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## Comprehensive Lipidomic Profiling by Plasma Separation Cards

Lauren M. Bishop,

Oliver Fiehn

University of California Davis, Department of Chemistry, Davis, California, USA

University of California Davis, West Coast Metabolomics Center, Davis, California, USA

### Abstract

Large-scale lipidomic analyses have been limited by the cost and accessibility of traditional venipuncture sampling. Microsampling techniques offer a less-invasive and more accessible alternative. From a single drop of blood, plasma separation cards (PSC) deliver two volumetric dried plasma samples which are studied here for profiling endogenous blood lipids. Six lots of EDTA-treated human whole blood were used to compare PSC, dried blood spot analyses (DBS), and classic wet plasma extractions. Six replicate extractions were performed for each lot. Nontargeted lipidomics was performed by liquid chromatography-high resolution tandem mass spectrometry. Lipids were annotated by accurate mass/retention time matching and MS/MS spectral library matching using peak intensities for quantitation. 498 compounds covering 24 lipid subclasses were annotated. Inter-lot repeatability was evaluated by the percent relative standard deviation (%RSD) for each lot, giving median %RSD values across the lots at 14.6% for PSC, 9.3% for DBS, and 8.6% for wet plasma. Strong correlations of lipid peak intensities between wet plasma and PSCs were observed, but less for DBS. Lipid recovery and stability were comparable between the PSC and DBS samples, with roughly 60% of annotated lipids stable at room temperature after 28 days. Overall, PSCs provide a better alternative for quantitative blood lipidomics analyses compared to dried blood spots. However, problems with lipid stability for samples handled and shipped at room temperature are currently unavoidable outside of a clinical setting. Data transferability and comparability to standard plasma is lipid and lipid class dependent.

### Keywords

lipidomics; microsampling; method validation; mass spectrometry

### Introduction

Lipidomics focuses on the high-throughput profiling and quantification of lipids. As essential molecules, lipids participate in many biological functions and the analysis of these endogenous lipids serves to expand our understanding of disease pathologies [1].

Corresponding author: Oliver Fiehn, Address: UC Davis Genome Center, 451 Health Sci Drive, Davis, CA 95616, ofiehn@ucdavis.edu.

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Nontargeted lipidomic assays are becoming more robust by liquid-chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS), with possible applications in clinical research such as Alzheimer's Disease and cardiovascular diseases [2,3,4].

For clinical applications, traditional venipuncture sampling and analysis of blood specimen (plasma or serum) are mostly used. However, classic clinical and epidemiological studies face multiple obstacles. Recruitments and retention of patients is time consuming and costly, especially for healthy controls that are less likely to present at a clinic. Secondly, populations that live in remote areas, have reason to mistrust institutions, or people in adverse socioeconomic circumstances are difficult to convince to undertake the efforts necessary to participate. Third, longitudinal studies and studies with homebound patients are increasingly challenging to perform in classic clinical settings.

For these reasons, subject participation should be empowered by at-home blood sampling. Here, microsampling techniques have provided a less invasive, more accessible, and cheaper alternative. Whole blood microsampling is most commonly used, involving minimally invasive techniques such as finger-prick or heel-stick. Dried blood spots (DBS) are the most common form of microsampling devices. While they have served as a mainstay in newborn screening for the last several decades, recent applications of dried blood spots have included therapeutic drug monitoring, targeted metabolite screenings, and lipidomic analyses [5,6,7]. Nontargeted lipidomics by DBS has been generally successful; however, significant challenges remain to meet the demands for accurate quantification [8]. The most notable disadvantage of DBS is hematocrit, or the ratio of red blood cells in whole blood, which can vary between subjects and impact the quantity of specific lipids. Although there have been efforts to measure and correct for hematocrit variations, there are still no standardized approaches to overcoming this issue [9]. Additionally, the non-volumetric aspect of DBS impedes straightforward translation to in-vivo concentrations, which is an important prerequisite for clinical application [10].

In response to these limitations, several volumetric microsampling devices have been introduced, though the complexity of whole blood remains a concern. Telimmune Plasma Separation Cards (PSC), formerly Noviplex, were introduced in 2012 [11] and produce two, 3.2 $\mu$ L-volume dried plasma spot samples that are highly reproducible and stable at ambient temperatures. Figure 1 illustrates the principle schema of such plasma separation cards. PSC devices have shown promising results in their application to toxicological studies and targeted metabolite quantitation [11,12]. Considering the independence from hematocrit and volumetric compatibility to traditional venipuncture plasma, application of the PSC is an exciting prospect for the advancement of human blood lipidomic analyses. The primary goal of our work is to validate PSCs as an acceptable alternative to venipuncture plasma for nontargeted lipidomic profiling. Following standard lipidomics workflows for sample preparation and data analysis [8,13], evaluations of inter-subject repeatability, intensity correlations, analyte recovery, and storage stability are made to assess the performance of the sampling methods. Additionally, we here compare the PSC devices to DBS samples to determine whether they offer an improved approach to quantitative lipidomics.

