Feasibility study for microplastics detection via human biomonitoring

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CONTENT

1 INTRODUCTION

Plastics are an integral part of modern life, yet their environmental persistence has increased concerns about their impact on human health. Among these concerns, microplastics (MPs), particles up to 5 mm in size, have gained significant attention due to their widespread presence and potential health implications [1-3]. Microplastics (MPs) are solid plastic particles smaller than 5 mm that consist of mixtures of polymers, functional additives, and residual impurities. Smaller particles ranging from 1 to 100 nanometers or 1 to 1000 nm are called nanoplastics, depending on the definition used. Currently, there is no consensus on the exact definition, so within the framework of this report, the definition of nanoplastics, ranging between 1 and 1000 nm, is used.

Micro- and nanoplastics (MNPs) are a novel class of environmental pollution. MNPs have arisen due to the enormous growth of plastics in the past 70 years and the waste that is spread in the environment. MNPs originate from different sources and processes. Primary MNPs are intentionally produced at the micro- or nanoscale for use in specific industrial or consumer products, such as exfoliating scrubs, toothpaste, industrial abrasives, and plastic powders used in paints or coatings. Additionally, particles released directly into the environment from tire wear and synthetic textile fibres, when they fall within the micro- or nanoscale size range, are also classified as primary MNPs. In contrast, secondary microplastics refer to small plastic fragments that originate from the degradation of larger plastic materials. Both types can be found in various parts of the environment, including water, soil, and air [3-5]. Due to their small size, these particles can enter the human body primarily through ingestion, inhalation, and dermal exposure [6-9]. Food sources, such as seafood, drinking water, and packaged food, have been identified as important pathways for MP exposure via ingestion [1, 5, 7, 10]. The most abundant commercial plastic polymers are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (PA), polyvinylchloride (PVC), and polyethylene terephthalate (PET) [11]. These polymer types are frequently found in food packaging [12]. Despite recognising these sources and polymer types, MPs are very diverse and complex, and the determination of environmental and health risks is challenging.

The reliable measurement of the quantity and types of MPs in human samples as a biomarker of internal exposure is essential to estimate human risks. The development of reliable measurement methods is a major challenge due to the diversity of shapes, sizes, composition, and origin of the particles. Several laboratories are working on developing promising measurement techniques. Such analytical methods can be of great value for analysing the effects through human biomonitoring (HBM), but for this, a good understanding of the most cost-efficient techniques and the factors that are important in the collection, storage, and processing of biological samples must be obtained. The knowledge of human exposure and health effects is still very limited. HBM is an ideal instrument to investigate human exposure levels and relate them to MP-related changes in biological mechanisms and health. Insights gained through HBM research can contribute to taking measures to protect public health. The challenge is to identify more validated methods for sampling and analysis so that MP research can be implemented. As

such, this study investigated the feasibility of implementing MP analyses in the Flemish HBM campaign for young people

2 OBJECTIVES

To investigate the feasibility of implementing MP analysis in the Flemish HBM campaign, we developed the following objectives:

(i) A **comprehensive analysis of the current literature** to identify methodological limitations, evaluate research quality, and gather information on the most frequently detected polymer types and their concentrations/particle number in human samples. While several recent reviews have already explored the presence of microplastics in the human body, they tend to focus on specific aspects. For example, Abbas Q. et al. (2024) [13], Mariano S. et al. (2021) [14], and Shao K. et al. (2025) [15] focused on various detection methods used to identify polymers in human samples. In contrast, Hermsen E. et al. (2018) [16] and Roslan N. et al. (2024) [17] assessed the overall quality of studies by evaluating the risk of bias across key domains such as study design, sampling, analysis, and reporting. By integrating both methodological and quality-focused perspectives, a more holistic overview of microplastic detection in humans can be achieved, highlighting what is known and where further improvements are needed.

(ii) A **pilot study** to assess the feasibility of **microplastics detection in human blood by two complementary methods** – a mass-based method (Pyrolysis GC/MS) and a microscopy-based method (Fluorescence Microscopy).

(iii) **Technical procedures, questionnaire information**, and **considerations for the implementation** of microplastics detection in Flemish human biomonitoring program.

3 LITERATURE OVERVIEW

3.1 LITERATURE SEARCH CRITERIA

A systematic search was conducted via PubMed for studies published between 2021 and April 2025 on MPs in human samples, finalized on 28 March 2025. Keywords included “microplastics”, “MPs”, “micro- and nanoplastics”, and “MNPs”, combined with terms like “amniotic fluid”, “blood”, “feces”, “lung”, “placenta”, “urine”, and other body sites. Only English, open-access papers were considered. Papers were included if: (1) full text was available; (2) information about collected human biological samples and their origins were provided; (3) the method of preparation, extraction, and analysis of the selected samples were described; and (4) methodological procedures for identification of MPs in the samples was described. Data extracted included sample type/size, predigestion method, detection technique, use of reference materials, recovery rates, detection of polymers (PA, PE, PET, PP, PS, PVC, other), polymer mass/particle count, QA/QC measures, and LOD/LOQ. Methodological quality was assessed via a risk of bias (RoB) tool across six domains: sampling, pre-processing, reference materials, recovery, QA/QC, and LOD/LOQ transparency. A three-level color code (green=low risk, yellow=intermediate risk, red=high risk) was used to rate each criterion based on the completeness and clarity of reporting. Studies with comprehensive documentation were rated low risk, while those with missing or poorly reported information were rated high risk. A scoring system was applied to rate the overall risk of bias in the study. For the sampling method, reference materials, recovery, and LOD/LOQ, a score of 2 was assigned if there was low risk, 1 for intermediate risk, and 0 for high risk. For pre-processing and QA/QC, a score of 1 was assigned if there was a low risk, 0.5 for intermediate risk, and 0 for high risk. The entire scoring criteria are presented in Table 1.

Table 1. Assumptions for the risk of bias analysis – Assumptions are defined for each risk level per category to evaluate the included articles.

	Low Risk	Intermediate Risk	High Risk
Sampling method	Clearly described sampling procedure with details on the type of sample and volume/mass collected Mention of precautions taken to minimize contamination during sampling (e.g., using appropriate containers and materials, no plastic containers or tools).	Sampling method is described, but some details are lacking (e.g., specific container material, volume/mass not precisely stated) Limited mention of contamination control during sampling, or only general statements provided.	Sampling method is poorly described or lacks crucial details. No mention of contamination control during sampling.
Pre-processing	Detailed description of all pre-processing steps (e.g., digestion, filtration, density separation) with specific reagents, concentrations, and durations.	Pre-processing steps are described, but some details are missing (e.g., exact pore size of filters, digestion conditions not fully specified).	Pre-processing steps are poorly described or lack essential information.
Reference materials	For vibrational Spectroscopy & Mass Spectrometry: Studies used characterized reference materials (e.g., polymer standards of known size and type) for instrument	For Vibrational Spectroscopy & Mass Spectrometry: Reference materials are mentioned, but details on their characterization or source are limited. Spectral library matching is	For Vibrational Spectroscopy & Mass Spectrometry: No reference materials are used for calibration or polymer identification, relying solely on unverified spectra or

	calibration and/or spectral library matching. Details on the source and characterization of reference materials are provided. For Optical Microscopy (Polymer Identification): If polymer identification is attempted (e.g., using staining), appropriate controls and reference materials are used. If only morphology and size are assessed, this criterion might be less directly applicable (N/A)	used, but the library is not well-defined. For Optical Microscopy: Limited or no use of reference materials for polymer identification when attempted.	assumptions. For Optical Microscopy: No attempt at polymer identification is made, or methods used are unreliable without proper referencing.
Recovery	Recovery experiments are conducted by spiking blank matrices with known amounts of characterized MPs (representative of those expected in the samples). Reported recovery rates	Recovery experiments are mentioned, but details on the spiked MPs or the procedure are lacking. Recovery rates are not reported	No recovery experiments are conducted to assess potential losses during pre-processing and analysis.
QA/QC	Comprehensive QA/QC measures are described in material methods, including: Analysis of field and laboratory blanks. Use of positive controls and blanks (e.g., analysis of known MP standards). Personnel training and laboratory environment control to minimize contamination. Clear reporting of QA/QC results and how they were used to ensure data quality	Some QA/QC measures are mentioned, but the description lacks detail or some important aspects are missing (e.g., only laboratory blanks are analyzed, but not field blanks). Limited reporting of QA/QC results.	Minimal or no QA/QC measures are described. No blanks or controls are mentioned.
LOD/LOQ	LOD and LOQ are clearly defined and reported for the specific analytical method and MP characteristics The methods used to determine LOD/LOQ are appropriate and well-described (e.g., based on signal-to-noise ratio, standard deviation of blanks). MP concentrations or sizes reported in the study are generally above the LOQ, or if below, are clearly identified as such and interpreted with caution	LOD and/or LOQ are reported, but the definition or the method of determination is not entirely clear. LOD/LOQ might not be specific to different MP types or size ranges. A significant portion of the reported MPs are close to or below the LOD/LOQ, and the implications for data reliability are not fully discussed.	LOD and LOQ are not reported. MPs are reported without any consideration of the detection limits of the method. Interpretation of results does not acknowledge the limitations imposed by the sensitivity of the analytical method.

SCORING	**Low risk**	**Medium risk**	**High risk**
Sampling method	**2**	**1**	**0**
Pre-processing	**1**	**0.5**	**0**
Reference materials	**2**	**1**	**0**
recovery	**2**	**1**	**0**
QA/QC	**1**	**0.5**	**0**
LOD/LOQ	**2**	**1**	**0**

QA/QC=Quality assurance/Quality control, LOD/LOQ=Limit of detection/Limit of quantification, MP=microplastics

3.2 LITERATURE ANALYSIS

A total of 80 articles were evaluated and included in this study after applying predefined screening criteria. Since several studies analyzed multiple sample types, the number of studies according to sample type exceeds the number of cited studies. For instance, Abbasi S et al. (2021) analyzed skin, hair, and saliva samples from 2,000 subjects. In total, 98 sample types were examined, leading to a total of 98 studies according to sample type. To provide an overview of the cited studies included in this analysis, **Figure S1** presents each cited study and its corresponding sample size, categorized by sample type (fluids, tissues, or both). A vertical line is drawn at n=50 as a reference to distinguish smaller sample sizes from larger ones, as studies exceeding this threshold are more likely to produce statistically robust and clinically significant results. The distribution of the sample sizes varied widely, ranging from studies with fewer than 50 samples to a large epidemiologic study with 2,000 samples. Among the 80 cited studies, only 13 surpassed the threshold, including eight focusing on tissues, four on fluids, and one analysing both. The majority, 67 studies, fell below this threshold. Building upon the sample distribution, **Figure 1** presents the annual trends in the number of studies according to sample type from 2021 to 2025, categorized by sample type. The data reveals an increasing trend in the number of studies over time, peaking in 2024. Moreover, in 2024, there was a sharp rise in the number of analysed studies for both sample types, with fluids reaching 17 studies and tissues reaching 19 studies.

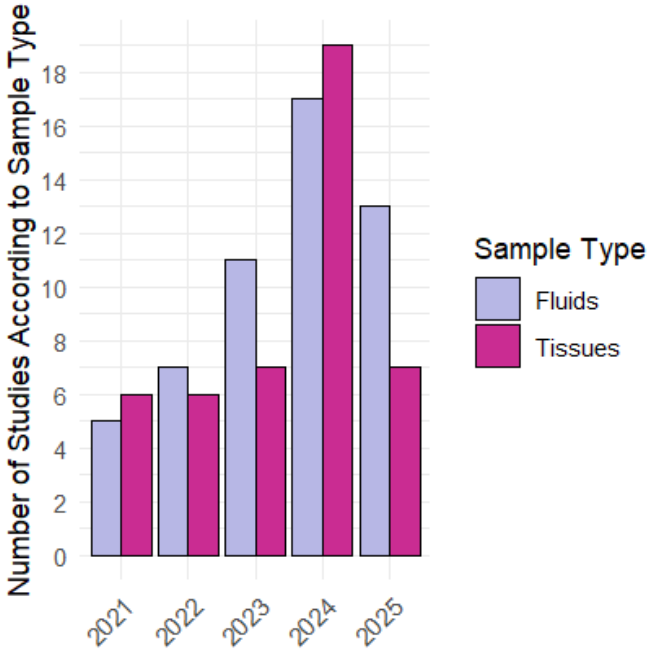
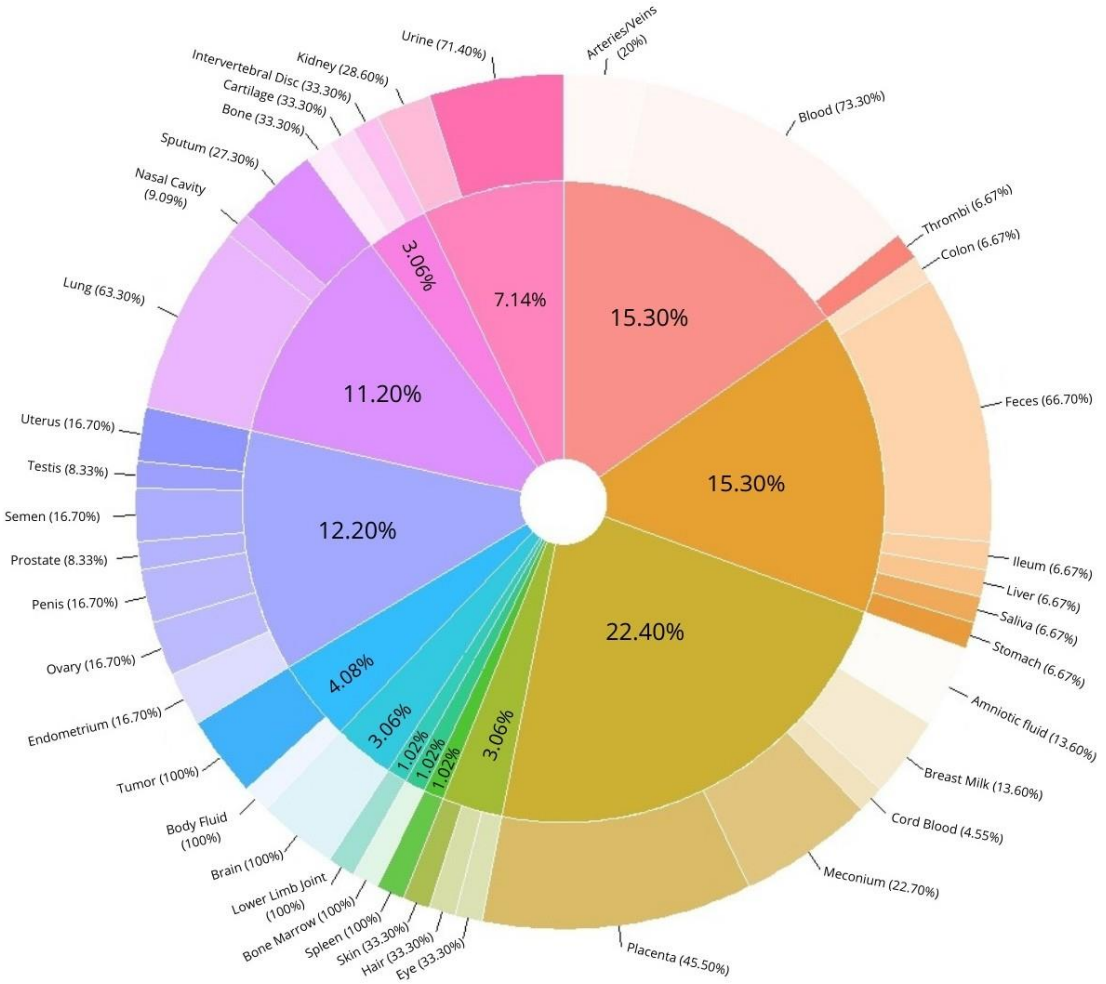


Figure 1: Distribution of studies by sample type and year – *Distribution of studies according to sample type (n=98) by publication year and primary sample type. Bars represent the number of studies utilizing fluids (lavender) or tissues (magenta) as the main sample matrix, published between 2021 and April 2025.*

3.2.1 Detection of microplastics in human body samples

The presence of MPs was investigated across all eleven human organ systems between 2021 and April 2025 (**Figure 2**), alongside "early life" and "other" categories. Within this comprehensive analysis, the most extensively studied systems, representing 76.40% of the total samples, were early life (22.40%), cardiovascular (15.30%), digestive (15.30%), reproductive (12.20%), and respiratory (11.20%) systems. Notably, the most frequently studied specific sample types were placenta, blood, faeces, and lung, representing key areas of investigation within these broader systems. While their polymer composition classifies microplastics, sample predigestion is necessary for their detection. The extraction methods employed for this crucial step exhibit significant variability, presenting distinct advantages and limitations. As illustrated in **Figure 3**, these methods fall into four main categories: chemical digestion (85.72%), enzymatic digestion (4.08%), and chemical and enzymatic digestion (4.08%). Notably, 6.12% of the analyzed studies did not provide details regarding the predigestion method when considered by sample type. Within chemical digestion, the specific methodologies commonly involved H_2O_2, HNO_3, and KOH, either used individually or in combinations. Conversely, a greater diversity of specific methodologies was observed for both enzymatic and combined digestion approaches.



