Clinical Proteomics

REVIEW

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From blood drops to biomarkers: a scoping review of microsampling in mass spectrometry-based proteomics

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Abstract

Background Microsamples are simple blood sampling procedures utilizing small blood draws. Although microsamples are regularly used in some disciplines, proteomic analysis of these samples is an emerging field. Currently, it is unclear whether the quantitative precision and proteome coverage achieved in microsamples is comparable to plasma or serum. As a consequence, microsamples are not used in proteomics to the same degree as more traditional blood samples.

Objectives The objective of this scoping review was to report the applications of microsamples within clinical mass spectrometry-based proteomics. This was accomplished by describing both proof-of-concept and clinical proteomics research within this field, with an additional evaluation of the newest advances regarding clinical proteomics.

Inclusion criteria Original scientific literature was included where bottom-up mass spectrometry was used to analyze endogenous proteins from human microsamples.

Methods Relevant publications were sourced through three scientific databases (MEDLINE, EMBASE and Scopus) in addition to backward and forward citation searches through Scopus. Record screening was performed independently by two separate authors. The review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) guidelines.

Results A total of 209 records were screened for inclusion from database searches and 3157 records were screened from forward and backward citation searches, resulting in 64 eligible studies. An evaluation of proof-of-concept research within this field revealed that although microsamples are amenable to high-throughput proteomics using a variety of targeted and untargeted acquisition methods, quantification remained a relevant issue. Microsampling practices were heterogeneous, and no standard procedure existed for protein quantification. Clinical studies investigated protein expression in numerous disease or experimental groups, including hemoglobinopathies and immunodeficiency disorders.

Conclusion The use of microsamples is increasing within the proteomics field and these samples are amenable to standard bottom-up workflows. Although microsamples present a clear advantage in terms of sampling procedure, both the sample collection and quantification procedures remain to be standardized. However, there is an incentive to address the remaining issues, since microsampling would greatly reduce the resources necessary to sample large cohorts within clinical proteomics, a field that currently lacks large discovery and validation cohorts.

Keywords Clinical proteomics, Microsampling, Dried blood spots, Scoping review, Mass spectrometry

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Background

Precision medicine aspires to treat patients according to their genetic and lifestyle risk factors. Biomarkers are an important aspect of this new era of medicine, as they indicate the presence or absence of disease, response to treatment or exposure, or the risk of relevant outcomes [1]. Blood-based biomarkers are readily accessed and provide a wealth of information pertinent to the individual's overall health status, reflecting systemic changes in the body as well as tissue leakage from disease sites [2]. While less invasive than a tissue biopsy, a traditional blood sample still requires trained personnel for the blood draw, necessitating a visit to a hospital or health clinic. Additionally, most blood biomarkers are calibrated with serum or plasma as the analytical matrix, requiring centrifugation and allocation into secondary testing tubes before analysis. Transport and storage of blood or blood products requires cold-chain logistics, which are demanding with regards to both cost and energy use. Microsamples are a simple alternative to traditional blood sampling, where < 100 µL of blood is collected [3]. While not widely used in healthy adult populations, microsamples from a heel prick are routinely used for newborn screening in many countries to test for inborn errors of metabolism [4]. One common microsample is the dried blood spot (DBS), where a lancet is used to prick the finger and capillary blood is deposited onto filter paper and allowed to dry. The simple sampling procedure can allow self-testing outside of a hospital setting, which has advantages regarding both economic resources and patient comfort. Transport and storage of microsamples at room temperature is also common, facilitating straightforward postal shipment to a laboratory for analysis, although analyte stability depends on the target peptides [5]. The use of microsamples in research has increased steadily over the past two decades, reflected by an increase in publication activity (Fig. 1A) [6].

However, significant barriers exist for mainstream use of microsamples in clinical proteomics. The amount of blood in one drop undoubtedly varies and cannot be precisely controlled for some microsamples, such as DBS. Additionally, sample collection by non-medical personnel could potentially result in incorrect or substandard sample quality, leading to increased technical variation. Using a punch to obtain the same area from each DBS sample does not result in the same volume of blood for each sample due to variations in hematocrit level. High hematocrit increases blood viscosity, with the consequence that the amount of blood sampled from patients with high hematocrit values will be higher than the amount of blood sampled from patients with lower hematocrit values when using the same size punch for all samples [7]. Lack of knowledge of the precise blood volume of a sample poses a problem for absolute quantification, often a requirement for clinical assays. Several strategies have been developed to account for the above concerns. For example, hematocrit can be estimated through the measurement of potassium, and this has been applied to correct quantification values in small molecules [8, 9]. Another possibility is the use of a linear model using surface area and hematocrit to estimate blood volume [10]. However, these concepts have not yet been applied to mass spectrometry-based proteomics.

Proteins are important biomolecules responsible for virtually all cellular functions, and dysregulated protein expression is often the causative element behind a disease state [11]. Proteins are also the largest biomarker subset, in one example comprising 42% of routine clinical analyses performed in a hospital setting [12]. The investigation of proteins associated with disease is advantageous for developing a biological understanding of disease pathogenesis as well as for the development of multi-protein biomarker panels for high diagnostic or prognostic accuracy. Mass spectrometry (MS) is a natural choice for the aforementioned proteomics applications due to high throughput and a lack of reliance on antibodies, allowing the analysis of any protein via MS-based methods, at least theoretically. Bottom-up proteomics analysis is the most common approach [13], where proteins are enzymatically digested to peptides prior to analysis. Alternative high-throughput proteomics technologies, such as proximity extension assay or aptamer-based technologies, still require definition of target proteins and therefore a priori knowledge, whereas MS allows true hypothesis-free testing. For applications where proteins of interest are known, targeted MS assays can be developed. Recent advances in sample preparation, acquisition methods, and data processing have increased the sensitivity of this technology to the single-cell level, opening up new possibilities for MS-based analyses [11, 14, 15]. These advances are also expected to have a large impact within clinical proteomics by facilitating the development and validation of high quality MS assays for routine use [16]. On the other hand, blood is highly complex and blood-based proteins demonstrate a difference in concentration range greater than 10 orders of magnitude, and this huge dynamic range is a challenge for mass spectrometers [17]. High-abundant plasma proteins, including albumin, immunoglobulins, and complement factors, comprise the bulk of total plasma protein and whole blood contains the additional complication of an abundance of hemoglobin from erythrocytes. The presence of very high-abundant proteins can reduce the overall proteome coverage and sensitivity of MS analysis, especially when data-dependent acquisition is used [18].