## Materials and Methods

### Materials

UltimateSPLASH ONE lipidomics standards and supplemental standards of oleic acid-D9, arachidonic acid-D11, cholesterol-D7, and C17-sphingosine were purchased from Avanti Polar Lipids. Additional supplement standards of decanoyl-L-carnitine-D3, dodecanoyl-L-carnitine-D3, and octadecanoyl-L-carnitine-D3 were purchased from Cambridge Isotope Laboratories, and palmitic acid-D3 was purchased from CDN Isotopes. All reagents used were of LC-MS grade.

Telimmune DUO plasma separation cards were obtained from Novilytic, now Telimmune. Whatman® 903 Protein saver cards purchased from Sigma-Aldrich were used for dried blood spot sampling. Human whole blood was obtained through BioIVT. Six individual matrix lots treated with K<sub>2</sub>EDTA and another lot treated with 3.8% sodium citrate were used for the initial analysis. An additional lot of K<sub>2</sub>EDTA-treated human whole blood was purchased from BioIVT to evaluate storage stability.

### Sample Preparation for Lipidomic Analyses

75 µL of human whole blood was applied by pipette to each Telimmune DUO Plasma Separation Card. After three minutes, the top layer of the plasma separation card was removed, exposing two collection discs each containing 3.2 µL of the plasma fraction. The collection discs were dried for approximately 15 minutes and placed into the original packaging with desiccant for later extraction. Similarly, 75 µL of K<sub>2</sub>EDTA-treated whole blood was added onto Whatman® 903 Protein saver cards for each individual dried blood spot. The cards were dried for approximately three hours and stored with desiccant in an air-tight bag. The remaining whole blood was left at room temperature for 45 minutes and centrifuged at 3400rpm for 15 minutes to isolate the wet plasma. No hemolysis was observed. All samples were stored overnight at -20°C before extraction.

To evaluate recovery, a spiked solution was prepared using the following internal standards: 1 mL of UltimateSPLASH ONE mix, 0.5 µL decanoyl-L-carnitine-D3, 1.0 µL dodecanoyl-L-carnitine-D3, 1.5 µL octadecanoyl-L-carnitine-D3, 75 µL palmitic acid-D3, 25 µL arachidonic acid-D11, and 50 µL each of oleic acid-D9, cholesterol-D7, and C17-sphingosine. All supplemental standards were pulled from a 1 mg/mL stock solution. The mix was then dried down and resuspended with 1mL of methanol. 2 µL of the standard solution was aliquoted directly onto the dried spots, dried for approximately 20 minutes and extracted immediately.

For sample extraction, one collection disc containing 3.2 µL of dried plasma was placed in a 1.5 mL polypropylene tube. Dried blood spot samples were collected using a 6mm punch to replicate the diameter of the plasma separation card disc. Additionally, 3.2 µL aliquots of wet plasma were used to maximize comparability between the sample types. Blank spots in replicates of six from both the PSC and DBS cards were used in addition to extraction method blanks to evaluate potential contaminants. Protocols for recovery solution preparation and sample extraction are included in the Supplemental Data.

### Sample Preparation for Storage Stability Evaluation

Briefly, an additional lot of K<sub>2</sub>EDTA-treated whole blood was used to prepare samples to evaluate short-term storage stability at room temperature and –20°C conditions. Following the sample preparation protocols previously stated, samples were prepared in replicates of six for time points of 1 day, 7 days, and 28 days for each storage condition. Plasma separation card and dried blood spot samples were stored in air-tight bags with desiccant while wet plasma samples were pre-aliquoted into 1.5 mL polypropylene tubes. Day zero and blank card samples were extracted on the same day of preparation to provide baseline data. Method blank and bioreclamation plasma samples were also extracted at each time point to provide quality control. All stability test samples were extracted and prepared for analysis as described above.

