


# Clinical Evaluation of the Pre-Analytical Capabilities of Hemostasis Instrument

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## Abstract

**Objective:** Evaluate the technical performance of the pre-analytical hemolysis-icterus-lipemia (HIL) check module on the ACL-TOP-750. **Methods:** 8433 routine coagulation samples were evaluated for HIL, the presence of clotting and low sample volume by both visual inspection and the pre-analytical HIL check module on the ACL-TOP-750. **Results:** 7726 samples were in agreement with both methods and 707 were not consistent. 356 samples with low volume were identified by visual inspection and 920 by the instrument (2.7 mL threshold). Visual inspection identified 56 lipemic samples while 13 of those with moderate or high lipemia were identified by the instrument. Visual inspection identified 47 hemolyzed samples while 7 with moderate or high hemolysis were identified by the instrument. Both visual inspection and the instrument identified 36 icteric samples. For triglyceride concentration and bilirubin concentration, there was good correlation between the ACL-TOP-750 and the DXC800 biochemistry analyzer. Among 30 samples with varying amounts of clotting, 27 were discovered by visual inspection and 3 were discovered by the instrument. **Conclusion:** The pre-analytical check module on the ACL-TOP-750 improved the detection rate of samples below the target 2.7 mL volume, and the accuracy in detection of HIL. However, the automated method could not replace visual assessment of clotting in samples.

## Keywords

coagulation test, pre-analytical, hemolysis, icterus, lipemia

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## Introduction

Routine coagulation tests are the most common and basic screening test for thrombosis and hemostasis; the tests include prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB-C), D-dimer (DD) and antithrombin (AT). They are mainly used for the evaluation of hemostatic function and monitoring of anticoagulant therapy and are useful for identifying pathological changes of the coagulation system.<sup>1</sup> Timely access to accurate results is important for diagnosis and therapy. In clinical practice, many factors may influence results. The quality of samples (hemolysis, icterus, lipemia, undue clotting and low sample volume) is one of the most crucial group of factors.<sup>2,3</sup> Visual inspection of samples is the main method of pre-analytical quality control. However, it has some limitations: 1. Subjectivity: it is difficult to accurately judge some samples with atypical appearance and even experienced clinical

laboratory technicians may misjudge. 2. Efficiency: large numbers of samples need a lot of time for manual assessment, which directly affects laboratory turnaround time. Some hemostasis instruments feature a pre-analytical check module, which can improve the efficiency of clinical testing and further improve quality in the laboratory.

The aim of this study was to assess the technical performance of the pre-analytical HIL check module on the ACL-TOP-750 compared with visual inspection and to evaluate

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the effectiveness of automation in detection of pre-analytical problems.

## Materials and Methods

### Sample Collection

Results were obtained from a total of 8433 patient samples referred to the laboratory for routine coagulation testing by the Clinical Laboratory, Peking University First Hospital in October 2020. These samples were collected from 24 departments without considering the patient's age, gender, department and health conditions. The study was approved by the Ethics Committee of Peking University First Hospital (2018 - 231).

### Hemostasis Analyzer and Reagents

Samples were analysed using the ACL-TOP-750 (Instrumentation Laboratory, Bedford, USA) with matching reagents, RecombiPlasTin-2G (PT), SynthASil (APTT), Thrombin Time (TT), Fibrinogen-C XL (FIB-C), D-Dimer HS (DD) and Liquid Antithrombin (AT).

### Sample Processing

**Sample Collection.** Venous blood (2.7 mL) was collected in 3.0 mL vacuum blood collection tubes containing 0.3 mL of a 0.109 M sodium citrate (Belliver Industrial Estate, Belliver Way, Roborough, Plymouth Devon, PL6 7BP, United Kingdom), with a 1:9 ratio between anticoagulant and blood. Samples were centrifuged at 2300 *g* for 15 min at room temperature.

**Evaluation of Unqualified Samples by Visual Inspection.** The samples were visually checked before centrifugation following the standard operating procedures of the laboratory. The inspection included sample fill volume and clotting. The tube we used was marked with a filling line representing 2.7 mL and a tube containing 2.0 mL of water was used to guide the sample volume assessment. The samples with clots or the sample volume less than 2.7 mL were recorded. After centrifugation, the plasma color and turbidity were assessed for hemolysis, icterus and lipemia, and the degree of any abnormality recorded. A colorimetric card made by our laboratory which divides anomalies into five grades from 1+ to 5+ was used as the criterion for judgement. The standard was as follows (1+, 2+, 3+, 4+, 5+): hemolysis (50, 100, 150, 300, 500 mg/dL), icterus (5, 10, 20, 25, 30 mg/dL), lipemia (100, 200, 500, 1000, 1300 mg/dL).