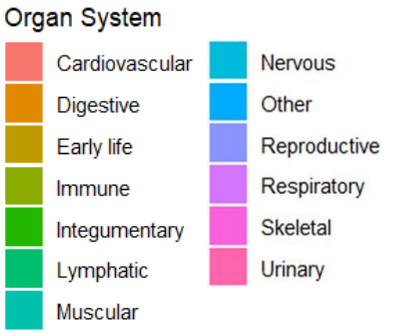


Figure 2: Distribution of studies by organ system and sample type *– Distribution of studies according to sample type (n = 98) categorized by organ system and specific sample type. The inner ring segments represent the percentage distribution of studies across the organ systems. The outer ring segments show the breakdown of specific sample types used within each organ system. Percentages in the outer ring indicate the proportion of studies within the organ system that utilized the specific sample type*

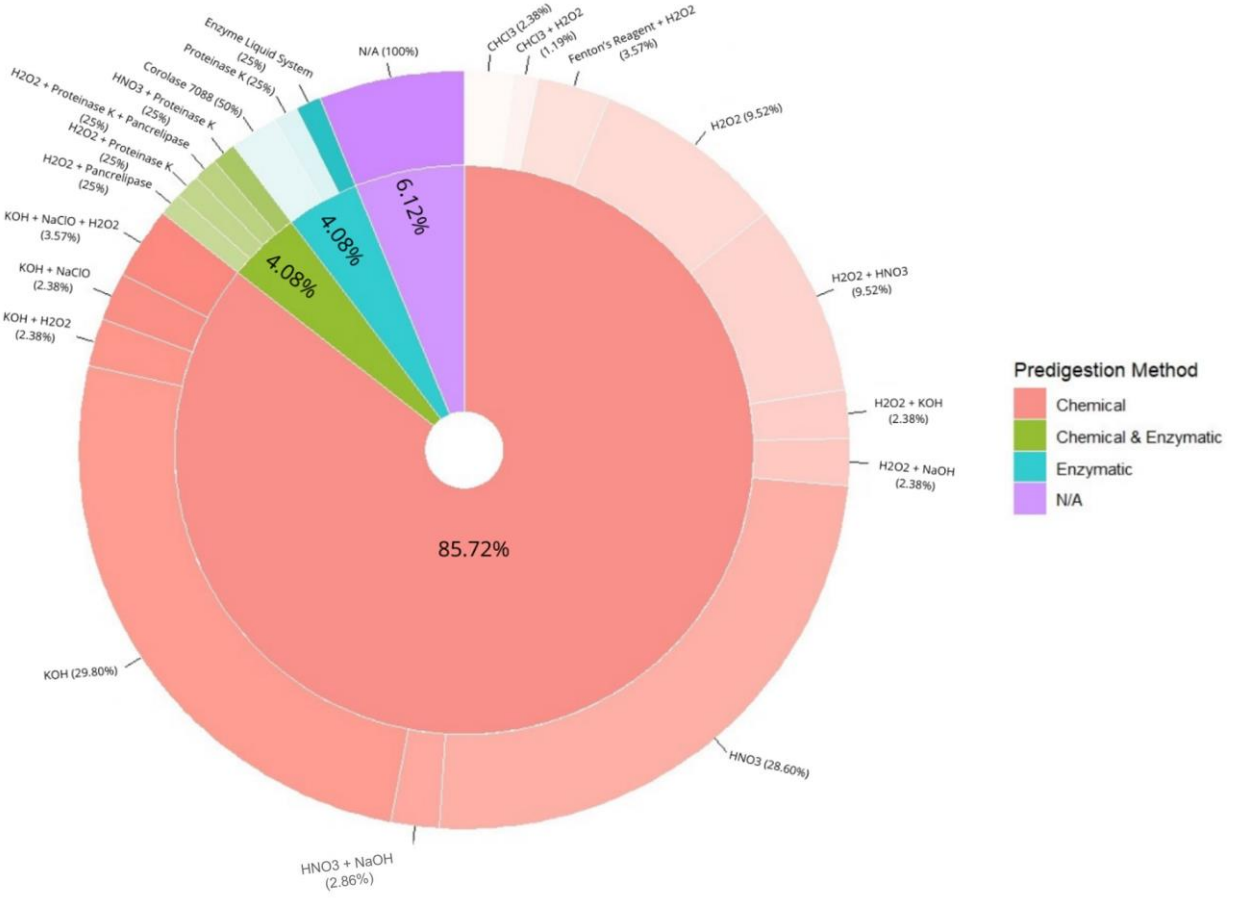


Figure 3: Distribution of studies by predigestion method *– Distribution of studies according to sample type (n=98) categorized by predigestion methods. The inner ring illustrates the overall percentage distribution of studies based on the type of method used. The outer ring segments specify the individual reagents, enzymes, or combinations used within each method type. The percentages shown in the outer ring represent the relative frequency of each specific protocol within its corresponding broader method.*

After predigestion, MP detection in the analyzed samples relied on three primary methods (**Figure 4A**). Vibrational spectroscopy was the dominant technique used for 60.60% of the samples, while optical microscopy and mass spectrometry accounted for 21.20% and 18.20%, respectively. Analyzing the evolution of these methods from 2021 to 2025 (**Figure 4B**) indicates a growing trend in applying both optical microscopy and vibrational spectroscopy. A particularly significant increase was observed for mass spectrometry between 2023 and 2024, with the number of studies according to sample type rising from 2 to 12. This growth continued in 2025, positioning mass spectrometry as more common than optical microscopy. Nevertheless, vibrational spectroscopy consistently remained the most frequently applied detection method throughout the studied years. The specific techniques employed within each detection method reveal nuanced trends over the study period (**Figure 4C**). Within Mass Spectrometry, Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS) stands out with a notable increase in its application, particularly between 2023 and 2024, where it saw a substantial rise in the number of studies. Notably, Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS) was only utilized in 2021. The optical microscopy category shows an increasing trend, with light microscopy (LM) consistently contributing to a significant number of studies. Scanning electron microscopy (SEM) experienced a marked increase in 2024, suggesting a growing reliance on this technique. Fluorescence microscopy (FM) had a more limited presence within this category, used only in 2021, 2022, and 2024. Vibrational spectroscopy consistently remained the most popular overall method. Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopy (RS) were the dominant techniques within this category throughout the entire period, both showing a general upward trend in their use. Laser direct infrared imaging (LDIR) had a consistent presence from 2022 onwards, while Near-infrared spectroscopy (NIR) was only present in 2024. The sustained popularity of FTIR and RS likely reflects their established effectiveness and versatility in identifying microplastic polymers.

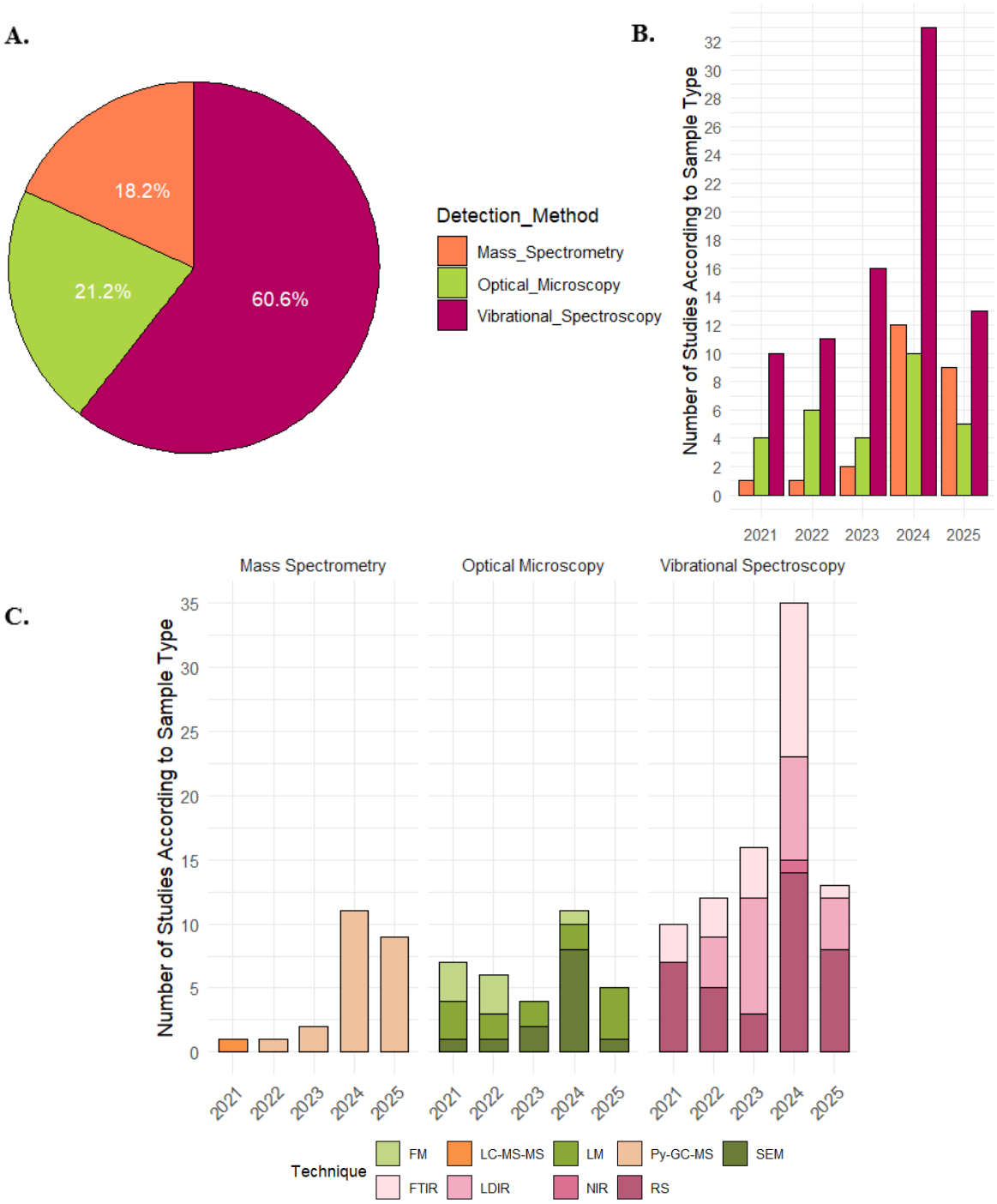


Figure 4: Overview of detection methods employed in included studies *– Analysis of detection methods used across the studies according to sample type (n=98).* ***(A)*** *Overall distribution of studies by primary detection method category: Vibrational Spectroscopy (60.6%), Optical Microscopy (21.2%), and Mass Spectrometry (18.2%).* ***(B)*** *Number of studies published per year (2021-2025) grouped by the primary detection method category.* ***(C)*** *Yearly distribution (2021-2025) of studies employing specific analytical techniques within each primary detection method category (Mass Spectrometry, Optical Microscopy, Vibrational Spectroscopy).*

Figure 5 illustrates the six most abundant polymer types identified in the analysed studies according to sample type. PP and PE were the most prevalent, found in 73 and 72 samples, respectively, followed by PS (64 samples), PVC (7 samples), PET (54 samples), and PA (44 samples). 75 samples revealed other, less common polymer types, which were not further evaluated in this study.

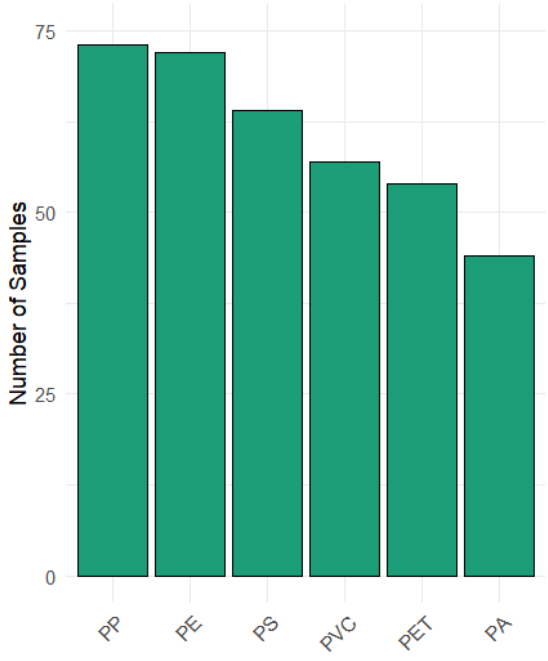


Figure 5: Frequency of reported polymer types in included studies *– Frequency distribution of polymer types reported across the studies according to sample type (n=98). Each bar represents the number of samples that identified the specified polymer type. The 'Other' category includes studies that report polymers that are not specified or are less common.*

Looking at detected concentrations of the six different polymer types, **Table 2** presents the mean concentrations (±SD) of the six most abundant polymer types found in different sample types that were expressed in particles and mass units. If the SD=0.00, this indicates that the results were coming from one single study. In the colon, PP showed a concentration of 28.10 particles/g (±0.00). Analysis of faeces revealed relatively higher particle concentrations of PS (3.64 ±3.74 particles/g) and PA (3.54 ±4.36 particles/g). When expressed per sample of faeces, PA had a mean concentration of 0.30 (±0.19) particles/sample. In terms of mass concentration in faeces, PE and PVC exhibited a notably high mean of 208.1 µg/g (±0.00) and 321 µg/g (±0.00), respectively, followed by PET at 113.0 µg/g (±0.00). The study analysing the ileum reported a particle concentration of 2.67 ±0.00 particles/g for PP. In the single study examining saliva, PET was found at a concentration of 35.0 (±0.00) particles/sample. Finally, the single study on the liver reported a PS concentration of 19.0 (±0.00) particles/sample.

