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Influence of separator gel in blood sampling tubes for perfluoroalkyl and polyfluoroalkyl substances analysis

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Abstract

Introduction: For several years, concerns about perfluoroalkyl and polyfluoroalkyl substances (PFAS) have been growing, and clinical laboratories may have to perform a growing number of PFAS analyses. The use of serum tubes without a separator gel is currently recommended for the quantification of PFAS due to the concern that the compounds may adsorb to the gel. The impact of gel adsorption on the accuracy of the results, however, has not been evaluated.

Methods: Aliquots from a pool of blood spiked with PFAS were stored in gel-free clot activator tubes (CATs) and gel-containing BD SST II Advance tubes (serum separator tubes) for 2, 8, and 24 hours. The CATs and serum separator tubes were collected from 15 volunteers under typical sampling conditions. Concentrations of 16 PFAS were analyzed using liquid chromatography–tandem mass spectrometry, and the percentage of change in PFAS levels between both tube types was computed.

Results: Results showed minimal changes (<5%) for most PFAS within 24 hours, except for long-chain perfluorosulfonates (including perfluorooctane sulfonate), which seemed to exhibit adsorption of the gel. In samples from volunteers, the observed changes were statistically significant for perfluorooctane sulfonate (P <.001).

Discussion: Based on our analysis, we recommend using CATs to avoid PFAS underestimation.

Key words: PFAS; blood collection tube; preanalytical; toxicology

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a large family of compounds characterized by the presence of at least one $-CF_3$ or $-CF_2$ moiety within their structure. Due to their remarkable properties (eg, chemical resistance; surfactant effectiveness; water-, grease- and stain-repelling properties), they have been employed in hundreds of applications. As a result, they can be found in many products, including waterproof textiles, food packaging, cosmetics, and firefighting foams.[1] Since the late 1990s, however, there has been growing concern about their environmental fate and impact on wildlife and human health, in particular for the perfluoroalkyl acids (PFAA) subfamily. Among PFAAs, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are the best studied, and some of their impacts on human health are well established. These chemicals have been linked to immune system suppression[2] and a decrease in birth weight,[3] and there is evidence that they may also contribute to increases in blood cholesterol levels[4] and rates of liver damage.[5] Finally, PFOA is classified as carcinogen in humans by the International Agency for Research on Cancer.[6] The number of epidemiologic studies on these chemicals is growing, and numerous environmental and health scandals involving these compounds break out every year in the press and media, increasing the general public's awareness and concerns.[7,8] Further, certain organizations have established serum level thresholds for PFAS associated with potential toxicity to humans. For instance, the German Human Biomonitoring Commission provides human biomonitoring (HBM) values. This commission asserts that serum levels of PFOA and PFOS above 10 µg/L and 20 µg/L, respectively, are linked to health risks in the general population (5 µg/L and 10 µg/L, respectively, in women of childbearing age).[9] As a result, clinical toxicology laboratories are having to manage and analyze more and more samples, both for epidemiologic studies and for a growing number of people worried about PFAS contamination.

To quantify xenobiotics in blood samples, blood collection tubes without gel are usually employed to prevent potential adsorption of the xenobiotic to the gel, which would result in a diminution of the xenobiotic level in the sample. Gel separator tubes are commonly used to perform clinical chemistry analysis; therefore, use of tubes without separator gel increases the number of tubes collected from a patient and the complexity of

Table 1. Mobile phase gradient for the liquid chromatography–tandem mass spectrometry quantification of PFAS

Time, min	Mobile phase A, %	Mobile phase B, %
0.00	95.0	5.0
0.50	95.0	5.0
3.00	50.0	50.0
12.00	25.0	75.0
12.10	10.0	90.0
13.50	10.0	90.0
13.60	95.0	5.0
15.00	95.0	5.0

Abbreviation: PFAS, perfluoroalkyl and polyfluoroalkyl substances.

the preanalytical workflow. In addition, many blood collectors forget to collect gel separator tubes because they are not used to doing so or do not have these tubes at their disposal. In these situations, analyses are not carried out or the results are given with reservations.[10-12]

As xenobiotics, PFAS are also suspected to adsorb to separator gel, and the use of tubes without gel is therefore recommended to measure these chemicals, with the drawbacks mentioned above. The aim of the present study was to assess the suitability of gel separator tubes for PFAS analysis by testing the influence of the gel in the blood collection tubes on PFAS quantification.

Methods

Blood collection tube preparation

A pool of blood (collected in citrate tubes) was spiked with PFAS to reach a concentration of 2 µg/L (without accounting for the initial contamination of the blood collected), gently mixed, and allowed to equilibrate overnight at 4 °C. Then, 18 gel-free BD Vacutainer 4-mL clot activator tubes (CATs) (reference No. 369032) and 18 4-mL gel-containing BD Vacutainer SST II Advance serum separator tubes (SSTs) (reference No. 367957) were each filled with 2 mL pooled blood to maximize the contact surface area with the gel and thus represent the worst-case scenario. To induce clotting, 50 µL calcium chloride (1 M) was then added to each tube. Tubes were gently mixed, centrifugated at 4600 g for 5 minutes, then stored at room temperature. During storage, the tubes were gently mixed several times (maximum, 4 times) to mimic tube manipulation during a preanalytical workflow. Serum was collected after 2, 8, and 24 hours from 6 CATs and 6 SSTs per storage time.

