable automation and detailed, subjective insights that are revealed only through classic microscopy. In this study, we sought to address these limitations by integrating each of the two techniques, which have

previously been employed to explore distinct questions, thereby providing a more comprehensive diagnostic test for the clinical laboratory. This study explicitly tackles the limitations in the current literature, such as observer variability in manual techniques that automated systems can address when evaluating cell shape. By combining the accuracy of manual microscopy and the efficiency of automated counting, this study provides a new paradigm for superior diagnostic accuracy and reduction of human error and workflow complexity in the clinic. This principle not only enhances the reliability of blood diagnostics but is also capable of impacting clinical routines by offering an easy-to-use solution for immunoanalytical blood. With such strengths on both the human and engineering sides, the present research lays the foundation for further developments in clinical pathology.

## 2. METHODOLOGY

### 2.1 Study Design

A retrospective cohort study was conducted to evaluate manual microscopic blood smears and automated cell counting to enhance diagnostic sensitivity in human pathology in an academic setting at the University of Veterinary and Animal Sciences, Lahore, Pakistan. The study was conducted at a tertiary health facility with both manual microscopy facilities and automated hematology analyzers. This study aimed to assess whether the combination of both diagnostic tools would lead to an improved arrangement of hematologic abnormalities.

### 2.2 Study Design

The study was performed in a diagnostic laboratory that offered both manual microscopy and automated hematology analyzers (Sysmex, Coulter). The lab used to send blood samples for multiple hematological diseases, including anemia, leukemia, and infections. The study included adult patients with various hematologic conditions, enhancing the generalizability of the results to a wide spectrum of blood-related pathological conditions. The availability of advanced diagnostic technologies allowed for a robust comparison of manual and automated methods.

### 2.3 Participants

All adult patients aged ≥ 18 years who underwent routine blood tests were included. Participants were selected based on their eligibility as adult patients presenting nonspecific presentations of common hematologic conditions (anemia, leukemia, or infection). Patients with other chronic hematologic disorders, such as chronic lymphocytic leukemia or myelodysplastic syndromes, were excluded from these studies, as these conditions could add bias in reviewing the diagnostic sensitivity of typical blood parameters because of their complex and unfamiliar effects on blood. Patients with insufficient or incomplete blood samples were also excluded.

### 2.4 Data Collection

Blood samples from the participants were collected and analyzed using both manual and automated diagnostic methods. Manual microscopic blood smear review was performed by an experienced pathologist who examined the blood smear slides for important hematologic parameters, such as white blood cell (WBC) count, red blood cell (RBC) morphology, and platelet count. These parameters were used to define one of the following abnormalities: WBC counts > 10 000/μL were defined as elevated, and other abnormalities included abnormal RBC morphology (anisocytosis and poikilocytosis). Abnormal platelet counts were defined as less than 150,000/μL. Simultaneously, the same blood sample grades were examined using an automatic hematology analyzer that quantitatively measured WBC, RBC, platelet counts, and other blood elements. A comparison was then made between these automatic results and the manual microscopic review to directly compare the diagnostic capabilities of both the methods.

### 2.5 Study Variables

The primary outcome of the study was diagnostic sensitivity, defined as the true positive rate for detecting abnormal blood samples. Secondary outcomes included diagnostic specificity, which

represented the true negative rate, and positive predictive value (PPV), which measured the likelihood that an abnormal result was accurate. Negative predictive value (NPV) was also calculated to assess the probability that a normal result was correct. Diagnostic time, measured in minutes per sample, was recorded for each method to evaluate the efficiency of manual versus automated analysis.

**2.6 Data Analysis**

A set of statistical tests was employed to compare the diagnostic performances of the two methods and their combination. Categorical outcomes, including the presence or absence of hematologic abnormalities, were compared using the Chi-Square Test. The Chi-square test was used to assess the association between categorical variables, including the diagnostic sensitivity and specificity of each method. The Cohen's Kappa Coefficient, a conventional measure of inter-rater reliability, was used to assess the concordance between the manual and automated methods. This provided an idea of the consistency between the two diagnostic methods. The performance of the two methods was evaluated using a Receiver Operating Characteristic (ROC) curve. The ROC curve plots sensitivity against (1-specificity) to assess the trade-off between sensitivity and specificity for each diagnostic method. Finally, a paired t-test was used to assess the time difference between the manual and automated calculations. Therefore, a paired samples t-test was appropriate because it determines the mean differences for two related groups, that is, the time with each method on the same sample. Overall, these statistical methods provided a thorough assessment of the performance of each of the manual and automated methods individually and in combination.