In blood, relatively high particle concentrations per gram were observed for PE (30 ±0.00 particles/g). PE had a mean of 2.12 ±1.42 particles/mL when considering particles per milliliter of blood. Notably, PVC showed a high mean of 110 (±0.00) particles/sample in blood. In terms of mass concentration, PVC exhibited a mean of 34.08 ±1.97 µg/g, while PET had the highest mean mass concentration per milliliter at 4.89 ±5.10 µg/mL. In urine, PE showed a mean of 0.63 ±0 particles/mL and 2.0 ±0 particles/sample. The highest mass concentration in urine was again observed for PE at 1.96 ±0 µg/g.

Table 2. Concentrations of polymers found according to a selection of sample types *– Concentration distribution of polymer types reported across the studies according to a selection of sample types (n=31), represented as mean ± SD.*

		PE	PP	PET	PS	PVC	PA
Blood	Particles						
	/g	30.0 (0.00)	10.0 (0.00)	20.0 (0.00)	4.0 (0.00)	10.0 (0.00)	
	/mL	2.12 (1.42)	1.17 (0.23)	0.30 (0.00)	1.15 (0.56)	0.94 (0.00)	0.96 (0.93)
	/sample	0.24 (0.00)	0.13 (0.00)			110 (0.00)	33.03 (0.00)
	Mass						
	µg/g	12.4 (0.00)	0.64 (0.00)	12.4 (0.00)	4.71 (3.96)	34.1 (1.97)	3.61 (0.00)
	µg/mL	4.54 (2.14)	2.58 (2.34)	4.89 (5.10)	1.69 (2.20)	2.39 (1.91)	
Urine	Particles						
	/g		0.02 (0.00)	0.01 (0.00)			
	/mL	0.63 (0.00)	0.01 (0.00)	0.07 (0.00)	0.09 (0.00)	0.06 (0.00)	0.08 (0.00)
	/sample	2.00 (1.00)	1.50 (0.50)		1.00 (0.00)	1.00 (0.00)	
	Mass						
	µg/g	1.96 (0.00)				0.12 (0.00)	0.02 (0.00)
Saliva	Particles						
	/sample		23.0 (0.00)	35.0 (0.00)	3.0 (0.00)	1.0 (0.00)	
Colon	Particles						
	/g		28.1 (0.00)				
Faeces	Particles						
	/g	1.29 (0.35)	3.07 (1.77)	1.96 (0.87)	3.64 (3.74)	0.97 (0.80)	3.54 (4.36)
	/sample	0.04 (0.03)	0.06 (0.04)	0.15 (0.13)	0.01 (0.00)	0.08 (0.80)	0.30 (0.19)
	Mass						
	µg/g	208 (0.00)	6.66 (3.53)	113 (170.04)	6.47 (2.74)	321 (0.00)	
Ileum	Particles						
	/g	1.19 (0.00)	2.67 (0.00)	0.94 (0.00)	1.27 (0.00)	0.25 (0.00)	
Liver	Particles						
	/sample		1.00 (0.00)	8.00 (0.00)	19.0 (0.00)		

3.2.2 Quality of research

The risk of bias was assessed for the 80 included studies across several domains. Detailed results are presented in **Table 3**. Overall, eight studies were judged to have a low risk of bias across all assessed domains, represented by a score of ≥8. 33 studies had an overall score below five, suggesting a high risk of bias. However, 39 studies obtained an overall score between five and eight, suggesting an intermediate risk of bias. Particularly regarding recovery procedures (52 studies high risk) and the reporting or determination of LOD/LOQ (42 studies high risk) should be noted. Intermediate risk was prevalent in assessing reference materials (60 studies) and QA/QC procedures (48 studies). Regarding other key methodological steps, potential bias related to the sampling method was rated as intermediate in 38 studies. For pre-processing procedures, 31 studies were assessed as having an intermediate risk, while three were rated as high risk.

Table 3 Results of the risk of bias adopted to this study *– The RoB displays the evaluation scores for each of the six domains. A red rating signifies a high risk of bias, a green rating indicates a low risk of bias and a yellow rating indicates an intermediate risk of bias.*

	Sampling method	Pre-processing	Analysis - reference materials	Analysis - Recovery	QA /QC	LOD / LOQ	Overall
Abbasi S et al. 2021	1	1	0	0	1	1	**3.5**
Amato-Lourenco et al. 2021	2	1	1	0	1	1	**5.5**
Braun T et al. 2021	2	1	1	0	1	1	**5.5**
Ibrahim YS et al. 2021	2	1	1	0	1	1	**5.5**
Luqma A et al. 2021	1	1	1	0	0	2	**4.5**
Ragusa A et al. 2021	1	1	1	0	1	1	**4**
Wibowo AT et al. 2021	1	1	1	0	1	2	**5**
Zhang J et al. 2021	1	1	1	2	1	1	**5**
Zhang N et al. 2021	1	1	1	0	1	1	**4**
Amereh F et al. 2022	2	1	2	0	1	1	**7**
Horvatits T et al. 2022	2	1	1	0	1	1	**5**
Huang SM et al. 2022	1	1	1	1	1	1	**5**
Jenner LC et al. 2022	2	1	1	0	1	1	**6**
Jiang Y et al. 2022	1	1	1	0	1	1	**4**
Leslie HA et al. 2022	2	1	2	2	1	2	**10**
Liu S et al. 2022	2	1	1	1	1	1	**6**
Martinez BC et al. 2022	2	1	1	0	1	1	**5**
Ragusa A et al. 2022	2	1	2	0	1	2	**8**

	Sampling method	Pre-processing	Analysis - reference materials	Analysis - Recovery	QA /QC	LOD / LOQ	Overall
Yan Z et al. 2022	2	1	1	0	1	1	**6**
Guan Q et al. 2023	2	1	1	0	1	1	**6**
Halfar J et al. 2023	2	1	2	0	1	0	**6**
Ke D et al. 2023	1	1	1	2	1	2	**7.5**
Li Z et al. 2023	1	1	1	2	1	2	**7.5**
Liu S et al. 2023	1	1	1	2	1	1	**7**
Pironti C et al. 2023	2	1	1	0	1	0	**4.5**
Qiu L et al. 2023	1	1	2	2	1	1	**7.5**
Rotchell JM et al. 2023	1	1	1	0	1	1	**4.5**
Salvia R et al. 2023	1	1	1	0	1	1	**4.5**
Wang s et al. 2023	1	1	1	2	1	2	**7.5**
Wu D et al. 2023	1	1	1	0	1	0	**4**
Zhao Q et al. 2023	1	1	1	0	1	0	**3**
Zhu L et al. 2023	1	1	1	2	1	0	**5.5**
Amato-Lourenco LF et al. 2024	1	1	1	0	1	0	**4**
Brits M et al. 2024	2	1	2	2	1	2	**10**
Codrington J et al. 2024	2	1	2	0	1	0	**5.5**
Demirelli E et al. 2024	2	1	1	0	1	0	**5**
Deng C et al. 2024	2	1	2	2	1	0	**7.5**
Dong C et al. 2024	2	1	1	0	1	0	**5**
Garcia MA et al. 2024	1	1	2	1	1	1	**6.5**
Guo X et al. 2024	1	1	1	2	1	0	**5**
Hartmann C et al. 2024	2	1	2	0	1	2	**7.5**
Lee DW et al. 2024	1	1	1	1	1	0	**4.5**
Leonard SVL et al. 2024	1	1	1	2	1	2	**8**
Li N et al. 2024	2	1	1	0	1	0	**4.5**
Li Z et al. 2024	1	1	1	0	1	0	**3.5**
Liu S et al. 2024	1	1	1	1	1	0	**4**
Massardo S et al. 2024	1	1	1	1	1	0	**4**
Min HJ et al. 2024	1	0	1	0	1	0	**2.5**
Ozsoy s et al. 2024	2	1	1	0	1	0	**5**
Qin X et al. 2024	2	1	1	0	1	0	**5**
Rotchel JM et al. 2024	1	1	1	0	1	1	**5**
Santini S et al. 2024	1	1	1	2	1	0	**5.5**
Saraluck A et al. 2024	1	1	0	0	0	0	**1.5**

	Sampling method	Pre-processing	Analysis - reference materials	Analysis - Recovery	QA /QC	LOD / LOQ	Overall
Song X et al. 2024	2	1	1	0	1	0	**4.5**
Sun H et al 2024	1	1	0	0	1	0	**2**
Sun J et al. 2024	2	1	1	0	1	0	**4.5**
Wang T et al. 2024	2	1	1	2	1	1	**8**
Xie J et al. 2024	1	1	2	2	1	1	**7.5**
Xu H et al. 2024	2	1	1	0	1	0	**4.5**
Xu Ho et al. 2024	2	1	1	0	1	0	**4.5**
Xue J et al. 2024	2	1	1	0	1	0	**4.5**
Yu H et al. 2024	2	1	1	2	1	2	**9**
Zhao J et al. 2024	2	1	0	0	1	2	**6**
Zhu M et al. 2024	2	1	1	2	1	0	**7**
Gu X et al. 2025	2	1	2	0	1	0	**5**
He S et al. 2025	1	0	1	0	1	0	**2.5**
Jahedi F et al. 2025	1	1	1	0	1	0	**3**
Li J et al. 2025	1	1	2	2	1	2	**8.5**
Momeni MK et al. 2025	1	1	1	1	1	0	**4.5**
Montano L et al. 2025	1	1	1	1	1	0	**4**
Ni D et al. 2025	2	1	1	0	0	0	**3.5**
Nihart AJ et al. 2025	2	1	1	0	1	1	**5**
Rauert C et al. 2025	2	1	2	2	1	2	**10**
Tian J et al. 2025	2	1	1	0	1	1	**5**
Wang M et al. 2025	2	1	1	0	1	0	**4**
Wang S et al. 2025	1	0	0	0	0	0	**1**
Wu F et al. 2025	2	1	1	0	1	0	**5**
Yang Q et al. 2025	2	1	1	0	1	0	**4.5**
Zang S et al. 2025	2	1	0	0	1	0	**3**
Zhang K et al. 2025	2	1	1	2	1	0	**6**

RoB=Risk of Bias, QA/QC=Quality assurance/Quality control, LOD/LOQ=Limit of detection/Limit of quantification

3.2.3 Conclusion of literature review

The literature review on microplastics (MPs) detection in human matrices reveals a strong alignment with broader trends in the field. Vibrational spectroscopy emerges as the most widely used technique, followed by optical microscopy and mass spectrometry, reflecting patterns observed in recent reviews [13-15, 17, 18]. However, the analysis underscores significant methodological limitations, particularly regarding inconsistent reporting of recovery efficiency and limits of detection/quantification (LOD/LOQ), which undermine the reliability and comparability of exposure estimates.[16, 19-21].

Polyethylene (PE) and polypropylene (PP) are the most frequently detected polymers, consistent with their global production volumes and widespread use in food-related plastics. such as packaging and cutting boards [22, 23]. However, this must be interpreted cautiously. Mass-based detection methods may underrepresent or misidentify certain polymers like PE and PVC due to sensitivity issues and matrix interference, introducing detection bias [24-28]. This highlights the necessity of multimodal approaches—combining vibrational spectroscopy, optical microscopy, and mass spectrometry—to balance their respective strengths and limitations for more accurate and comprehensive detection. Vibrational spectroscopy provides molecular identification but can struggle with sensitivity for small particles. Optical microscopy offers high spatial resolution for more prominent MPs but lacks chemical identification capabilities. Mass spectrometry provides sensitive quantification but can be hindered by matrix interference [14]. Combining these techniques can leverage their complementary strengths for more comprehensive detection of polymer types in human samples.

The literature review also emphasises a critical need for standardised detection protocols, including quality assurance/quality control (QA/QC) measures and validated reference materials. Without such standardisation, exposure data remain difficult to interpret or compare across studies. Future research should aim to develop harmonised and complementary methodologies with robust validation and transparent reporting practices to advance the reliability of MP exposure assessments in human health studies.

To note: Several limitations of this literature review should be acknowledged. The literature review focused on studies published between 2021 and 2025, potentially excluding relevant earlier work.

4 PILOT STUDY FOR MICROPLASTICS DETECTION USING COMPLEMENTARY METHODS

4.1 STUDY DESIGN

Peripheral blood was collected from 25 women from the ENVIR*ON*AGE cohort during a regular checkup at the maternity ward. Blood samples were collected in 10 mL glass heparin tubes with anticoagulant using venipuncture blood drawing with a needle and blood tube holder, where the tube could be directly in contact with the glass tube. After blood withdrawal, the blood sample was flicked ten times and kept at room temperature until storage. The sample was first stored for 24h at 4°C, followed by freezing at -20°C, until further processing. Because the same sample had to be processed by two different labs (**Figure 6**), including duplicate and triplicate measurements, aliquots were prepared by the following procedure:

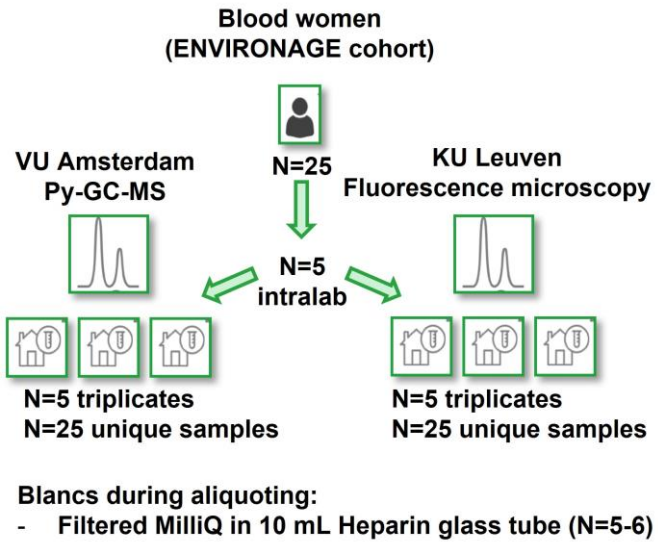


Figure 6 – Schematic overview of the study design of the pilot study for microplastics detection.

(1) Blood samples were thawed at 4°C overnight
(2) The lab assistant wore a 100% cotton lab coat, washed hands thoroughly with soap and disinfectant, followed by wearing nitrile lab gloves for aliquoting.
(3) A biosafety cabinet was cleaned with 70% ethanol, and only a 1000 µL pipette and its corresponding pipette tips were allowed in the cabinet, an aliquot of pre-filtered and sterilised MilliQ water in a glass container, and the glass tubes for aliquoting. Pre-cleaned glass sample vials for Pyr-GC/MS were kindly provided by the researchers of VU Amsterdam, and 4 mL glass blood tubes without anticoagulant were used to aliquot the samples for the Fluorescence Microscopy-based method of KU Leuven.
(4) Thawed blood samples were transferred to the biosafety cabinet
(5) Aliquots were prepared under a laminar flow cabinet by first homogenising the blood sample by flicking ten times, followed by transferring 1 mL of blood to the corresponding glass vial. For each 5-10 blood samples, a blank with MilliQ water was prepared in a 10 mL glass Heparin tube, and left open for the time needed to aliquot the blood samples, followed by transferring 1 mL to the corresponding aliquot vial.
(6) Aliquots were kept at 4°C for 24h, followed by freezing at -20°C
(7) Samples were kept frozen during transport on dry ice.

