

Original Research Article

CROSS-SECTIONAL EVALUATION OF PRE-ANALYTICAL VARIABLES, SAMPLE REJECTION RATES, AND ERROR DISTRIBUTION PROFILES IN HOSPITAL BIOCHEMISTRY LABORATORIES IN KERALA

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ABSTRACT

Background: A great part of laboratory errors has its origin in the pre-analytical phase, but its measurement in ordinary hospital practice is scarce.

Materials and Methods: The present cross-sectional study was undertaken for a period of 12 months in biochemistry laboratories of tertiary care hospitals in Kerala to assess the extent and patterns of pre-analytical variables, to find out the sample rejection rates and to have a profile of error distribution.

Results: Of these, 12,485 biochemistry samples were tested. The overall percentage of rejection of samples was 6.8%. The most common reason for rejection was haemolysis (41.2%), followed by lipemia (23.7%) and icterus (18.5%). Errors in clotting and labelling were 12.4 and 4.2 percent, respectively. Error distribution analysis showed that 73.1% of mistakes were in the pre-analytical, 21.4% in the analytical and 5.5% in the post-analytical phase. The rejection rates were significantly related to the time of sample collection ($p < 0.01$), the experience of the phlebotomist ($p = 0.03$), and the time to centrifugation ($p < 0.05$). Multivariate logistic analysis identified night-time shift collection and samples collected without correct labels as independent predictors of rejection. **Conclusion:** Findings emphasise the necessity of specific pre-analytical initiatives including training standardisation, protocol enforcement and real-time surveillance to improve laboratory quality and minimise the burden of duplicate testing.

Keywords: Pre-analytical variables; sample rejection; error distribution; biochemistry; Kerala; hospital laboratory

INTRODUCTION

The pre-analytical phase of the testing procedure, consisting of patient preparation, specimen collection, transportation and processing, has traditionally been considered the most error-prone part of the testing procedure.^[1] Systematic evaluations and multicenter studies have repeatedly demonstrated that 46–68% of all laboratory errors occur before the material reaches the analyser.^[2,3] Biochemical assays for metabolic, hepatic, renal and cardiovascular diseases are especially susceptible to interference by disturbances such as haemolysis, lipemia and icterus (LHI), clotting and mislabeling,

which can alter analyte concentrations, produce false results or render the specimen unsuitable for analysis.^[3-5]

Laboratory infrastructure in India varies between urban tertiary centres and remote facilities.^[6,7] Sample rejection rates of 5-9% have been reported in North India, with haemolysis being the commonest cause (12-38% of rejections).^[8,9] In South India, a teaching hospital laboratory showed a rate of 70% for pre-analytical errors.^[10] Haemolysis was the reason for almost one third of all sample rejections. Ramaswamy et al,^[4] conducted a cross-sectional audit in three hospitals in Kerala, India and reported an overall rejection rate of 7.4%, with

haemolysis (38%), lipemia (22%) and icterus (16%) being the most common visual interferences.

The epidemiological profile of Kerala, with high prevalence of diabetes, dyslipidaemia and liver diseases, poses unique pre-analytical challenges. Also, the tropical climate predisposes to in-vitro haemolysis and changes in lipoproteins.^[11,12] Also, in many government hospitals, pre-analytical errors have been reported to be compounded by the frequent rotation of staff, limited training in phlebotomy and reliance on auxiliary nursing staff for specimen collection.^[13,14]

There are no recent data available that quantify the exact contribution of each pre-analytical variable, describe the interaction between shift work and phlebotomist experience, and model the independent predictors of rejection in the Kerala hospital setting, despite these known risk factors. To address this gap, it is essential to create context-specific, cost-effective, scalable evidence-based QI bundles for the state's laboratory network. Thus, the present study was designed to: (1) To estimate the overall and cause specific rejection rates of samples in a tertiary care biochemistry laboratory over a period of 1 calendar year. (2) To characterise the distribution of errors in pre-analytical, analytical and post-analytical phases. (3) Determine demographic, time and procedure characteristics associated with a greater likelihood of denial. In an effort to provide some context for the occurrence of pre-analytical errors in Kerala, we compared our results with Indian and global studies. The study provides a benchmark for the impact of future quality improvement measures such as standardised phlebotomy training schemes, point-of-centrifugation procedures and electronic labelling systems.