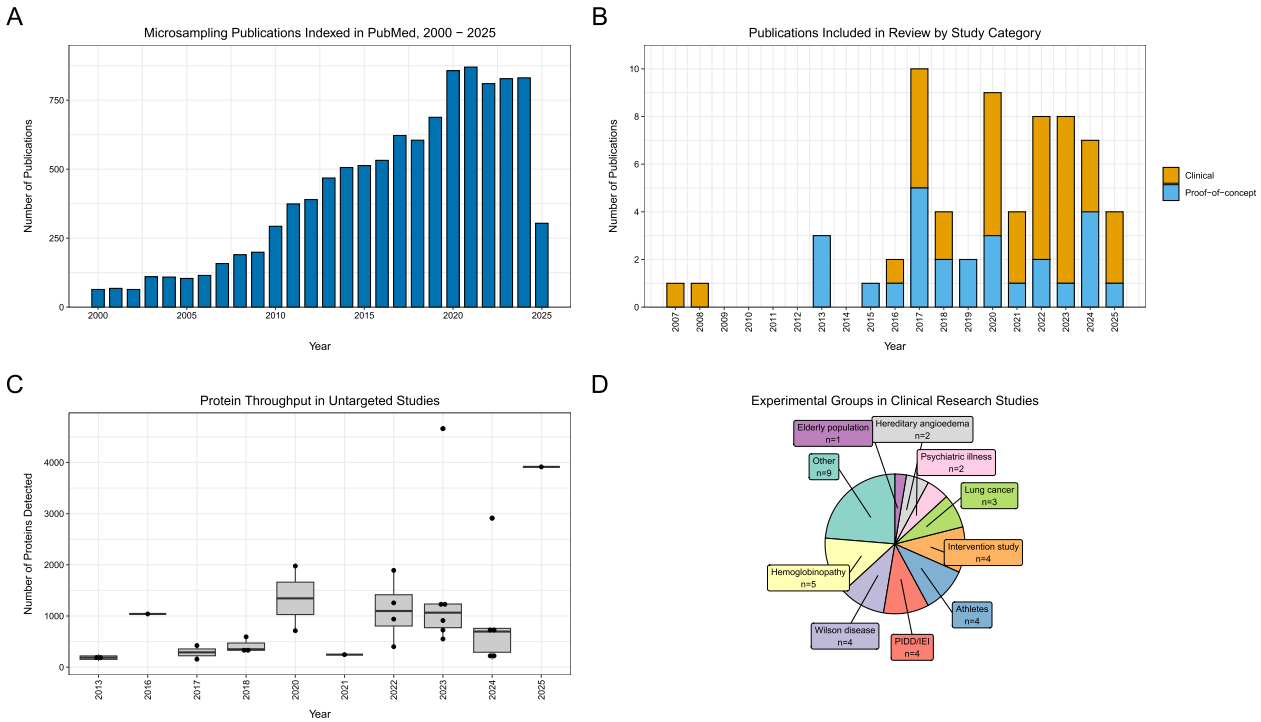

Fig. 1 Overview of publications included in the review. **A** Publications involving microsamples over time. PubMed was searched for '"dried blood spot*" OR "dry blood spot*" OR "volumetric absorptive microsampl*" OR "neonatal blood spot*" OR "newborn blood spot*" OR "microsampl*" OR "finger prick*"' on April 23rd, 2025. The search was restricted to the years 2000–2025. **B** Bar graph of studies included in the review, according to year and divided according to whether studies were categorized as proof-of-concept or clinical research. Publications from 2025 were included up until April 22rd, 2025. **C** Box plots of protein throughput in untargeted studies included in the review, according to year. For studies including multiple experiments, only the experiment with the highest throughput in microsamples was represented. Only years in which untargeted studies were published were represented. **D** Pie chart of experimental and disease groups included in the 37 clinical research studies. PIDD/IEI: primary immunodeficiency disorders or inborn errors of immunity

Presently, microsamples remain a largely untapped and poorly characterized resource for proteomics due to sample heterogeneity and normalization issues, despite the fact that successful MS analysis of microsamples is already feasible and proteome coverage has improved greatly in recent years [19, 20]. Therefore, a scoping review is necessary to clarify what can currently be accomplished within this new field and to determine whether microsamples are a viable alternative to traditional blood sampling within clinical proteomics. The purpose of this scoping review was to systematically describe research which has combined blood microsampling in humans and bottom-up mass spectrometry. Within this area, the specific objectives of this review were to:

I. Summarize proof-of-concept research to define relevant limits and advantages. Proof-of-concept research encompassed blood microsamples from healthy donors or a pool of healthy donors, demonstrating feasibility of microsampling in proteomics, but without a pertinent clinical context.
II. Identify clinical research applications and describe their typical usage. Clinical research papers included analysis of microsamples from one or more disease states or experimental groups.
III. Evaluate the most recent scientific papers (since 2020) within objective II with regards to the specific advances these papers present in terms of methodology and results.

Methods

Study design

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) guidelines [21]. All required reporting items from the PRISMA-ScR checklist were included (Supplementary Table 1). The research protocol was defined a priori and uploaded to the Open Science Framework (https://osf.

io/2tz8h/) using the JBI protocol guideline for scoping reviews [22].

Eligibility Criteria

Included articles were original scientific literature which analyzed human microsamples using bottom-up proteomics to study endogenous proteins. Several article types were excluded, such as preprints, protocols, conference abstracts, and review articles. Inclusion and exclusion criteria were defined in Table 1. Microsamples were defined as noninvasive blood sampling techniques drawing < 100 µL blood [3] or mimics of these techniques in the case of proof-of-concept research and method development. As described in the research objectives, articles were categorized as either proof-of-concept or clinical research. Proof-of-concept research involved analysis of samples from healthy donors or a pool of healthy donors, while clinical research involved analysis of samples from at least one disease state or experimental group. Articles not written in English were excluded during title and abstract screening.

Database searches and information sources

The three databases MEDLINE, EMBASE, and Scopus were searched on October 3rd, 2024 and updated on April 22nd, 2025. MEDLINE and EMBASE were accessed through Ovid. The search strategy was designed with the aid of a research librarian (Supplementary Table 2). Title and abstract screening was performed independently by two reviewers (AJC and NBP) with Covidence software (Veritas Health Innovation, Melbourne, Australia). Discrepancies were addressed with discussion and mutual agreement. Full-text assessment of studies was performed by one reviewer (AJC) in Covidence. Forward and backward citation searches were also used to source additional relevant studies. For each eligible study identified by the database search, forward and backward citation searches were performed with Scopus on October 16th, 2024 and April 23rd, 2025. These studies were initially screened based on the title, and studies with relevant titles were assessed for inclusion according to the same eligibility criteria as for database searches (Table 1). If multiple studies were published using the same patient cohort, the first published study was included and later studies were excluded.

Data collection and analysis

Full-text data extraction was performed by one reviewer (AJC) in Microsoft Excel 2016. The variables for data extraction were determined prior to extraction through discussion and agreement between three of the reviewers (AJC, HCB and NBP). Data extraction variables were refined in a similar manner by the same three reviewers after an initial pilot test where data for six studies was collected. The collected variables were described in Supplementary Tables 3 and 4. The original research objectives and protocol were followed as written (https://osf.io/2tz8h/), and papers described in research objective I were referred to as 'proof-of-concept' instead of 'method-based' to ensure clear terminology. The research objectives necessitated the division of studies into two main subgroups. To address research objectives I and II, tables describing either proof-of-concept or clinical studies were developed along with a quantitative analysis based on frequency counts and a narrative analysis describing the studies, including recurring themes in the literature and potential impact. Objective I included two tables, one descriptive and result table of proof-of-concept studies and one table describing the technical parameters assessed by the studies in more detail. Objective II included two tables, one descriptive and result table of clinical studies and one table describing the disease or experimental groups studied. Frequency data regarding experimental groups in clinical studies was represented in a pie chart. To address research objective III, the narrative text was based on a published framework to evaluate the quality of protein biomarkers [23]. An additional post-hoc subgroup analysis was included for studies utilizing untargeted acquisition methods to provide an overview of the acquisition parameters and proteome coverage within these studies, producing a bar chart and additional variables for the result tables. All tables listed studies in the order of year published and alphabetically by first author within each year. All data visualizations were generated using the tidyverse in R (v. 4.2.1) through the RStudio environment (v. 2022.7.1.554) [24–26].

