INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2024.79037
Int J Med Biochem 2025;8(1):39–44

Technical Report

Evaluation of the hemolysis threshold for the measurement of serum lipase on Roche Cobas systems

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Abstract

Objectives: Following the release of an informational bulletin, Roche Diagnostics adopted a more restrictive hemolysis index (100 HI) for the release of serum lipase results on all Cobas systems. This study aimed to evaluate the interference threshold for serum lipase hemolysis on Cobas C501/311/701/Integra 400 systems using a total allowable error set by the Royal College of Pathologists of Australasia (RCPA).

Methods: To assess the influence of hemolysis on lipase, the parameter was quantified in serum pools spiked with escalating concentrations of a hemolysis interferent. The lipase assay was performed using the colorimetric lipase method (LIPC), and the HI was determined by absorbance measurements of diluted samples in accordance with the system protocol.

Results: The Cobas Integra 400 and Cobas C311 showed the greatest interference of lipase with hemolysis (≤300 HI). The Cobas C501 and C701 demonstrated less sensitivity to hemolysis (≤1300 HI).

Conclusion: The results of this study demonstrate that interference limits may vary between different Roche systems, even when the same reagent is used. Our study indicated that the lipase hemolysis threshold (100 HI) currently set by the manufacturer was excessively restrictive. This finding highlights the necessity of verifying manufacturers' information bulletins to provide better medical care.

Keywords: Hemolysis, interference, lipase, threshold

How to cite this article: Ilardo C, Al Muhanna B, Lamarti C. Evaluation of the hemolysis threshold for the measurement of serum lipase on Roche Cobas systems. Int J Med Biochem 2025;8(1):39–44.

Lipase is a glycoprotein that reduces triglycerides and diglycerides to free fatty acids and glycerol by catalyzing the hydrolysis of ester bonds [1]. Lipases are primarily present in pancreatic secretions but can be found in several tissues, including hepatic lipase in the liver, hormone-sensitive lipases in adipocytes, lipoprotein lipase in endothelial cells, and pancreatic lipase in the small intestine [2]. Clinically, a lipase test may be used to help diagnose disorders of the pancreas, most often acute pancreatitis. Other pathologies can cause an increase in blood lipase levels, such as pancreatic cancer, gallbladder issues, chronic kidney disease, intestinal problems, cancer of a salivary gland, peptic ulcer disease, primary biliary cirrhosis, and certain drugs [3]. The quantitative determination of lipase in human serum and plasma on the Roche Cobas c system is based on the enzymatic hydrolysis of 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester, followed by monitoring the production of resorufin, measured photometrically between 570 and 583 nm. The intensity of the red color developed is directly proportional to lipase activity. Hemolysis involves the release of hemoglobin into the extracellular compartment [4]. Oxyhemoglobin and deoxyhemoglobin have maximum absorption at 415 nm, with a detection range between 320 and 450 nm and 540 and 589 nm, respectively [5]. Thus,

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Submitted: July 01, 2024 **Accepted:** August 26, 2024 **Available Online:** November 07, 2024

colorimetric assays using absorbance measurements within these ranges may be susceptible to spectral interference [6, 7]. According to Ishiguro et al. [8], spectral interference is linked to the additivity of the Beer-Lambert law and will be observed for parameters whose final measurement is spectrophotometric and carried out at wavelengths close to hemoglobin's main absorption wavelengths (415, 540, and 578 nm). Following an informational bulletin, Roche's quality control center evaluated the potential for interference in serum lipase results due to hemolysis. The hemolysis index (HI) threshold for Cobas C501/311/502/701/702 instruments was adjusted from 1000 to 100, while the Cobas Integra 400 plus and Cobas C111 systems were adjusted from 300 to 100 [9]. This change could result in more lipase results being rejected. The aim of this study was to evaluate the interference threshold for serum lipase hemolysis on Cobas C501/311/701/Integra 400 systems, using a total allowable error set by the RCPA as the limit [10].

Materials and Methods

Ethical approval

The laboratory investigations were conducted in accordance with the General Data Protection Regulation (EU Regulation 2016/679 and Directive 95/46/EC) and the French data protection law (Law 78–17 of 6 January 1978 and Decree 2019–536 of 29 May 2019). The reuse of human body elements and products for medical or scientific purposes other than that for which they were removed or collected is permitted (Article L.1211-2 of the Public Health Code) and does not require ethics committee review.

