OXFORD UNIVERSITY PRESS | Clinical Chemistry

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Journal:	*Clinical Chemistry*
Manuscript ID	Draft
Manuscript Type:	Article
Date Submitted by the Author:	n/a
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Keywords:	Lipids, Mass Spectrometry, Liquid Chromatography

Ex vivo instability of lipids in whole blood – preanalytical recommendations for clinical lipidomics studies

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Running head: *Ex vivo* stability of lipids in whole blood

Keywords: Clinical lipidomics, blood, sample collection, preanalytical, lipid stability

Abbreviations: ACN, acetonitrile; AUC, area under the curve; Cer, ceramide; CV, coefficient of variation; DG, diacylglycerol; EDTA, ethylenediaminetetraacetic acid; FA, free fatty acid; HexCer, hexosylceramide; HRMS, high resolution mass spectrometry; IPA, isopropanol; ILS, International Lipidomics Society; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LSI, lipidomics standards initiative; MeOH, methanol; MTBE, tert-butyl methyl ether; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; QC, quality control; ROC, receiver operating characteristics; SE, cholesterol ester; SM, sphingomyelin; SOP, standard operating procedure; TG, triacylglycerol; UHPLC-MS, ultra-high-performance liquid chromatography-mass spectrometry.

Abstract

BACKGROUND

Reliability, robustness, and inter-laboratory comparability of quantitative measurements is critical for clinical lipidomics studies. Lipids' different *ex vivo* stability in blood bears the risk of misinterpretation of data. Clear recommendations for the process of blood sample collection are required.

METHODS

We studied by UHPLC-high-resolution mass spectrometry, as part of the "Preanalytics interest group" of the International Lipidomics Society, the stability of 417 lipid species in EDTA-whole blood after exposure to either 4°C, 21°C, or 30°C at six different time points (0.5h to 24h) to cover common daily routine conditions in clinical settings.

RESULTS

In total, >800 samples were analyzed. 325 and 288 robust lipid species resisted 24h exposure of EDTA whole blood to 21°C or 30°C, respectively. Most significant instabilities were detected for FA, LPE and LPC. Based on our data, we recommend cooling whole blood at once, persistent, and separate plasma within 4h, except the focus is solely on robust lipids. Lists are provided to check the *ex vivo* (in)stability of distinct lipids and potential biomarkers of interest in whole blood.

CONCLUSIONS

Our results contribute to the international efforts towards reliable and comparable clinical lipidomics data paving the way to the proper diagnostic application of distinct lipid patterns or lipid profiles in the future.

Introduction

The general interest in clinical lipidomics to study lipid profiles and find new lipid biomarkers is continuously increasing, along with the collection of blood for this purpose. However, the procedures used to collect a blood sample for lipidomics analyses are not always well-defined and are pretty diverse, since preanalytical situations differ between individual hospitals or study wards. In many cases, samples are collected following standard operating procedures (SOP) that are suitable for robust clinical routine parameters but not necessarily for quantifying a broad range of lipid species in blood. Not unexpectedly, discrepancies in published lipidomics data and more general issues of irreproducibility have been recognized and are debated *(1, 2)*. Whether these differences are due to pre-, post-, or analytical issues remains unclear in most cases. To increase the inter-laboratory comparability of quantitative lipid profiles, the Lipidomics Standards Initiative (LSI) and the International Lipidomics Society (ILS) aim to design guidelines for the major lipidomics workflows on a community-base, - not only for the analytical and data processing/reporting phases but also for preanalytics *(1, 3-5)*. ILS aims to pave the way for future reliable diagnostic applications of distinct lipid patterns or lipid profiles. Effects of prolonged exposure of plasma/serum samples to adverse conditions on the stability of lipids had already been extensively studied and described *(6-14)*. However, plasma/serum samples comprise the less vulnerable preanalytical phase because they are the cell-free supernatant of whole blood after centrifugation, i.e., obtained at the end of the entire blood collection, handling, and transportation processes. In contrast, whole blood samples before centrifugation are a “liquid tissue” containing trillions of cells. These metabolically active cells can alter the abundance of distinct lipid species to various extents *ex vivo*. Consequently, handling whole blood is the most critical preanalytical step for clinical lipidomics *(15)*. Up to now, studies investigating the stability of lipid patterns in whole blood are rare and provide only limited and incomplete information for implementation into clinical practice *(7, 16-18)*. Uniform preanalytical recommendations about conditions and timespans for whole blood

handling after collection are missing so far, but would be desirable and could be one step toward eliminating discrepancies and issues of irreproducibility in published lipidomics data.

To target preanalytical errors in lipidomics, we studied in a considerable number of samples the stability of 417 lipid species representing 13 lipid classes in EDTA-whole blood at six different time points after exposure to either 4°C or 21°C or 30°C in comparison to blood samples processed at once. Three short time points (up to 1.5h) and three longer exposures (2h, 4h, and 24h) were investigated in blood samples of 27 and 56 individuals, respectively. In addition to reporting the differences in the stability of the 417 lipid species exposed to these conditions, we discovered a potential quality control lipid triplet for the detection of sampling artifacts in the preanalytical phase from blood collection until centrifugation and separation of plasma/serum. Our data may contribute to recommendations for preanalytical guidelines for clinical lipidomics to harmonize and standardize the blood collection process.

Materials and Methods

Standards and Reagents. HPLC-grade methanol (MeOH), acetonitrile (ACN), and isopropanol (IPA) were purchased from Merck (Darmstadt, Germany), HPLC-grade tert-butyl methyl ether (MTBE) and ammonium acetate from Sigma−Aldrich (St. Louis, USA), HPLC-grade chloroform ($CHCl_3$) from Duksan (Ansan-si, South Korea), and ultrapure water was prepared by a Milli-Q system (Millipore, USA). All internal standards were purchased from Avanti Polar Lipids (Alabaster, USA), except FA 22:0-d4 (ten Brink, Amsterdam, The Netherlands) and TG 15:0/15:0/15:0 (Sigma-Aldrich, USA).