**Evaluation of Samples by ACL-TOP-750.** After centrifugation, the samples were tested for the requested coagulation assays on the ACL-TOP-750, which incorporates the pre-analytical check module, hereinafter referred to as the instrument method. The check module includes three functions: HIL detection, tube fill volume verification, and abnormal sample aspiration (tiny

clots and bubbles). The threshold value for stopping each test was as follows (data expressed as: hemolysis (mg/dL), bilirubin (mg/dL), lipemia (milliAbsorbance)): PT (500, 30, 4886), APTT (500, 26, 4886), TT (500, 24, 1650), FIB-C (375, 21, 3549), DD (500, 18, 1650) and AT (500, 40, 11502). If sample volume was less than 2.7 mL, the instrument gave an alarm signal.

**Unqualified Samples.** Samples with significant abnormality discovered by visual inspection and the instrument method were both recorded as unqualified samples. The laboratory would not reject samples with light or moderate hemolysis, icterus, lipemia (1+ - 3+), and low sample volume (2.0 mL - 2.7 mL). This kind of sample would be reported with a relevant comment as having hemolysis, icterus, lipemia, or low volume but was acceptable for analysis. For samples with severe hemolysis, icterus, lipemia (4+ - 5+), low sample volume (< 2.0 mL) or clotting, when noting the words "hemolysis", "icterus", "lipemia", "low volume" or "clotting" in the remarks, a request was added for sample re-collection after communication with the clinic.

### Statistical Analysis

Statistical analyses were performed by SPSS statistical software v.21.0 (SPSS Inc., Chicago, USA). Cohen's Kappa test was used for consistency evaluation. The coincidence rate analysis was used to compare the visual inspection and the automatic checking system. The difference was regarded as statistically significant with  $P < 0.05$ .

## Results

### Detection of Unqualified Samples

In this study, 8433 samples were observed. 7344 were classified as qualified by both visual and instrument methods, and 382 were classified as unqualified. Of the 7726 samples (91.6%) in agreement with both methods, 707 samples (8.4%) were inconsistent, as shown in Table 1. For the inconsistent samples, the largest proportion was low sample volume, accounting for 79.8%, followed by lipemia (6.1%), hemolysis (5.7%), icterus (4.2%) and clotting (4.2%).

Of the 8433 samples, 519 were classified as unqualified by visual inspection and 952 were classified as unqualified by instrument method. The proportion of types of unqualified samples detected by visual inspection were as follows: low sample volume (68.6%), lipemia (10.8%), hemolysis (9.1%), icterus (6.4%), clotting (5.2%). And the proportion of those detected by the instrument method were as follows: low sample volume (96.6%), lipemia (1.4%), icterus (0.9%), hemolysis (0.7%), and clotting (0.3%).

**Table 1.** Number of Unqualified Samples Detected by Manual Visual and Instrumental Methods.

	Visual-Normal n (%)	Visual-Abnormal n (%)	Total
Instrument-normal n (%)	7344 (87.09%)	137 (1.62%)	7481 (88.71%)
Instrument-abnormal n (%)	570 (6.76%)	382 (4.53%)	952 (11.29%)
Total	7914 (93.85%)	519 (6.15%)	8433 (100%)

### Low Sample Volume

920 samples with low sample volume were discovered by the instrument method, accounting for 10.9% of total samples. A total of 356 samples were discovered by visual inspection, accounting for 4.2% of total samples, and all of them were also discovered by the instrument method. 564 samples were discovered by the instrument but not by visual inspection. It is clear that the detection rate of the instrument for low sample volume is higher than visual inspection.

### Lipemia

A total of 56 samples were identified by visual inspection and the instrument method. The results were as follows: visual inspection 1 + lipemia (24 instrument normal, 0 instrument abnormal), 2+ (13, 0), 3+ (6, 4), 4 + (0, 6), 5+ (0, 3).