Table 1 Inclusion and exclusion criteria for studies

Inclusion criteria	Exclusion criteria
• Microsamples drawing < 100 µL	• Preprints, protocols, conference abstracts, reviews
• Human blood samples	• Analysis of spiked-in or exogenous proteins
• Proteomics analysis with bottom-up mass spectrometry	• Analysis of recombinant or therapeutic proteins

Results

Study selection and inclusion

The database searches resulted in 209 records for title and abstract screening after removal of duplicates, with 46 studies eligible for inclusion after full-text screening (Fig. 2). Forward and backward citation searches sourced 18 additional studies, resulting in a total of 64 studies included in the review. An overview of included studies and their key findings can be found in Supplementary Table 5. Studies were published from 2007 to 2025, and most research was relatively recent (Fig. 1B). Overall, 26 (41%) studies were defined as proof-of-concept, demonstrating method development or technical optimization of microsampling and proteomics based exclusively on samples from healthy donors. A total of 38 (59%) studies were categorized as clinical research, indicating that these studies applied microsampling to a clinical proteomics context including disease or experimental groups. Although microsampling is a newer sampling technique, this indicated that clinical proteomics research involving microsamples already existed and actually comprised the majority of published research. Twenty-seven (42%) studies described untargeted experiments, 33 (52%) studies described targeted experiments, and 4 (6%) studies described both targeted and untargeted experiments. Several of the included studies also investigated other biomolecules in microsamples, such as the transcriptome or metabolome. These results are beyond the scope of this review and will therefore not be discussed. Experimental results based on traditional blood sampling will only be described in the context of comparison to microsampling results.

Acquisition parameters for untargeted discovery proteomics

Non-targeted data acquisition is ideal for hypothesis-generating experiments and exploratory studies, although complete proteome coverage is generally not attained, especially in complex samples such as blood [17]. Untargeted acquisition methods were included in 31 of the 64 studies (48%) (Tables 2 and 4). Most studies used data-independent acquisition (19, 61%), while ten studies (32%) used data-dependent acquisition, one study used both acquisition strategies, and one study used matrix-assisted laser desorption/ionization combined with a time-of-flight analyzer (MALDI-TOF). Improvements in many aspects of the plasma proteomics workflow in recent years have increased protein coverage in plasma and serum, with several hundred proteins detected on average in neat plasma [17], while

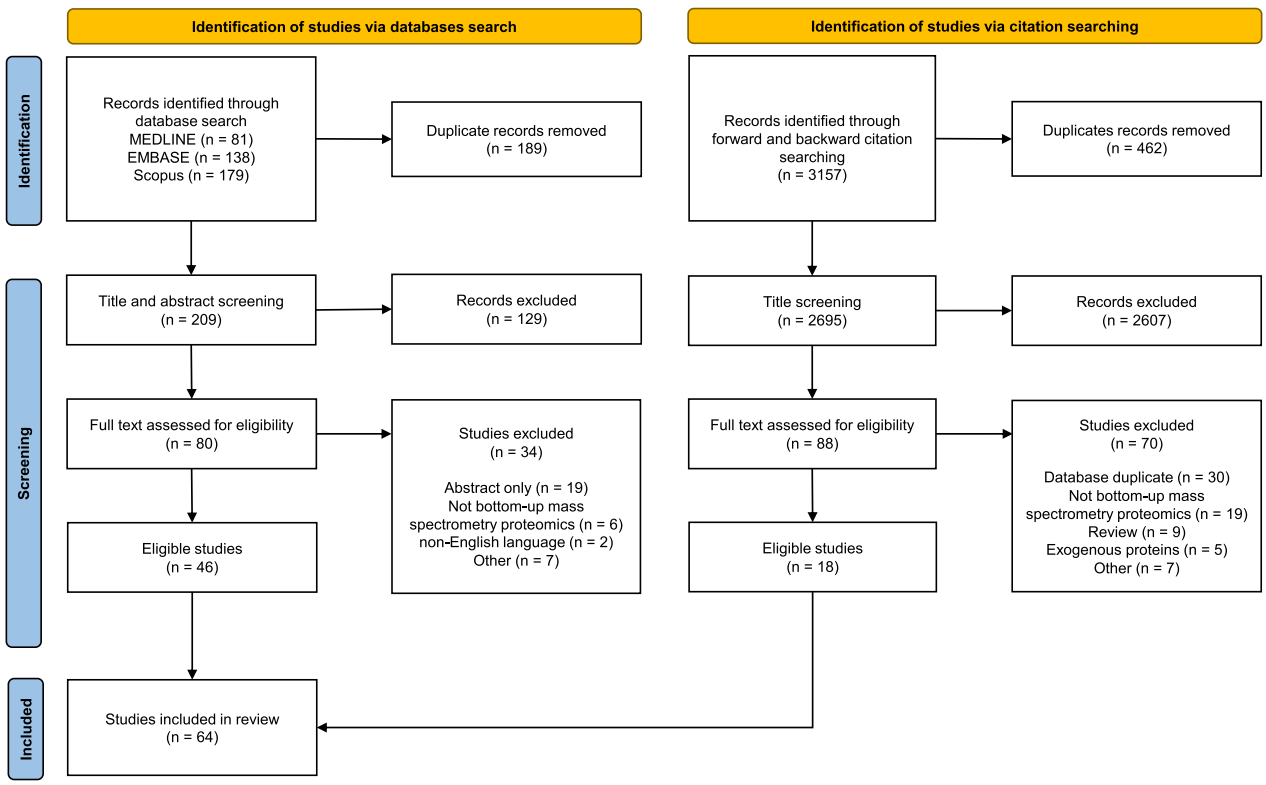

Fig. 2 PRISMA flow chart representing the inclusion and exclusion of information sources

extensive depletion and fractionation allows detection of thousands of proteins [27]. This general trend within blood-based proteomics was also reflected in the studies included in this review (Fig. 1C). The first two publications describing untargeted protein detection in microsamples were published in 2013, with Martin et al. being the earliest. These studies demonstrated that DBS samples could be used for data-dependent acquisition, identifying 120 and 253 proteins in three or ten DBS samples, respectively [28, 29]. In contrast, ten years later, Whelan et al. identified 4,661 proteins in VAMS devices and plasma, which was the highest number of proteins identified in a single experiment in this review [30]. This was achieved with data-independent acquisition and a pan-human spectral library, using volumetric absorptive microsampling (VAMS) devices, which use capillary action to sample a fixed volume of blood with less than 5% volume variation [31]. This demonstrated that while untargeted acquisition in microsamples has been possible for a long time, recent improvements in both MS and sampling procedures have increased proteome coverage, and microsampling workflows achieve comparable coverage to traditional plasma proteomics. The length of the liquid chromatography gradient, where the proportion of organic solvent in the mobile phase increases, also affects coverage. Longer gradients with low flow rates typically provide better peptide separation and higher protein coverage [32]. The average gradient length was 60 min. Four of the studies used untargeted acquisition for protein identification alone. All remaining studies used varying strategies to achieve label-free quantification.

Accounting for the hematocrit bias

One issue specific to DBS microsamples is the hematocrit bias, where varying hematocrit levels between individuals affects blood viscosity, thereby altering the diffusion of blood across filter paper [5]. This means that both the total amount of blood in the sample and the amount of blood in a predetermined area of the sample are unknown. This is a significant quantification issue, since the utility of external standards is limited when the initial blood volume is not known. There was no consensus among studies regarding how to address the hematocrit bias. Many of the studies which used DBS samples circumvented the issue of unknown blood volumes by deposited a known amount of venous or capillary blood onto DBS samples, usually with a pipette. This allowed protein extraction from the entire spot of known volume, although some studies still used a punch. Two papers normalized the abundances of target proteins relative to selected internal proteins. Razavi et al. first defined baseline samples from a cohort of longitudinal samples and then selected stable peptides from five proteins with correlated abundances, namely albumin, plasminogen, hemopexin, C3, and IgM. The average peak area ratio (PAR) of endogenous to stable isotope-labeled standards for each of the five peptides was then used as a scaling factor. For all target peptides, PARs were corrected by division with the subject-specific scaling factor. Z-scores were calculated as an additional normalization step using baseline mean and standard deviations, which allowed the calculation of a "personalized" z-score specific to each participant [33]. Bassini et al. employed a comparable strategy using only albumin for scaling [34]. This type of internal standard could potentially adjust for the variation in blood sampling volumes. A similar alternative is the use of peptide or protein ratios for relative quantification, since the difference in abundance between two peptides is assumed equal regardless of blood volume [35].