Sample Collection. For this preanalytical study, 10 ml of EDTA whole blood were drawn from 83 randomly selected subjects by a longstanding, experienced team at the ward for metabolic studies at the University Hospital of Tuebingen. After the drawing of blood, samples were immediately divided into aliquots according to the scheme shown in Figure 1. Centrifugation to separate EDTA-plasma from blood cells was either performed immediately or after 0.5h, 1h,

1.5h (short-term stability analysis, n= 27 subjects), or after 2h, 4h or 24h (long-term stability analysis, n = 56 subjects) at 4°C (cooled at once), or 21°C (room temperature) or 30°C (summer time conditions). At the end of the respective exposure time, whole blood was centrifuged at 4°C (3100×g for 7 min), and EDTA-plasma was stored at once at −80°C in 100 µl aliquots until further use. In total, 829 samples were studied (one missing sample at 2h; 30°C). Informed written consent was obtained from all participants, and the ethics committee of the university of Tuebingen approved the protocol (ref. 247/2017BO1) according to the Declaration of Helsinki of 1964 and its later amendments.

Sample Preparation. Plasma samples were extracted using MTBE/MeOH/H_2O as described previously *(19)*. 50 µL of plasma were mixed with 300 µL of MeOH containing internal standards (per 300µl: 1250 ng PC 15:0/15:0, 500 ng LPC 19:0, 100 ng LPC 15:0, 100 ng PE 15:0/15:0, 25 ng PG 15:0/15:0, 500 ng SM d18:1/12:0, 100 ng Cer d18:1/17:0, 100 ng DG 15:0/18:1-d7, 1 µg TG 15:0/15:0/15:0, and 250 ng FA 22:0-d4) and deproteinized (further details, see supplement).The extracted lipids were reconstituted in 30 µL of $CHCl_3$/MeOH (2:1, v/v), followed by dilution with ACN/IPA/H_2O (65:30:5, v/v/v) containing 5 mM ammonium acetate. Quality control (QC) samples were prepared by pooling equal amounts of lipid extracts from each sample and were analyzed after every 10th sample in a sequence.

Nontargeted UHPLC-HRMS-based lipidomics. A UHPLC-Q Exactive MS system (Thermo Fisher Scientific, Rockford, USA) was operated in both positive and negative ion modes using a 2.1 × 100 mm ACQUITY^{TM} 1.7 µm BEH C8 column (Waters, Ireland). The mobile phases were ACN/water (60:40, v/v) and IPA/ACN (90/10, v/v) both with 10mM ammonium acetate. Further chromatographic and MS details are provided in the supplement and the attached reporting checklist of the LSI. The analytical performance of this approach is illustrated in Figure S1.

Data pre-processing. Lipid identification was achieved according to our prior study *(20)*, meaning MS/MS fragment, exact m/z, and retention time in combination with LipidSearch software

(Thermo Fisher Scientific, Waltham). Peak areas of the detected lipids were obtained using Thermo TraceFinder and normalized by corresponding internal standards. Coefficients of variation (CV) were calculated for all peaks in QC samples to evaluate the quality of the data. Lipids with a CV > 30% were excluded from subsequent analysis.

Statistical analysis. All statistical studies and visualization were conducted using R software (v4.1.0) unless otherwise noted. Wilcoxon matched-pairs signed rank tests was performed with MATLAB (R2014a, MathWorks, Natick, MA). False discovery rate for multiple testing was calculated with the Benjamini-Hochberg method. Significant alteration of a lipid species level was defined as >10% change in signal intensity ($p < 0.05$; FDR < 0.05) in comparison to fresh, i.e., at once processed blood samples. R was also used for heatmap visualization and GraphPad Prism to generate boxplots. For heatmap generation, missing values were imputed with one-tenth of the minimal value for every variable. Then the fold-changes of different time points at different temperatures compared to fresh, i.e., at once prepared samples (time point 0h), were calculated and log-transformed fold-change values were visualized. The strategy applied for the detection of possible sample quality control biomarkers is illustrated in Figure S2 and described in detail in the supplement.

Results

The stability of the lipidome in EDTA whole blood, i.e., possible alterations during the timespan from blood drawing until centrifugation and separation of plasma, was investigated in a profile of 417 lipid species from 13 classes in a semi-quantitative manner at six exposure times from 0.5 to 24h under three different temperature conditions (4°C, 21°C and, to reflect summertime, 30°C; Fig. 1). The time points and exposure conditions were chosen based on our practical experiences in everyday clinical situations for collection, handling, and transportation of whole blood either for diagnostic or research purposes.

We detected a vast range of stabilities, reaching from very stable (24h at 30°C) to very unstable lipids (< 0.5h at non-chilled conditions). Table 1 shows the stability of each lipid class at a glance, and Fig. 2 illustrates these findings by a heatmap. A complete list of all covered lipid species is provided in Table S1.

Our first interest was identifying lipids that are very stable in whole blood. No alterations at all conditions studied were detected for three classes (Table 1 and S1), PI (17 species covered), HexCer (7 species covered), and SM (34 species covered). Furthermore, 123 of 132 covered TG were stable. In total, 288 lipid species did not display any changes in their levels at the most challenging condition (24h exposure of EDTA whole blood to 30°C). At room temperature, six out of 13 lipid classes (PE, PG, PI, Cer, HexCer, SM) and 325 lipid species levels were unaffected after 24h, including 124 out of 132 TG (note: only two PG species were covered).