The results of plasma triglyceride concentration (TG) detected by the automatic biochemical analyzer DXC800 (Beckman Coulter Inc., Fullerton, CA, USA) were used as a reference to compare the detection by visual inspection and the instrument method for lipemia. When the levels of TG were detected in 38 of 56 samples (the other 18 samples were excluded from the analysis because of assay failure), the interpretation of lipemia concentration by visual inspection was consistent in 18 cases (47.4%) and lower in 20 cases (52.6%). For the instrument method, 28 cases (73.7%) were consistent with the DXC800 TG results, 7 cases (18.4%) were low, and 3 cases (7.9%) were high. Cohen's Kappa consistency test was conducted, and the Kappa value was 0.5103, showing moderate consistency.

### Hemolysis

A total of 47 hemolyzed samples were discovered by visual inspection and the instrument method. The standard of visual inspection was as follows (1+, 2+, 3+, 4+, 5+): hemolysis (50, 100, 150, 300, 500 mg/dL). The threshold value for stopping each test was (hemolysis mg/dL): PT 500, APTT 500, TT 500, FIB-C 375, DD 500 and AT 500. The combined visual and instrument results are - visual inspection 1+ hemolysis (29 instrument normal, 0 instrument abnormal), 2+ (10, 3), 3 + (1, 1), 4+ (0, 2), 5+ (0, 1).

### Icterus

36 icterus samples were discovered by visual inspection and the instrument method. The results were as follows: visual

inspection 1+ icterus (10 instrument normal, 0 instrument abnormal), 2+ (10, 0), 3+ (6, 3), 4+ (1, 3), normal (N/A, 3).

The results of total bilirubin concentration (TBIL) measured by the DXC800 were used as reference to compare the interpretation of icteric samples by visual inspection and the instrument in 32 samples that had sufficient remaining plasma. The interpretation of bilirubin concentration by visual inspection was consistent with the biochemical results in 6 cases (18.8%), higher in 22 cases (68.8%) and lower in 4 cases (12.5%). For the instrument, bilirubin concentration in samples showed a linear relationship with plasma TBIL results, with a formula of  $y = 1.0278x + 28.672$ ,  $R^2 = 0.9679$ , and a correlation coefficient of 0.9838.

### Clotting in Samples

There were 30 samples with clots, of which 27 were classified as abnormal by visual inspection but not by the instrument, and the other 3 samples were reported with "abnormal sample suction" by the instrument, but not detected by visual inspection. After checking, we found fine coagulation filaments in the plasma of these 3 samples, but they were not found by visual inspection in the first time.

### Discussion

Routine coagulation test results have important guiding significance for disease diagnosis, and the quality of samples directly affects accuracy of results. More than half of test errors come from poor sample quality before analysis.<sup>4</sup> Therefore, it is necessary to strengthen monitoring and management of sample quality before analysis in order to improve accuracy of results.

This study shows that consistency between visual inspection and the instrument method in judgment of unqualified samples was fair (91.6%). The unqualified samples refer to the samples that are flagged for inspection but are potentially still acceptable for reporting. The detection rate of unqualified samples by the instrument (11.3%) was higher than that of visual inspection (6.2%).

When the volume of blood collected is too low, the ratio of blood and anticoagulant will be out of proportion, thus affecting test results. Therefore, whether the tube fill volume reaches the standard is the first step to check sample quality in routine coagulation tests. Among the 8433 samples in this study, the detection rate of the instrument for low sample volume (10.9%) was significantly higher than that of visual inspection (4.2%). There were no unqualified samples with visual inspection but qualified by the instrument, which indicates that the instrument

was more sensitive in judging low sample volume. The target value for both methods is 2.7 mL. In the instrument method, the volume sensing function of the sample needle is used to judge whether the tube fill volume is correct or not, and the tube fill volume less than 2.7 mL gives an alarm. The instrument method specifically distinguishes sample volume at or above 2.7 mL from those below 2.7 mL, but does not identify samples with a mildly reduced volume that are acceptable (2.0–2.6 mL). Previous studies have shown that APTT and FIB-C are the most affected by sample volume.<sup>5</sup> In this study, the instrument only sets one alarm threshold, which cannot distinguish between the two types of samples. It is regretted that we did not record the numbers of samples in the two volume ranges (2.0 mL–2.7 mL and less than 2.0 mL) separately for samples detected by visual inspection, too. Therefore, it is impossible to determine whether and to what extent the low sample volume had an impact on routine coagulation tests. The ACL-TOP-750 has the function of setting two alarm thresholds that require the sample needle to work twice. But the setting process is tedious. It is recommended that the manufacturer update the software to make it easier to provide more than one alarm threshold of tube fill volume as this will be more useful for clinical applications.