### LC-MS/MS Data Acquisition

For nontargeted lipidomics analysis, 5 µL of the resuspended non-polar phase was injected into a ThermoFisher Scientific Vanquish UHPLC+ liquid chromatography system coupled to a Q-Exactive HF orbital ion trap mass spectrometer. The LC system was equipped with a Waters Acquity UPLC CSH C18 column (100 mm × 2.1 mm; 1.7 µm) and Waters Acquity VanGuard CSH C18 precolumn (5 mm × 2.1 mm; 1.7 µm). The column compartment and mobile phase preheater were set at 65°C, and the mobile phase flow rate was 0.6 mL/min. As demonstrated previously [14], different mobile phases were employed for positive mode and negative mode analysis to improve lipid coverage. The positive mobile phase A was acetonitrile/water (60/40, v/v) with 0.1% formic acid and 10 mM ammonium formate as modifiers and mobile phase B consisted of isopropanol/acetonitrile (90:10, v/v) with 0.1% formic acid and 10 mM ammonium formate. Mobile phase A for negative mode analysis was prepared using acetonitrile/water (60/40, v/v) with 10 mM ammonium acetate while mobile phase B consisted of isopropanol/acetonitrile (90/10, v/v) with 10 mM ammonium acetate. Both modes shared the same gradient with B started at 15%, increasing to 30% between 0–2 min. B was brought from 30% to 48% between 2–2.5 min, 48% to 82% between 2.5–11 min, and 82% to 99% from 11–11.5 min. 99% B was maintained between 11.5–12 min and then brought back to 15% between 12–12.1 min and held there between 12.1–14.2 min for re-equilibration. The injection needle was washed for 10s before and after each injection with isopropanol.

Positive mode and negative mode electrospray ionization (ESI) used a spray voltage of 3.6 kV, capillary temperature of 300°C, sheath gas flow rate of 60 units nitrogen, and auxiliary gas flow rate of 25 units nitrogen. Data were collected from 0 to 13 min of the LC gradient in scan range 120–1700 m/z using data-dependent acquisition (DDA) with the top four ions from each MS1 scan being selected for MS/MS fragmentation. DDA MS/MS was acquired with a stepped normalized collision energy of 20%, 30%, and 40%. MS1 spectra were collected with a resolving power setting of 60,000, and MS/MS spectra were collected at a resolving power setting of 15,000. To increase the total number of MS/MS spectra, five consecutive runs were made using the R package “IE-Omics” [15] for both positive and negative electrospray conditions. All spectra were stored in centroid, “.raw” format.

## Data Analysis

The data were converted from “.raw” format into “.abf” format using the Analysis Base File converter. Deconvolution, peak picking, alignment, and compound identification were completed through open source software MS-DIAL v4.60 [16]. Compounds were annotated by matching retention times, accurate precursor masses, and MS/MS fragmentation patterns against the LipidBlast library [17]. The primary result data matrix was processed with MS-FLO software to identify ion adducts, duplicate peaks, and isotopic features [18]. Peak height was used as spectral intensity for all data analysis. Samples were normalized first by CUDA internal standard intensities followed by Systematic Error Removal by Random Forest (SERRF) [19] to correct for instrument signal drifts. Internal standards added during extraction were used to assess injection quality and demonstrated a relative standard deviation of less than 6% across all matrix-containing samples after normalization. For lipids that were detected in both acquisition modes, values with the lowest relative standard deviation in quality control samples were kept. The quantitative results of lipids were calculated using the peak heights and the known concentrations of the spiked internal standards and then normalized to sample volume.

## Results

### Lipidome Coverage

We identified 498 unique lipids with 237 of these knowns characterized by accurate mass, retention time, and MS/MS spectral matching. These annotations are detailed as 357 lipid species and 67 labeled internal standards confidently detected and characterized in ESI positive mode, compared to 306 known compounds and 54 labeled internal standards annotated in ESI negative mode. Roughly 80% of the known compounds were found in all three sample types. The remaining 20% of annotations were either unique to a specific sample type or were measured at contaminant levels in one of the microsampling devices. A complete list of the classifications can be found in Supplemental Table S1. To compare the analytical performance of each sample type, 315 compounds that were found in at least 95% of matrix-containing samples were utilized for data analysis. As shown in Figure 2, these lipids are represented by 17 subclasses of 5 main lipid classes [20]: 10% Fatty Acyls (FA), 16% Glycerolipids (GL), 50% Glycerophospholipids (GP), 21% Sphingolipids (SP), and 3% Sterol Lipids (ST).