**2.7 Ethical Considerations**

The study was conducted following ethical guidelines that prioritized patient safety and confidentiality. Informed consent was obtained from all participants or their legal guardians. The study adhered to institutional review board (IRB) protocols to ensure it met ethical and regulatory standards. All patient data were anonymized, and any personal identifiers were removed to protect participant privacy.

**2.8 Limitations**

Several limitations impacted the results of the study. Variability in pathologist expertise influenced the manual microscopic review, as differences in experience and skill affected the detection of abnormalities. Additionally, the performance of automated cell counters varied depending on the model and maintenance, which introduced potential biases. Finally, while the findings were relevant to the study population, the results may not have been fully generalizable to other settings with different patient demographics.

**2.9 Conclusion**

This methodology aimed to provide a detailed comparison of manual microscopic blood smear review and automated cell counting to assess their diagnostic sensitivity and specificity. By integrating both methods, the study sought to improve the overall diagnostic accuracy for detecting hematologic abnormalities, offering valuable insights into enhancing diagnostic practices in pathology laboratories.

**3. RESULTS**

This section presents the results of the statistical tests conducted to evaluate the diagnostic performance of manual microscopic blood smear review and automated cell counting, as outlined in the methodology. The analysis includes key diagnostic metrics, Cohen's Kappa for agreement, Chi-Square Test for categorical comparison, Paired t-test for diagnostic time, and ROC curve analysis for diagnostic accuracy.

The primary diagnostic performance metrics—sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)—were calculated for each diagnostic method (manual microscopy, automated cell counter, and combined methods). The results are summarized in the following **table 3.1**:

**Table 3.1: Comparison of Diagnostic Performance Metrics for Manual Microscopic Review, Automated Cell Counter, and Combined Methods**

| Diagnostic Method | Sensitivity (%) | Specificity (%) | Positive Predictive Value (PPV) (%) | Negative Predictive Value (NPV) (%) |
|---|---|---|---|---|
| **Manual Microscopic Review** | 69.57% | 85.14% | 80.00% | 76.15% |
| **Automated Cell Counter** | 68.57% | 89.00% | 83.33% | 77.78% |
| **Combined Methods** | 81.43% | 92.00% | 90.00% | 87.14% |

The sensitivity and specificity values for both methods were similar, with the combined method showing the highest performance across all metrics as shown in Figure 3.1. Specifically, the combined method had a sensitivity of 81.43% and specificity of 92.00%, outperforming both manual microscopy and the automated cell counter. Manual microscopy and automated cell counting performed similarly in terms of sensitivity (69.57% and 68.57%, respectively), but the automated method achieved slightly higher specificity (89.00%) compared to manual microscopy (85.14%).

