The Impact of Pre-analytical Variables on the Accuracy of Biochemical Tests: A Hospital-Based Study on Sample Handling, Storage, and Timing

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Abstract

The accuracy of biochemical tests in clinical laboratories is highly influenced by pre-analytical variables, including sample handling, storage conditions, and timing. This study aimed to investigate the impact of these factors on the accuracy of glucose, potassium, lactate dehydrogenase (LDH), and alanine transaminase (ALT) test results in a hospital setting. A total of 500 blood samples were analyzed to assess the effects of improper handling (e.g., hemolysis), prolonged storage, and delays in processing. The findings revealed that improper sample handling significantly elevated potassium and LDH levels, while glucose and enzyme levels degraded with prolonged storage at room temperature. Additionally, delays in processing resulted in lower glucose and higher potassium levels, emphasizing the importance of timely sample processing. These results underscore the need for strict adherence to pre-analytical protocols to ensure accurate test results and enhance patient care.

Keywords: Pre-analytical variables, biochemical test accuracy, sample handling, storage conditions, processing delays, hemolysis, clinical laboratory

Introduction

Biochemical tests are critical tools in diagnosing and monitoring various diseases, guiding clinical decision-making and patient management. The accuracy of these tests is paramount, as even small inaccuracies can lead to misdiagnosis or inappropriate treatment. However, the accuracy of biochemical test results is not solely dependent on the analytical phase; pre-analytical variables, such as sample handling, storage conditions, and timing, play a significant role in ensuring reliable outcomes.

Pre-analytical variables encompass all factors that affect the sample before it undergoes laboratory analysis. These include patient preparation, sample collection, transportation, and storage conditions (Simundic and Lippi, 2012). Errors introduced during this phase can account for up to 70% of total laboratory errors (Lippi et al., 2011). For example, improper handling, such as excessive agitation, can cause hemolysis, leading to falsely elevated potassium levels (Lippi et al., 2013). Additionally, inadequate storage conditions, such as exposure to room temperature for prolonged periods, can degrade sensitive analytes like enzymes and hormones, compromising test accuracy (Guder et al., 2008).

Timing is another critical pre-analytical variable. Delays between sample collection and analysis can lead to significant changes in analyte concentrations. For instance, glucose levels in blood samples can decrease by 5-7% per hour if not promptly processed, due to ongoing glycolysis(Gambino and Bruns, 2013). These pre-analytical variables introduce variability in test results, making it challenging to differentiate between true pathological changes and errors introduced by the sample handling process.

Despite the recognized importance of pre-analytical variables, their impact on the accuracy of biochemical tests is often underestimated in clinical practice (Narayanan, 2000). Therefore, this study aims to explore how factors such as sample handling, storage conditions, and timing affect the accuracy of biochemical test results in a hospital setting. By identifying critical points where errors are most likely to occur, this study seeks to inform best practices that can enhance the reliability of biochemical testing and ultimately improve patient care.

Literature Review

The accuracy of biochemical test results is critically influenced by the pre-analytical phase, which encompasses all procedures performed on the sample before its analysis. Errors in this phase can significantly impact clinical decisions, often leading to misdiagnosis or inappropriate treatment. Several studies have explored the various pre-analytical variables that affect test outcomes, highlighting the importance of proper sample handling, storage, and timing.

1. Sample Handling: Improper sample handling is one of the most common sources of pre-analytical error. Mishandling during collection or transport can lead to hemolysis, which is the breakdown of red blood cells, releasing intracellular components into the plasma or serum. Hemolysis is a significant cause of error in laboratory tests, particularly in the measurement of electrolytes such as potassium and enzymes like lactate dehydrogenase (LDH) (Simundic and Lippi, 2012). Lippi (2013) highlighted that hemolysis could falsely elevate potassium levels, leading to misdiagnosis of hyperkalemia. Other studies have emphasized the need for standardized procedures for handling and transporting samples to minimize variability in test results (Lippi et al., 2011).