4.2 METHODOLOGY USED FOR MICROPLASTIC DETECTION IN HUMAN BLOOD USING PYR-GC-MS

An optimised Pyrolysis-Gas Chromatography-Mass Spectrometry (Pyr-GC-MS) method from VU Amsterdam was used for microplastic detection and quantification in human blood, building on the method described in Brits *et al.* 2024 [29] with key modifications:

4.2.1 Sample Preparation

1 mL blood samples underwent thawing, mixing, protein digestion with Proteinase K, and filtration through 0.7 µm filters. Filter residues were placed in pyrolysis cups. 25 ng poly(4-fluoro)styrene was added as an injection standard.

4.2.2 Pyr-GC-MS Analysis

Analysis was performed using a new Py-GC-MS system with a septum-free inlet and full scan acquisition mode (vs. SIM in Brits et al. 2024 [29]). Optimised indicator compounds were used for quantification: 1-eicosene (PE), 1,2-dihydronaphthalene (PVC), and diphenyl (PET) (**Table 4**). Full scan mode enabled broader detection and library-searchable spectra. A reduced carrier gas flow rate post-pyrolysis (1.4 mL/min) was employed for gas savings. Signal ratios of indicators to the injection standard corrected for instrument sensitivity variations.

4.2.3 Quality Assurance and Quality Control (QA/QC)

Each analytical batch included procedural blanks (n=4), unspiked matrix controls (n=2), and spiked matrix controls (n=2) from a homogenised donor blood pool to assess contamination, recovery, and matrix effects. Blank results informed LOD/LOQ. The LOD is 3 times the standard deviation of the blank values found (n=9), and the LOQ is 3.3 times the LOD.

Table 4. Pyrolysis Products for Polymer Identification and Quantitation

Polymer	Indicator compound	RT (min)	*m/z* (Q1)	*m/z* (Q2)	*m/z* (Q3)
PMMA	Methyl methacrylate	2.96	100	69	41
PP	2,4-Dimethyl-1-heptene	4.23	126	83	70
PS	Styrene trimer	14.26	91	117	194
PE	1-Eicosene	12.05	83	97	111
PVC	1,2-Dihydronaphthalene	7.10	130	129	115
PET	Diphenyl	8.82	154	153	76
IS	Poly(4-fluoro)styrene	4.48	122	121	96

4.3 METHODOLOGY USED FOR MICROPLASTIC DETECTION IN HUMAN BLOOD USING FLUORESCENCE MICROSCOPY

A fluorescence microscopy-based method, recently published for the detection of microplastics in bottled water [30], is under development by the Roeffaers lab at KULeuven to quantify and identify microplastic particles in anonymised blood samples. The method is based on chemical digestion, vacuum filtration on 1 µm pore size filters, and fluorescence microscopy using NileRed.

4.3.1 Digestion

The blood samples, initially weighed at approximately 500 mg each, were diluted with 2.5 mL of 0.22 µm filtered MilliQ water. This was followed by incubation of the samples under elevated temperature to deactivate enzymatic activity. Following this, a multi-step digestion process was performed, involving oxidative and alkaline treatments to break down organic components and enable further analysis. The protocol included sequential steps using common laboratory reagents, with temperature-controlled incubations over multiple days to ensure adequate sample transparency and filterability. Due to the proprietary nature of this protocol, specific reagents, concentrations, and incubation parameters are not disclosed at this time. Requests for more detailed information can be made to Roeffaers Lab at KU Leuven.

Remarks:
A subset of samples (n=6) was subjected to an alternative version of the digestion protocol, which incorporated a different oxidative reagent. This variation unintentionally introduced particles that could not be completely removed, leading to elevated background signals in those samples. These samples were flagged ("sampleID_F") accordingly and should be interpreted with caution.

4.3.2 Vacuum filtration

Vacuum filtration of the digested blood samples was conducted using silica filters (Smartmembranes) with a pore size of 1 µm for microplastic extraction. The vials and funnels were rinsed with 1.5 mL of MilliQ water to ensure all left-over sample material is also captured on the filters. Due to the pore size restriction, only particles larger than 1 µm were retained on the filters.

4.3.3 Fluorescence microscopy

Images were acquired via the same protocol as described in Aslam *et al.,* 2025 [30].
Staining: Nile red solution (10 µg/mL) in PBS and DMSO was used to stain the microplastics retained on the filter. Staining was performed for 5 minutes on the filtration set-up. The staining solution was washed away with 10 drops of 0.22 µm filtered MilliQ water.
Imaging: Stained filters were imaged using a Leica TCS SP8 FALCON confocal microscope. Nile Red-stained microplastics were excited with a 488 nm wavelength from the White Light Laser

(WLL). Emission was captured across five separate 40 nm-wide channels using the system's five available detectors (500 nm – 700 nm). The entire circular filter area (diameter = 8 mm) was imaged using the tilescan function.

4.3.4 Image analysis by MATLAB

Fluorescence images were analysed in MATLAB (PLASTECTAPP machine learning application: developed in Roeffaers' lab) to classify different microplastic types and distinguish them from non-plastic particles, using a model trained on silica and carbonate particles, described in Aslam *et al.,* 2025 [30]. Due to software limitations, analysis was based on 10 randomly selected tiles within the filter area (cfr. ISO 16094-2), which were consistently used across samples—unless a tile was visibly obstructed by biological residue, in which case an adjacent tile was selected. Particle counts were then extrapolated to represent the full filter area, and results were corrected to report the number of particles per gram of blood.

4.3.5 Quality assurance

Measures to minimize contamination during sample preparation were:

- Sample handling in a biosafety cabinet
- All glassware (vials, Pasteur pipettes, filtration funnels, storage vials, …) used was thoroughly cleaned and calcined at 450°C for a minimum of 8 hours
- Weight-based sampling was performed (Pasteur pipette)
- All reagents for digestion were kept in cleaned and calcined glass bottles without a plastic cap after syringe filtration (0.22 µm pore size)
- Filters were cleaned by sonicating them for 5 seconds in Hellmanex lll 2% and rinsing 3x with prefiltered MilliQ water.
- Vials were capped with aluminium foil, and parafilm was used for sealing during incubation steps

Each analytical batch included procedural blanks (n=3), using MilliQ to assess contamination. Blank results informed LOD/LOQ.

4.4 MICROPLASTICS DETECTION IN BLOOD SAMPLES USING COMPLEMENTARY METHODS

4.4.1 Microplastics detection in blood samples using the PYR-GC/MS method

Within this pilot study, the concentration of microplastic polymers was assessed in blood samples using a mass-based method (ng/mL), namely Pyrolysis GC-MS. This approach allows comparison of the relative contribution of different polymer types to the overall microplastic mass burden. The reported values have been corrected for the average blank value (n=9) that was calculated based on 4 procedural blanks (included during the analysis) and 5 blanks that were incorporated during aliquoting of the samples. The average of the blank values was subtracted from the value found in the sample, and the result was then compared to the Limit of Detection (LOD) and Limit of Quantification (LOQ) values to see if the result is <LOD, >LOD but <LOQ, or >LOQ.

Polymer	LOD (ng/mL)	LOQ (ng/mL)
PMMA	1	3.3
PP	15	49.5
PVC	90	297
PET	43	142
PE	273	901
PS	15	49.5

Based on the pilot study results, most of the detected microplastic concentrations were <LOD or between LOD and LOQ values (Figure 7).

PMMA - Polymethyl methacrylate (PMMA) was undetectable in nearly all samples, with the exception of one sample (S_2316), which showed a concentration between the LOD and LOQ (~1.8 ng/mL). However, its duplicate did not show a detect of PMMA. This result shows the low prevalence of PMMA.

PVC - Polyvinyl chloride (PVC) was frequently detected and quantified above LOQ in most samples. Concentrations ranged from just above LOD to nearly 350 ng/mL, with the majority of samples between 100 and 250 ng/mL. This confirms PVC as a consistently present microplastic in the bloodstream, but with very low concentrations.

PE - Polyethylene (PE) was detected at more modest mass concentrations ranging from just around LOD to approximately 600 ng/mL in a single sample (S_2335) and in only 5/22 samples.

PP - Polypropylene (PP) showed many non-detects and if detected variable detection across samples, with 1 sample (S_2335) exceeding the LOQ reaching nearly 60 ng/mL.

PET - Polyethylene terephthalate (PET) was detected 3/22 samples with concentrations generally around or slightly above 50 ng/mL. One sample (S_2316) showed a higher concentration approaching 130 ng/mL. The relatively low number of positive detections suggests more episodic

exposure or individual uptake, but in some cases, PET may contribute significantly to the total microplastic mass.

PS - Polystyrene (PS) was only clearly detected in a single sample (S_2306), with a concentration just under 40 ng/mL.

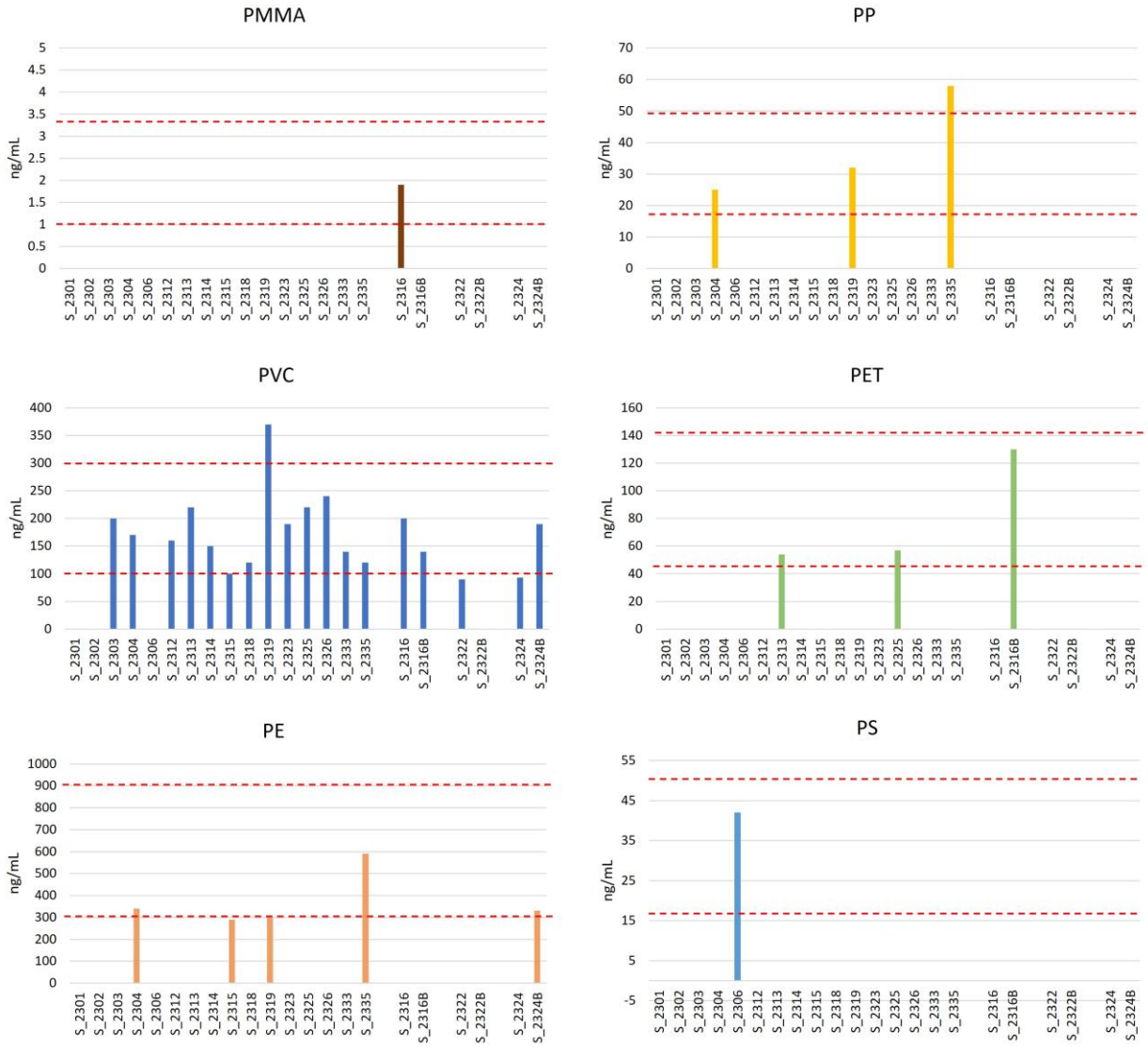


Figure 7 – Microplastic concentrations (ng/mL) in pilot study blood samples for each polymer type. *Red dotted lines represent LOD and LOQ values calculated per polymer type.*

The total microplastic mass (ng/mL), which is the sum of the microplastic concentrations detected in each blood sample, and the distribution of polymer types (%) in each blood sample are represented in **Figure 8**. The total microplastic mass was variable between blood samples, going from 0 to 768 ng/mL, with duplicate measurements having a considerable difference of 68.1 to 427 ng/mL total microplastic mass. The distribution of polymer types showed the highest prevalence of PVC across blood samples, followed by PE, PET, and PP. Duplicate measurements, however, showed different polymer distributions for the same blood sample. These results should be interpreted with caution, given that most measurements were below LOQ values.