Our subsequent interest was to find the best timespan and condition for whole blood handling and transportation, aiming to preserve the level of all covered lipids. At 4°C, no significant change of any covered lipid was detected for up to 4 h, and even in blood chilled for 24 h before plasma separation, only 19 out of the 417 lipids were altered in their levels (Table 1 and S2).

Next, we focused on expected less stable lipids, i.e., the question of which lipids are vulnerable *ex vivo* in whole blood during the preanalytical phase. Very rapid, after 0.5h at 30°C, 14 out of 417 lipids changed, most strongly 12 LPC species (Table 1 and S3). After 24h at 30°C, one-third of all lipid levels were altered (48 decreased, 81 increased; Table 1 and S2). Particularly pronounced alterations (>3-fold increases) were detected for 14 lipids, most of which were LPC (Table S2). Decreases were dominated by PC (20 out of 80), PE (13 out of 26), and TG (7 out of 132). Exposure of blood at room temperature for 4 h, a situation not unusual in hospitals, led to changes of 41 lipid species in whole blood from the classes of FA (10 out of 40), LPC (22 out of 30), LPE (3 out of 8), DG (3 out of 11), and TG (3 out of 132).

Figure 3 shows representative examples of an unstable and a very stable species and illustrates the pronounced differences in the stability of distinct lipids. LPC, frequently reported as biomarkers in diagnostic patterns, show very rapid and pronounced changes, as demonstrated by LPC O-18:1 (Figure 3A). For LPC O-18:1, a more than 5.6-fold increase after 24h exposure to 30°C was detected (Table S2). Noteworthy, this increase can be prevented for up to 4h if the whole blood is directly cooled after drawing (Fig. 3A). Even after 24h at 4°C, the increases in LPC O-18:1 level amounted to only 13.4% (Table S2). In contrast to LPC, the box plots of Cer d18:1/24:0 in Figure 3B underline the preanalytical robustness of other lipids in whole blood under all studied conditions. Cer d18:1/24:0 is one of four ceramides described as a novel diagnostic tool in cardiology *(21, 22)*.

Next, we aimed to identify a lipid pattern allowing to detect samples of questionable quality (or lipidomics data obtained from such samples) caused by shortcomings during blood collection and transportation. The stepwise applied detection strategy is illustrated in Figure S2, and a detailed description is given in the method section. We identified a lipid triplet, namely LPC 16:1, LPC- O- 18:1, and LPE 20:4, as a potential QC pattern for lipidomics studies, possibly allowing to assess the occurrence of sampling artifacts during blood collection. In a first evaluation step the performance by an average area under the receiver operating characteristic (ROC)-curve (AUC) analysis was studied. For this evaluation, all samples generated under conditions leading to no significant changes in the total covered pattern of 417 lipids were classified as samples of good (n = 220) and the others as of questionable quality (n=526). The lipid triplet achieved an AUC of 0.959 based on a 5-fold cross-validation (10 repeats) (Figure S3). Next, we compared the performance of the lipid triplet with one of the few lipid-based, published sample quality control tools, the ratio at lipid class level of LPC/PC *(14)*. Figure 4 shows that very similar AUCs were achieved (0.962 for the lipid triplet and 0.951 for the ratio) for the detection of the 526 samples having a quality less suitable to achieve valid lipidomics data.

Discussion

Reliability, robustness, and inter-laboratory comparability of quantitative measurements is critical for lipidomics analyses, primarily if the application of distinct lipids or patterns in blood and other body fluids is intended for clinical diagnostics. Currently, international efforts by the ILS to collect data for the generation of guidelines are on the way, aiming to harmonize and standardize all steps of the lipidomics workflow. A minimal reporting checklist has already been published *(23)*. From our data, achieved as part of the "Preanalytics interest group" in a considerable number of samples, recommendations can be derived for the handling and transportation of whole blood. In addition, the comprehensive results in Table S2, can be of practical use to check the instability of distinct lipids including potential biomarkers of interest. Furthermore, we identified a triplet of lipids (LPC 16:1, LPC O-18:1, and LPE 20:4) potentially suitable to detect whole blood sampling artifacts, which is covered by most lipidomics approaches.

While processing plasma/serum for clinical lipidomics is generally well standardized and tightly controlled, this is not the case for blood collection. In our study, we provide clear evidence that cooling at once after blood drawing stabilized all covered 417 lipids for up to 4h. Even after 24h the levels of 398 lipids were stable in at once cooled blood. Thus, we recommend to cool blood at once and persistently until separation of plasma/serum, which should ideally be completed within 4h, except in cases where the analytical focus is exclusively on very stable lipid species. Our recommendation is in good agreement with previous publications, which, however, provided only incomplete information for clinical lipidomics *(7, 16-18, 24)*. The group of G. Liebisch described a rapid increase of LPA, S1P, and SA1P in blood stored at room temperature within 1h and its prevention by cooling *(7)*. At 4°C, selected PUFA were stable in EDTA-whole blood samples up to the longest studied time point of 2h *(17)*. Hahnefeld and co-authors showed that most of the lipids covered in their profile were stable for up to 4h at 4°C

in blood, except the endocannabinoids (N-acetylethanolamides and monoacylglycerols), which were only stable for 20 min *(18)*. They recommended at once cooled whole blood to be processed for the measurement of endocannabinoids within 1h and for LPA, sphingolipids, and non-targeted profiling within 2h *(18)*. Kamlage et al. recommended processing blood samples as quickly as possible based on data from metabolite profiles, including lipids *(16)*. Thus, for both lipidomics and metabolomics studies, cooling at once and processing within 4h is a consistent recommendation *(15, 24)*.