2. Storage Conditions: Storage conditions, such as temperature and duration, have a profound impact on the stability of biochemical markers. Some analytes are particularly sensitive to temperature fluctuations and may degrade if not stored under appropriate conditions. For example, enzymes and hormones are prone to degradation when exposed to room temperature for extended periods. Guder et al. (2008) demonstrated that inadequate storage conditions could significantly alter the concentrations of various biomarkers, affecting the accuracy of diagnostic results. Additionally, the use of incorrect anticoagulants or preservatives during storage can lead to erroneous test outcomes, emphasizing the importance of following standardized storage protocols.

3. Timing: The timing of sample analysis, particularly the delay between sample collection and testing, is another crucial factor affecting the accuracy of biochemical tests. Prolonged delays can lead to changes in analyte concentrations due to ongoing biological processes. For instance, glucose levels in blood samples decrease over time due to glycolysis if not promptly processed. Bergmeyer et al. (1974) found that glucose levels could drop by 5-7% per hour without the addition of glycolysis inhibitors (Narayanan, 2000).. Similarly, the stability of other analytes, such as lactate and certain hormones, can be compromised if there are significant delays before analysis (Da Rin, 2009).

4. Importance of Standardization: To minimize the impact of pre-analytical variables on test accuracy, several studies have advocated for the standardization of pre-analytical procedures. Lippi et al. (2011) emphasized that total quality in laboratory diagnostics depends heavily on controlling pre-analytical variables through standardized protocols. This includes proper training of healthcare professionals involved in sample collection, transportation, and storage, as well as the implementation of quality control measures. Despite these recommendations, variability in pre-analytical practices remains a significant challenge in clinical laboratories, leading to inconsistent test results and compromised patient care.

5. Gaps in the Literature: Although considerable research has been conducted on individual pre-analytical variables, there is still a need for comprehensive studies that examine the combined effects of multiple factors, such as handling, storage, and timing, on biochemical test accuracy. Furthermore, most studies have been conducted in controlled environments, leaving a gap in understanding how these variables impact test results in real-world hospital settings. Addressing these gaps can help develop more effective strategies for minimizing pre-analytical errors and improving the overall quality of biochemical testing.

The literature underscores the critical role of pre-analytical variables in determining the accuracy of biochemical test results. Sample handling, storage conditions, and timing are all key factors that need to be carefully controlled to ensure reliable diagnostic outcomes. Despite existing knowledge, there is still a need for further research, particularly in hospital settings, to better understand how these variables interact and how their impact can be minimized through standardized protocols.

Methodology

Study Design: This study employed a cross-sectional observational design conducted over a six-month period at the biochemistry laboratory of a tertiary hospital. The aim was to investigate the impact of pre-analytical variables, including sample handling, storage conditions, and timing, on the accuracy of biochemical test results. The study focused on commonly ordered biochemical tests, such as glucose, potassium, and enzyme levels, which are known to be sensitive to pre-analytical factors.

Study Setting: The study was conducted in the biochemistry laboratory of a tertiary hospital. a large tertiary-care hospital with a well-established laboratory department. The laboratory serves a diverse patient population from various departments, including emergency, outpatient, and inpatient units. The hospital follows standard operating procedures (SOPs) for sample collection, handling, and processing, which were reviewed as part of the study.

Study Population

The study included samples collected from 500 patients admitted to the hospital during the study period. The patients were selected based on convenience sampling, and the sample types included serum, plasma, and whole blood. The inclusion criteria were:

1. Patients undergoing routine biochemical testing (e.g., glucose, potassium, enzyme levels).
2. Samples collected from both inpatients and outpatients.
3. Only samples with complete documentation of handling, storage, and timing details were included.

The exclusion criteria were:

1. Samples with incomplete documentation of pre-analytical variables.
2. Samples collected from patients undergoing emergency procedures that required immediate testing.

Data Collection: Data were collected from patient records, laboratory information systems (LIS), and direct observation of sample handling processes. The following pre-analytical variables were recorded for each sample:

1. **Sample Handling:** Details on the method of sample collection (e.g., venipuncture, capillary puncture), labeling, and transport conditions (e.g., whether the sample was transported on ice, at room temperature, or under controlled conditions).
2. **Storage Conditions:** The temperature and duration of storage before analysis. Samples were categorized based on storage duration (e.g., processed within 1 hour, 1-4 hours, >4 hours) and storage conditions (e.g., refrigerated, room temperature, frozen).
3. **Timing:** The time elapsed between sample collection and analysis. The impact of delays was analyzed by categorizing samples into groups based on the time interval between collection and processing (e.g., <1 hour, 1-3 hours, >3 hours).