However, this simple normalization only addresses unknown blood volumes, not differing hematocrit levels between samples. If hematocrit is known, cellular blood components can be adjusted for to estimate a target analyte's concentration in plasma [36]. If hematocrit is not known or not sufficiently adjusted for, quantification can be affected. For example, within untargeted, label-free proteomics, quantification is typically achieved by normalization of each precursor ion to the total precursor abundance. When samples have different hematocrit levels, the differing proportions of proteins from cellular and soluble components will contribute to and affect the normalization factor. This means that although the amount of a soluble protein would not change when measured in plasma, this protein would be measured at a higher level in a whole blood sample with low hematocrit compared to a sample with high hematocrit. Additionally, the actual extent to which variations in hematocrit lead to changed blood sampling volumes, with the consequence of increased variation in protein abundances, has not been experimentally assessed. Until a standard normalization procedure is developed, proteomics studies where quantitative precision is crucial can use microsamples where blood volume is exact, such as VAMS devices. Several of the papers included in this review assessed novel blood sampling devices that removed the cellular blood components, thereby successfully removing the hematocrit bias. One study described a device that used the differing sedimentation properties of blood particles in a laminar flow to isolate 2 µL of plasma from a whole blood sample, while another study used lateral flow in combination with a membrane to separate blood fractions [37, 38]. A third study used custom-made pipette tip-based devices and centrifugation to isolate 1 µL of plasma from 5 µL of whole blood [39]. Isolating plasma

Table 2 Descriptive and result table of 26 studies categorized as proof-of-concept research

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Notable sample preparation	Acquisition method		Effective gradient length *(min)*	Throughput Quantification
				Targeted	*Untargeted*		
Chambers [28] *2013a*	DBS/DPS/DSS *- 15 µL - Entire spot - 903 Protein Saver (Whatman)* Plasma Serum WB	Venous blood deposited onto DBS	*NA*	*NA*	DDA Orbi-q-IT	90	253 proteins (1549 peptides) No quantification
Chambers [50] *2013b*	DBS *- 15 µL - Entire spot - 903 Protein Saver (Whatman)*	Venous blood deposited onto DBS	*NA*	MRM	*NA*	*NA*	37 proteins (40 peptides) Calibration curve with SIS peptides
Martin [29] *2013*	DBS *- Ahlstrom grade 226 filter paper (ID Biological Systems)*	Finger prick	Liquid extraction surface analysis to extract proteins	*NA*	DDA Orbi-q-IT	30	120 proteins No quantification
Chambers [49] *2015*	DBS *- 15 µL - Entire spot - 903 Protein Saver (Whatman)*	Venous blood deposited onto DBS	*NA*	MRM	*NA*	*NA*	97 proteins (169 peptides) PAR relative to SIS peptides *LLOQ: 190 ng/mL (cholinesterase)*
Geyer [39] *2016*	Plasma *- 1 µL - Custom pipette tip-based centrifugal devices*	Finger prick	Basic reverse-phase fractionation (high throughput method)	*NA*	DDA Orbi-q-Orbi	15 100	347 proteins (short gradient) 1040 proteins (long gradient) LFQ
Cox [46] *2017a*	DBS *- 20 µL - Entire spot - DMPK-C (Whatman)*	Venous blood deposited onto DBS	Buffer washing to enrich membrane proteins	PRM	*NA*	*NA*	4 proteins (4 peptides) PAR relative to SIS peptides
Henderson [44] *2017*	DBS *- 65 µL - 1 × 3.2 mm - 903 Protein Saver (Whatman)* Plasma	Finger prick by trained personnel Venous blood deposited onto DBS	*NA*	PRM	*NA*	*NA*	3 proteins (6 peptides) Peak area integration of transitions *LLOQ: 8.3 mg/dL (apolipoprotein B)*
Ozcan [53] *2017*	DBS *- 1 × 3 mm - 903 Protein Saver (Whatman)* Serum	Finger prick	*NA*	MRM	*NA*	*NA*	82 proteins (156 proteins) PAR relative to SIS peptides
van den Broek [88] *2017*	Plasma VAMS *- 10 µL - Mitra (Neoteryx)*	VAMS dipped into venous blood	*NA*	SRM	DIA SWATH-MS	60	SRM: 6 proteins (10 peptides) PAR relative to SIS peptides DIA: 423 proteins (1661 peptides) LFQ

Table 2 (continued)

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Notable sample preparation	Acquisition method		Effective gradient length *(min)*	Throughput Quantification
				Targeted	*Untargeted*		
Wouters [89] *2017*	DBS *- 10 µL - Entire spot - Human ID Blood stain Card BFC 180 (Whatman)*	Finger prick	Immobilized enzyme reactor for digestion	*NA*	DDA TOF-q-TOF	45	156 proteins No quantification
Forchelet [37] *2018*	Microsample *- 20–25 µL resulting in 2 µL sample - Custom microfluidic device* Plasma Serum	Finger prick	*NA*	*NA*	DIA SWATH-MS	*ND*	312 proteins LFQ
Rosting [90] *2018*	DBS *- Entire spot - Ahlstrom grade 226 (ID Biological Systems)*	Finger prick	Liquid surface extraction analysis to extract proteins	*NA*	DDA Orbi-q-IT	30	350 proteins (1065 peptides) No quantification
Samenuk [91] *2019*	NBS *- ¼ of spot*	*ND*	*NA*	*NA*	MALDI-TOF	*ND*	30 peptides No quantification
Vidova [52] *2019*	DBS *- 1 × 3 mm - 903 Protein Saver (Whatman)* Serum WB	Finger prick	*NA*	SRM	*NA*	*NA*	7 proteins (13 peptides) PAR relative to SIS peptides *LLOQ: 0.8 nM (serum amyloid A4)*
Eshgi [41] *2020*	DBS *- 50 µL - 5 × 6 mm - 903 Protein Saver (Whatman)* WB	Venous and capillary blood deposited onto DBS	*NA*	MRM	*NA*	*NA*	245 proteins PAR relative to SIS peptides *LLOQ: 0.2 fmol*
Nakajima [42] *2020*	DBS *- 2 × 3.2 mm - Blood sampling paper (Advantec)*	Finger prick	Sodium carbonate precipitation to reduce soluble proteins	*NA*	DIA Orbi-q-Orbi	75	1977 proteins LFQ
van den Broek [92] *2020*	VAMS *- 10 µL - Mitra (Neoteryx)*	Finger prick, both self-sampled and by trained personnel	*NA*	SRM	*NA*	*NA*	11 proteins (22 peptides) PAR relative to SIS peptides
Cox [47] *2021*	DBS *- 20 µL - Entire spot - DMPK-C (Whatman)*	Venous and capillary blood deposited onto DBS	Buffer washing to enrich membrane proteins	PRM	*NA*	*NA*	4 proteins (4 peptides) PAR relative to SIS peptides *LLOQ: 0.135 nM (CD71)*
Mc Ardle [93] *2022*	Plasma VAMS *- 10 µL - Mitra (Neoteryx)*	VAMS dipped into venous blood	*NA*	*NA*	DIA Orbi-q-Orbi	21 60	939 proteins LFQ

Table 2 (continued)