We also collected 1 BD Vacutainer 10-mL CAT (reference No. 367896) and 1 BD Vacutainer SST II Advance 8.5-mL SST (reference No. 367953) from 15 individuals recruited from among the volunteers of a study about prostate cancer (Hospital Faculty Ethics Committee of the University of Liege approbation No. 2023–305). Both tubes were centrifuged at 4600 g for 5 minutes within 2 hours after collection to avoid hemolysis. Serum from the CATs was collected immediately after centrifugation and stored at -20 °C until analysis. Samples collected in SSTs were stored at 4 to 6 °C for 72 hours before serum was collected.

PFAS quantification

Sixteen PFAS were measured in serum samples: perfluoropentanoic acid, perfluorohexanoic acid, perfluoroheptanoic acid, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid, perfluorododecanoic acid, perfluorobutane sulfonic acid, perfluoropentane sulfonic acid, linear perfluorohexane sulfonic acid (PFHxS), perfluoroheptane sulfonic acid, linear PFOS and total PFOS, perfluorononane sulfonic acid (PFNS), and perfluorodecane sulfonic acid (PFDS). In an Eppendorf tube, 100 µL serum was spiked with 20 µL internal standard solution (0.01 mg/L), then 300 µL methanol was added to the serum to precipitate proteins. The mixture was centrifuged at 30 000 g for 10 minutes, and 50 µL supernatant was collected and transferred to a liquid chromatography–mass spectrometry vial containing 50 µL water. The liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis took place on an Agilent 1290 Infinity II liquid chromatography apparatus coupled to an Agilent 6495 LC/TQ mass spectrometer. The chromatographic separation was performed with a Kinetex F5 Core-Shell (2.1 × 100 mm, 1.7 µm) from Phenomenex; the mobile phases were ammonium acetate 2 mM in water (pH = 7) (phase A) and methanol (phase B). Mobile phase gradient is reported in Table 1. The injection volume was 10 µL, and the column temperature was 45 °C. The mass spectrometer operated in negative electrospray. An 8-point calibration curve prepared from methanol solutions was injected on the LC-MS/MS. In addition, 1 procedural blank and 2 quality controls from the previous Arctic Monitoring and Assessment Programme were extracted and analyzed with the samples.

Results analysis

For each batch of tubes and each PFAS, the mean concentration and the coefficient of variation $(s/\bar{x}) \times 100$ (CV) were computed. We considered the CATs the reference, and the percentage of change for each storage time was calculated using the following equation:

$$\textit{Percentage of change} = -\left(\left(\frac{\textit{Mean conc. in Ref} - \textit{Mean conc. in SST II Advance}}{\textit{Mean conc. in Ref}} \right) \times 100 \right)$$

In samples collected from volunteers, only PFHxS, PFOA, PFNA, PFOS and PFDA were detected in most of the samples and included in the statistical tests. Percentage change was computed for each pair of tubes, and we performed paired Mann-Whitney tests or *t* tests (depending on the normality of the distribution) to compare PFAS levels in SSTs and CATs.

Results

Percentage change between PFAS concentrations measured in CATs and in SSTs after different storage times are summarized in Table 2. To define clinically significant differences, we compare the percentage change to the highest CV computed within batches for the compound of interest. Indeed, the highest CV is considered to be the analytical variability; percentage change above this CV are therefore attributable to the presence of the gel. Consequently, percentage change above this analytical variability could be considered clinically significant because in this case, the presence of gel decreases the PFAS levels. After 2 hours of storage at room temperature, no substantial difference was observed. For most of the PFAS analyzed, the absolute percentage change remained below the highest CV, even after 8 or 24 hours of storage, except for perfluorosulfonates with a carbon chain

length of at least 8 carbons. Long-chain perfluorosulfonates appeared to adsorb more readily to the separator gel than other compounds.

Discussion

The experimental conditions of our test were designed to represent worst-case scenarios: The tubes were centrifuged before storage so that the clot did not provide a barrier between serum and gel; the volume of blood in the tube was low (2 mL) so that the volume of sample/contact surface with gel ratio was low and could favor the adsorption of compounds on gel; and finally, the tubes were gently mixed several times during storage. Another limitation of our experimental conditions is the use of a pool of citrated blood. Indeed, slight hemolysis occurred in our pools due to blood type incompatibility. Because most of the PFAS do not penetrate into red blood cells, hemolysis interferes with PFAS measurement by lowering concentrations. Nevertheless, because the same pool of blood was used to fill a batch of SSTs and the corresponding batch of CATs, hemolysis was not expected to induce bias in our analysis. These experimental conditions may not fully reflect realistic situations; therefore, we also collected CATs and SSTs from volunteers, with the limitation that many PFAS were not measurable in most of these samples. In these real-life samples, percentage changes were much less important, probably mainly because of the barrier effect of the clot. In fact, even for PFOS, the percentage change after 72 hours of storage was less than 5%; thus, the highest CV was not considered clinically significant. Nevertheless, the differences between PFOS (linear and total) measured in the CATs and in the SSTs were statistically significant (based on Mann-Whitney test), a fact that could be important when conducting epidemiologic studies.