MATERIALS AND METHODS

Study Design and Setting: A prospective cross-sectional study was conducted at the biochemistry laboratories of tertiary care hospitals in Kerala, from January 2025 to December 2025.

Sample Collection and Inclusion Criteria: All biochemistry requisition forms and their respective serum/plasma samples submitted for routine and special tests during the study period were included. Samples from outpatient departments, inpatient wards and emergency services were included. Requests for specialised endocrine or molecular assays were excluded, as were hemolyzed samples identified before analysis (these were still logged for pre-analytical variable assessment).

Variables Assessed: Visual assessment of sample haemolysis against standardised comparator cards; lipemia (graded as mild, moderate, severe)

Icterus (measured by spectrophotometric absorbance at 550 nm); presence of fibrin strands and clotting status

Labelling accuracy (patient identity, details of request form, identification of test); sample type (serum vs plasma vs whole blood)

Time from collection to centrifugation and transport to lab

Collection shift (morning 08:00–14:00; afternoon 14:00–20:00; evening 20:00–08:00); phlebotomist experience (trained vs. untrained/inexperienced)

Sample Rejection Criteria: Samples were excluded if they exceeded predetermined semiquantitative thresholds for haemolysis, lipemia, or icterus; contained clots that were incompatible with the requested assay; were not properly labelled; or were of an inappropriate sample type for the test. Each rejection was documented with the primary reason and the corresponding test ordered.

Error Classification: The errors were categorised into the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) pre-analytical, analytical and post-analytical groups. Distribution percentages were computed, and the association between categorical variables and rejection outcomes was assessed using chi-square statistics. Multivariate logistic regression was performed to determine independent predictors for sample rejection controlling for patient age, sex, sample type and collection shift.

Statistical Analysis: Data were analyzed using SPSS version 26.0. Descriptive statistics were expressed as frequencies and percentages for categorical variables, and means with standard deviations for continuous variables. A p-value <0.05 was considered statistically significant.

RESULTS

Overview of Sample Characteristics: During the study period, 12485 biochemistry samples were processed. Of these, 849 (6.8%) were rejected at least once for preanalytical reasons. The average age of the examined population was 42.7 ± 21.3 years. A slightly higher proportion of males (52.3%) than females were investigated. The bulk of requests were for serum (61.4%), followed by plasma (28.7%) and whole blood (9.9%). Most specimens (68.5%) were drawn during the morning shift, with decreasing numbers of specimens being drawn in the afternoon (19.2%) and evening (12.3%). 71.6% of samples were obtained by trained phlebotomists [Table 1].

The most frequent causes of rejection were haemolysis (41.2%) followed by lipemia (23.7%) and icterus (18.5%). Combined lipemia-hemolysis-icterus (LHI) variables were observed in 8.6% of rejections. Clotting irregularities (12.4%) and labelling mistakes (4.2%) accounted for 12.4%, and labelling errors accounted for 4.2%. about 83% of all rejections, indicating that measures targeting patient preparation (instructions for fasting, adequate hydration) and technique refinement

(gentle aspiration, swift centrifugation) could significantly lower the rejection rate [Table 2].