Sample Processing: Biochemical tests were conducted using standardized automated analyzers (e.g., Roche Cobas 6000) following the manufacturer's instructions. Quality control measures were strictly followed to ensure the reliability of the results. Samples were tested for the following analytes: glucose, potassium, lactate dehydrogenase (LDH), and alanine transaminase (ALT). These tests were chosen due to their known sensitivity to pre-analytical factors.

Statistical Analysis

Data were analyzed using SPSS version 26. Descriptive statistics were used to summarize the study population and the distribution of pre-analytical variables. The impact of sample handling, storage conditions, and timing on the accuracy of biochemical tests was assessed using multivariate regression analysis. The dependent variable was the test result for each analyte, and the independent variables were the pre-analytical factors (e.g., handling method, storage temperature, time to processing). A p-value of <0.05 was considered statistically significant.

Ethical Considerations

Ethical approval for the study was obtained from the ethics committee. Informed consent was waived as the study involved the analysis of de-identified routine laboratory data without any direct patient intervention. Data confidentiality was maintained by using anonymized patient identifiers, and all data were stored securely in password-protected systems.

Findings

The study analyzed 500 samples collected from both inpatient and outpatient settings. The primary focus was on examining the influence of sample handling, storage conditions, and timing on the accuracy of biochemical test results for glucose, potassium, lactate dehydrogenase (LDH), and alanine transaminase (ALT).

1.Sample Handling: Samples that were mishandled during collection or transport showed significant deviations in test results, particularly for potassium and LDH levels. Hemolysis was observed in 8.4% of the samples, which had a statistically significant impact on potassium levels ($p < 0.01$).

Handling Category	Number of Samples	Hemolysis Rate	Mean Potassium Level (mmol/L)	p-value
Proper Handling	458	2.6%	4.1	-
Mishandling (e.g., Hemolysis)	42	100%	6.5	<0.01

Hemolysis was associated with falsely elevated potassium levels, which could have led to misdiagnosis if not identified. LDH levels were also affected by hemolysis, with mean values increasing by approximately 25% in hemolyzed samples.

2. Storage Conditions: Storage conditions played a critical role in the stability of the biochemical markers tested. Samples stored at room temperature for more than 4 hours showed significant degradation of glucose and enzyme levels. The following table illustrates the effect of different storage durations on glucose levels:

Storage Duration	Number of Samples	Mean Glucose Level (mg/dL)	Standard Deviation	p-value
<1 hour	173	92.1	±8.2	-
1-4 hours	212	89.6	±9.4	0.12
>4 hours	115	80.2	±12.3	<0.01

Glucose levels were significantly lower in samples stored for more than 4 hours at room temperature ($p < 0.01$), indicating ongoing glycolysis. Enzyme levels, including LDH and ALT, were also found to be unstable when stored improperly.

3. Timing: The timing of sample processing had a noticeable impact on the accuracy of test results. Samples that were processed within 1 hour of collection generally showed stable and accurate results. However, delays of more than 3 hours led to deviations in glucose, potassium, and enzyme levels.

Time to Processing	Number of Samples	Mean Glucose Level (mg/dL)	Mean Potassium Level (mmol/L)	p-value (Glucose)	p-value (Potassium)
<1 hour	210	91.8	4.2	-	-
1-3 hours	180	88.7	4.3	0.08	0.15
>3 hours	110	79.5	4.6	<0.01	0.03

Glucose levels were particularly sensitive to delays, with a significant decrease in levels observed in samples processed after more than 3 hours ($p < 0.01$). Potassium levels also showed a statistically significant increase with longer delays ($p = 0.03$).

Discussion

The findings of this study highlight the significant impact of pre-analytical variables, such as sample handling, storage conditions, and timing, on the accuracy of biochemical test results. Understanding these factors is critical for improving diagnostic accuracy in clinical laboratories, as even small deviations in these variables can lead to misinterpretation of patient data and potentially incorrect clinical decisions.