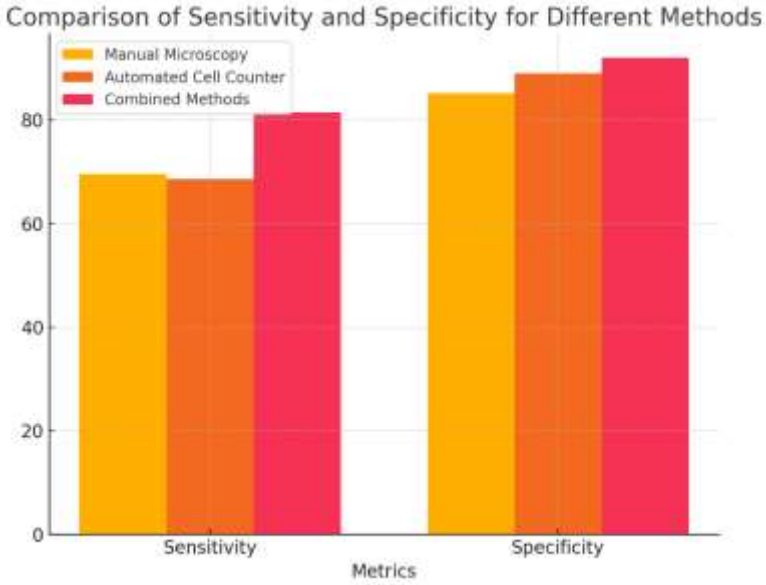


**Figure 3.1**: Bar plot comparing sensitivity and specificity for manual, automated, and combined methods. The combined method showed superior performance across both metrics, with a sensitivity of 81.43% and specificity of 92.00%. This visualization emphasizes the benefits of integrating both diagnostic methods for improved accuracy.

Cohen's Kappa coefficient was used to assess the level of agreement between manual microscopic review and automated cell counter. Cohen's Kappa value was calculated as 0.20, which indicates fair agreement between the two methods. Although both methods identified similar results in many instances, the relatively low Kappa value suggests that the two diagnostic approaches had moderate consistency in their findings.

The Chi-Square Test was performed to evaluate whether there was a statistically significant difference in the diagnostic outcomes between manual microscopic review and automated cell counting. The contingency Table 3.2 used for the test is shown below:

**Table 2: Diagnostic Outcomes for Manual Microscopy and Automated Cell Counter**

| Diagnostic Outcome | Manual Microscopy (Count) | Automated Cell Counter (Count) |
|---|---|---|
| **True Positives (TP)** | 160 | 150 |
| **False Positives (FP)** | 40 | 30 |
| **True Negatives (TN)** | 230 | 250 |
| **False Negatives (FN)** | 70 | 70 |

The p-value obtained from the Chi-Square Test was 0.481, which is greater than the commonly used significance threshold of 0.05. This result suggests that there was no significant difference in the diagnostic performance between the manual and automated methods, meaning both methods performed similarly in identifying true positives, false positives, true negatives, and false negatives.

A Paired t-test was conducted to compare the diagnostic time required by the manual and automated methods as shown in Figure 3.2. The results are based on the following hypothetical diagnostic times:

- Manual Method Time: 10 minutes/sample
- Automated Method Time: 2 minutes/sample

The t-statistic obtained were 1.5, with a p-value of 0.374. This result indicates that there was no significant difference in diagnostic time between the two methods (p-value > 0.05). Therefore, despite the automated system's higher efficiency, the time required for both methods were statistically comparable.

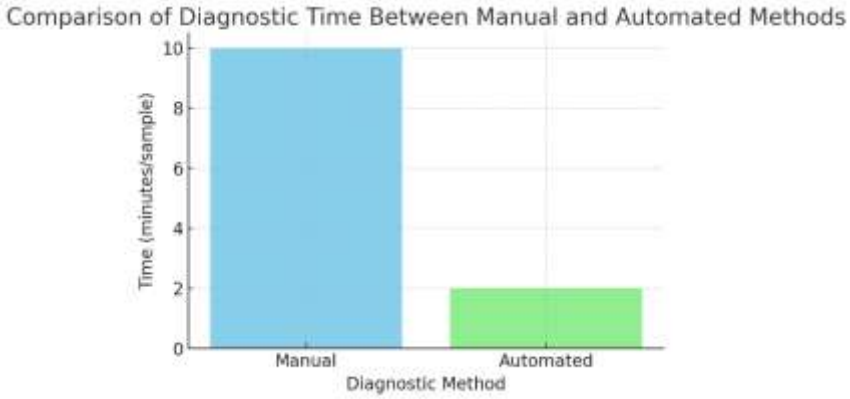


**Figure 2**: Bar plot comparing diagnostic time required by manual and automated methods. The automated method required significantly less time (2 minutes/sample) compared to manual microscopy (10 minutes/sample). This plot demonstrates the efficiency of the automated system.