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Notable sample preparation	Acquisition method		Effective gradient length *(min)*	Throughput Quantification
				Targeted	*Untargeted*		
Schneider [94] *2022*	DBS *- 50 µL - 1 × 3 mm - 903 Protein Saver (Whatman)*	Venous blood deposited onto DBS	*NA*	*NA*	DIA Orbi-q-Orbi	90	~400 proteins (~ 3000 peptides) LFQ, peptide ratios
Whelan [30] *2023*	Plasma VAMS *- 10 µL - Mitra (Neoteryx)*	Finger prick, self-sampled VAMS dipped into venous blood	*NA*	MRM	DIA Orbi-q-Orbi	60	MRM: 60 proteins (114 peptides) PAR relative to SIS peptides DIA: 4661 proteins No quantification
Brockbals [35] *2024*	DBS *- 20 µL - 1 × 3 mm - 903 Protein Saver (Whatman)*	Venous and capillary blood deposited onto DBS	*NA*	MSMS with inclusion list	*NA*	*NA*	3 proteins (11 peptides) Peptide ratios
Richard [95] *2024*	DBS *- 20 µL - 4 × 6 mm - 903 Protein Saver (Whatman)*	Finger prick	*NA*	MRM	*NA*	*NA*	250 proteins (319 peptides) PAR relative to SIS peptides
Shen [96] *2024*	VAMS *- 10 µL - Mitra (Neoteryx)*	Finger prick, self-sampled	Biphasic MTBE extraction to precipitate protein pellet	*NA*	DIA SWATH-MS	43	291 proteins LFQ
Sun [51] *2024*	Blood cells DBS *- 100 µL - Entire spot - FTA card (Qiagen)* Plasma WB	Venous blood deposited onto DBS	*NA*	*NA*	DDA Orbi-q-Orbi	78	758 proteins (7348 peptides) LFQ
Vegesna [97] *2025*	DBS *- 903 Protein Saver (Whatman)* VAMS *- Mitra (Neoteryx)* Plasma	VAMS dipped into whole blood or plasma Venous blood deposited onto DBS	*NA*	*NA*	DIA Orbi-q-Orbi	60	*ND* LFQ

Both microsample and standard blood sample types were listed, and additional details were described for only for microsamples, including the volume of blood used in the microsample if measured precisely, the punch size (DBS samples only), and the microsample collection device. Any sample preparation steps outside of reduction/alkylation, digestion and sample cleanup were reported. The acquisition strategy was reported. For untargeted studies, the effective gradient was described, defined as the length of time from 2 to 5% organic solvent to 20–45% organic solvent where peptides were actively eluting from the column during reverse-phase high-performance liquid chromatography. Throughput described the maximum number of proteins and/or peptides identified based on analysis of microsamples. Quantification strategies were specified and the LLOQ was reported if assessed

DBS: dried blood spot; DDA: data-dependent acquisition; DIA: data-independent acquisition; DPS: dried plasma spot; DSS: dried serum spot; IT: ion trap analyzer; LLOQ: lower limit of quantification; LQF: label-free quantification; MRM: multiple reaction monitoring; MTBE: methyl tert-butyl ether; NA: not applicable; NBS: newborn dried blood spot; ND: not described; Orbi: Orbitrap analyzer; PAR: peak area ratio; PASEF: parallel accumulation—serial fragmentation; PRM: parallel reaction monitoring; q: quadrupole isolation; SIS: stable isotope-labeled standard; SRM: single reaction monitoring; SWATH-MS: Sequential Windowed Acquisition of all Theoretical Mass Spectra; TOF: time-of-flight analyzer; VAMS: volumetric absorptive microsampling

has the advantage of removing hemoglobin and the quantification issues described above, however the removal of the whole blood component also excludes the analysis of proteins from blood cells. Whether these proteins are of interest depends on the goal of the experiment and their relevance regarding disease pathology.

Insights from proof-of-concept studies within microsampling proteomics

The 26 papers categorized as proof-of-concept research described development and validation of proteomics methods in the context of microsampling, but without the inclusion of disease or experimental groups. The studies were described in Table 2, and were almost evenly split with regards to acquisition strategy, with 13 (50%) studies using untargeted acquisition, 11 (42%) using targeted, and 2 (8%) using both. The most popular microsample was DBS, with 16 (62%) studies using DBS as the sole microsample type. These samples are low cost as well as easy to collect and store, making them widely used within microsampling applications, also outside of a proteomics context [6]. Likely due to the precise sample volume, VAMS devices were also common, and were the sole microsample type in 5 (19%) studies. Many studies compared one type of microsample to a traditional blood sample such as plasma or serum. This is important to establish the comparability between microsampling and traditional blood sampling, but does not clarify the advantages and disadvantages of different microsample types. One study compared multiple microsamples of the same type, identifying 253 proteins in DBS samples compared to 186 and 196 in dried plasma or serum spots, respectively, with an overlap of 96 proteins [28]. Although some studies used multiple microsample types, no studies compared microsamples of different types directly, such as a comparison between DBS and VAMS, so whether one type of microsample is more suited for proteomics cannot currently be determined. A few studies also used custom microsampling devices [37, 39]. These devices can be used to demonstrate a new sampling method or address a very specific sampling issue, however these studies cannot be readily replicated by other laboratories, and it is difficult to directly compare the results of these studies to others. Many types of microsamples exist, and only a few of them were seen in the studies included in this review. For the interested reader, a comprehensive overview of microsampling types can be found in Baillargeon et al. [6].

The microsample collection strategy varied greatly among studies. In an ideal use case, microsamples are obtained through a finger prick which replaces the more invasive blood draw. However, 14 (54%) studies included microsamples from venous blood, where microsamples were produced from an aliquot of whole blood obtained through a traditional blood draw. Although not the intended scenario for microsampling, these mimics of microsampling techniques are important for establishing this new area of research and providing proof-of-principle of microsample proteomics. Of the 16 studies which only used DBS microsamples, five placed an aliquot of venous blood onto the DBS card, often with a pipette at a predetermined sample volume. Seven studies used capillary blood collected through a finger prick, while four studies used multiple blood collection methods. Capillary and venous blood are not identical in composition, therefore this affects the comparability of studies [40]. In general, there was a paucity of information regarding blood collection. Most studies which used finger prick-derived samples did not specify whether the sample was taken by trained personnel or the subject themselves, a crucial detail. Details regarding the sampling procedure, such as the DBS card used or volume of blood added, were also occasionally omitted. All of the above details are important for comparison of results between different studies and sampling procedures should be described in full. The heterogeneity in the types of microsample and blood collection methods used also reflects the varied purposes of the included studies. For example, the goal of one study was to assess the potential of a 60-biomarker health surveillance panel, therefore VAMS devices were used to allow study subjects to precisely sample a 10 µL volume of their own capillary blood [30]. Another study used 50 µL of venous or capillary blood pipetted onto DBS filter paper to validate a targeted assay for > 200 proteins by testing linear range, repeatability, stability, and other parameters [41]. Whether a user-friendly sampling strategy is a priority depends on the goal of the experiment, and must be balanced with the reality of scientific research and the need for precise quantification. As mentioned previously, Whelan et al. was a proof-of-concept study able to demonstrate the highest proteome coverage, at 4661 proteins from VAMS devices containing either venous or capillary blood as well as plasma [30]. Another proof-of-concept study detected 1,997 proteins using data-independent acquisition with gas-phase fractionation and sodium carbonate precipitation, which removed most of the hemoglobin along with other hydrophilic, high-abundant proteins [42]. Several targeted studies assessed the lower limit of quantification (LLOQ), with a few studies demonstrating sensitivity down into the nano-molar range and a single study demonstrating an LLOQ of 0.2 fmol [41]. Therefore, protein detection in microsamples can be optimized for either highly specific and sensitive protein quantification on par with plasma and serum samples.