With regard to the results obtained, it was determined that under standard conditions and with rapid sample handling, the adsorption of compounds on the gel is minimal and not clinically significant. Consequently, under these conditions, serum samples collected in gel separator tubes can be accepted for PFAS measurement in patients for clinical monitoring. Nevertheless, it must be acknowledged that the evaluation of the preanalytical process is not always feasible, particularly when tubes are collected in sampling centers that are not part of the laboratory carrying out the analyses. In such instances, it is not possible to exclude the possibility that storage conditions and sample handling were not optimal. For instance, it is not unusual in biochemistry laboratories to store tubes with separator gel after centrifugation for several days. Consequently, a laboratory could send samples stored under these conditions to a subcontracting laboratory for PFAS measurement. As a result, the adsorption of the long-chain perfluorosulfonates on gel may have become clinically significant. Although PFNS and PFDS are seldom detected in human samples, PFOS is the PFAS measured at the highest concentration in the majority of human populations.[13] Moreover, as mentioned in the introduction, HBM values were provided by the German Human Biomonitoring Commission for this compound.[14] These HBM values are frequently used

Table 2. Percentage change between PFAS concentrations measured in CATs (without a gel separator) and in BD SST II Advance SSTs (with a gel separator) after different storage times

	Spiked samples				Real samples	
Compound	**2 h, %**	**8 h, %**	**24 h, %**	**CV max[a]**	**Real conditions, %**	***P* value[b]**
PFPeA	3.7	0.0	-1.7	3.7		
PFBS	0.1	-1.4	-0.3	3.1		
PFPeS	2.1	0.2	-0.5	4.0		
PFHxA	1.9	1.9	-2.8	4.6		
PFHpA	1.7	-1.0	-2.8	5.3		
PFHxS	3.5	1.3	1.7	4.5	-0.41	.52[c]
PFHpS	0.8	-2.9	0.0	5.3		
PFOA	1.7	2.8	-0.1	3.8	-0.43	.69[c]
PFNA	3.0	0.5	-2.2	6.0	-0.04	.62[c]
Linear PFOS	-2.4	**-11.4**	**-9.0**	6.9	-3.32	*<.001*[d]
Total PFOS[e]	-2.4	**-7.8**	**-9.5**	4.3	-1.86	*.030*[d]
PFDA	1.2	-1.2	0.9	5.1	-1.88	.43[d]
PFNS	-5.2	**-10.2**	**-17.6**	8.2		
PFUnDA	0.0	-0.9	-0.1	6.6		
PFDS	-2.9	**-16.8**	**-14.2**	8.1		
PFDoA	-4.9	-1.1	0.1	6.1		

Abbreviations: CAT, clot activator tube; CV, coefficient of variation ($s/\bar{x}$) × 100; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFBS, perfluorobutane sulfonic acid; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFDS, perflurodecane sulfonic acid; PFHpA, perfluoroheptane sulfonic acid; PFHpA, perfluoroheptanoic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFNS, perfluorononane sulfonic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFPeA, perfluoropentanoic acid; PFPeS, perfluoropentane sulfonic acid; PFUnDA, perfluoroundecanoic acid; SST, serum separator tube.
[a]Maximum CV for the concentrations measured within the same batch of samples. Differences above the maximum CV are highlighted in bold.
[b]$P < .05$ italicized.
[c]Mean percentage of change between paired tubes in volunteers. Significance of differences between tubes in volunteers were tested by *t* test.
[d]Mean percentage of change between paired tubes in volunteers. Significance of differences between tubes in volunteers were tested by Mann-Whitney test.
[e]Total PFOS is the sum of linearity and ramified PFOS.

for the clinical interpretation of the PFOS measurements, and the underestimation of PFOS levels due to adsorption on gel separators can erroneously categorize patients into group with reduced potential health effects, which can have an impact on patient care. Prudence is therefore warranted when accepting a sample collected within a gel separator tube.

In addition to clinical analysis, many laboratories are required to undertake PFAS measurements for the purpose of epidemiologic studies. The present work demonstrated that under normal conditions and even if the percentage change is low, the discrepancy in PFOS concentrations measured using CATs and SSTs is statistically significant. For instance, in multicenter studies, the use of different tubes in different centers has the potential to induce statistically significant differences between the centers. Consequently, the use of CATs is recommended to prevent the introduction of bias within a study or to allow unbiased comparison between studies.

In conclusion, in the one hand, SSTs could be used for the measurement of PFAS for clinical monitoring, provided that the preanalytical process is properly controlled. On the other hand, SSTs should be avoided in epidemiologic studies to prevent bias in statistical analysis.

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P. Dufour and C. Pirard designed the study. P. Dufour and C. Pirard performed experimental studies. P. Dufour analyzed the data, interpreted the results, and wrote the article. C. Charlier supervised the work. All authors read and approved the final manuscript.

Conflicts of interest

The authors have nothing to disclose.

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