### Data Comparability

The performance of the PSC and DBS sampling devices were evaluated qualitatively through inter-subject repeatability and quantitatively by average lipid peak intensities as compared to wet plasma. Median percent relative standard deviation (%RSD) was calculated for each of the 315 lipids using 6 replicates prepared from the same whole blood lot. For the EDTA-treated samples, the median %RSD of the six individual lots were used to visualize overall repeatability in the three sample types, as shown in Figure 3a. The median %RSD across all lipids for the wet plasma, PSC, and DBS samples is 8.6%, 14.6%, and 9.3% respectively. The PSC samples show elevated variability in contrast to the other sampling methods due to notably increased variation of three lipid subclasses: free fatty acids (FFA), diglycerides (DG), and ceramides (Cer). We therefore investigated whether this increase was

due to the citrate anticoagulant used in PSCs, or if the variation was being influenced by blood separation mechanisms during PSC usage. We did not find major differences between the standard wet plasma and PSC-treated samples that were prepared using BioIVT citrated whole blood, but in both cases, we found the same trend of increased lipid subclasses (Figure 3b) compared to EDTA plasma. This finding suggests that citrate anticoagulants induce this difference in lipid subclass abundance compared to EDTA-plasma rather than the mechanisms of separation within the PSC itself. Indeed, the same three lipid subclasses largely contributed to the increase in median %RSD observed between PSCs and wet plasma or DBS (Figure 3a). When comparing EDTA- and citrate-derived plasma samples, the median %RSD increased by an average of 7.8% for free fatty acids, diglycerides, and ceramide lipids.

Quantitative potential was evaluated through correlations between average lipid peak intensities, or peak heights, and were assessed by one-to-one comparisons of the microsampling devices to wet plasma. Figure 4a shows the correlations of lipid intensities between the wet plasma and PSC samples derived from EDTA-treated whole blood. With an  $R^2=0.9851$ , these two sample types show a very strong correlation across all lipid classes, although the PSC samples presented an overall median percent change of -34% in peak intensities compared to the wet plasma extracts of the same volume. A weaker linear correlation was observed at  $R^2=0.9115$  in the comparison of wet plasma and DBS intensities, as shown in Figure 4b. Interestingly, the change in median lipid intensities was more than twice as high at 118% (Supplemental Table S2). This observation was expected due to the additional red blood components present in whole blood (as used for DBS) compared to wet plasma, in addition to different volumes used between DBS and PSC methods. It is important to note that fatty acyls, glycerophospholipids and sphingolipids species were significantly increased in the DBS samples compared to PSC and standard plasma (Figure 4b), making direct comparisons of DBS data to clinical human cohort data difficult. In Figure 4c, the average intensities from the citrate-treated samples are compared. Similar to figure 4a, the citrate-treated PSC and wet plasma samples are strongly correlated across all lipid classes with a median percent change of -26%. This value is consistent across most lipid subclasses, with an exception of diglyceride species which exhibit average intensities 46% higher in the PSC samples than the citrate-treated plasma samples.

Additionally, the quantitative performance of each sampling method was evaluated through absolute quantitation by labeled internal standards. 28 endogenous lipids were selected and paired with a matching deuterated internal standard. Using the known concentrations of the standards and the peak heights obtained during analysis, concentrations in nmol/mL were calculated for all three sample types and compared between the microsampling devices and wet plasma (Supplemental Figure 1). The PSCs show strong correlation in absolute concentration for all 28 lipids without any additional corrections; however, the DBS samples require further volume normalization for direct comparison to wet plasma. The internal standard levels were not adjusted for recovery values in the PSC and DBS samples.

## Recovery

Recovery experiments were performed by extracting an internal standard solution directly from the dried card samples. Upon evaluation of 61 representative internal standards, peak intensities decreased by an average of 1.42 fold in PSC recovery samples and 1.34 fold in DBS recovery samples. The change in internal standard response for the PSC samples is illustrated in Figure 5a. While the 1.42-fold change is a representative average of the responses, the fold changes measured in the PSC recovery samples were calculated for each specific lipid subclass represented by the added internal standards (Figure 5b). When applying these fold change values as correction factors to the corresponding classes of the endogenous lipids, much of the previously observed deviation in the PSC samples with respect to wet plasma was absolved, with an improved median percent change of -6% and an  $R^2=0.993$  (Figure 5c).

## Stability

Short-term stability was evaluated for room temperature and -20°C storage conditions at 1 day, 7 days, and 28 days after initial sample preparation. In this experiment we annotated 554 unique lipid species. 344 lipids were present in at least 5 of 6 replicates at each time point at < 30%RSD on average for each sample type. These 344 lipids were used to evaluate stability. Stable compounds were defined as having < 30% change in average peak intensity from the baseline. Figure 6a shows the proportions of stable compounds across both microsampling devices at -20°C and room temperature as compared to the standard plasma at -20°C. Samples stored at -20°C proved most stable, with 95.9%, 92.2%, and 94.5% of the compounds stable after 28 days for wet plasma, PSC, and DBS, respectively. For samples kept at room temperature, only 58.7% of compounds were stable after 28 days in PSC samples, and 55.5% in DBS samples. Trends between the PSC and DBS samples were overall consistent at room temperature conditions with both sample types identifying 72.7% of compounds as stable after 7 days.