Instruments

The HI and lipase activities were determined using four different analytical systems (Cobas C501/311/701/Integra 400, Roche Diagnostics, Mannheim, Germany). The lipase assay was performed using the colorimetric lipase method (LIPC),

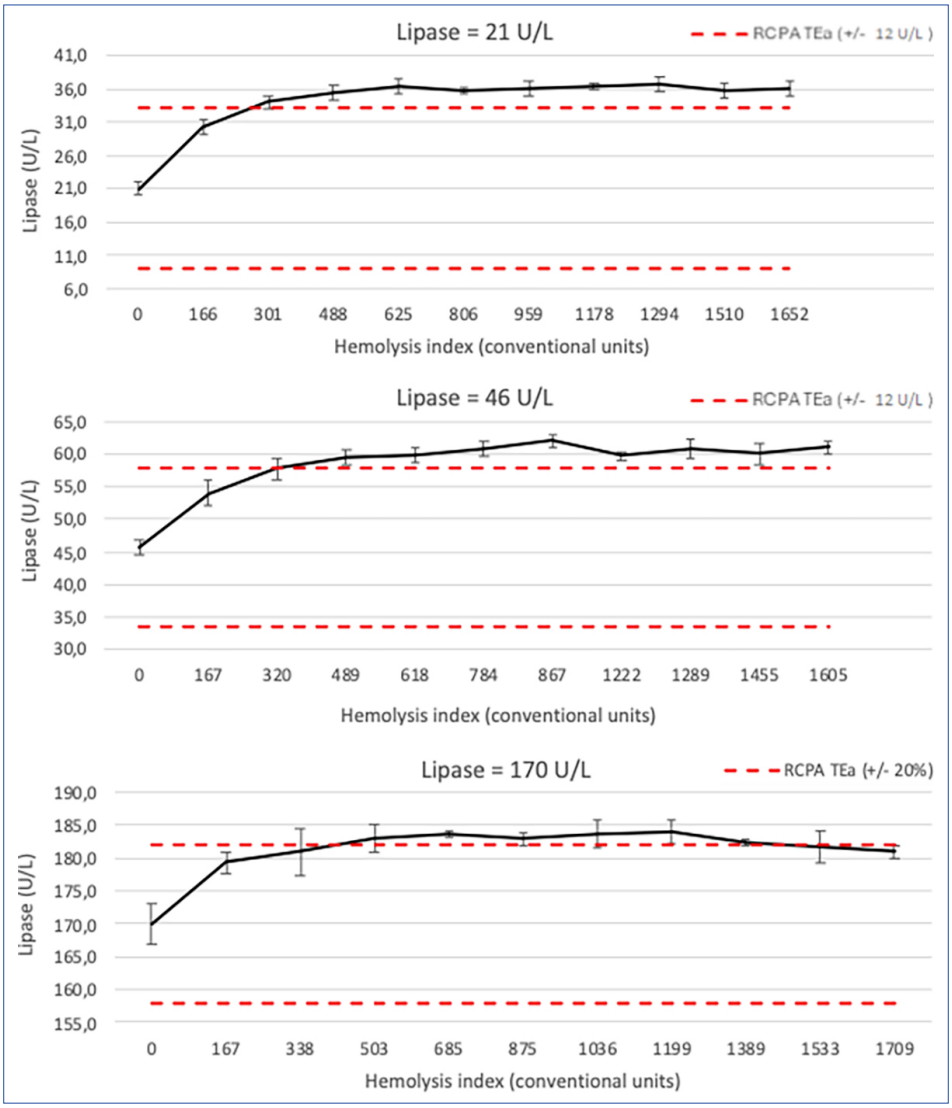

Figure 1. The lipase measured from the Cobas C311 according to the hemolysis index.
RCPA: Royal College of Pathologists of Australasia; TEa: Total error allowable.