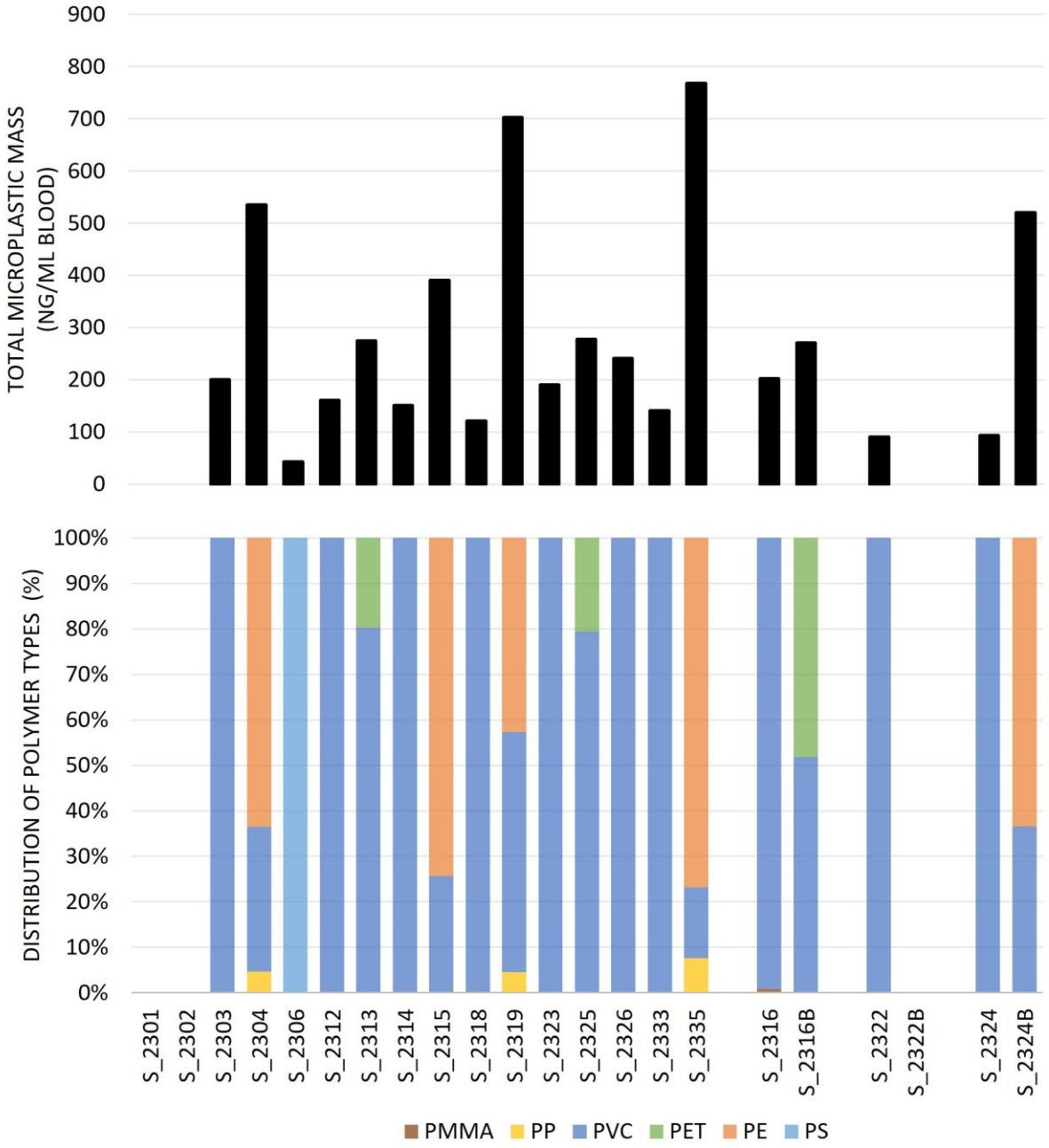


Figure 8 – Total microplastic mass (ng/mL blood) and distribution of polymer types (%) across blood samples using the Pyr-GC/MS method.

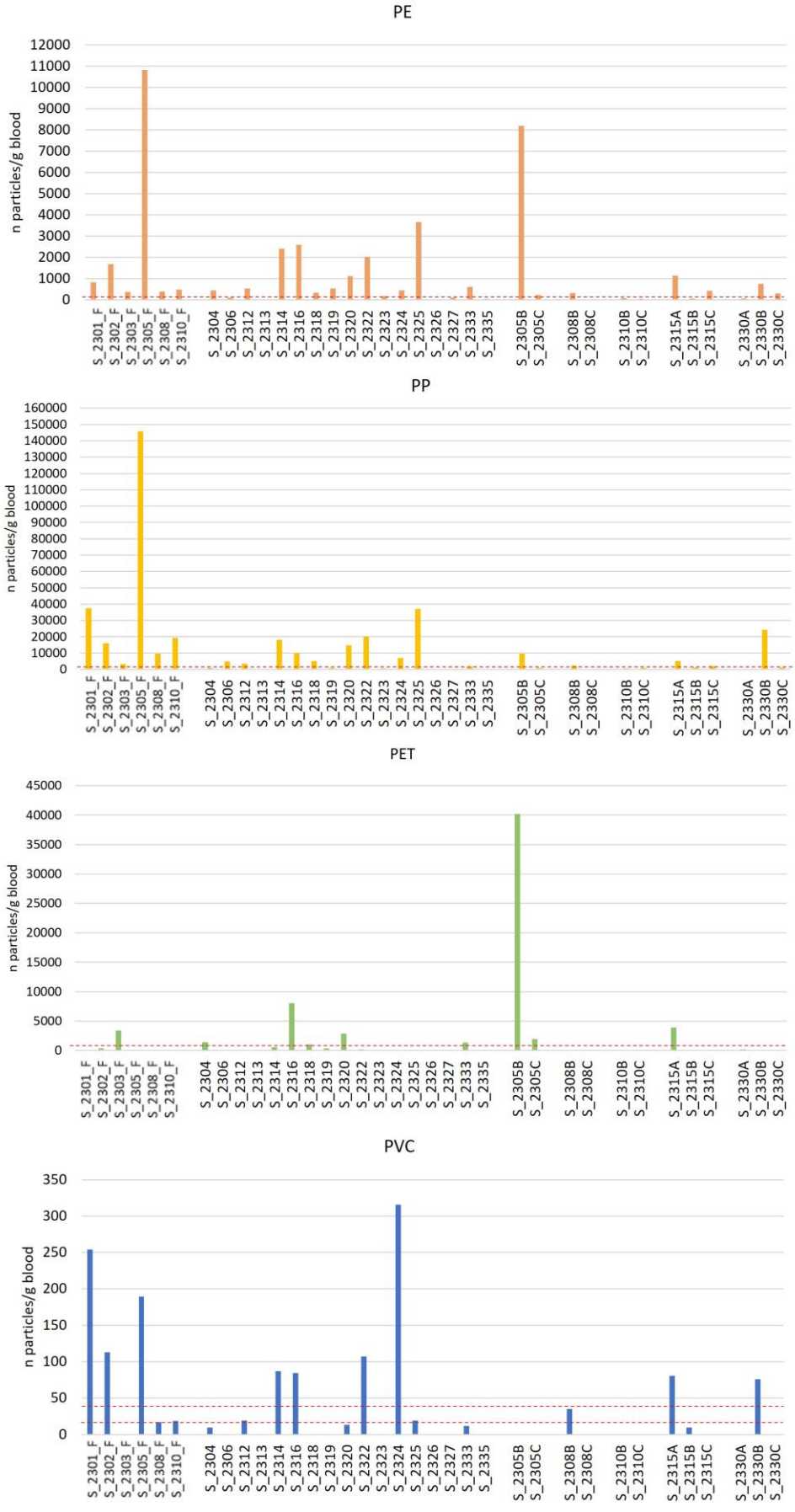
4.4.2 Microplastics detection in blood samples using the Fluorescence Microscopy-based method

In this pilot study, the presence of microplastic particles in human blood was analyzed across multiple polymer types, including PMMA, PVC, PE, PA, PP, PET, PS, and PTFE. Quantification was reported in units of particles per gram of blood, with biological replicates included for some individuals to assess reproducibility. The reported values have been corrected for the average blank value (n=6) that was calculated based on 3 procedural blanks (included during the analysis)

and 3 blanks that were incorporated during aliquoting of the samples. The average of the blank values was subtracted from the value found in the sample, and the result was then compared to the Limit of Detection (LOD) and Limit of Quantification (LOQ) values to see if the result is <LOD, >LOD but <LOQ, or >LOQ. The LOD was calculated by the mean + 3 times the standard deviation, and the LOQ by the mean + 10 times the standard deviation.

Polymer	**LOD (n particles/ g blood**	**LOQ (n particles/ g blood**
PE	56.2	159.3
PP	166.6	451.3
PET	92.9	276.9
PVC	12.1	36.9
PS	73.2	216.9
PTFE	76.1	217.3
PA	5.8	17.7
PMMA	NA	NA

PE - Polyethylene demonstrated a high prevalence and particle count among all analysed polymers. Several samples contained particle counts exceeding 1,000 particles/g, with some reaching levels above 3,500. The widespread detection of PE across the samples suggests its environmental ubiquity and relevance as a major contaminant.
PP - Polypropylene was also found in high numbers, with several samples demonstrating particle counts over 10,000 particles/g. The widespread detection of PP across the samples suggests its environmental ubiquity and relevance as a major contaminant.
PET - Polyethylene terephthalate was generally found at lower levels, with most samples below 5,000 particles/g. One sample (S_2305B) exhibited a pronounced spike (~40,000 particles/g), suggesting an isolated event of high exposure. However, its biological replicate did not show this peak. Overall, PET appears to be less prevalent but capable of reaching high numbers under certain conditions.
PVC - Polyvinyl chloride was detected in a moderate number of samples, with 9/35 samples having particle counts >LOQ, and 4/35 samples between LOD and LOQ. The presence of PVC in replicate samples varied between non-detected and >LOQ values, showing high variability.
PS - Polystyrene was detected at moderate levels across a wide number of samples. Particle counts ranged between 100 and 500 particles/g, with some variable detection across replicates.
PTFE - Polytetrafluoroethylene (PTFE) or teflon was present in nearly all samples, with particle counts typically between 100 and 500 particles/g.
The consistency of PS and PTFE detection and The consistency of PVC, PS and PTFE detection and the relatively narrow range of concentrations point toward steady-state exposure.
PA – Polyamide, commonly known as nylon, was detected at relatively low to moderate particle counts. While the majority of samples fell below the LOD, several showed notable elevations, including one sample (S_2304) with a count exceeding 400 particles/g.
PMMA - Polymethyl methacrylate was not detected in the analysed blood samples. Particle counts for this polymer remained at zero across all subjects, including replicates, suggesting either a true absence or particle counts below the analytical LOD.


PE
n particles/g blood
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
S_2301_F
S_2302_F
S_2303_F
S_2305_F
S_2308_F
S_2310_F
S_2304
S_2306
S_2312
S_2313
S_2314
S_2316
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S_2325
S_2326
S_2327
S_2333
S_2335
S_2305B
S_2305C
S_2308B
S_2308C
S_2310B
S_2310C
S_2315A
S_2315B
S_2315C
S_2330A
S_2330B
S_2330C
PP
n particles/g blood
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
PET
n particles/g blood
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
PVC
n particles/g blood
350
300
250
200
150
100
50
0

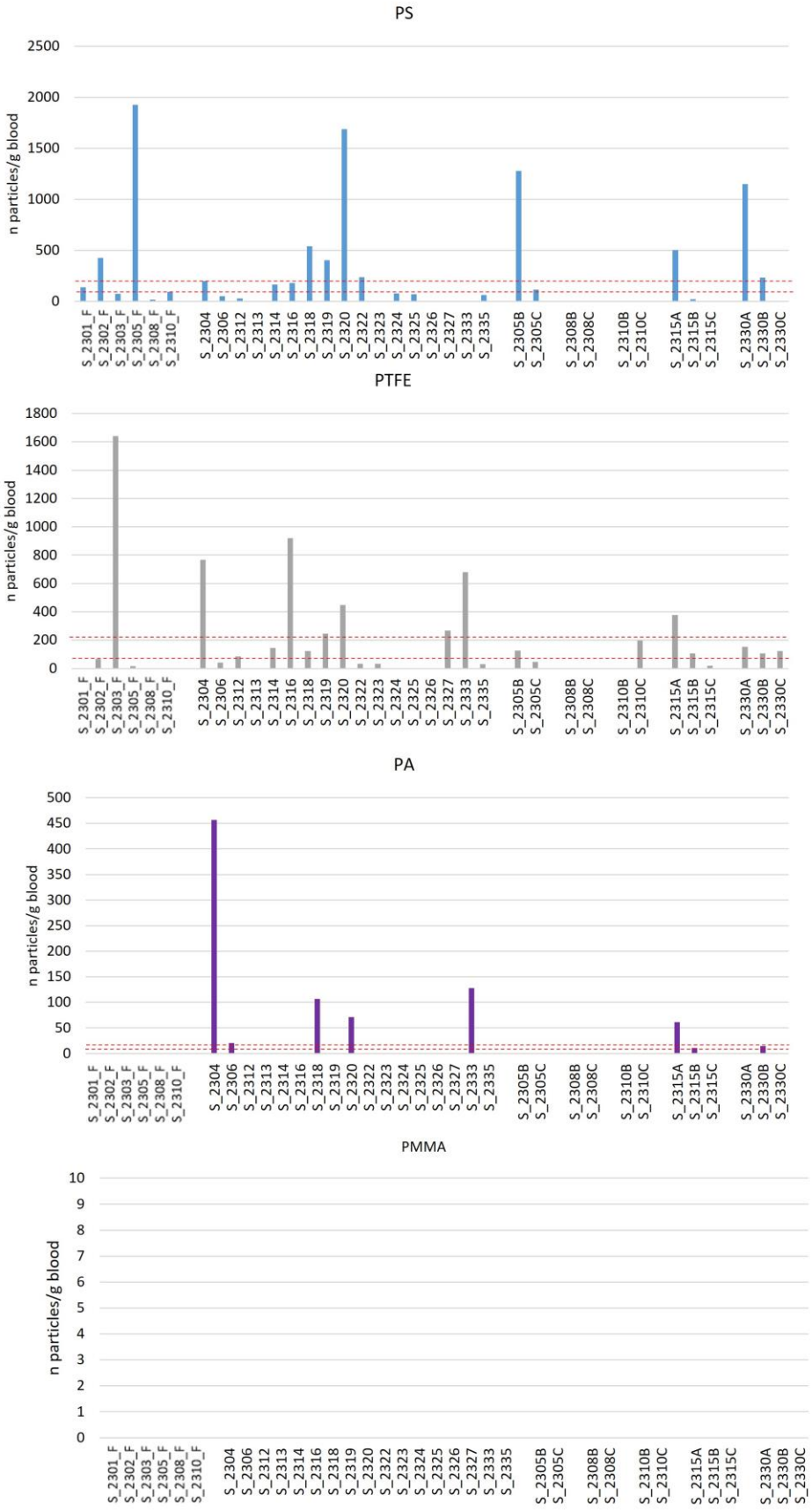


Figure 9 - Microplastic particle counts (n particles/g blood) in pilot study blood samples for each polymer type. *Red lines represent LOD and LOQ values calculated per polymer type.*

The total microplastic count (n particles/g blood), which is the sum of the microplastic particle counts detected in each blood sample, and the distribution of polymer types (%) in each blood sample are represented in **Figure 10**. The total microplastic count was variable between blood samples, going from 0 to 158,635.7 particles/g blood, with duplicate or triplicate measurements having a low to high difference of 78.9 to 56,199.3 particles/g blood. The contribution of polymer types showed the highest prevalence of PP, followed by PET, PTFE, PE, and PS. PVC and PA were also detected in 18/35 samples and 8/35 samples, respectively, but the number of particles detected was considerably lower than several other polymer types. For example, the average ± SD particles detected for PVC were 41.7 ± 75.4 particles/g blood, and for PA, 24.8 ± 81.2 particles/g blood, whereas PP had 11,563.6 ± 25,413.7 particles/g blood and PET 1,892.6 ± 6,858.2 particles/g blood. Duplicate and triplicate measurements also showed different polymer distributions for the same blood sample.