Of the 26 proof-of-concept studies, 18 (69%) included description of parameters for assay characterization and validation, including precision, stability, comparison between sample types, etc., summarized in Table 3. Many studies used some form of the coefficient of variation (CV), which can be calculated at different analytical steps to determine separate types of assay precision. Precision was comparable to what can be achieved

with plasma or serum, with most studies reporting CVs from 10 – 30%. Although this level of precision is often acceptable for research purposes, routine clinical analysis requires much lower CVs [43]. For example, Henderson et al. designed an assay to evaluate HbA1C and apolipoproteins B and A-1 to replace measurements of lipoprotein particles. The assay used DBS for parallel reaction monitoring with stable isotope-labeled standard peptides and a single-point calibrator for HbA1c to achieve CVs of 10–11%. However, this could not compete with current routine analysis, where nephelometry was used to measure apolipoproteins B and A-1 and liquid chromatography with UV detection was used to measure HbA1c, resulting in CVs ranging from 2 to 5%. A higher bias was also observed in DBS samples compared to plasma [44]. An instance where microsample proteomics could prove to be more appropriate is the use of DBS for detection of doping in competitive sports, and the World Anti-Doping Agency published a technical document regarding the collection, storage, and testing of DBS samples in 2021 [45]. Several proof-of-concept papers included in this review investigated the use of proteomics within this area, primarily to determine cell type for detection of immature reticulocytes, a cell population which expands upon blood doping. These targeted methods demonstrated CVs of less than 15% and proteins were stable for up to 29 days at room temperature, which was well-suited for doping testing, where both frequent testing and shipment to an accredited analysis facility is necessary [46, 47].

Protein stability was tested at a range of temperatures and time durations, and proteins were generally found to be stable in microsamples short-term with peptide-specific differences. A notable exception was the measurement of HbA1c, where glycation increased already after three days at room temperature, suggesting ex vivo glycation in the microsample [48]. Chambers et al. tested a maximum storage duration of 154 days at several temperatures (-20 – 37 °C) for over 160 peptides. Nearly all measured peptides were stable after two days, however after 154 days, approximately half of the peptides remained stable regardless of temperature [49]. Therefore, stability must be tested separately for each assay to ensure that the target peptide is stable with the specific collection and storage method applied.

The agreement between traditional blood samples and microsamples is also an important factor to consider. Since DBS samples are dried whole blood, it is not surprising that the similarity between these two sample types has been confirmed in several studies, with an estimated protein overlap of 96% by one study [50–52]. Although protein abundances were correlated between DBS and whole blood, protein abundances were on average higher in DBS samples compared to whole blood, suggesting that peptide-specific correction factors could be used to directly convert between DBS and whole blood concentrations [41, 49]. Protein similarity between DBS and plasma or serum was lower at 32%, reflecting the removal of the cellular components of blood during production of plasma and serum [51]. Despite this, good correlation between protein abundances in DBS and serum has been demonstrated in medium- and high-abundant proteins [53]. These proof-of-concept studies have established that high proteome coverage and sensitive protein detection is attainable in microsamples despite the prevalence of hemoglobin from erythrocytes. These sample types are also well-suited for research purposes since precision comparable to plasma was achieved. Although these studies did not include experimental groups, many studies were aware of the clinical implications of microsamples and laid important groundwork for clinical proteomics research.

Microsampling applications in a clinical proteomics context

Clinical research included 38 studies, where a disease or experimental group was investigated in the context of microsample proteomics. These studies were tabulated in Table 4. With regards to acquisition method, 15 (39%) studies used untargeted acquisition, 22 (58%) studies used targeted acquisition, and one (3%) study used both. This trend contrasted with the results for proof-of-concept research, where a relatively even split was seen between targeted and untargeted acquisition methods (Table 2). The increase in proportion of targeted studies in clinical research reflects the different purpose of the studies within this research category, since much of clinical proteomics is focused on developing assays to quantify known protein targets. The earliest two studies included in this review, from 2007 and 2008, were targeted clinical assays for quantification of peptides from high-abundant proteins, demonstrating that clinical applications were included in microsampling proteomics research from its inception [54, 55].

Microsampling practices in clinical research overwhelmingly favored DBS samples, with 26 (68%) studies using DBS and three (8%) studies using newborn DBS (NBS) samples as the sole microsample. Two studies used dried plasma spots, three studies used VAMS devices, and four studies used multiple types of microsample. The dominance of DBS and NBS samples likely reflects the wide availability and low cost of these sampling types. In many countries, NBS samples are collected in newborn screening programs, and these samples can occasionally be accessed for secondary research purposes

Table 3 Table of technical parameters from 18 studies categorized as proof-of-concept research

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Precision	Other parameters
Chambers [50] *2013b*	DBS *- 15 µL* *- Entire spot* *- 903 Protein Saver (Whatman)*	Venous blood deposited onto DBS	Avg CV 6.4%, < 15% for all peptide targets	- High correlation between DBS and WB samples with an R^2 of 0.97 for 33 proteins - 35/38 peptides were stable after 5 days of storage at varying temperatures (– 20/4/37 °C), most were stable after 10 days - Linear dynamic range observed for 40/44 peptide targets
Chambers [49] *2015*	DBS *- 15 µL* *- Entire spot* *- 903 Protein Saver (Whatman)*	Venous blood deposited onto DBS	- Intra-assay precision < 20% for > 97% of peptides - Inter-assay precision < 20% for 83–91% of peptides	- Peptide-specific correction factors necessary to compare WB and DBS - 52–66% of peptides stable after 154 days - Peptide stability not affected by temperature - 89–97% of peptides were within 20% abundance when comparing fresh and frozen samples
Geyer [39] *2016*	Plasma *- 1 µL* *- Custom pipette tip-based centrifugal devices*	Finger prick	- Intra-assay variability 67% of proteins with CV < 20% - Analytical variability 71% of proteins with CV < 20% - Intra-individual variability 55% of proteins with CV < 20% - Inter-individual variability 19% of proteins with CV < 20%	*NA*
Cox [46] *2017a*	DBS *- 20 µL* *- Entire spot* *- DMPK-C (Whatman)*	Venous blood deposited onto DBS	- Intra-assay precision was ≤ 15.9% CV at varying Hct levels - Intra-individual variation of CD71 measurements over 8 weeks was 17.1–38.7%	- Higher recovery observed for DBS compared to WB - Assay was stable at RT for 9–15 days
Henderson [44] *2017*	DBS *- 65 µL* *- 1 × 3.2 mm* *- 903 Protein Saver (Whatman)* Plasma *- 10 µL*	Finger prick by trained personnel Venous blood deposited onto DBS	Total CV of HbA1c quantification was 11% after adding an single point calibrator	- Stable for 14 days up to a temperature of 25 °C - Linearity was ≥ 0.96 for all three proteins
Ozcan [53] *2017*	DBS *- 1 × 3 mm* *- 903 Protein Saver (Whatman)* Serum *- 5 µL*	Finger prick	Median CV was 13.2% in DBS and 8.8% in serum	High correlation between DBS and serum (r = 0.72, rho = 0.80)

Table 3 (continued)