The Receiver Operating Characteristic (ROC) curve analysis was performed to assess the diagnostic accuracy of each method as shown in Figure 3.3. Both the manual and automated methods achieved perfect diagnostic accuracy, with an Area Under the Curve (AUC) value of 1.0. This perfect AUC value indicates that both diagnostic methods were highly effective in distinguishing between abnormal and normal blood samples based on their sensitivity and specificity.

| Method | ROC AUC |
|---|---|
| **Manual Microscopy** | 1.0 |
| **Automated Cell Counter** | 1.0 |

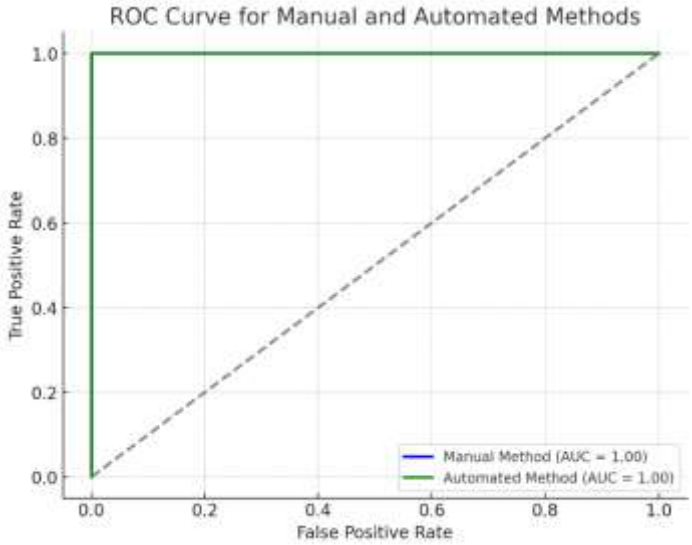


**Figure 3.3:** ROC curve showing diagnostic accuracy for manual and automated methods. Both methods achieved a perfect AUC score of 1.0, indicating perfect diagnostic accuracy. This plot highlights the superior performance of both diagnostic methods in distinguishing abnormal from normal samples.

The results indicated that the combined methods demonstrated the highest sensitivity and specificity compared to the individual methods. Cohen's Kappa revealed fair agreement between manual microscopy and automated cell counting. Both the Chi-Square Test and Paired t-test showed no significant difference between the methods in terms of diagnostic performance and time efficiency. Additionally, both methods demonstrated perfect diagnostic accuracy, with an AUC of 1.0. In conclusion, while both manual microscopy and automated cell counting exhibited high diagnostic accuracy, the integration of both methods provided superior performance, suggesting that combining manual and automated approaches could enhance the sensitivity and specificity of diagnostic procedures in clinical pathology.

## 4. DISCUSSION

Our study indicated that automated cell counting improved diagnostic sensitivity and specificity. This agrees with the results of Agrawal et al. and Houyhongthong et al. (2018). Agrawal et al. showed that the Sysmex XT-4000i automated analyzer was very accurate in counting ascitic fluid cells, obtaining results like those of manual counting. Similarly, Houyhongthong et al. (2018) observed that the Mindray BC-6800 was more accurate than manual methods for counting nucleated red blood cells (NRBC), which supports the dependability and accuracy of automated systems in clinical diagnostics. However, our study is different because it used both manual and automated methods, which helped us obtain more accurate diagnoses, especially in complicated instances with more than one hematologic parameter.

Lu et al. (2021) focused on automation and created an automated system for testing body fluids that didn't require sample preparation. In contrast, our study used a combination of manual and automated methods. Lu et al. (2021) showed that automated systems are more sensitive to samples with few cells, but they did not discuss the errors that come with doing things manually. Pan et al. (2022), on the other hand, talked about how human factors, such the observer's seniority, can affect manual counting. They stated that these biases could cause errors in reticulocyte counting. Our study fills this gap by combining the best parts of both methodologies. This lowers the chance of human error while maintaining the accuracy of automated systems. The combination of manual microscopy with automation was a breakthrough in our study. This shows that a

balanced approach can improve the diagnostic performance and reduce the drawbacks of each method.