After a detailed investigation of the performance of each lipid class within the PSC and DBS samples at room temperature, we found that free fatty acids were the least stable lipid subclass for both sampling devices (Figure 6b). While 18 of 27 free fatty acids from DBS samples were stable after one day, only 5 of 27 remained stable after the 28-day time point. Similarly, only 14 of 27 free fatty acids were stable after one day at room temperature in the PSC samples, decreasing to 10 species determined as stable after 28 days of storage under room temperature. Each free fatty acid species labeled as unstable in PSCs showed an increase in intensity between day 0 and day 28, with 6 compounds exhibiting greater than a 3-fold change, including oleic acid (FA 18:1), linoleic acid (FA 18:2), and arachidonic acid (FA 20:4). This finding supports the notion that increases in free fatty acids are likely caused by lipase activities in blood samples, but not by oxidation through exposure to residual air during storage. From the glycerolipids, 11 diglyceride species were unstable at each time point in the PSC samples with all but one showing intensities between 2 and 3 fold higher than the baseline extracts. Consistent decrease in intensity was observed across all time points for nearly half of the annotated glycerophospholipids with, most notably, all 14 phosphatidylethanolamine (PE) species labeled as unstable following the day 1 time point

for both PSC and DBS. In contrast, roughly 90% of sphingolipids remained stable through 28 days at room temperature for both sample types.

## Discussion

Plasma sampling by venipuncture remains the gold standard for nontargeted lipidomic analyses. However, the detection and characterization of nearly 500 unique lipids by plasma separation cards indicates a promising future for accessible lipidomic profiling. While the overall data quality of the PSCs fared well against wet plasma, the use of citrate anticoagulant and its impact on specific lipid subclasses is a concern. Our observations of increased variability in the free fatty acids, diglycerides, and ceramide lipids is supported by previous findings that the higher pH and ionic strength of sodium citrate can influence the ability to reliably measure specific lipids [21]. Conversely, other work has shown that citrate improves inter-subject repeatability for glycerophospholipids and sphingolipids [22]. From a quantitative perspective, diglycerides were the only subclass significantly impacted by the citrate additive with substantially elevated responses throughout the PSC samples. The high ionic strength of citrate is known to promote lipid release from lipoproteins and is likely a factor in the ion enhancement of specific lipids, such as the diglycerides in our analysis [21]. Nevertheless, most lipids extracted from PSCs appeared unaffected by the citrate and shared comparable repeatability and intensity levels to the control wet plasma.

Overall, we established that recovery was the largest contributing factor in data comparability to traditional wet plasma. Similar to most microsampling devices, both PSC and DBS sampling methods must overcome challenges in analyte recovery in order to provide accurate data. Previous studies have acknowledged the limitations of extracting dried spot samples with traditional methods, especially in the addition of internal standards during extraction [10]. However, treating dried matrix spots directly with internal standards is a time-consuming and often tedious task. Nonetheless, our analysis of directly-applied internal standards and the implementation of subclass-specific correction factors was effective in amending deviation caused by lipid recovery. We recommend similar methods be employed to evaluate recovery in future applications of PSCs to ensure quantitative accuracy.

The quantitative compatibility and performance of PSCs proved superior to DBS with most lipid classes showing exceptional correlation to plasma peak intensities. While the comparison of wet plasma and DBS also yielded a positive correlation, the difference in matrix constitution prevented true assessment of the quantitative performance of DBS. Attempts of improving quantitative analysis by DBS have included normalization by hemoglobin, automated hematocrit analysis, and use of reference standards though further validation of these methods is needed before regular application [6,23,24,25]. With the larger goals of accessibility and clinical utility, our analysis shows the volumetric and hematocrit-independent PSC provides quantitative lipidomic results without the additional corrections that are required for DBS analyses.