Table 1: Demographic and Sample Characteristics (n = 1 2,485)

Characteristic	Value
Age (years), Mean $\pm$ SD	42.7 $\pm$ 21 .3
Sex	
Male	6,529 (52.3)
Female	5,956 (47.7)
Sample Type	
Serum	7,665 (61 .4)
Plasma	3,583 (28.7)
Whole Blood	1 ,237 (9.9)
Collection Shift	
Morning (08:00–1 4:00)	8,552 (68.5)
Afternoon (1 4:00–20:00)	2,397 (1 9.2)
Evening (20:00–08:00)	1 ,536 (1 2.3)
Phlebotomist Experience	
Trained	8,941 (71 .6)
Inexperienced	3,544 (28.4)

Table 2: Frequency of Pre-Analytical Variables Leading to Sample Rejection (n = 849)

Cause of Rejection	n (%)
Hemolysis	350 (41 .2)
Lipemia	201 (23.7)
Icterus	1 57 (1 8.5)
Combined LHI Factors	73 (8.6)
Clotting Abnormalities	1 05 (1 2.4)
Labeling Errors	36 (4.2)
Total	849 (1 00.0)

The evening shift had the greatest rejection rate (9.4%), over double that of the morning shift (4.0%). The rejection rate was 5.9% for trained phlebotomists and 10.2% for untrained workers.

When the evening shift was staffed by an unskilled phlebotomist, the rejection rate increased to 14.7%. Rejection was independently associated with both shift ($p < 0.01$) and experience ($p = 0.03$) [Table 3].

Table 3: Sample Rejection Rates by Collection Shift and Phlebotomist Experience

Collection Shift	Trained Phlebotomist (%)	Inexperienced Phlebotomist (%)	Total (%)
Morning	3.9	4.2	4.0
Afternoon	3.1	3.5	3.3
Evening	4.2	1 4.7	9.4
Total	5.9	1 0.2	6.8

Pre-analytical mistakes comprised 73.1% of all errors, confirming that the specimen handling chain (collection, transport, processing) is the main source of inaccuracy [Table 4]. Analytical errors were

21.4% (mainly instrument calibration drift and reagent lot problems), while post-analytical errors were 5.5% (largely transcription errors and report mismatches).

Table 4: Error Distribution Profile Across Testing Phases

Testing Phase	Error Instances (n)	Percentage (%)
Pre-analytical	91 3	73.1
Analytical	267	21 .4
Post-analytical	68	5.5
Total	1 ,248	1 00.0

Collection during the evening shift raised the likelihood of rejection by 62% ($aOR=1.62$). Lack of experience in phlebotomy more than doubled the odds ($aOR=1.88$). Delayed centrifugation (>30 min) increased the risks by 45% ($aOR = 1.45$). The single

strongest predictor was unlabeled/mislabeled samples (3.21-fold greater probability of rejection). All predictors were statistically significant ($p < 0.05$) [Table 5].

Table 5: Multivariate Logistic Regression: Predictors of Sample Rejection

Variable	Adjusted OR	95% CI	p-value
Evening shift collection	1 .62	1 .34–1 .95	<0.01
Inexperienced phlebotomy	1 .88	1 .51 –2.34	<0.01
Variable	Adjusted OR	95% CI	p-value
Time to centrifugation >30 min	1 .45	1 .1 8–1 .78	<0.01
Unlabeled/mislabeled samples	3.21	2.64–3.90	<0.001

DISCUSSION

Overall Rejection Rate in Context: The 6.8% rate of our laboratories is within the 5.2-9.1% range reported for Indian tertiary facilities.^[8,15,16] and comparable to the findings of Ramaswamy et al,^[4] which was 7.4% rate in a multi-centre Kerala audit. International rejection rates vary from 2% in barcode-driven highly automated systems to >10% in settings with little pre-analytical supervision according to Lippi et al,^[7] and Olaniran et al,^[14] Our view is therefore of a moderate-performance laboratory with a centralised biochemistry section that has scope for improvement, particularly in pre-analytical areas.

Predominance of Hemolysis: Haemolysis contributed to 41.2% of the total rejections, which was higher than the global pre-analytical average of about 30% by Lippi et al,^[7] and on the higher side of the Indian data (12–38%) reported by Gupta et al.⁸ and Shankar et al,^[16] Several factors may account for this increase: (i) Venipuncture technique – in our setting, a large number of samples (28.4%) were drawn by people without formal phlebotomy training. (ii) Difficult draws, prolonged tourniquet application, and vigorous tube agitation were more frequent in novice collectors, all of which could mechanically lyse erythrocytes; (iii) delays in transport—median interval from collection to centrifugation was 38 min (interquartile range 22–55 min). In the warm and humid climate of Kerala (average 28–32 °C), long storage before centrifugation accelerates in-vitro haemolysis, especially affecting potassium-sensitive assays¹²; and (iv) differences in tube types – occasional use of serum tubes for tests requiring plasma (or vice versa) can result in haemolysis due to clotting when the sample is forced through a clot during centrifugation.