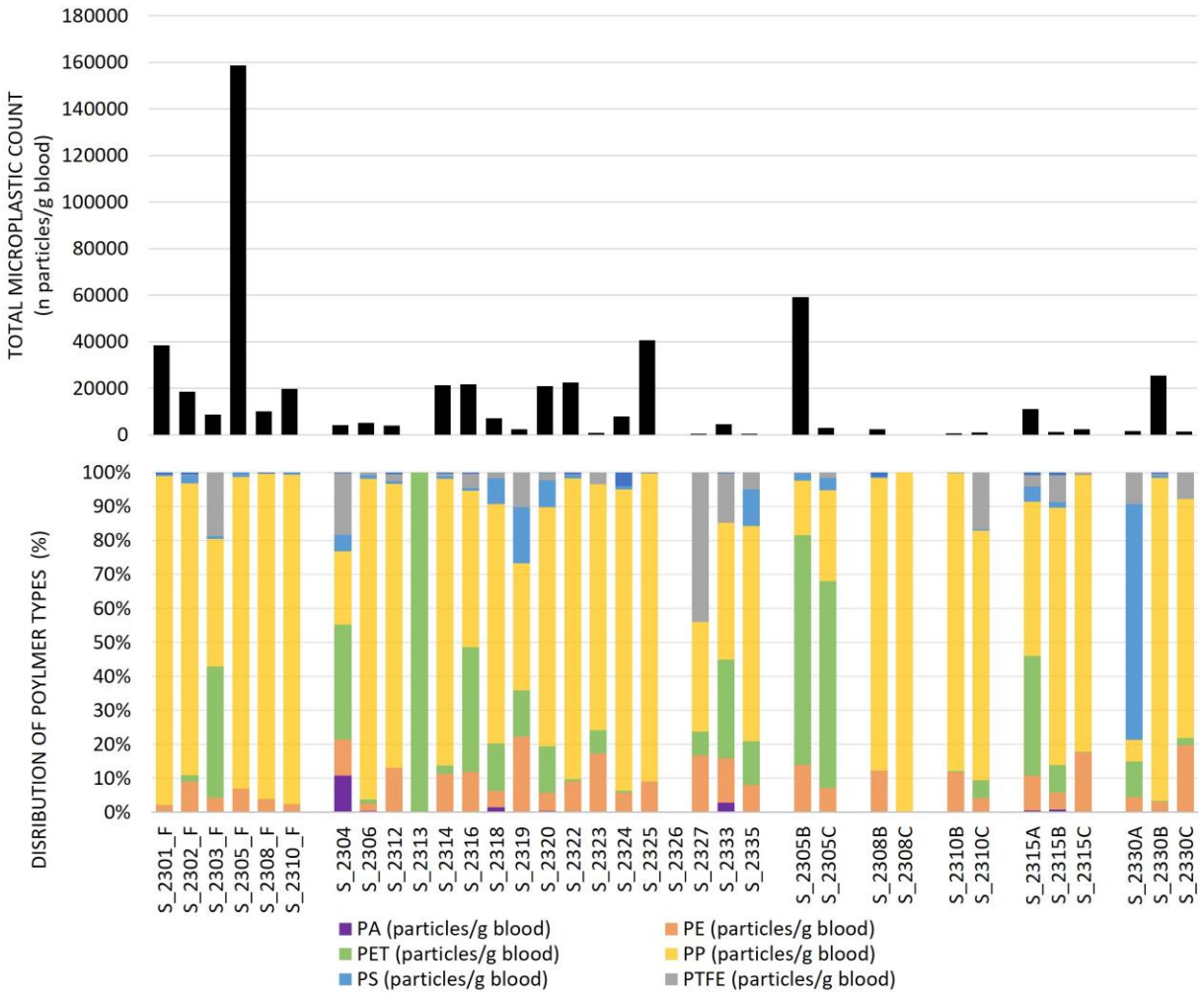


Figure 10 – Total microplastic count (n particles/gram blood) and distribution of polymer types (%) across blood samples using the fluorescence microscopy-based method.

4.4.3 Conclusion of pilot study

Mass-based quantification of microplastic polymers in human blood revealed a heterogeneous distribution across polymer types. **PVC** emerged as the most consistently detected polymer, with most samples exhibiting concentrations well above the LOD but below the LOQ, reaching up to ~350 ng/mL. **PE**, **PP** and **PET** showed intermediate detectability, with selected samples exceeding the LOD but less widespread presence across all individuals. **PS** and **PMMA** were either undetected or present only in isolated samples at concentrations near or just above the LOD. These findings suggest that PVC and PE are the primary contributors to the microplastic mass burden in blood. While the possibility of matrix interference affecting mass-based quantification cannot be entirely ruled out, the in-house method was specifically optimised to minimise such interference.

The particle-based analysis highlighted a distinct pattern, where **PE** and **PP** were the most abundant polymers by number of particles, often reaching thousands of particles per gram of blood. PET also showed considerable numbers of particles present in a selected number of samples. **PVC** and **PS** were moderately abundant, typically with tens to hundreds of particles per gram. **PA** appeared sporadically, with notable spikes in a few samples. **PMMA** was undetectable in all samples.

While **PE** and **PP** dominated the particle counts, **PVC** contributed most to the mass-based microplastic burden. This divergence should be further explored to determine this discrepancy. The high particle counts of **PE** and **PP** might suggest frequent environmental exposure or biological uptake, yet their relatively lower mass concentrations could imply smaller or less dense particles. In contrast, **PVC**, despite lower particle counts, consistently exhibited higher mass, which might indicate the presence of fewer but larger or denser particles. However, no information was available for certain particle characteristics, such as particle size, limiting interpretation capabilities. Nevertheless, these findings emphasise the necessity of integrating complementary detection strategies to fully characterise microplastic exposure and accumulation in human blood. Additionally, the variability observed between duplicate and triplicate measurements warrants further investigation to assess analytical reproducibility, although it may be partially attributed to different factors, including differences in exposure distribution, uptake and transfer rates, particle characteristics, and analytical sensitivity.

5 IMPLEMENTATION OF MICROPLASTICS DETECTION IN HUMAN BIOMONITORING

The Flemish Environment and Health Study (FLEHS) is a human biomonitoring program that measures exposure to environmental chemicals and the possible associated health effects in the general population of Flanders. Human samples should be easily obtained from healthy participants without surgery. Therefore, to discuss the technical procedures, 47 articles were selected from the literature analysis concerning microplastic measurements in matrices that can be readily obtained.

5.1 SELECTION OF THE MATRIX

From the 80 articles in the literature overview, 47 articles could be identified measuring MPs in readily available human matrices (**Figure 11**). The distinction between internal and external exposure was based on whether the **microplastic (MP) crossed a biological barrier** (e.g., lung, intestinal, or skin barrier).

- Matrices available in all members of the **general population** include blood (10 articles), urine (3 articles) and sputum (3 articles), which reflect internal dose, and hair (1 article), saliva (1 article), nasal cavity (1 article), feces (10 articles) and skin (1 article), reflecting external dose.
- Matrices available in **women or pregnant women** only include placenta (10 articles), meconium (5 articles), amniotic fluid (3 articles), breast milk (3 articles), cord blood (1 article) and endometrium (1 article).
- Matrices available in **men** only include semen (1 article).

Selection of the matrix depends on the target population and on the interest in external or internal exposure to microplastics. Human biomonitoring studies are usually designed to assess internal exposure to environmental chemicals, very often in combination with assessing associations with biomarkers of health effects. From the abovementioned matrices, blood, cord blood, urine, sputum, placenta, meconium, amniotic fluid, breast milk, endometrium and semen are considered to reflect internal dose of MPs, while hair, saliva, nasal cavity, faeces and skin are considered to reflect mostly external MP dose. A motivation is given in **Table 5**.

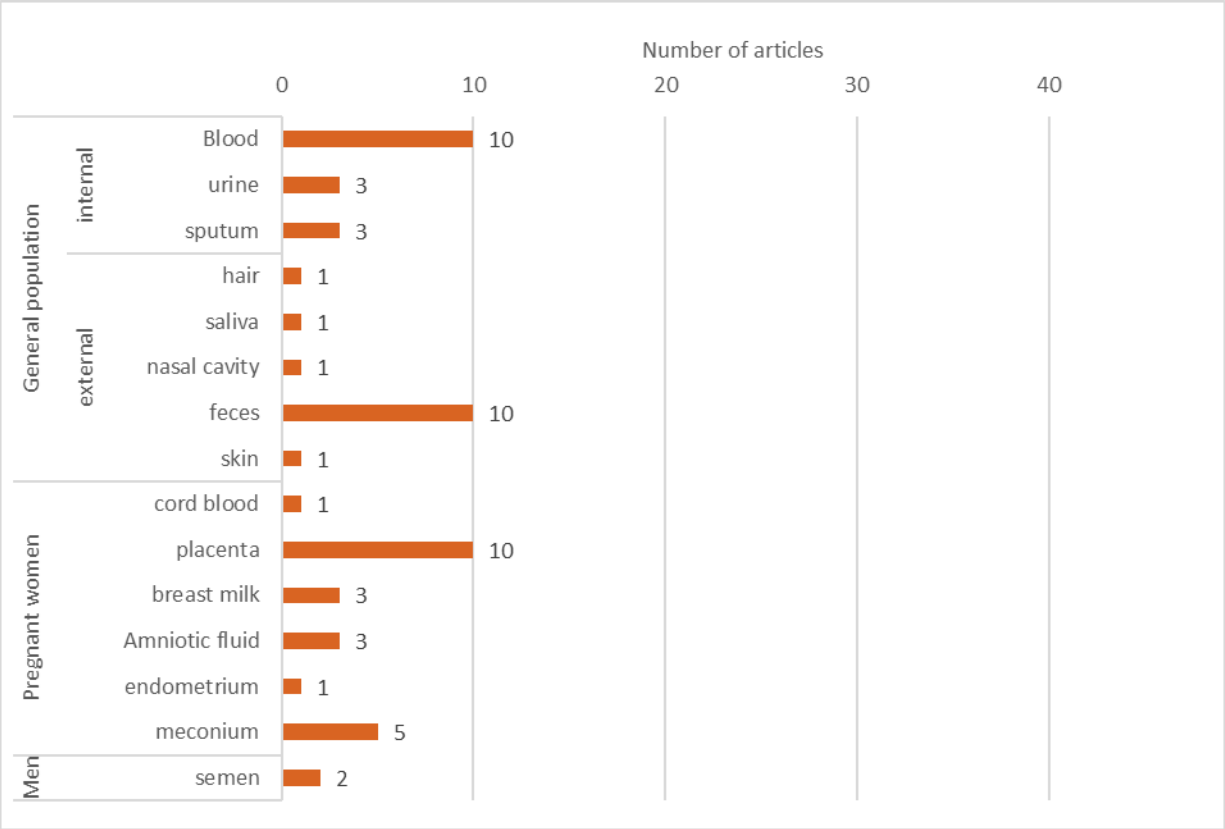


Figure 11: Overview of the different matrices addressed in the 47 selected articles concerning microplastics in a readily available human matrix.

Table 5: Motivation of matrices reflecting internal or external dose of MPs based on barrier crossing.

Matrix	Motivation
Reflecting internal dose	
Blood / cord blood	MPs in blood represent the MPs that were taken up by the body through ingestion (passing the gastrointestinal barrier), inhalation (passing the pulmonary barrier) or dermal contact (passing the skin barrier). For measurements in cord blood the MPs also passed the placenta barrier.
Urine	Urine is the product from the kidneys from filtering the blood. MPs in urine reflect the MPs that entered the body through ingestion, inhalation or dermal contact and were filtered from the blood into the urine. Some of the MPs in blood could remain in the blood or in the kidney tissue.
Sputum	Sputum is a thick liquid produced in the lungs and respiratory tract, protecting the airways.
Placenta	The placenta, meconium and amnionic fluid are tissues that protect the unborn child during pregnancy. MPs present in these tissues result from uptake by the mother's body through ingestion, inhalation or dermal contact.
Meconium	
Amnionic fluid	
Breast milk	Microplastics in breast milk are MPs that have entered the bloodstream or lymphatic system and were excreted in the human milk by the mammal glands. Therefore, these measurements reflect internal exposure to MPs.
Endometrium	The endometrium is the inner lining of the uterus. Microplastics in the endometrium indicate that these particles have entered the body and reached reproductive tissues.
Semen	Microplastics measured in semen reflect MPs that enter the body through ingestion, inhalation, or dermal absorption, and then travel via the bloodstream and cross the blood-testis barrier.
Matrix	**Motivation**

Reflecting mostly external dose	
Hair	Hair is constantly exposed to the environment, making it a natural collector of airborne microplastics. These particles can settle on the hair from dust and air pollution, personal care products or textile fibers. Because hair is not a living tissue and lacks blood supply, it does not directly reflect internal bodily processes like blood or organs do. Therefore, most microplastics found on hair are likely deposited from the environment. It might be possible that very small microplastics that enter the body can potentially be excreted through sweat or sebum, which could then deposit on hair.
Saliva	While saliva is produced internally, the microplastics detected in it are not typically the result of internal bodily transport (e.g., from the bloodstream). MPs in saliva can originate from MPs present from oral intake, that were not yet ingested, for example from chewing gum or contaminated food.
Nasal cavity	The nasal cavity is part of the external respiratory interface, and MPs found there most likely reflect larger particles from air born sources that were not able to enter the body.
Faeces	Faeces are undigested remains of food that pass through the gut. MPs measured in faeces are therefore not taken up by the body and reflect part of the external exposure levels via ingestion.
Skin	Microplastics that are sampled from the skin surface have not yet entered the body and therefore reflect external exposure.

Preferred matrix

When the objective of a human biomonitoring study is to investigate the internal load of microplastics that can interfere with body processes, blood would be the preferred matrix to sample in the general population. MPs in blood reflect particles that entered the body through ingestion, inhalation or dermal contact, passed the gastrointestinal, pulmonary and skin barrier to enter the bloodstream and can be transported to all body tissues.

Urine and sputum are also available for all age groups of the general population. MPs in urine, however, reflect only those particles that passed the filtering process of the kidneys. While MPs in sputum mainly reflect particles accumulated in the respiratory tract.

5.1.1 Sampling procedure for peripheral blood

To define a sampling procedure for implementing microplastic measurements in human biomonitoring studies, we rely on reported procedures in literature and on experiences in the pilot study.

The human matrices collected in the FLEHS-studies in Flanders since 2002 consist of blood, cord blood, urine and hair samples. Belgium also participated in several WHO human milk surveys, in which exposure to POPs was monitored in breast milk. In the previous session, blood samples were identified as the preferred matrix to measure exposure to microplastics in the general population. Therefore, we focus the information about sampling procedures on these matrices.

5.1.1.1 Information from literature

An overview of reported procedures in the literature for the collection of blood, urine, hair and breast milk samples is presented in **Table 6**.