Lipemia is one of the common factors influencing sample quality. Patients' diets or drug treatments may lead to production of lipemia and turbidity in blood, which will increase plasma turbidity, especially for tests based on immune turbidimetry, such as DD.<sup>6</sup> The results of this study show that for judgment of mild (1+, 2+) and severe (4+, 5+) lipemia samples, visual inspection was consistent with the instrument method, while for the moderate lipemia samples, the methods were significantly inconsistent. The ACL-TOP-750 determines the degree of lipemia by detecting the absorbance of the sample at three different wavelengths, as follows: 405 nm for turbidity, cell-free hemoglobin and total bilirubin; 535 nm for turbidity and cell-free hemoglobin; 671 nm for turbidity. Measurement at the three wavelengths creates three equations, which can then be solved mathematically to determine the levels of the three different interfering substances.<sup>7</sup> To determine which method is more accurate, TG concentrations were measured on lipemic samples and used as reference. The results showed that the ACL-TOP-750 detected lipemia more accurately (73.7%) than visual inspection (47.4%). Thus, for moderate lipemia samples, the instrument method is more accurate, while visual inspection may lead to unnecessary sample re-collection and reduce efficiency.

During hemolysis, red blood cells are destroyed and their contents are released into the blood, which can interfere with multiple coagulation tests. For example, hemoglobin will affect the results of chromogenic substrate assays, such as AT. Tissue factor and proteases activate the exogenous pathway of the coagulation system, resulting in depletion of coagulation factors and affecting PT and APTT.<sup>8,9,10</sup> The results of this study show that for mildly hemolyzed (1+) and severely hemolyzed (4+, 5+) samples, visual inspection was consistent with the instrument, while for moderately hemolyzed

(2+, 3+) samples, the two methods were significantly inconsistent. The ACL-TOP-750 determines the degree of hemolysis by detecting the absorbance of the sample at 405 nm and 535 nm wavelengths.<sup>7</sup> Therefore, it is more objective and accurate in detecting hemolyzed samples, which can reduce unnecessary review.

For icteric samples, a high concentration of bilirubin interferes with optical-based coagulation tests.<sup>5,11</sup> The results of this study show that for mildly icteric (1+, 2+) samples, visual inspection was consistent with the instrument method, while for the moderately icteric (3+) and severely icteric (4+, 5+) samples, the two methods were significantly inconsistent, and that visual inspection may miss individual samples. The instrument can be used to quantitatively detect the concentration of bilirubin in plasma samples. To determine which method is more accurate, plasma TBIL were measured in icteric samples, and the results showed good correlation between the instrument and plasma TBIL. However, only 18.8% of cases determined to have icterus by visual inspection were consistent with plasma TBIL, and the visual results were generally graded as higher than the actual level, which could easily lead to unnecessary repeated tests. Therefore, the instrument method is more objective and accurate. Previous studies also showed that most icteric samples can be detected by the ACL-TOP-750<sup>6</sup> although mild icterus will not have important implications for the test result.

Among 30 samples with clotting present, 27 were detected by visual inspection without alarm by the instrument. Further analysis showed that there were large blood clots in all of them. The clots sank to the bottom of the tubes after centrifugation, leading to failure of detection by the instrument. 3 samples were reported as "abnormal sample aspiration" by the instrument but not detected by visual inspection. After checking, we found fine coagulation filaments in the plasma of these 3 samples. This indicates that visual and instrument methods have their own advantages in identification of undue clotting. The instrument senses the change of plasma through a needle baroreceptor during sample suction. Once the pressure change is sensed, an alarm is triggered indicating that there may be a clot present. Therefore, the ACL-TOP-750 can detect small clots or filament in plasma that are difficult to detect by visual inspection, but is not sensitive to large clots.

In conclusion, the pre-analytical stage is a significant source of laboratory errors. More accurate and efficient pre-analytical sample quality checks are a great improvement for the quality of the laboratory. In general, the pre-analytical check module on ACL-TOP-750 has improved accuracy and efficiency of sample quality verification for routine tests. There are still limitations, which require visual inspection of samples and a review of test results.

#### Author Contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

## Ethical Approval

Research complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board (Ethics Committee of Peking University First Hospital).


## Declaration of Conflicting Interests

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