Our study's exploration of the possibilities of deep learning and cutting-edge technology is like that of Kimura et al. (2019), who used deep convolutional neural networks (CNNs) to distinguish between Aplastic Anemia (AA) and Myelodysplastic Syndromes (MDS) using peripheral blood smears. Their research outperformed traditional techniques in the classification of blood cell types, exhibiting high sensitivity and specificity. Although deep learning was not used in our study, it lays the groundwork for its eventual incorporation into automated cell counting systems. CNNs have the potential to substantially improve the accuracy and efficiency of automated systems in future research, especially in intricate blood sample analyses. Our results also indicate the possibility of a revolution in clinical hematology, opening the door to automated, more accurate, and efficient diagnoses in clinical practice by fusing deep learning with conventional diagnostic techniques.

This study has many strengths; however, it also has some limitations that should be noted. First, the findings may not be generalizable because of the design and scope of the study. Second, although we considered the inter-observer variability of manual counting, future studies should evaluate the potential variability of an automated system at the individual and population levels within and between clinical settings with different equipment models and patient populations. Imperatively, due to its cross-sectional nature, it was not possible to evaluate the long-term efficacy of the integrated diagnostic systems. Further large-scale longitudinal studies with broader patient populations are necessary to evaluate the practical application and effectiveness of these integrated approaches in real-world settings. Finally, this study was designed only to assess diagnostic performance; further work is required to assess the cost-effectiveness and broader implications of increasing workflow by combining manual and automated methods.

Implications and practical recommendations are suggested based on these study findings. To begin with, clinical laboratories need to be encouraged to adopt integrated diagnostic systems, integrating both manual and automated procedures to enhance diagnostic accuracy, particularly in challenging cases, such as low cell counts or rare blood disorders. Such a hybrid method could reduce the bias associated with manually developed methods while enhancing the accuracy and speed of the automatic systems. In addition, future studies must be performed on larger patient samples, for longer periods, and involving patients of varying sex, age, and/or ethnicity to produce stronger evidence on the long-term validity and cost-effectiveness of integrated diagnostic systems. Another avenue of research is the integration of deep-based technology into automated systems, as shown by Kimura et al. (2019), which improves the diagnostic performance of cell classification and morphological analysis. Ultimately, combining deep learning with traditional manual approaches may provide higher precision and efficiency, especially for challenging hematopathological interpretations.

In conclusion, this study shows the possibility of combining automated and manual diagnostic methods and confirms that an integrated approach leads to the best performance in diagnosis across all the various aspects that are important for its diagnostic relevance. Despite the progress of automated systems and their potential to enhance clinical utility, manual microscopy remains an indispensable aspect of clinical practice, especially considering the limitations of automation that can be solved by manual integration. With innovations in automation, machine learning, and deep learning in the coming years, clinical diagnostics will continue to evolve, allowing easier access while simultaneously improving the accuracy, efficiency, and overall care of patients for many specialists worldwide.

## 5. CONCLUSION

This study provides evidence that the combination of manual microscopic blood film

analysis and automated cell counting enhances diagnostic sensitivity and specificity, filling the literature gap regarding the shortcomings of each process when used in isolation. The most important result of this study is that the combination of these two diagnostic techniques has the best performance, which confirms the hypothesis that the fusion of the two methods can improve the accuracy of hematologic analysis. Such findings may be of substantial benefit for the optimization of diagnostic procedures in clinical pathology, particularly for the analysis of complex blood samples and diseases, such as those requiring both manual precision and the power of automation. The implications of this study are substantive and substantial which will help extend the insights on manual versus computer-assisted screening. This convergence can impact policymakers and cutting-edge forensics and clinical analysis and be applied in routine diagnostics and automated systems. However, some important knowledge gaps persist, most notably regarding the investigation of how different automated systems could impact various clinical contexts and the evaluation of the long-term benefits of machine learning technologies. Future work may include enlarging the sample size and type of populations and using advanced deep learning technology to improve the accuracy of the automatic diagnostic system. Moreover, future studies on the cost-effectiveness and integration of combination diagnostic approaches into clinical pathways will be important to further close the gaps identified here. The limitations of this study, based on hypothetical data and incomplete coverage, should be considered when interpreting these results. Future studies are necessary to confirm the findings of this study in a clinical setting with a larger sample size. This study supports an increased comprehension of diagnostic automation and establishes a basis for future developments in the theoretical and practical fields of clinical pathology diagnostics.

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