Although cooling at once and timely transportation of whole blood can be stipulated in guidelines, SOPs and protocols, there is no guarantee of its permanent fulfillment during a daily routine in hospitals. Hence, a reliable diagnostic tool should ideally contain only very robust lipid(s) to avoid that the period until centrifugation of whole blood is a critical issue when it is prolonged. We detected 325 and 288 robust lipid species in EDTA-whole blood samples showing for 24h at 21°C or even at 30°C no changes, respectively (Table S1). Such lipids guarantee valid results even when samples are transported in an extended hospital area or by mail within 24h. Useful (in)stability data for many published and potential future diagnostic lipid biomarkers are provided in Table S1 and S2, including e.g. a quartet of ceramides on the verge of being used in cardiologic diagnostics *(21, 25, 26)*. Two of these ceramides are stable for 24h at 30°C (Cer d18:1/24:0, and Cer d18:1/24:1) and the other two for 24h at 21°C (Cer d18:1/16:0, Cer d18:1/18:0), hence common preanalytical issues will not compromise the achievement of valid results with this lipid quartet, at least in internal clinical use. However, among the numerous novel plasma/serum lipid biomarkers published so far for possible diagnostic use *(27-29)* are also lipids less suited from the preanalytical point of view. One recent example is a pattern of 7 lipids, including LPC 20:0, identified by Alshery et al., which improves the prediction of cardiovascular events in type 2 diabetes *(30)*. Based on our data, LPC 20:0 shows a continuous, temperature-dependent increase in whole blood (e.g. > 2.5-fold after 24h at 30°C; Table S2), which may lead to false-high concentrations and consequently

misinterpretations if preanalytical issues occur. To achieve valid results with those less stable lipids requires greater preanalytical efforts and needs, more precisely, to cool blood samples at once and process them within 4h. This is recommended for unstable diagnostic lipid biomarkers and blood collected for non-targeted lipidomics projects. Based on our findings, FA and LPC should be interpreted with caution in all circumstances, except the preanalytical process was tightly controlled and followed the strictest recommendations of immediate cooling and processing.

The risk of misinterpretation, particularly for the less stable lipids, can be reduced or minimized by applying a QC marker to detect and exclude samples or results of questionable quality. Only very few studies have identified promising quality control biomarkers reflecting deviations in sample processing *(14, 16, 31-33)*. However, their applicability in lipidomics approaches is limited to some extent because they are either difficult to analyze, a complex pattern is suggested *(14, 16)*, the marker is not covered in typical analysis *(33)*, or it is not at all included in lipid profiles *(31, 32)*. We identified a possibly suited, simple lipid triplet, consisting of LPC 16:1, LPC O-18:1, and LPE 20:4, included in most data sets of standard lipidomics procedures. The ROC-curve AUC performance of this possible QC tool to identify sampling artifacts is comparable to a published lipid-based QC marker (the ratio at lipid class level of LPC/PC *(14)*), but its application is much easier. Applying this potential QC triplet for identifying samples containing lipids altered by delays during whole blood processing or interruption of the cold chain could be feasible, but further extensive validation is needed (e.g. robustness, definition of cut-off levels, exclusion of effects of diseases, etc.). Regular application of sample QC markers may also contribute to increased comparability and reproducibility of clinical lipidomics data *(3)*.

Some limitations of our study merit consideration: a) valid conclusions for a transport by mail exceeding 24h are not possible, b) stability statements are valid for the 417 covered lipids but not necessarily for other lipids of the same class, and c) for analytical reasons no

recommendation for very unstable lipid classes (e.g., oxylipins, eicosanoids, endocannabinoids, etc.) can be provided. Furthermore, if, in contrast to our approach, no HRMS is applied, the interpretation in samples of inferior quality can be more challenging because of overlapping isomeric and isobaric species, particularly in direct infusion lipidomics analyses.

To conclude, reproducible and quantitatively concordant data requires different layers of quality assurance and quality control measures, as stated by the "Lipidomics Standards Initiative" in their position paper *(3)*. Aiming to fulfill these requirements for the phase of whole blood handling, we recommend to cool blood samples at once and persistently and to complete centrifugation and plasma separation within 4h. This allows to achieve valid and reproducible profiles for, at least, all lipids covered in our approach. Furthermore, quality control, i.e., searching for sampling artifacts during whole blood collection, should regularly be performed using QC markers. We provide information about 325 and 288 lipids with a very high *ex vivo* robustness in blood at room and higher temperatures, respectively. Lipids from this list could be particularly well suited for diagnostic purposes since sample treatment can be performed as for most of the other patient samples in the hospital with no need for cooling or timely processing. Overall, our findings, generated within the LSI "Preanalytics interest group," may contribute to the international efforts to reach reliable and comparable clinical lipidomics data in the near future, independent from the place where they were generated.

Acknowledgments

We gratefully acknowledge Inge Fink and Heike Runge for their technical assistance (Institute for Clinical Chemistry and Pathobiochemistry, Department for Diagnostic Laboratory Medicine, University Hospital Tübingen, Tübingen, Germany).