While PSCs appear to be suitable for lipidomic analyses, regular implementation of microsampling devices continues to be limited by analyte stability. Our results revealed

deficient stability following storage at room temperature with less than 60% of annotations remaining stable through 28 days for both PSC and DBS samples. Other studies have also encountered difficulty retaining endogenous compounds when dried samples are left unattended at ambient temperatures, with cumulative degradation attributed to enzymatic reactions, chemical hydrolysis, and oxidation [26,27,28]. For example, degradation of glycerolipids and glycerophospholipids during sample handling has been associated with phospholipase activity promoting the hydrolysis of glycerol backbones, leading to elevated levels of lysoglycerophospholipids and free fatty acids [29]. Additionally, the enzyme-catalyzed hydrolysis of triglycerides could explain our observations of elevated diglycerides and free fatty acid species. Successful efforts to overcome residual enzyme activity in dried matrix samples have included heat-treatments and flash freezing, though these methods are not accessible outside of a laboratory setting [29,30]. New strategies for increasing stability of at-home sampling devices at room temperature have focused extensively on additives. Sample pretreatments of non-specific enzyme inhibitors and antioxidants such as phenylmethanesulfonyl fluoride (PMSF) and butylated hydroxytoluene (BHT) have proved effective in reducing the degradation of specific lipids [31,32]. Obstacles remain with incorporating additives to microsampling methodologies, but their usage could potentially improve the stability of PSCs in nonoptimal conditions. Moreover, in its current state we recommend storage at  $-20^{\circ}\text{C}$  for PSC samples kept for longer than 7 days.