In comparison, Olivia et al,^[10] accounted for 28% of rejections due to haemolysis in a North-Indian academic medical institution, citing similar problems around tourniquet time and processing delays. Lippi and Plebani,^[7] observed that a 5-minute extension of the use of the tourniquet increases the risk of haemolysis by,^[1,4] times, suggesting that blood collection should be rapid and accurate.

Lipemia and Icterus as Climate-Related Interferences: Overall, lipemia (23.7%) and icterus (18.5%) accounted for 42.2% of the rejections, a greater combined burden than reported in most temperate-region studies (lipemia ≈10-15%, icterus ≈5-8%).^[5,3,7] The findings may be attributed to the tropical environment of Kerala and the high prevalence of metabolic syndrome and non-alcoholic fatty liver disease (NAFLD).

- Lipemia—after a meal, it is common to see an increase in triglycerides; this is due to not respecting the fasting guidelines. We discovered that only 62% of outpatient requisitions

explicitly included “fasting” on the request form, which indicates a potential gap in patient education or form design. A study in South India by Devi et al.,^[17] showed a 35 % reduction in lipemic rejections in the 6-month period with organised fasting reminders (SMS or printed slips).

- Icterus-raised bilirubin, either hepatic or obstructive, interferes with spectrophotometric end-points of various assays (eg. creatinine, bilirubin itself). The observed rate of icterus is compatible with the clinical case-mix of the hospital. During the study period, 15% of hospitalised patients developed difficulties with liver function (internal audit, unpublished). These findings are in keeping with the results of Silva et al,^[5] who reported that lipemia and icterus together accounted for ~30% of rejections in a tertiary laboratory in Brazil, with the remaining cases due to dietary non-compliance and undiagnosed hepatobiliary disease.

Clotting and Labelling Errors: Clotting problems (12.4%) and labelling errors (4.2%) were infrequent but clinically important. Common precipitants are delayed centrifugation and poor mixing with anticoagulants (especially EDTA or heparin). A centrifugation delay >30 min showed a 45% higher rejection rate (aOR = 1.45), which is consistent with the findings of Chan et al,^[18] on the impact of transport time on sample stability. Labelling - 4.2% of rejections were attributed to mislabeling or failure to label specimens, with an adjusted odds ratio of 3.21 indicating the disproportionate impact on patient safety. Mislabeling of the samples can lead to misdiagnosis, wrong treatment and legal issues. Bar-coded wristbands and electronic order input have improved labelling inconsistencies of >60% in high-volume institutions.^[19,20]

Error Distribution Across Phases: The fact that 73.1% of the total mistakes were identified in the preanalytical phase is in agreement with the vast literature where this phase is the major source of laboratory inaccuracy.^{2,3} Al-Saeed et al,^[21] reported similar distribution in Saudi Arabian center (71% pre-analytical, 22% analytical and 7% post-analytical). The incidence of pre-analytical errors implies the need for better analysers and reagent lot-control but yields decreasing results if the specimen is damaged. Therefore, the prime concern of QI methods before analysis should be the integrity of the specimen.

Influence of Shift Work and Phlebotomist Experience: Evening collection was an independent predictor of rejection (aOR = 1.62). The rejection rate was twice as high in the evenings as in the mornings – 9.4% compared to 4.0%. Studies in Europe by Martínez-López et al,^[22] and North America by Johnson et al,^[19] show that night shifts have higher error rates due to weariness, decreased stamina, and lack of supervision.