Funding sources

This work was supported by the foundation (No. 21934006) from the National Natural Science Foundation of China to G.X., DFG/NSFC Sino-German mobility program (M-0257) to GX and RL, Chinese Academy of Sciences (CAS)-President´s International Fellowship Initiative (Grant No. 2019VBA0038) to RL, the foundation from the Youth Innovation Promotion Association CAS (2021186) to XL, Innovation program (DICP I201918) of Science and Research from the DICP, and the German Federal Ministry of Education and Research (BMBF) to the German Centre for Diabetes Research (Grant no. 01GI0925)

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Table 1. Summary of the stability of 417 lipids from 13 different classes in whole blood after collection. The numbers in each column correspond to lipids defined as unstable according to the following criteria: $p < 0.05$ and FDR < 0.05 and >10% change in signal intensities in comparison to samples prepared at once (0.5–1.5h, n= 27 per time point; 2h–24h, n= 56 per time point). Details of each significantly altered lipid species after 24h exposure (e.g. significance level, percentage of alteration) are provided in supplemental Table S2. FA, free fatty acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; Cer, ceramide; HexCer, hexosylceramide; SM, sphingomyelin; SE, cholesterol ester; DG, diacylglycerol, and TG, triacylglycerol.

Lipid class	Cooled at once 4°C						Room temp. 21°C						Summer time conditions 30°C						
	0.5	1	1.5	2	4	24	0.5	1	1.5	2	4	24	0.5	1	1.5	2	4	24	Time [h]
FA (n=40*)						10		1	2	2	10	25		2	4	5	14	29	
LPC (n=30)						5		5	11	12	22	27	12	19	23	25	27	29	
LPE (n=8)								1	1		3	6	1	3	3	3	5	7	
PC (n=80)												9					2	21	
PE (n=26)																		13	
PG (n=2)																		1	
PI (n=17)																			
Cer (n=21)																		2	
HexCer (n=7)																			
SM (n=34)																			
SE (n=9)												6					1	7	
DG (n=11)						1				1	3	11		2	3	6	11	11	
TG (n=132)						3					3	8	1				1	9	
sum						19		7	14	15	41	92	14	26	33	39	61	129	
(%)						(4.6%)		(1.7%)	(3.4%)	(3.6%)	(9.8%)	(22.1%)	(3.4%)	(6.2%)	(7.9%)	(9.4%)	(14.6%)	(30.9%)	

* total number of covered lipid species in the lipid class

Figure captions

Figure 1

Illustration of the study design. Short term" means exposure of whole blood from 27 individuals for 0.5h, 1h and 1.5 h to either 4°C, 21°C or 30°C. "Long term" means exposure of whole blood from 56 individuals for 2h, 4h and 24h to either 4°C, 21°C or 30°C.

Figure 2.

Heatmap showing the time-dependent (in)stability of all 417 covered lipids in EDTA-whole blood exposed to different temperatures. Six exposure times from 0.5h to 24h and three different temperatures (4°C, 21°C, and 30°C) were investigated, based on typical conditions during the timespan from blood drawing until centrifugation and separation of plasma in hospitals or study wards.

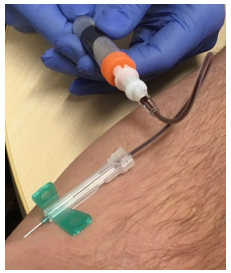
Figure 3.

Representative examples illustrate pronounced differences in the stability of distinct lipid species in whole blood. (A) LPC O-18:1 is an unstable, and (B) Cer 18:1/24:0 is a very stable lipid species. Time points up to 1.5h, n = 27 individuals; time points 2h, 4h, and 24h, n= 56 subjects (at time point 2h (30°C), one sample is missing). *= $p < 0.05$

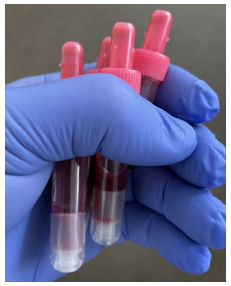
Figure 4.

Comparison of the classification performance to assess the sample and/or data quality in clinical lipidomics studies by a lipid triplet (LPC 16:1, LPC O-18:1, and LPE 20:4) as a possible quality control biomarker pattern and an already published lipid-based QC marker, the ratio at lipid class level of LPC/PC. The classification performance is shown by an area under the receiver operating characteristic (ROC) curve (AUC) analysis.