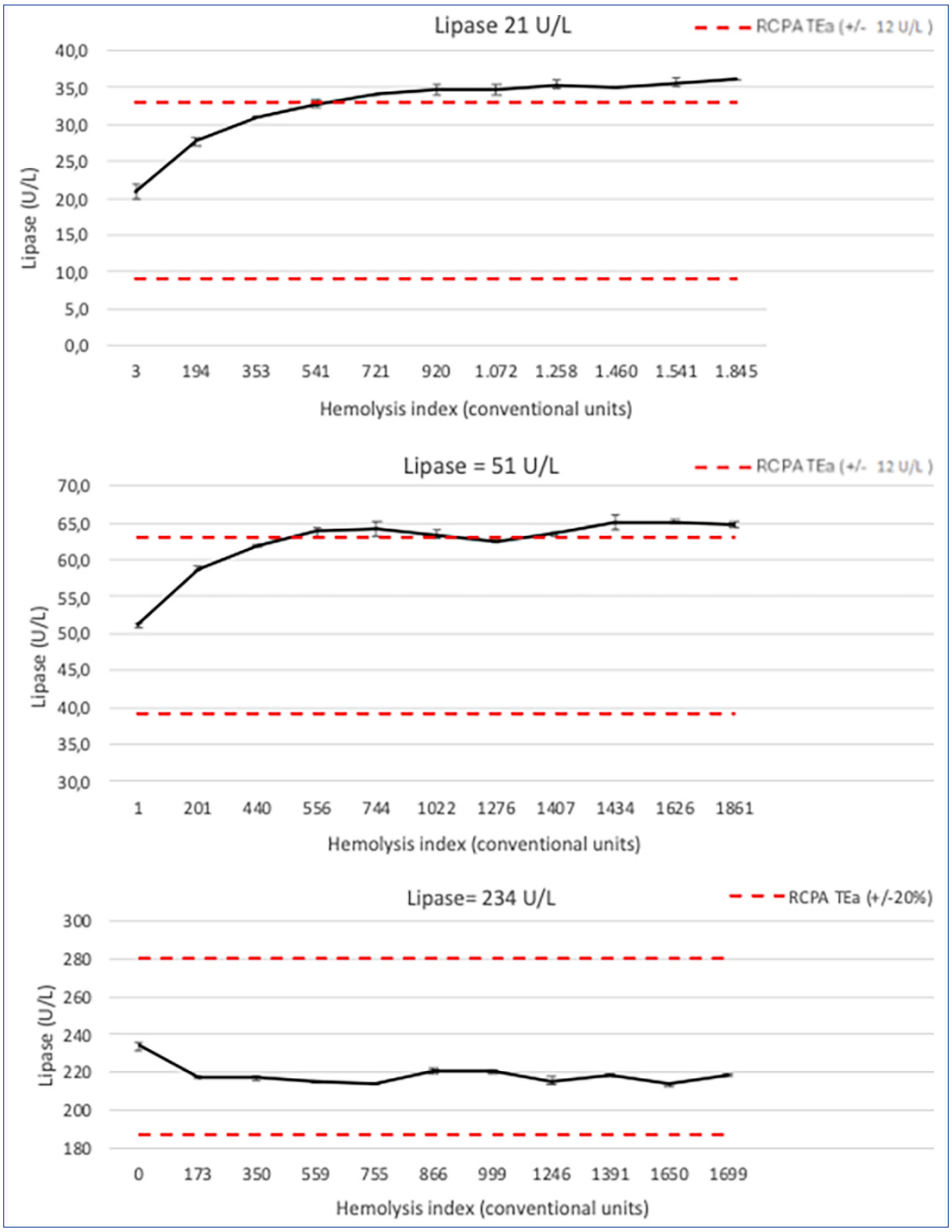

Figure 2. The lipase measured from the Cobas Integra 400 according to the hemolysis index.
RCPA: Royal College of Pathologists of Australasia; TEa: Total error allowable.

and the HI was determined by absorbance measurements of diluted samples in accordance with the system protocol. The expanded uncertainty for lipase was estimated at 15.4%, with a 95% confidence interval (CI) between 10.9% and 26.1%. Technical data sheets recommended an HI cutoff of 1000 for Cobas C501/311/701 and 300 for Cobas Integra 400, based on the requirement for a recovery of ±10% of the initial value. The HI unit is equivalent to a hemoglobin concentration of approximately 1 mg/dL, as stated by the manufacturer. Regarding the hemolysis index, the expanded uncertainty was estimated at 18.8%, with a 95% CI between 12.4% and 38.3%.

The Cobas Integra employs dual measurement at 589/659 nm, while the Cobas C311, C501, and C701 perform dual measurement at 570/700 nm.