Impact of Sample Handling: The study found that improper handling, particularly hemolysis, led to significant deviations in potassium and lactate dehydrogenase (LDH) levels. Hemolysis, which occurs when red blood cells rupture during or after sample collection, is a well-documented pre-analytical error known to falsely elevate potassium levels and alter enzyme measurements . In this study, hemolyzed samples showed significantly higher potassium levels, with a mean increase of over 2 mmol/L compared to properly handled samples. This elevation could have serious clinical implications, leading to misdiagnosis of conditions such as hyperkalemia, which can result in inappropriate treatment interventions.

The findings are consistent with previous research, which has shown that hemolysis can cause spurious hyperkalemia and elevated enzyme levels . To minimize this error, strict protocols for sample collection and transport must be adhered to, such as using appropriate gauge needles, avoiding prolonged tourniquet use, and ensuring gentle handling of samples during transport . Additionally, implementing routine checks for hemolysis before analysis can help laboratories flag and discard compromised samples.

Influence of Storage Conditions: Storage conditions were another critical factor affecting the accuracy of biochemical test results. The study showed that glucose levels were particularly sensitive to storage duration, with significant degradation observed in samples stored for more than 4 hours at room temperature. This decline in glucose levels is attributed to ongoing glycolysis in unprocessed blood samples, a phenomenon that has been extensively reported in the literature . Proper storage, such as refrigeration or immediate processing, is necessary to prevent glycolysis and maintain accurate glucose measurements.

Enzyme levels, including LDH and alanine transaminase (ALT), were also affected by improper storage, as enzymatic activity can be compromised by prolonged exposure to room temperature. These findings underscore the importance of timely and appropriate storage conditions, particularly for tests sensitive to temperature and time. Laboratories should enforce strict guidelines for sample storage, including immediate refrigeration of samples that cannot be processed within an hour of collection.

Effect of Timing on Sample Processing: Timing was another key factor influencing test accuracy. The study found that processing delays of more than 3 hours resulted in significant alterations in glucose and potassium levels. Glucose levels decreased with processing delays, likely due to continued glycolysis, while potassium levels increased, possibly due to cell lysis or leakage of intracellular potassium . These findings are consistent with earlier studies that highlight the importance of timely processing to ensure the accuracy of test results .

The implications of delayed processing are particularly relevant in hospital settings where logistical challenges may lead to processing delays. To mitigate these effects, strategies such as point-of-care testing, decentralized laboratory services, or the use of glycolysis inhibitors in blood collection tubes can be considered . These approaches could help reduce the time between sample collection and analysis, preserving the integrity of the test results.

Clinical Implications

The clinical implications of these findings are significant. Inaccurate biochemical test results can lead to incorrect diagnoses and inappropriate treatments, potentially compromising patient safety. For example, falsely elevated potassium levels due to hemolysis can lead to unnecessary interventions such as the administration of potassium-lowering agents, which can have harmful side effects. Similarly, inaccurate glucose measurements can result in the mismanagement of diabetes, leading to either under- or overtreatment of hyperglycemia .

To improve diagnostic accuracy, it is essential for clinical laboratories to prioritize minimizing pre-analytical errors. This can be achieved through continuous education and training of healthcare professionals on proper sample handling techniques, implementation of stringent storage protocols, and optimization of laboratory workflows to minimize processing delays.

Limitations of the Study

This study had several limitations. First, the convenience sampling method may introduce selection bias, as the samples were not randomly selected. Second, the study was conducted at a single hospital, limiting the generalizability of the findings to other settings. Future research should include multi-center studies with larger sample sizes to confirm these results and explore other potential pre-analytical variables that may influence test accuracy.

Conclusion

This study demonstrates the critical impact of pre-analytical variables on the accuracy of biochemical test results in a hospital setting. Sample handling, storage conditions, and timing were all shown to significantly affect test outcomes, highlighting the need for strict adherence to protocols to minimize pre-analytical errors. By addressing these factors, clinical laboratories can enhance diagnostic accuracy, improve patient outcomes, and reduce the risk of errors in clinical decision-making.

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