## Conclusion

From our analysis, we can conclude that the plasma separation cards are an acceptable alternative to traditional venipuncture plasma for nontargeted lipidomic analyses. Nevertheless, complete data transferability and comparability with standard plasma is lipid and lipid class dependent. When compared to DBS, the overall data quality was consistent for both sampling devices, however, there are clear quantitative advantages to using PSCs, especially in clinical settings. The volumetric capabilities, independence from hematocrit variation, and commercial availability indicate a promising path forward for these plasma microsampling cards in lipidomic analyses. Further validation with clinical cohorts is recommended before regular clinical application.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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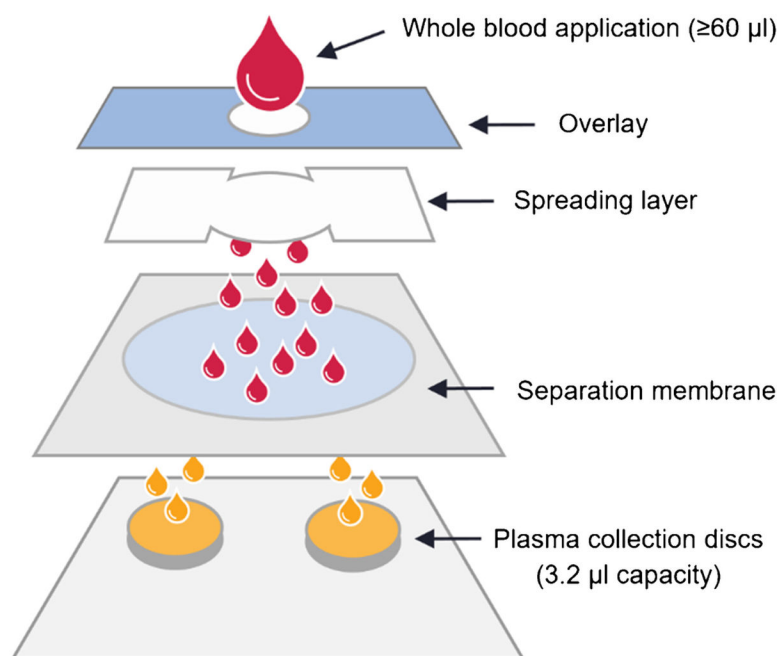
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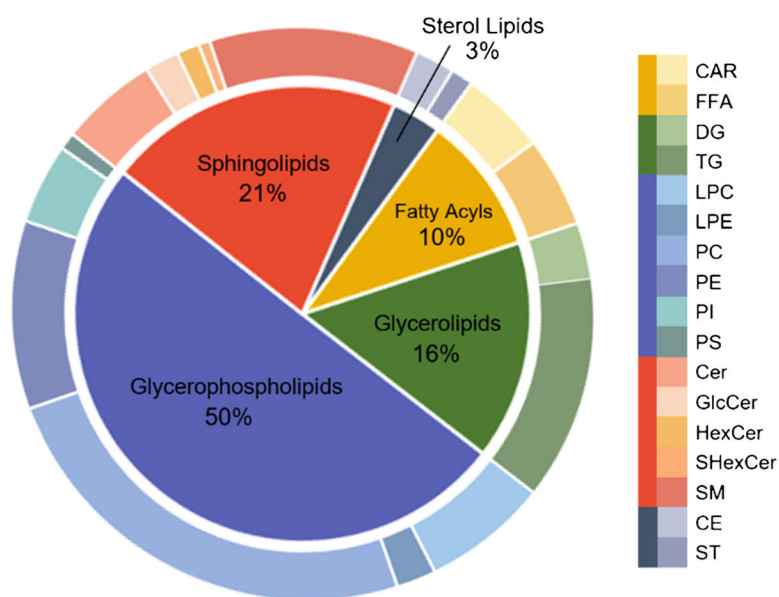
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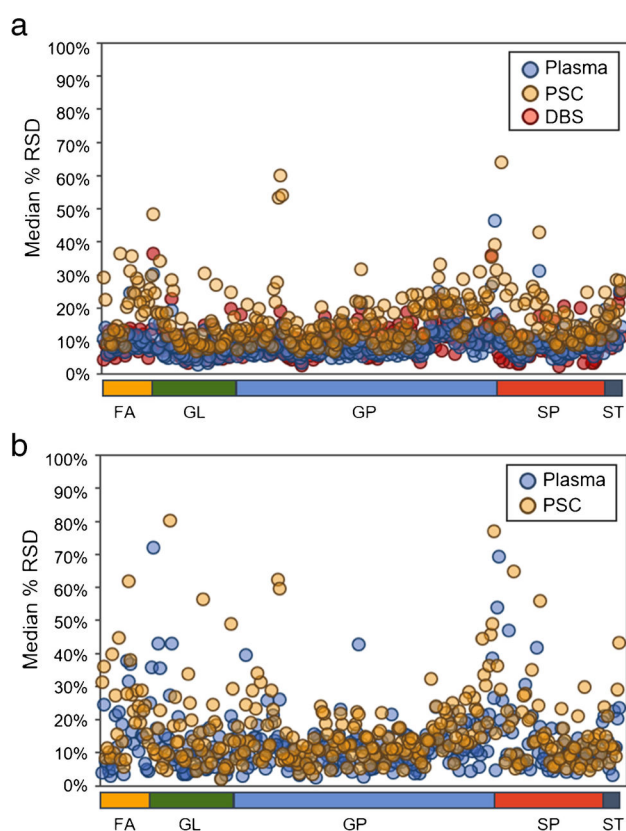
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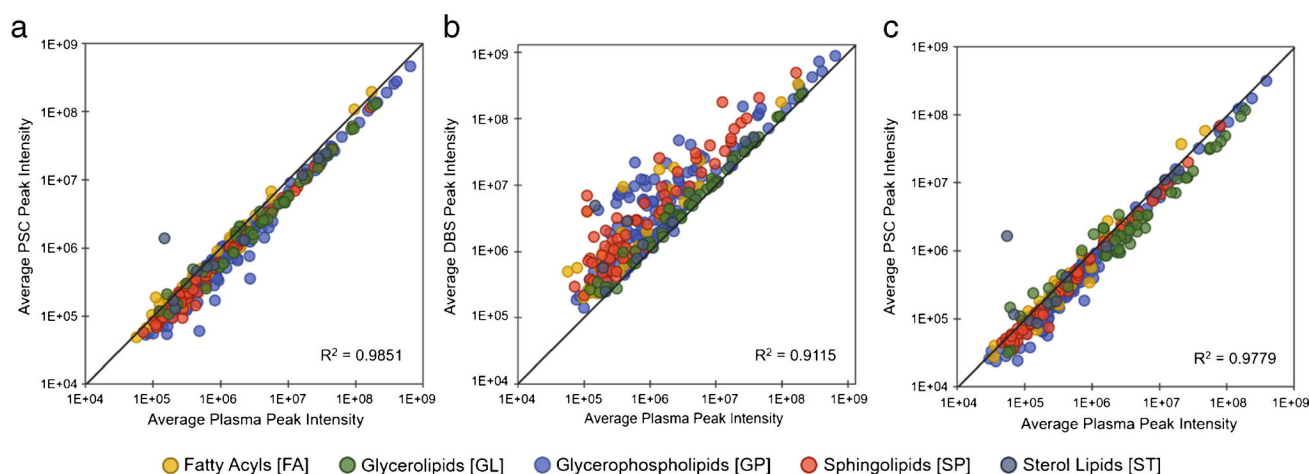
**Fig. 1.**  
Illustration of a commercial plasma separation card