Preparing the hemolysate and serum pools spiked

Serum pools with an HI of less than 10 were prepared to obtain three concentration ranges of lipase: approximately 22 U/L (normal), 50 U/L (close to the clinical threshold), and 180 U/L (high). To assess the influence of hemolysis on lipase, the parameter was quantified in serum pools spiked with escalating concentrations of a hemolysis interferent. The discrepancy between the result and the non-spiked baseline value was evaluated against the RCPA total error allowable (TEa). The hemolysate was produced from whole blood samples collected in lithium-heparin blood tubes in accordance with CLSI recommendations by osmotic shock [11]. A blood sample from a healthy subject was spun at 2000 g for 10 minutes. The red blood cells were obtained

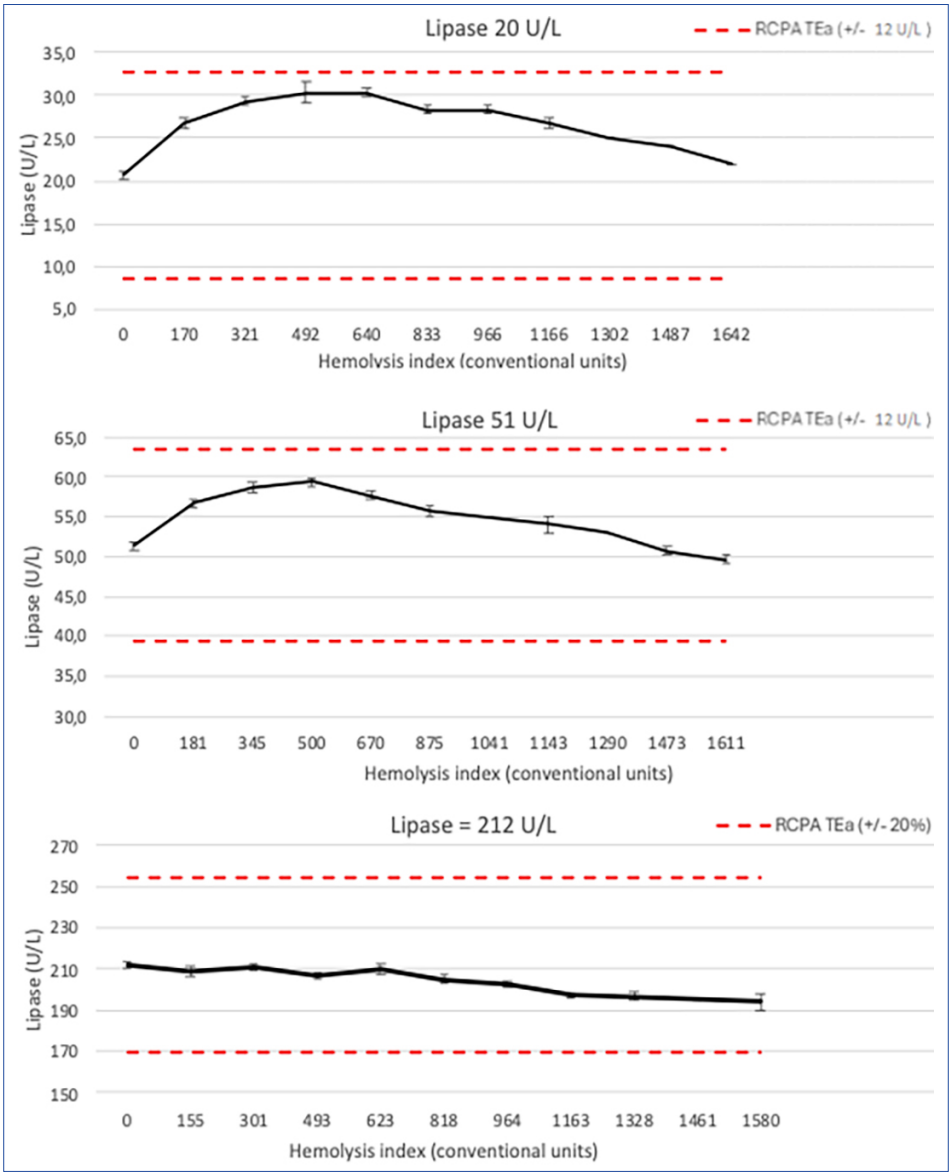

Figure 3. The lipase measured from the Cobas C501 according to the hemolysis index.
RCPA: Royal College of Pathologists of Australasia; TEa: Total error allowable.

after the plasma and buffy coat were removed, followed by three washes with physiological serum. The red blood cells were lysed by adding distilled water in a 1:1 ratio and centrifuged at 4000 g for 10 minutes to remove cellular debris. The hemoglobin content of the hemolysate was determined using the Siemens RP500® analyzer. The hemolysate was stored at -20°C until required.