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Precision	Other parameters
van den Broek [88] *2017*	Plasma VAMS *- 10 µL* *- Mitra (Neoteryx)*	VAMS dipped into venous blood	- Intra-day precision 3.2–10.4% CV - Inter-day precision 3.4–12.6% CV - Intra-operator precision 4.2–9.3% CV	- Peptides within 15% deviation after storage at -80 °C for 22 weeks - Avg. extraction recovery 100.3–111.8%
Forchelet [37] *2018*	Microsample *- 20–25 µL resulting in 2 µL sample* *- Custom microfluidic device* Plasma Serum	Finger prick	Median CV of chip-separated sample was 7.5%, compared to 10.9%/12.2% for plasma/serum when measuring 43 FDA-approved proteins	*NA*
Vidova [52] *2019*	DBS *- 1 × 3 mm* *- 903 Protein Saver (Whatman)* Serum WB	Finger prick	- CV was < 16.4% for DBS and < 10.2% for WB - Intra-day precision was < 16.4% while inter-day precision was < 14.3%	- Correlation coefficient between DBS and WB was 0.9973 - Correlation to immunonephelometry was high (R^2 = 0.7581–0.9987)
Eshgi [41] *2020*	DBS *- 50 µL* *- 5 × 6 mm* *- 903 Protein Saver (Whatman)* WB	Venous and capillary blood deposited onto DBS	- Median CV at LLOQ was 10% (3–20%) in 298 assays - 193/243 proteins had total CV < 20%	- Mean 1.26-fold (0.5–6.2) higher protein abundance in DBS samples compared to WB - 283/294 assays were stable at 4 weeks of storage at -80 °C or 2 freeze-thaws or 43 h in autosampler (10 °C), 95% proteins stable for 57 days at RT/-20 °C, 28 days at 40 °C - 225/258 assays exhibited parallelism
Nakajima [42] *2020*	DBS *- 2 × 3.2 mm* *- Blood sampling paper (Advantec)*	Finger prick	Pearson correlation coefficients ≥ 0.94 for 6 replicates	*NA*
Cox [47] *2021*	DBS *- 20 µL* *- Entire spot* *- DMPK-C (Whatman)*	Venous and capillary blood deposited onto DBS	- Intra-assay precision 6–14% - Inter-assay precision 5–15%	- No significant difference between venous blood and capillary blood collected using a TASSO device - Venous blood and capillary blood collected using a TAP device showed a small deviation for two proteins - Proteins were stable for 29 days at RT - Matrix interference was < 20% for samples with Hct 25–55%
Mc Ardle [93] *2022*	Plasma VAMS *- 10 µL* *- Mitra (Neoteryx)*	VAMS dipped into venous blood	- 87% of VAMS proteins had an inter-day CV < 30% using high-throughput, 78% mid-throughput	*NA*

Table 3 (continued)

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Precision	Other parameters
Whelan [30] *2023*	Plasma VAMS *- 10 µL* *- Mitra (Neoteryx)*	Finger prick, self-sampled VAMS dipped into venous blood	- In all sample types, CVs for the MRM assay were < 20% - Of the 122 FDA biomarkers detected with DIA, 30—38 of these markers had a CV < 20%	*NA*
Brockbals [35] *2024*	DBS *- 20 µL* *- 1 × 3 mm* *- 903 Protein Saver (Whatman)*	Venous and capillary blood deposited onto DBS	- Intra-day relative standard deviation was ≤ 15% for all peptides except one	- Samples were stable for 3 months at – 20 °C - 4 peptides were observed to have some carryover
Richard [95] *2024*	DBS *- 20 µL* *- 4 × 6 mm* *- 903 Protein Saver (Whatman)*	Finger prick	- Over 140 days, the median intra-individual relative standard deviation was 19.5%	*NA*
Shen [96] *2024*	VAMS *- 10 µL* *- Mitra (Neoteryx)*	Finger prick, self-sampled	Median CV 0.397 (0.149–1.728)	*NA*
Sun [51] *2024*	Blood cells *- 100 µL* DBS *- 100 µL* *- Entire spot* *- FTA card (Qiagen)* Plasma *- 100 µL* WB *- 100 µL*	Venous blood deposited onto DBS	*NA*	- 96% percent overlap in proteins identified in WB and DBS, 32% overlap between DBS and plasma, 26/591 significantly altered proteins in DBS compared to WB - ~ 4% out of 498 proteins were unstable in RT storage for 6 months

Both microsample and standard blood sample types were listed, and additional details were described for only for microsamples, including the volume of blood used in the microsample if measured precisely, the punch size (DBS samples only), and the microsample collection device. Evaluations of assay precision were described as well as other technical parameters assessed in the studies including correlations between sample type, peptide stability, extraction efficiency, linearity, etc.

C: Celsius; CV: coefficient of variation; DBS: dried blood spot; DIA: data-independent acquisition; Hct: hematocrit; hrs: hours; LLOQ: lower limit of quantification; RT: room temperature; VAMS: volumetric absorptive microsampling; WB: whole blood

Table 4 Descriptive and result table of 38 studies categorized as clinical research

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Notable sample preparation	Acquisition method		Effective gradient length *(min)*	Throughput Quantification
				Targeted	*Untargeted*		
Daniel [54] *2007*	DBS *- 35 µL - 1 × 3.2 mm - 903 Protein Saver (Whatman)* WB	Venous blood deposited onto DBS	*NA*	MRM	*NA*	*NA*	2 proteins (6 peptides) Peptide ratios, with correction by reference reagent
deWilde [55] *2008*	DBS *- 1 × 3 mm - 903 Protein Saver (Whamtan)*	*ND*	*NA*	MRM	*NA*	*NA*	1 protein (1 peptide) Calibration curve with SIS peptides *LLOQ: 7 mg/L (ceruloplasmin)*
Razavi [33] *2016*	DBS *- 1 x ¼ in - 903 Protein Saver (Whatman)*	Finger prick, self-sampled	SISCAPA	MRM	*NA*	*NA*	22 proteins (22 peptides) PAR relative to SIS peptides
Cooper [61] *2017*	NBS Serum	Newborn heel prick	*NA*	MRM	*NA*	*NA*	96 proteins (152 peptides) PAR relative to SIS peptides
Cox [98] *2017b*	DBS *- 20 µL - Entire spot - DMPK-C (Whatman)*	Venous blood deposited onto DBS	Buffer washing to enrich membrane proteins	PRM	*NA*	*NA*	2 proteins (2 peptides) PAR relative to SIS peptides
Jung [69] *2017*	DBS *- 70 µL - 20 × 3 mm - 903 Protein Saver (Whatman)*	Venous blood deposited onto DBS	Immunoaffinity enrichment	SRM	*NA*	*NA*	1 protein (1 peptide) PAR relative to SIS peptides *LLOQ: 27 pmol/L (ATP7B)*
Moat [70] *2017*	NBS *- 1 × 3.2 mm - 226 Spot Saver Cards (PerkinElmer)*	Newborn heel prick	*NA*	MRM	*NA*	*NA*	8 proteins (12 peptides) Peptide ratios
Yu [99] *2017*	DBS *- 1 × 3.2 mm - 903 Protein Saver (Whatman)*	Venous blood	*NA*	MRM	*NA*	*NA*	2 proteins (4 peptides) PAR relative to SIS peptides
Collins [67] *2018*	DBS *- 70 µL - 17 × 3 mm - 903 Protein Saver (Whatman)* NBS *- 5–6 × 3 mm*	Newborn heel prick Venous blood deposited onto DBS	Immunoaffinity enrichment	SRM	*NA*	*NA*	3 proteins (5 peptides) PAR relative to SIS peptides *LLOQ: 0.69 fmol (CD3-ε)*
Nieman [75] *2018*	DBS *- 903 Protein Saver (Whatman)*	Finger prick	*NA*	*NA*	DIA	*ND*	593 proteins LFQ
Collins [68] *2020*	DBS *- 70 µL - 2 × 6.35 mm - 903 Protein Saver (Whatman)*	Finger prick Venous blood deposited onto DBS	Immunoaffinity enrichment	SRM	*NA*	*NA*	7 proteins (8 peptides) PAR relative to SIS peptides *LLOQ:* 1.9 fmol (BTK)