Figure 1



4-30°C for 0-24h



EDTA whole blood

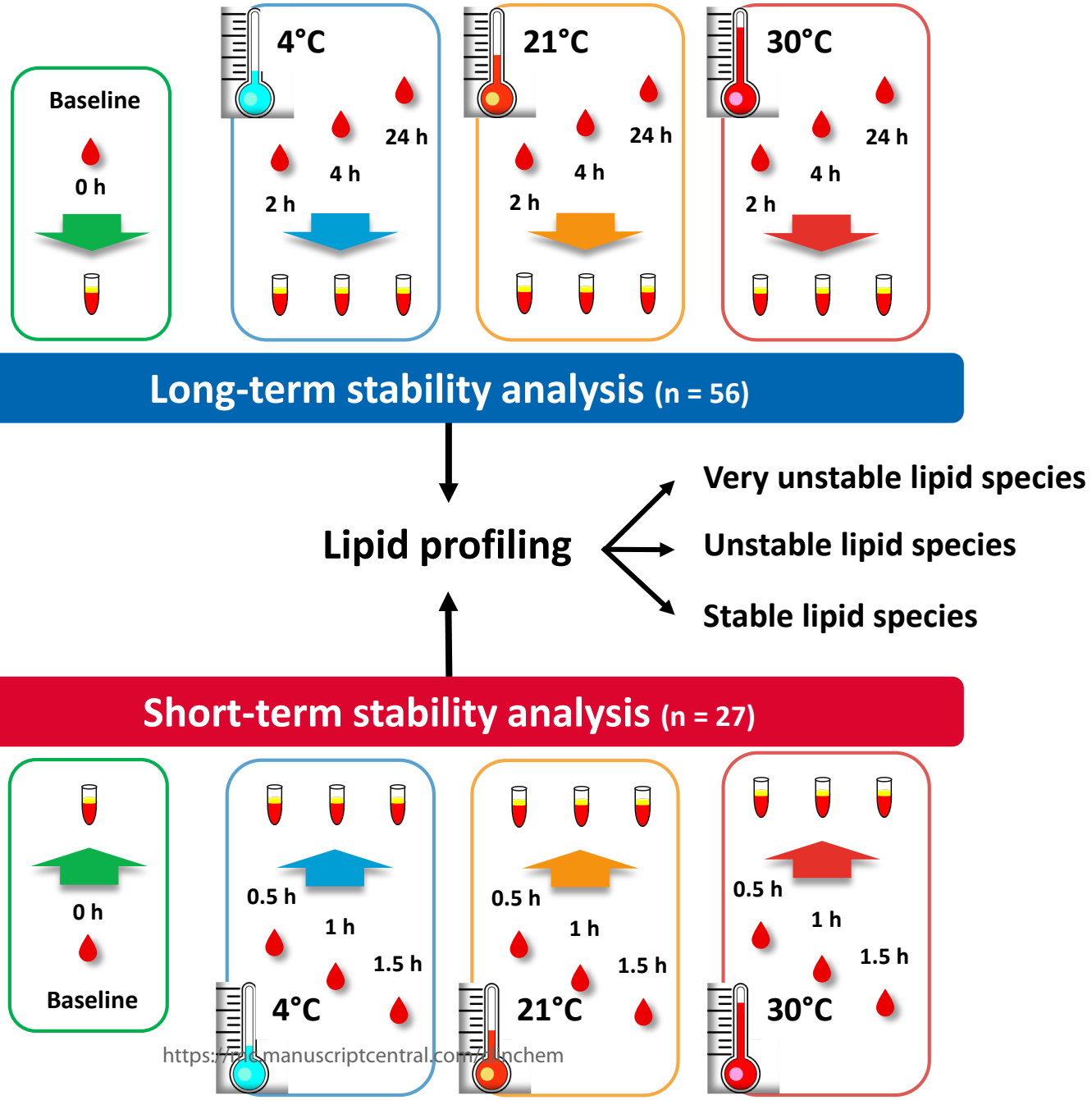