Data analysis

For each system, the impact of hemolysis on lipase was measured in pools of serum spiked with increasing concentrations of hemolysis interferent. The result was assessed against the RCPA TEa. The relative deviation is shown on the graphs, with the acceptability criteria (RCPA TEa) represented by horizontal dotted lines (+/- 12 U/L for lipase values below 60 U/L and +/- 20% for lipase values above 60 U/L).

Results

Cobas C311

For this instrument, regardless of the lipase concentration, positive interference occurred from a hemolysis index of 300. The observed overestimation did not exceed 15 U/L of the true value, irrespective of the hemolysis level (Fig. 1).

Cobas Integra 400

For this system, lipase activity measurement was positively affected by hemolysis but only for normal or borderline positive lipase activity. At the specified concentration levels, overestimation did not exceed 15 U/L of the true value, regardless of hemolysis degree. In contrast, for high lipase activities (approximately 240 U/L), a hemolysis index exceeding 170 HI resulted in a 6% reduction in the measured activity (Fig. 2).

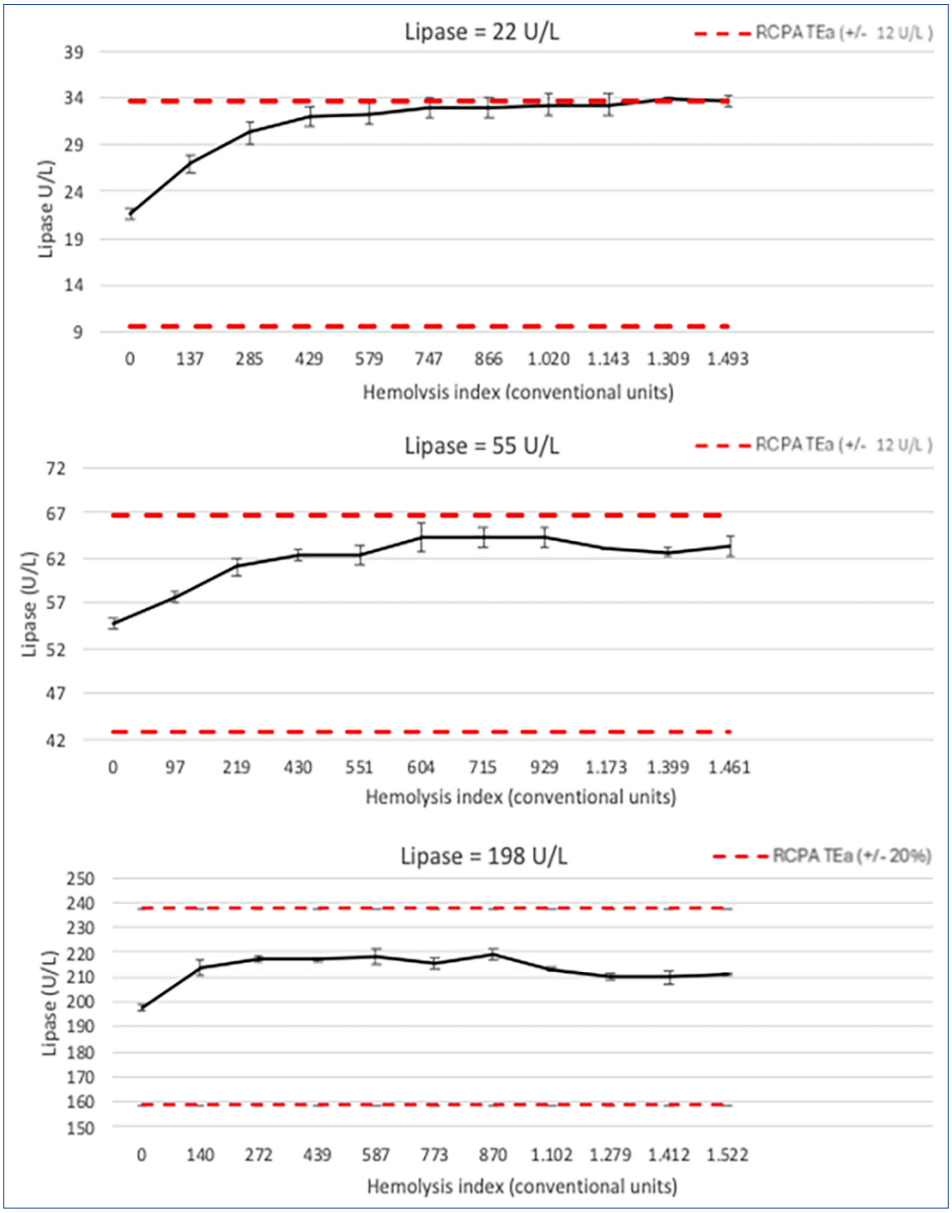

Figure 4. The lipase measured from the Cobas C701 according to the hemolysis index.
RCPA: Royal College of Pathologists of Australasia; TEa: Total error allowable.

Cobas C501

For this system, normal or borderline positive lipase activity was positively affected by hemolysis up to 500 HI (without exceeding the RCPA TEa). Thereafter, a decline was observed until values returned to approximate those of the non-spiked serum. For high lipase activities (approximately 212 U/L), a hemolysis index exceeding 1000 HI resulted in a 10% reduction in measured activity (Fig. 3).

Cobas C701

For normal lipase activity, measurement was positively influenced by hemolysis up to 1143 HI. For borderline high lipase activity, hemolysis caused a moderate increase in lipase (without exceeding the RCPA TEa) (Fig. 4).

Discussion

Despite the identicality of lipase assay methods, different interactions with hemolysis may be observed depending on the system used. The Cobas Integra 400 (previous generation) and Cobas C311 (latest generation) showed the greatest interference of lipase with hemolysis (from 300 HI). The Cobas C501 and C701 (intermediate generation systems) demonstrated less sensitivity to hemolysis when measuring lipase, with no significant interference up to 1000 HI. Differences in sensitivity to hemolysis among the Roche systems are unclear. Although measurement wavelengths differ slightly (589/659 nm for Cobas Integra 400), this difference does not account for hemolysis sensitivity, as the Cobas Pure, using identical measurement wavelengths to the Cobas C311 and C701 (570/700 nm), exhibited similar sensitivity.