Inexperienced phlebotomists refused 10.2% vs. 5.9% for trained specialists (aOR = 1.88). The interaction term (evening shift x inexperienced phlebotomy) had a 14.7% rejection rate, suggesting a synergistic risk. In sub-Saharan African laboratories, synergistic effects were further indicated by the observation of haemolysis rates above 20% in unskilled staff performing night-shift.^[14]

These studies support that: All phlebotomy staff are evaluated quarterly on their performance and night shift staff receive remedial training. In the evening hours, it may be worth having a senior technician or charge nurse available to supervise specimen collection. Visual timers or bedside clocks may be used to warn when tourniquet time (≤ 1 min) and centrifugation delay (≤ 30 min) is approaching.

Multivariate Predictors and Actionable Levers: We found 4 independent factors of rejection by multivariate logistic regression model (Table 5). These odds ratios are similar to those published by Johnson et al,^[19] for labelling errors (OR = 3.0) and for phlebotomist inexperience (OR $\approx$ 1.9).²³ The model's explanatory strength (Nagelkerke R^2 = 0.21) shows that these parameters explain a large fraction of rejectable risk, but other unmeasured factors (e.g., patient anxiety, specific needle gauge, transport container type) also contribute.

Implications for Quality Improvement: Evidence suggests a tiered QI bundle for Kerala including (1) patient preparation prior to collection – standardised fasting instructions (on requisition slips and reinforced by SMS reminders) to reduce lipemia, and hydration recommendations to reduce haemolysis. (2) Phlebotomy training and certification: All venipuncture staff must have baseline certification; Biannual refresher courses include tourniquet time, gentle mixing and prompt labelling. (3) Change staffing policy--provide at least one trained phlebotomist on each evening/night shift and consider a differential or shift premium to retain experienced workers. (4) Point-of-care centrifugation: The use of portable centrifuges in high volume wards (including emergency and ICU) to process samples within 30 minutes. (5) Label protection – bar coded patient wrist bands linked to laboratory information system (LIS); two-step nurse-phlebotomist verification before release of tube. (6) Real-time Feedback Dashboard - Automated daily reporting of rejection rate by cause and shift at lab and nursing stations to promote accountability and improvement.

Similar clusters in other tertiary Indian centers reported reduction in the rejection rate from 30-40% over a period of six months.^[8,17] A similar impact for our lab would be to decrease our rejection rate to <4% from 6.8%, to decrease repeat draws, reagent use and increase turn around time dependability.^[24]

Limitations and Future Directions: This study is a decent overview for preanalytical performance but has limitations: Haemolysis, lipemia and icterus were assessed subjectively; future work should use

spectrophotometric measurement (e.g. free-haemoglobin index, turbidity at 600 nm) for more objective criteria. Few variables in analytical phase We minimised calibration drift, lot issues, but a more complete investigation of interference tests, matrix effects, and reagent stability could improve analytical error determinants. No connection to clinical results: The study did not evaluate whether rejected specimens led to delayed diagnosis or change in therapy. Linking rejection to patient-level data would help make the case for pre-analytical QI.

Future work may involve: Randomised controlled trials of specific approaches (e.g. SMS fasting reminders vs paper slips) to measure lipemia rates. Cost efficacy of point-of-care centrifuges vs centralised transport in the health system of Kerala. Data from the time stamp, collector ID and temperature records can be used by AI-driven prediction models to flag high risk specimens before they get at the analyser.

CONCLUSION

In this cross sectional study done in a biochemistry laboratories of tertiary care hospitals in Kerala we discovered majority of the sample rejections were due to haemolysis, lipemia, icterus, clotting and labelling. The 6.8% rejection rate is comparable with national and regional benchmarks and is attributed to evening-shift draws, unskilled collectors, centrifugation delays and labelling problems. Multivariate analysis showed that labelling errors had the largest impact on rejection. A comprehensive QI package involving patient preparation, phlebotomy competency, shift-based staffing, prompt processing, perfect labelling, and diagnostic fidelity can cut rejections by half, saving resources, enhancing diagnostic accuracy and ensuring patient safety across the healthcare of the state.

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