- **Preparation**: To limit cross-contamination, several precautionary actions in the preparation phase are described in literature (Table 7). These consist of washing the sampling materials and glassware with distilled water, rinsing sampling materials with ethanol, preheating all glassware, working under laminar flow cabinets to prepare the sampling material, wearing dust free cotton laboratory coats, wearing nitrile or cotton gloves, sampling in a clean room with closed windows and doors, washing hands, cleaning hands with alcohol.
- **Blood/cord blood**: We identified 10 studies collecting blood and one study collecting cord blood. Most blood studies used venipuncture whole blood samples, one study collected coronary circulation blood. The needle is preferably directly connected to the vial to prevent contamination by plastic tubes. In all blood studies, glass vacutainers were used with anticoagulant. The collection vials were sealed immediately after collection. Seals consisted of rubber (isobutylene-isoprene rubber), polytetrafluorethylene or was not specified. Samples were stored at -20°C. In one study, samples were first stored at 4 °C overnight and subsequently at -20 °C until analysis to avoid breaking of the glass tubes. In the cord blood study, the cord blood was extracted with a syringe and released into a glass anticoagulant tube that was stored at -80°C until analysis.
- **Urine**: In the urine studies, midstream urine was collected in glass bottles and sealed after collection. Some samples were stored at 4°C, others were frozen (temperature not indicated).
- **Breast milk**: In the human milk studies, breast milk was collected through manual expression into glass containers and stored in the freezer (-20°C or -80°C).
- **Procedural blanks**: In order to be able to control for contamination during the sampling and sampling handling it is important to invest in procedural blanks. In most of the studies, procedural blanks consist of MilliQ water following the same procedure as the samples.

Sample Collection
Based on the described procedures in literature for sampling blood, cord blood, urine, hair and breast milk, the least risk of contamination of the samples is to be expected for peripheral blood samples. The blood can be directly collected with a sterile needle and collection tube, with minimal environmental contact (only during sealing of the collection device). Nevertheless, including procedural blanks remains important.

5.1.1.2 Experience from the pilot study

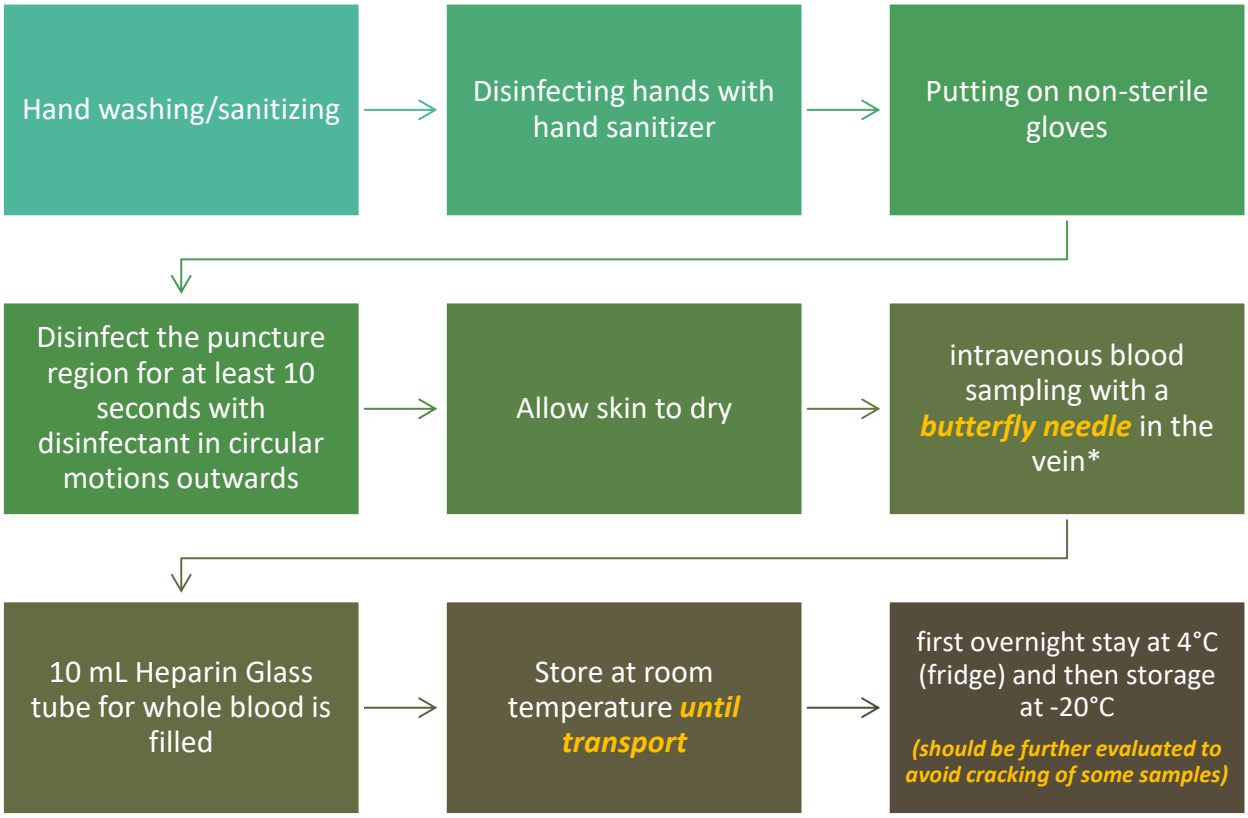
For the collection of blood samples, the use of glass 10 mL heparin tubes was recommended by the researchers of VU Amsterdam. The choice for these specific tubes was made during an initial testing phase of VU Amsterdam when setting up the method. As most blood tubes are made of plastic, glassware alternatives were considered to minimise potential contamination from the tubes themselves. The background of microplastics in the selected glass heparin tubes was tested at the time and found to be acceptable (data not shown).

These tubes performed well in the pilot study, as the tubes could collect peripheral blood directly by needle venipuncture using a blood tube holder, in which the needle was directly connected to the tube to prevent external plastic contamination. The pilot blank analyses also showed low background contamination of the tubes themselves, as well as during aliquoting under a laminar flow cabinet, by both the Pyr-GC/MS and Fluorescence Microscopy methods, supporting the use of the glass 10 mL heparin tubes for blood collection. Some tubes (10%), however, cracked during freezing, although a standard procedure of settling to room temperature, 24h at 4°C, followed by storage at -20°C was followed. This led to a small volume of leakage of blood during thawing and preparation of the sample vials, which is not preferred, and should be further evaluated in future studies to avoid the loss of samples during collection.

Although not recommended, it can still be considered to use plastic blood tubes; however, in that case, it is essential to demonstrate, prior to use, that the background contamination of these plastic tubes is comparable to or better than the glass heparin tubes currently employed. This requires a thorough analysis of blank tubes to ensure that there is no significant introduction of microplastics during the sampling and processing.

Sample collection
Based on the pilot study, the use of glass 10 mL heparin tubes is recommended because it allows the direct collection of blood with a sterile needle and collection tube, avoiding environmental contact. Blank analyses showed minimal background contamination of the tubes themselves, as well as during aliquoting. Nevertheless, the inclusion of procedural blanks remains important. The storage procedure, however, should be further evaluated, as 10% of the glass tubes cracked during freezing, which introduces a significant loss of samples.

5.1.1.3 Advised sampling procedure for blood samples



***For blood sampling in young children, or if a large volume of blood needs to be collected requiring multiple tubes for the analysis of various biomarkers. 3 to 5 procedural blanks should be added.**

Table 6: An overview of reported procedures in literature for collection of blood, urine, hair and breast milk samples

Study	Preparation	Collection device	Container	Storage	Blanks
Blood and cord blood					
Leslie HA et al. 2022 Netherlands		Venipuncture with surgical-grade sterile stainless-steel 21G needle, connected to the glass vacutainer under vacuum	10-ml glass heparinized vacutainer, sealed by a rubber seal (isobutylene-isoprene rubber)	freezer at - 20 ◦C	entire sampling system (including needle and vials) was tested for background contamination
Salvia R et al. 2023 USA		Peripheral blood samples	EDTA tubes	room temperature in agitation	
Brits M et al. 2024 Netherlands	glassware and new sample vials were washed with filtered water and covered with aluminium foil before use. Sample handling and preparation were performed in a laminar flow cabinet. Cotton laboratory coats, and nitrile gloves	Whole blood samples by venepuncture	10 mL glass heparinized vacutainer	at 4 °C overnight and subsequently at -20 °C until analysis to avoid breaking of the glass tubes	
Lee DW et al. 2024 Korea		Whole blood via venipuncture of the antecubital veins	25.7 ml of blood in a BD Vacutainer Heparin Glass tube	at −20 °C in a refrigerator for at least one week and at most eight weeks	contamination tests on the entire sampling system
Leonard SVL et al. 2024 Unied Kingdom			8.5 mL ± 10 % vacutainers containing 1.5 mL acid citrate dextrose solution A (sodium citrate, dextrose, citric acid and antimycotic (K sorbate) reagent)		10 procedural blanks, mimicking the production of a blood sample, opening the vacutainer, and transferring the sample into a clear, pre-cleaned Durham bottle for a similar length of time
Xu H et al. 2024 China	dust-free suit and sterile gloves glass containers used for sample collection were thoroughly cleaned with filtered ultrapure water and anhydrous ethanol and dried before use		sterile glass containers and sealed	-20 °C refrigerator	three sets of procedural blank control experiments
Yu H et al. 2024 China		a sterile syringe	6 ml of arterial blood in glass bottles sealed with polytetrafluorethylene (PTEE) lids	at -20℃	The entire sampling system (including artery sheath, syringe and bottles) was tested as blank or negative control

Study	Preparation	Collection device	Container	Storage	Blanks
Zhu M et al. 2024 China		Umbilical cord blood was extracted by a sterile disposable syringe	Collected cord blood was released into a glass anticoagulant tube	at −80 ◦C	
Gu X et al. 2025 China			Coronary circulation blood samples were collected in heparinized glass collection tubes		
Rauert C et al. 2025 USA?		Eclipse 21G needle with direct connection to the collection vial.	Glass 8.5 mL vacutainer tubes with anticoagulant acid citrate dextrose	At -20°C	Sampling blanks were prepared by drawing prefiltered Milli-Q water into a glass vacutainer tube (using the 21G needle), storing with the samples, and subsampling for analysis with every batch of blood samples
Zang S et al. 2025 China	Blood samples were collected between 8:00 and 11:00 am, centrifuged immediately sample collection glass device was calcined by an open flame All experimental consumables were made of glass and required to be rinsed with ethanol before use		sample collection glass device serum samples were divided into two glass bottles		after exposing the sample device in the sampling environment for 30 seconds, it was carefully placed into a storage container without interference from plastic particles. After siting for 24 hours, the container was opened and absolute ethanol was added to rinse the inner wall of the container and instruments. The resulting rinse solution was prepared for later use.
Urine					
Pironti C et al. 2023 Italy	A clean dedicated room		glass sampling tubes		
Massardo S et al. 2024 Italy	Glassware and instruments were washed using antiseptic detergent, rinsed with sterile distilled water and then with absolute ethanol. All the tools were stored in a laminar flow hood until use.		250–300 ml in glass jars, and KOH pellets were dissolved in. Jars were sealed with foil lids	at 60 ◦C for 5 days	procedural blanks were prepared simultaneously and following the same procedure as samples
Rotchel JM et al. 2024 United Kingdom		under anaesthesia, using a metal catheter	sterile universal containers	Frozen	
Study	**Preparation**	**Collection device**	**Container**	**Storage**	**Blanks**

Song X et al. 2024 China	Volunteers washed their hands, used disposable sterilized alcohol wipes to wipe the skin around the urethral orifice three times, wore disposable sterilized nitrile gloves, and a sterilized 1 L glass bottle, in a sampling room that met operating room cleaning standards		1 L glass bottle, midstream urine without making contact	at 4 ◦C	
Jahedi F et al. 2025	white cotton laboratory coats and gloves All glassware used for sampling and sample processing was cleaned with double-distilled water, sterilised with ethanol and heated in an oven at 450 ◦C before being used Participants were instructed to wash their hands thoroughly before sample collection	midstream urine in a clean, sterile and graduated glass cup which had remained sealed until the moment of collection	samples were transferred to 30 mL aluminium-capped glass bottles	short period of storage under refrigeration at 4 ◦C	controls consisting of double-distilled water
			Hair		
Abbasi S et al. 2021 Iran	Collection bottles pre-cleaned by triplicate washes with vacuum-filtered tap water participants were instructed to wash their hair at night and collect samples 24 h later washing head hair by researcher using filtered water into a 2 L bottle through a custom-built, 35-cm diameter stainless steel funnel Windows and doors remained closed and white cotton laboratory coats, single-use latex gloves and facemasks were worn throughout		wide-necked, screw-capped, silica glass bottles or jars		250 mL aliquots of filtered water were collected in glass jars after washing through one of the steel funnels
Study	**Preparation**	**Collection device**	**Container**	**Storage**	**Blanks**
			Breast milk		

Ragusa A et al. 2022 Italy		Breastmilk samples were collected 1 week after delivery. Patients were guided on a manual expression procedure, recommended by the World Health Organization.	Milk samples were placed into glass flasks.	Milk samples were weighed, and then stored at -20 °C until processing.	
Liu S et al. 2023 China	Mothers were asked to wash hands, rinse the nipple and wipe the breast with a cotton towel.		glass container	stored at −80 °C	
Saraluck A et al. 2024 Thailand	Sterile gloves were used, the first few drops were discarded, the breast was thoroughly cleansed with a 2% chlorhexidine solution before manually collecting the breast milk	breast milk was collected within 3 days postpartum using manual expression methods by the World Health Organization		stored at -20 °C	

5.2 QUESTIONNAIRE

Information concerning questionnaires to investigate possible sources of exposure to microplastics that were available in the publications on microplastics in blood, urine and breast milk was compared to the questionnaires of the H2020-AURORA project (which included a birth cohort and household study) and of FLEHS-5 (Table). Most of the questions addressed are already implemented in FLESH-5, and are comparable to the questions that are used for interpretation of internal exposure levels of bisphenols, phthalates, PFAS and flame retardants.