Spectral interference with lipase was not proportional to the extent of hemolysis and did not occur throughout the entire measurement range. This phenomenon is characteristic of spectral interference [12]. Interference was more pronounced for normal values and reduced or negligible for higher values, enabling reliable results with minimal risk of over- or under-estimation.

Data sheet hemolysis interference limits differed only for Cobas C311 (1000 versus 300), justifying a decrease in the hemolysis index alert threshold. For other systems, our findings aligned with data sheets, indicating no need to adjust the alert threshold.

A statistical analysis of the hemolysis indices of 1,260 samples on our platform revealed that 98.6% of samples showed hemolysis of less than 100 HI, 1.3% between 100 and 300 HI, and 0.08% above 300 HI. A rigorous application of the Roche bulletin could lead to the rejection of approximately ten lipase results per day, which would be unacceptable for clinical staff.

Study limitations

1. The method of cell lysis by the addition of water may differ from *in vivo* or *in vitro* hemolysis observed in samples received by the laboratory. Delgado et al. [13] demonstrated that the methodology used to prepare the hemolysate can contribute to data variability.
2. The hemolysis threshold may vary depending on the quality specifications chosen.

Conclusion

This study demonstrates that interference limits vary between different Roche systems. Results showed that the lipase hemolysis threshold (100 HI) proposed by Roche was unduly restrictive. By raising the threshold above the new Roche limit, sample rejection can be reduced without compromising result quality.

Authorship Contributions: Concept – C.I., B.A.M., C.L.; Design – C.I., B.A.M., C.L.; Supervision – C.I., B.A.M., C.L.; Funding – C.I., B.A.M., C.L.; Materials – C.I., B.A.M., C.L.; Data collection &/or processing – C.I., B.A.M., C.L.; Analysis and/or interpretation – C.I., B.A.M., C.L.; Literature search – C.I., B.A.M., C.L.; Writing – C.I., B.A.M., C.L.; Critical review – C.I., B.A.M., C.L.

Conflict of Interest: The authors declare that there is no conflict of interest.

Use of AI for Writing Assistance: No AI technologies utilized.

Financial Disclosure: The authors declared that this study has received no financial support.

Peer-review: Externally peer-reviewed.

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