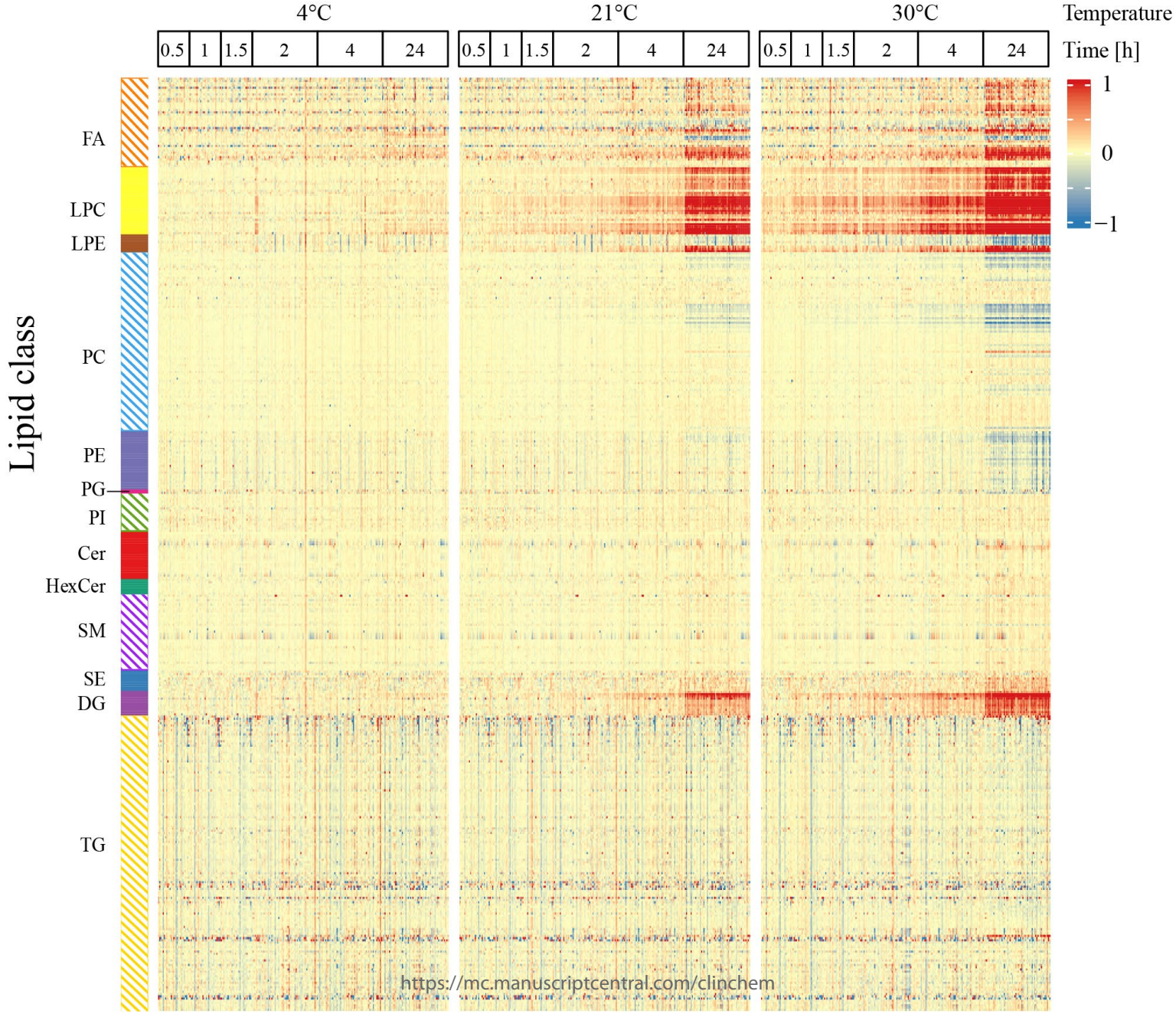
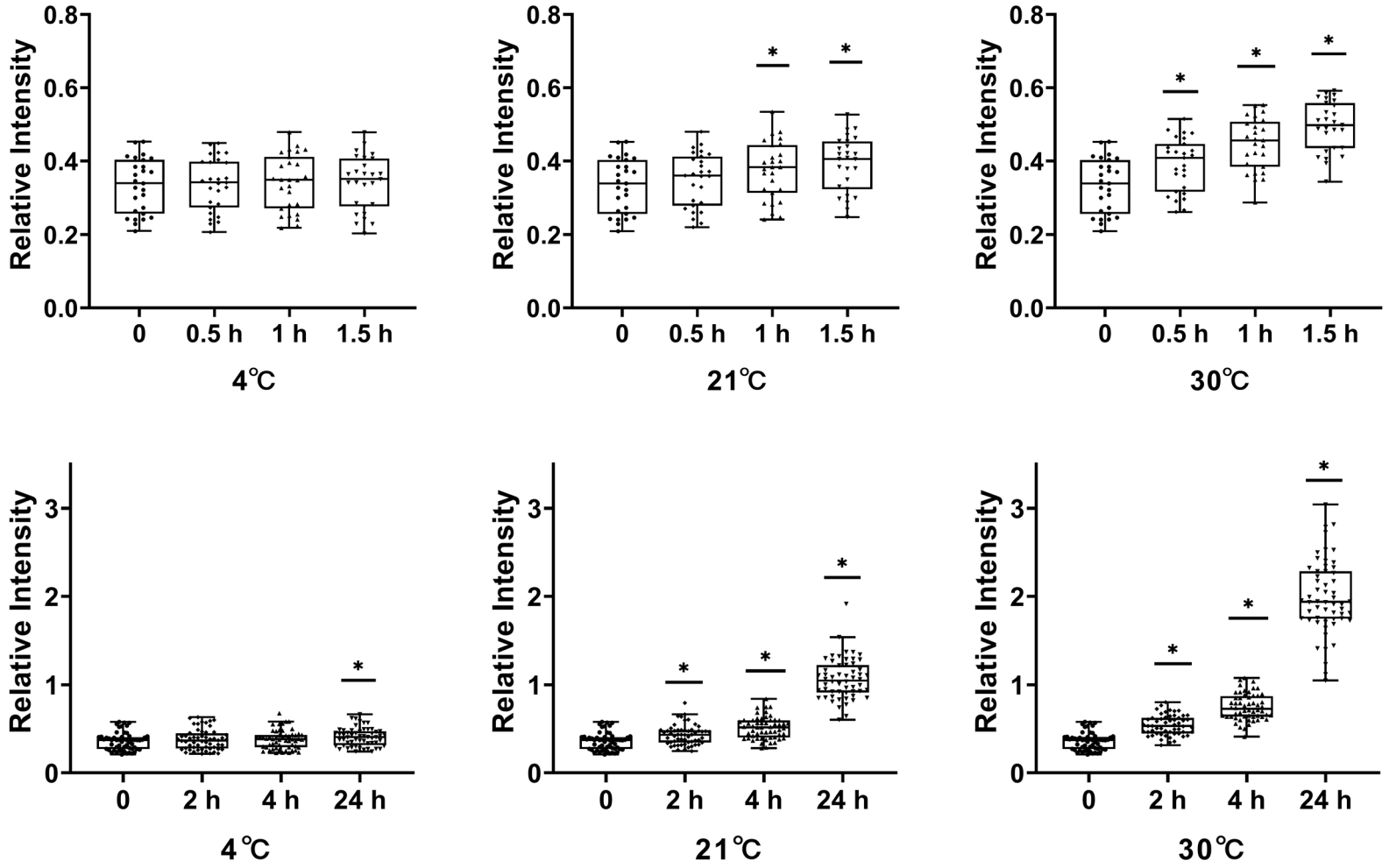