Table 8: Questionnaire information to investigate possible sources of exposure to microplastics that were available in the publications on microplastics in blood, urine and breast milk

	Lee DW et al. 2024	Xu H et al. 2024	Yu H et al. 2024	Zang S et al. 2025	Zhu M et al. 2024	Song X et al. 2024	Jahedi F et al. 2025	Ragusa A et al. 2022	Liu S et al. 2023	AURORA project (Household study)	FLEHS-5
General demographic characteristics											
Sex	X					X				X	X
Age	X			X	x	X	X		X	X	X
BMI					X	X			X	X	X
Education	X				x				X	X	X
Job	X						X		X	X	X
Income					x						X
marital status	X										
Parity					X						X
Mode of pregnancy					X						
Birth outcome					X						
Mode of delivery					X						
Sex of newborn					X						
Newborn weight					X						
smoking status	X					X	X		X	X	X
Passive smoking					X					X	X
alcohol consumption									X	X	X
physical inactivity	X									X	X
Living environment											
Place of residence			X			X	X			X	X
Sites of air pollution near the home					X						X
Distance residence to major transport routes					X						X
Plastic factory near the residence						X					X
Home floor level			X								
Plastic-related lifestyle questionnaires											
Plastic toys						X					
Plastic shoes											X
frequency of having ready-made meals	X									X	X
Home-cooked meals or take-out				X	X	X				X	X
Microwave food using plastic tableware					X				X	X	X
Plastic food packaging								X	X		X
Canned food									X	X	X
Water heater with plastic components										X	
Plastic cutting board for food preparation										X	
plastic containers	X									X	X

	Lee DW et al. 2024	Xu H et al. 2024	Yu H et al. 2024	Zang S et al. 2025	Zhu M et al. 2024	Song X et al. 2024	Jahedi F et al. 2025	Ragusa A et al. 2022	Liu S et al. 2023	AURORA project (Household study)	FLEHS-5
Discoloured plastic container	X										
Frequency of plastic bag usage			X								X
Frequency of disposable plastic lunch box use			X								
Plastic dinnerware						X			X		
Use of non-plastic food containers										X	X
Personal care products											
Frequency of using facial cleanser or toothpaste			X		X				X		
Daily time using a face mask					X	X					
Skin care products						X				X	X
Make-up						X				X	X
Sun screen										X	X
Dietary habits											
frequency of consuming vinyl-containing food	X										
frequency of consuming seafood	X				X			X	X		X
frequency of consuming fish								X			X
Source of drinking water			X	X	X	X			X		X
Drinking habits			X								
Drinking water in plastics cups/bottles					X				X	X	X
Drinking beverages in plastic bottles					X				X		X
Drinking boxed milk					X				X		
Milk tea consumption					X						
Tea consumption with tea bags					X				X	X	
Coffee with pads										X	
Chewing gum										X	
Breastfeeding				X							
Home characteristics											
Mechanical ventilation										X	X
frequency of indoor ventilation	X									X	X
Exposure to dust in living environment				X							
Frequency of using air purifiers at home					X						
Renovation of the residence within 1 year						X					X
Size of the residence										X	
Size of the living room										X	
Time spent in the residence										X	
Type of floor material in the living room / bedroom										X	X
Type of curtains in the living room / bedroom										X	
Frequency of cleaning the living room / bedroom										X	X
Type of material bed linen											
How is the laundry dried?										X	
Frequency of laundry drying										X	

5.3 MICROPLASTICS DETECTION IN HUMAN BLOOD

The following advice is based on the comprehensive literature analysis, the pilot study and the experience of VU Amsterdam and KU Leuven researchers, who have developed and executed microplastic analysis using Pyrolysis GC-MS or Fluorescence Microscopy, respectively, and is intended to optimise the measurement procedure and the interpretation of the results.

5.3.1 Volume of Blood Sample

While 1 mL of blood is often considered a standard volume for certain analyses, it is important to note that this volume does not represent a "gold standard" for microplastic analysis. Unlike dissolved compounds, microplastics are discrete particles that may be unevenly distributed within a blood sample. This heterogeneity can result in greater variability between duplicate or triplicate measurements compared to traditional analyses involving homogeneously distributed substances, as observed in the pilot study.

Using Fluorescence Microscopy, duplicate and triplicate measurements showed a high variability in the total number of microplastic particles and in the types of polymers detected. Using Pyr-GC/MS, most detected microplastics were at or below the limits of detection (LOD) and quantification (LOQ). Based on these preliminary results, it is possible that the variability in measurements arises from (i) preprocessing procedures (e.g. recovery of MPs during digestion and filtering), (ii) methodological constraints of the detection method (e.g. size of particles), or (iii) low concentrations or number of microplastics particles present in blood (e.g. <LOD or between LOD and LOQ). To gain a more accurate understanding of the microplastic burden, analysing a larger volume of blood can be considered. However, several factors must be taken into account:

1. **Blood Volume:** Standard blood collection tubes usually hold 5–10 mL of blood, allowing for the use of 2–3 mL in analysis while retaining sufficient material in case of technical issues.
2. **Processing Compatibility**: The selected blood volume must be compatible with existing sample vials and pre-processing procedures
3. **Filterability**: Larger blood volumes may present challenges in filtration.
4. **Analytical Considerations:** Analysing larger volumes may increase the risk of background contamination.

Blood Volume

Based on the pilot study, future studies should assess the feasibility of processing larger blood volumes and include thorough validation of the method to enable its routine application.

5.3.2 Blank Analysis and Detection Limits

The results of the pilot study blank analyses for Pyr-GC/MS are consistent with the blanks that have been measured in previous projects of the VU Amsterdam (data not provided). This suggests a stable background contamination over time. Based on this, we can conclude that the current limits of detection (LOD) and limits of quantification (LOQ) are comparable to their long-term data. Still, procedural blanks and detailed data on the specific LOD and LOQ values for the different polymer types should be included in future studies for reference.

For the Fluorescence Microscopy method, this pilot study was the first time the method was applied to human blood, as it was previously developed and validated to investigate the prevalence of microplastics in bottled drinking water [30]. As such, background contamination over time and across studies cannot be considered for this report. The pilot study blank analyses showed low amounts of external contamination (0.7 particles/g blood for PA up to 44.7 particles/g blood for PP), and is also supported by the considerable amount of detects above LOQ values. Particle count limits are currently set on the filter pore size of 1 µm to assess the number of particles.

Procedural blanks

The results of the pilot study and literature review highlight the relevance of including in each study at least 3-5 procedural blanks, and if needed, blanks during aliquoting, to appropriately determine LOD and LOQ values for each polymer type.

5.3.3 Microplastics detection method

The integrated findings from the literature review and pilot study underscore both the progress and ongoing challenges in the detection and characterization of microplastics (MPs) in human biological matrices. The literature review highlights multiple methods for MP detection — vibrational spectroscopy, optical microscopy, and mass spectrometry — each offering distinct advantages and limitations, and supports the integration of multimodal approaches to overcome sensitivity, specificity, and quantification challenges. These methodological insights are directly reflected in the pilot study, where complementary particle-based and mass-based analyses revealed divergent yet informative patterns in MP presence.

While the pilot study demonstrated that polyethylene (PE) and polypropylene (PP) dominate particle counts, polyvinyl chloride (PVC) accounted for the highest mass burden. These findings emphasise the need for information regarding MP characteristics, including particle size, which might critically influence detection outcomes. However, in the pilot study, there was a lack of information about particle sizes, which limited the ability to further explore. The detection inconsistencies and variability across replicates further highlight the need for

robust standardisation, including validated protocols, QA/QC measures, and transparent reporting practices, as discussed in the literature review.

MP detection

The literature review and empirical data strongly support the adoption of harmonised, multimodal detection strategies to improve accuracy, comparability, and interpretability of MP exposure data, alongside robust standardization of protocols, QA/QC, and reporting practices.

5.4 MARKET ANALYSIS & INTEGRATION OF MICROPLASTICS MEASUREMENT METHOD IN FLANDERS

5.4.1 Interview with experts & companies

At the start of the project, several online interviews were performed with scientific experts in the field of microplastics detection to gain insight into the progression of the field, the challenges that are encountered, as well as with a company to explore integration and development of a MP detection method for internal exposure in a human matrix.

The interview with **scientific experts** mainly highlighted the challenges with regard to MP detection. More specifically, sample preparation (e.g. diversity in predigestion methods) and the development of reference materials and internal standards for analytical methods were discussed. Also, the challenges with the detection of nanoplastics and the sensitivity of detection methods (e.g. variation in quantification) were addressed. These discussions showed the need to define the MP detection strategy at an early stage with respect to its advantages and limitations:

(I) Size resolution?
- Do you want to detect micro- and/or nanoplastics?

(II) Biological matrix?
- What does the matrix reflect, internal or external exposure, short- or long-term exposure?
- Is pre-digestion needed?
- Which pre-digestion method will be employed, and how does that influence the MP detection?
- MP Recovery rate after sample preparation?

(III) Vibrational spectroscopy-based, (optical) microscopy-based, and/or mass spectrometry-based method?
- Do you want to know polymer mass concentration, number of particles, size/shape?

(IV) Reproducibility and processing speed of the method?

(V) Avoidance/minimising external contamination?

Companies currently engaged in microplastic (MP) detection in environmental water were consulted regarding the feasibility of expanding their capabilities to include detection in human matrices, such as blood. These companies identified economic viability as a primary challenge, noting that the demand for direct internal human exposure assessment is significantly lower compared to environmental monitoring (e.g., water, soil). As a result, they expressed limited commercial incentive to invest in the development of such a detection method. Instead, they suggested that estimating internal exposure through extrapolation

from external dose measurements, such as those obtained from environmental samples or human excreta (e.g., faeces), may offer a more practical and cost-effective approach than performing direct internal exposure analyses.

5.4.2 Integration of the microplastics measurement method in Flanders

Several institutes and labs in Flanders were consulted to provide the following information if available:

Is an in-house microplastics detection method available? (yes/no)
If yes:

- Which method/technique?
- Developed for which type of sample(s)?
- How much time is needed to develop the method for human blood?
- Processing time?
- Indicative cost per sample?

The table below summarises the provided information and should be interpreted in the context of the laboratory's current expertise and experience with microplastic detection methods.

	Method/ Technique	**Sample Type(s)**	**Estimated time for (further) development**	**Processing time**	**Indicative cost**
VU Amsterdam	Pyr-GC/MS	Human blood	Published	30 samples/week	500€/sample
		Placenta	Not reported		
Roeffaers lab KU Leuven	Fluorescence Microscopy	Bottled water	Published	10 samples/week	120€/sample
		Placenta	Manuscript in preparation		
		Human blood	6-12 months		
BlueGrowth lab UGent	µFTIR	Environmental samples, including water and sediment	6 months, depending on the complexity of the matrix	Not reported	Upon request
Environment and Health KU Leuven	Raman	Airborne and environmental samples In vitro cells Fish tissue samples	Depending on the matrix. e.g. 1-2 weeks for environmental samples	2-36h, depending on scan region and resolution	Upon request

5.5 GENERAL CONCLUSION

The following table represents the recommendations for the feasibility of MP detection in the Flemish human biomonitoring program based on the literature review and pilot study.

Recommendations for MP detection in a human biomonitoring programme	
Preferred matrix	Human blood, as it reflects internal exposure, and the collection procedure has minimal risk for external contamination
Sample collection	10 mL glass Heparin tube, collected with a needle and tube holder, for minimising the risk of external contamination. If the direct collection poses a burden for the individual, a butterfly needle could be used, but the risk for contamination should first be evaluated. Nevertheless, 3-5 procedural blanks should be incorporated for each sampling collection.
Sampling procedure	See Section 5.1.1.3 - Advised sampling procedure for blood samples. Storage strategies should be further tested to avoid cracking of the glass Heparin tubes.
Sample test volume	Currently, a blood volume of 1 mL is commonly used, but reproducibility remains a concern and requires further investigation. Increasing the volume to 2–3 mL may help address variability across biological replicates and provide sufficient excess in case of technical issues. However, implementing this change would require revalidation of the method, and comparison with the current blood volume.
Detection method	Each microplastic detection method offers specific strengths and weaknesses, indicating that a multimodal approach is most effective for accurately assessing microplastic burden. However, to ensure consistency and reliability, research efforts should focus on harmonising these complementary methods. It is important to note that such integration and standardisation will lead to an increased cost per sample.
QA/QC and reporting	The following information should be available: Sampling procedure and preprocessing, Recovery rates (if applicable), Reference materials (if applicable), Internal Standards, Procedural Blanks, LOD and LOQ, Personnel training and laboratory environment control to minimise contamination**.**
Implementation in HBM program	Implementation in the Flemish human biomonitoring program depends on the progress of complementary method detection development and cost price per sample, with, in theory, the potential to integrate into research in the following 2-5 years, or only for biomonitoring purposes, >5 years.

6 SUPPLEMENTARY INFORMATION

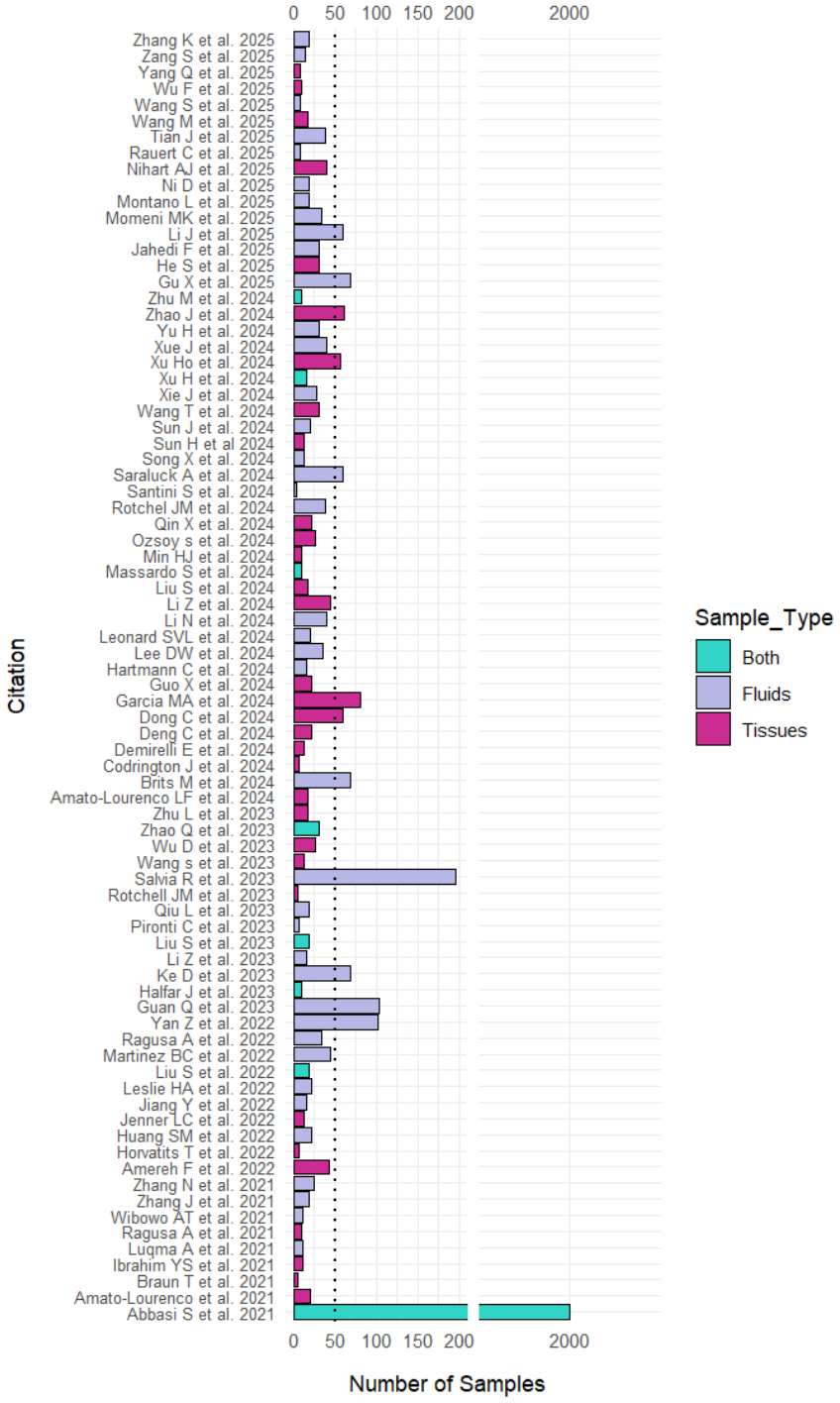


Figure S1: Overview of sample characteristics for each study *– Sample size and primary sample type reported by each study included (n=80). Studies are listed by citation on the vertical axis. The length of each horizontal bar corresponds to the number of samples analyzed in that study (horizontal axis), and the color indicates the sample type: Tissues (magenta), Fluids (light purple), or Both (teal). The vertical dotted line marks a threshold of n=50 samples.*

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