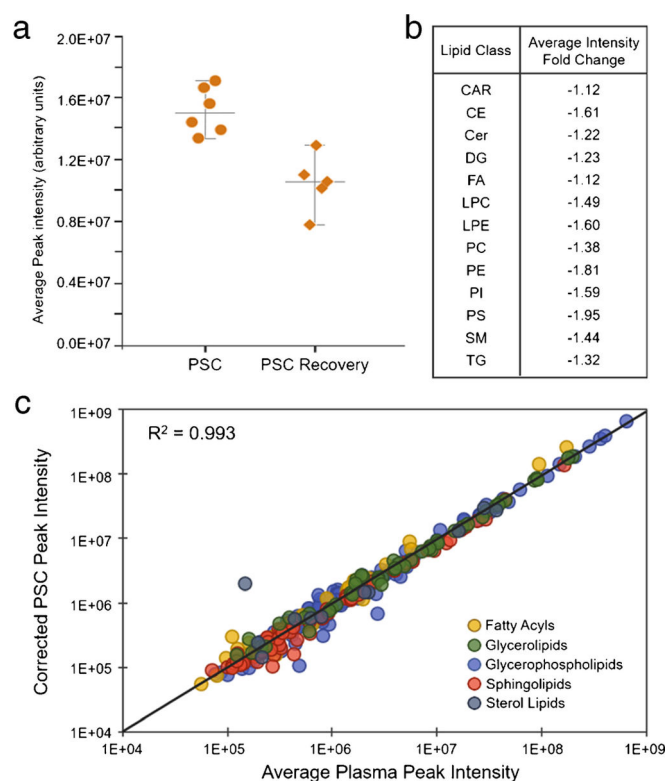
**Fig. 2.** Classification of lipids found in  $\geq 95\%$  of samples ( $n=315$ ). Abbreviations of lipid classes are given in the supplementary data.



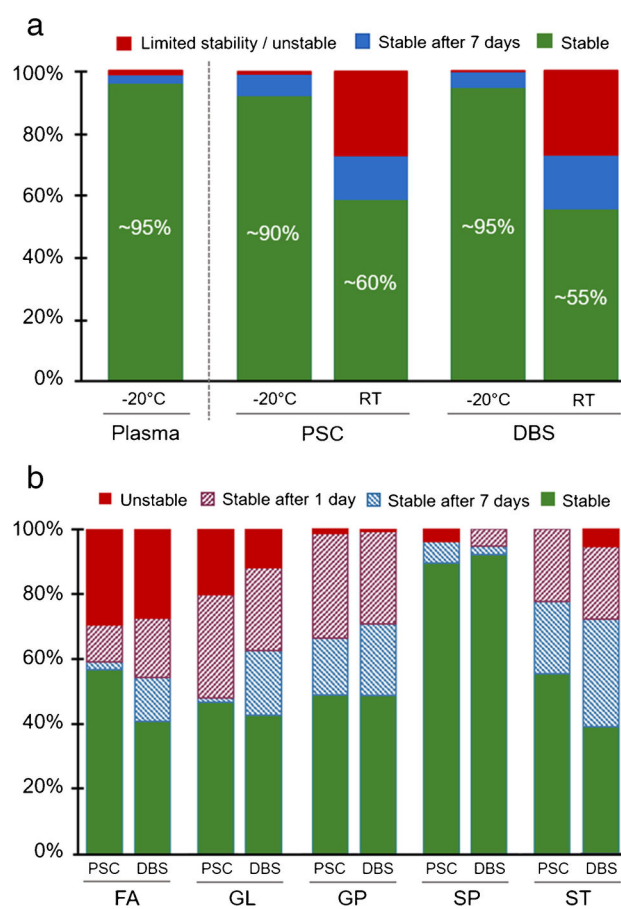
**Fig. 3.**  
**a** Median percent relative standard deviation (%RSD) values across all annotated lipids for EDTA-treated samples. Median %RSD for plasma, PSC, and DBS samples is 8.6%, 14.6%, and 9.3%, respectively. **b** Median %RSD of individual lipids for citrate-treated samples. Overall %RSD is 10.5% and 13.6% for plasma and PSC samples, respectively

**Fig. 4.**

**a** Correlation between average peak intensities in EDTA-treated PSC and EDTA plasma samples. **b** Correlation between average peak intensities in EDTA-treated DBS and EDTA plasma samples. **c** Correlation between average peak intensities in citrate-treated PSC and citrate plasma samples. The median percent changes of lipid intensities for the three sample types in comparison to plasma are -34%, +118%, and -26%, respectively

**Fig. 5.**

**a** Differences in peak intensities of regularly extracted lipid internal standards versus internal standards directly spotted onto the sample. **b** Intensity fold changes for each lipid class represented by the internal standards. **c** The fold changes applied as correction factors to the individual lipid intensities, improving the median percent change of PSCs to plasma to -6%

**Fig. 6.**

**a** Proportion of lipids at each temperature assigned to each stability category (n=344). Green indicates stability across days 1–28, blue indicates stability after 7 days of storage, and red indicates limited or no stability over the 28-day storage period. **b** Frequency of stability category per lipid class for PSC and DBS samples stored at room temperature