Figure 2



LPC O-18:1

Fig. 3A



Cer 18:1/24:0

Fig. 3B

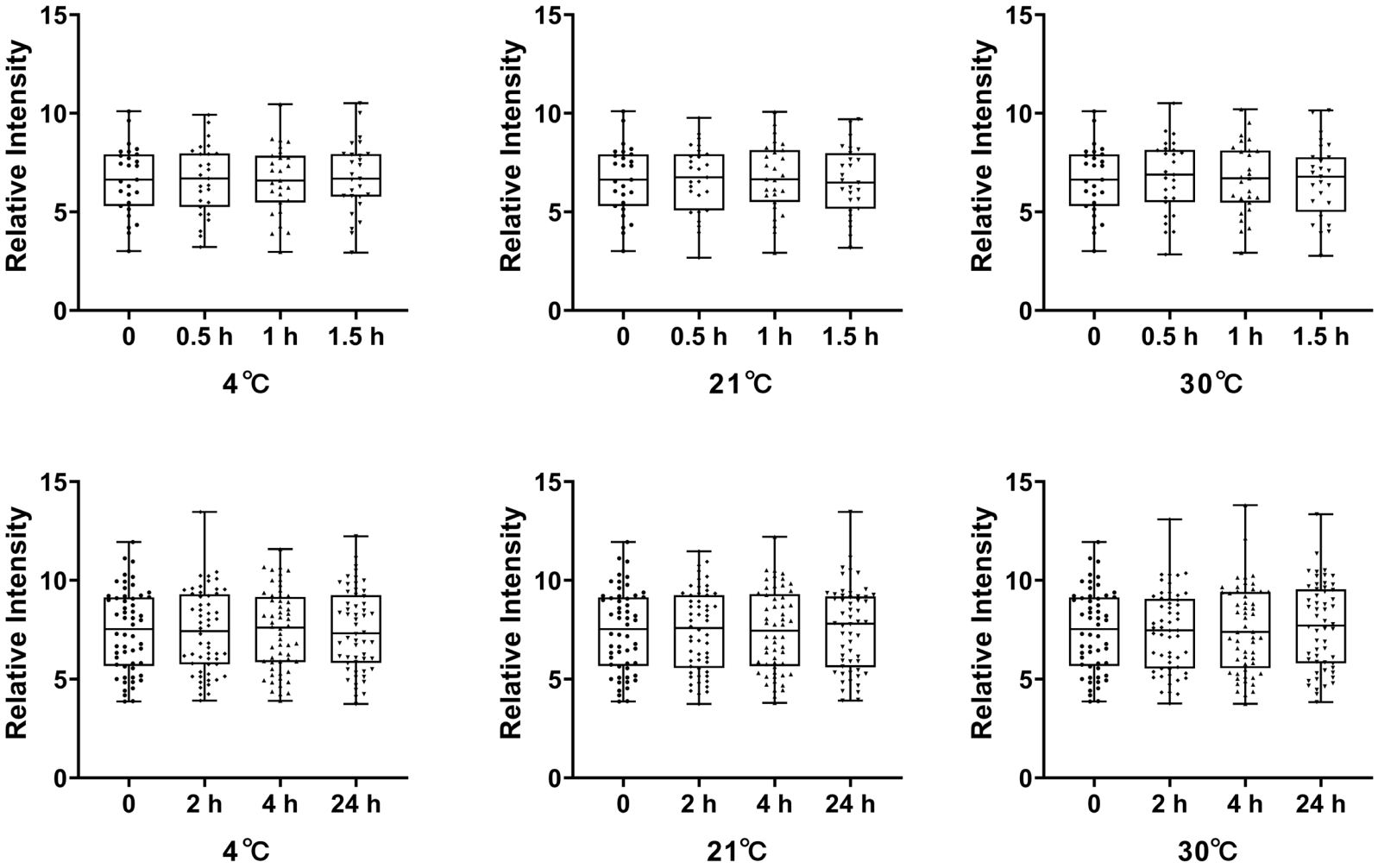
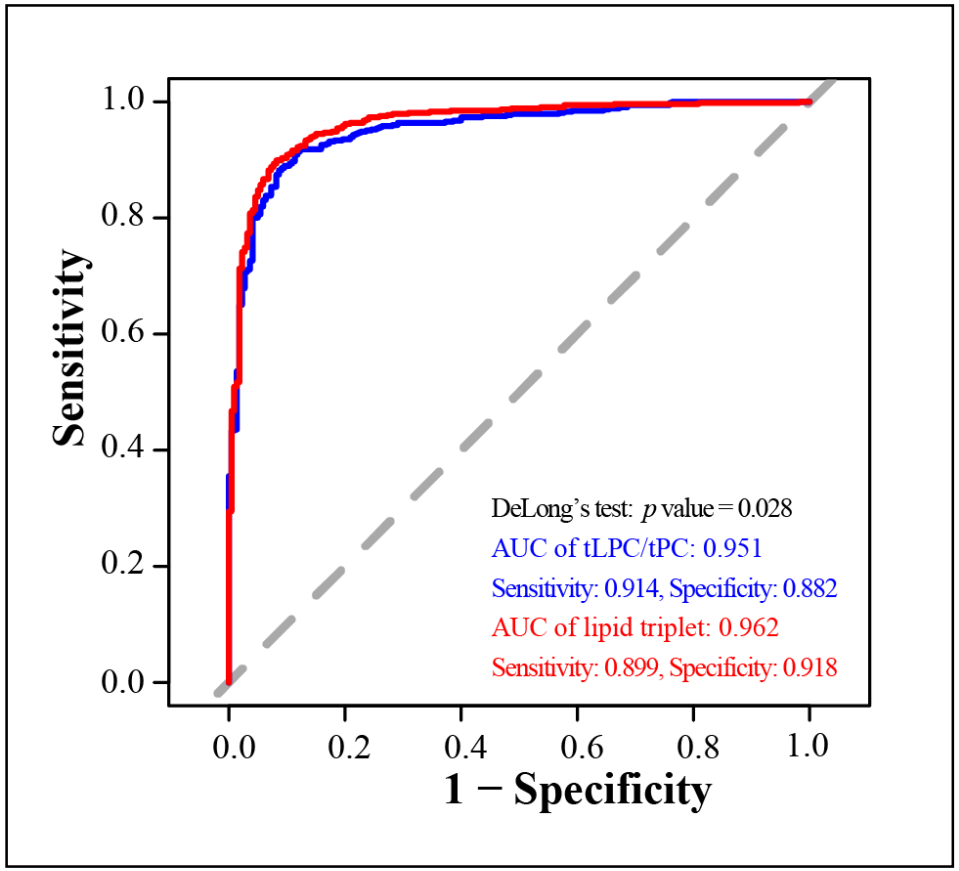


Fig. 4


1.0
0.8
0.6
0.4
0.2
0.0
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1 − Specificity
DeLong's test: p value = 0.028
AUC of tLPC/tPC: 0.951
Sensitivity: 0.914, Specificity: 0.882
AUC of lipid triplet: 0.962
Sensitivity: 0.899, Specificity: 0.918