Table 4 (continued)

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Notable sample preparation	Acquisition method		Effective gradient length *(min)*	Throughput Quantification
				Targeted	*Untargeted*		
Han [60] *2020*	DBS *- 1 × 3 mm - 226 Spot Saver Cards (PerkinElmer)*	Finger prick, self-sampled	*NA*	MRM	*NA*	*NA*	115 proteins (194 peptides) PAR relative to SIS peptides
Kaiser [38] *2020*	DPS *- 250 µL - 1.7 cm × 1.5 cm - Custom blood collection device* Plasma	Venous blood deposited onto DBS	Immunoaffinity depletion	MRM	*NA*	*NA*	2 proteins (2 peptides) PAR relative to SIS peptides
Knab [74] *2020*	VAMS *- Mitra (Neoteryx)*	Finger prick	*NA*	MRM	*NA*	*NA*	12 proteins PAR relative to SIS peptides
Nieman [59] *2020*	DBS *- 1 × 4 mm - Protein Saver 903 (Whatman)*	Finger prick, self-sampled	*NA*	*NA*	DIA Orbi-q-Orbi	40	712 proteins LFQ
Norris Bradley [76] *2020*	DPS *- 180 µL WB - 3 x ¼ in - AdvanceDx - Boston Microfluidics* Plasma/Serum *- 20 µL*	Finger prick, self-sampled Venous blood deposited onto DBS	*NA*	SRM	*NA*	*NA*	1 protein (5 peptides) PAR relative to SIS peptides
Collins [100] *2021*	DBS *- 70 µL - 1 × 6.35 mm - Protein Saver 903 (Whatman)*	Blood deposited onto DBS Finger prick	Immunoaffinity enrichment	SRM	*NA*	*NA*	4 proteins (5 peptides) PAR relative to SIS peptides *LLOQ: 7.14 pmol/L (ATP7B 887)*
Costa [101] *2021*	NBS *- ~ 25 mm^2 - Guthrie cards*	Newborn heel prick	*NA*	*NA*	DDA Orbi-q-IT	90	245 proteins LFQ
Kashirina [62] *2021*	DBS *- 40 µL - Entire spot*	Capillary blood deposited onto DBS	*NA*	*NA*	DIA PASEF	*ND*	1256 proteins LFQ
Lai [78] *2021*	DBS *- 60 µL - 1 × 3 mm - Protein Saver 903 (Whatman)*	Venous blood deposited onto DBS	*NA*	SRM	*NA*	*NA*	3 proteins (3 peptides) PAR relative to SIS peptides *LLOQ: 3.13 µg/mL (C1q)*
Bassini [34] *2022*	DBS *- 1 x ¼ in - Protein Saver 903 (Whatman)*	Finger prick	SISCAPA	MRM	*NA*	*NA*	MRM: 11 proteins (11 peptides) PAR relative to SIS peptides
Lima [48] *2022*	VAMS *- 10 µL - Mitra (Neoteryx)*	VAMS dipped into capillary blood VAMS dipped into venous blood	*NA*	*NA*	DIA Orbi-q-Orbi	19	DIA: LFQ, peptide ratios

Table 4 (continued)

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Notable sample preparation	Acquisition method		Effective gradient length *(min)*	Throughput Quantification
				Targeted	*Untargeted*		
Molloy [57] *2022*	DBS *- Entire spot - FTA DMPK-C (Whatman)* VAMS *- 30 µL - Mitra (Neoteryx)*	VAMS dipped into venous blood Venous blood deposited onto DBS	Washing with pulse centrifugation to remove high-abundant proteins	*NA*	DDA DIA Orbi-q-Orbi	90	DDA: 1642 proteins DIA: 1892 proteins LFQ
Nimer [65] *2022*	DBS *- 5 × 3.2 mm - 226 Spot Saver Cards (PerkinElmer)*	Venous blood deposited onto DBS	NA	MRM	*NA*	*NA*	1 protein (2 peptides) PAR relative to SIS peptides *LLOQ: 2 nM (both peptides)*
Zhang [66] *2022*	DBS *- 70 µL - 3 × 3.2 mm - Protein Saver 903 (Whatman)*	Finger prick Newborn heel prick Venous blood deposited onto DBS	Immunoaffinity enrichment	SRM	*NA*	*NA*	2 proteins (4 peptides) PAR relative to SIS peptides *LLOQ: 18.5 pmol/L (acid alpha-glucosidase)*
Iurașcu [77] *2023*	DBS *- 3 × 3.1 mm - CentoCard (Centogene GmbH)*	Venous blood deposited onto DBS	*NA*	MRM	*NA*	*NA*	2 proteins (2 peptides) PAR relative to SIS peptides *LLOQ: 11 nM (C4)*
Kashirina [102] *2023*	DBS *- Entire spot - 903 Protein Saver (Whatman)*	Finger prick	*NA*	*NA*	DIA PASEF	40	1219 proteins (7854 peptides) LFQ
Nieman [103] *2023*	DBS *- 1 × 4 mm - 903 Protein Saver (Whatman)*	Finger prick	*NA*	*NA*	DIA Orbi-q-Orbi	*ND*	725 proteins LFQ
Pastuhkova [104] *2023*	DBS *- Entire spot*	Finger prick	*NA*	*NA*	DIA PASEF	40	1239 proteins (6798 peptides) LFQ
Ren [79] *2023*	DBS *- 1 × 1.5 mm - 903 Protein Saver (Whatman)*	Venous blood	*NA*	MRM	DDA Orbi-q-IT	*ND*	DDA: 911 proteins LFQ MRM: 6 proteins (6 peptides) PAR relative to SIS peptides *LLOQ: 0.03 pmol/mL (hemoglobin ζ)*
Smith [105] *2023*	DBS *- 2 × 3 mm - 903 Protein Saver (Whatman)*	Venous blood deposited onto DBS	*NA*	*NA*	DDA	107	550 proteins LFQ
Vialaret [72] *2023*	DBS *- 1 × 6 mm - TFN Specimen Collection Card (SpotToLab)* Plasma	Capillary blood	*NA*	MRM	*NA*	*NA*	62 proteins PAR relative to SIS peptides

Table 4 (continued)

First author Year	Sample types *Microsample details:* - Volume of blood - Punch diameter or area (DBS only) - Collection device (manufacturer)	Microsample collection method	Notable sample preparation	Acquisition method		Effective gradient length *(min)*	Throughput Quantification
				Targeted	*Untargeted*		
Guedes [82] *2024*	DBS *- Entire spot*	Venous blood	*NA*	*NA*	DDA Orbi-q-Orbi	90	151 proteins (3953 peptides) 972 modified peptides LFQ
Kashirina [106] *2024*	DBS	Finger prick	*NA*	*NA*	DIA PASEF	*ND*	696 proteins LFQ
Shibata [107] *2024*	DBS/NBS *- 1 × 3.2 mm - Guthrie cards*	*ND*	Sodium carbonate precipitation to reduce soluble proteins	*NA*	DIA Orbi-q-Orbi	80	2912 proteins LFQ
Klippel [71] *2025*	DBS/NBS	*ND*	Immunoaffinity enrichment	SRM	*NA*	2.4	4 proteins (6 peptides) PAR relative to SIS peptides *LLOQ: 40.2 pmol/L (ATP7B)*
McMahon [108] *2025*	VAMS *- 30 µL - Mitra (Neoteryx)*	VAMS dipped into whole blood cell pellets	*NA*	*NA*	DIA Orbi-q-Orbi	62.5	3913.8 proteins (mean per sample) LFQ
Monteagudo-Vilavedra [109] *2025*	DBS	*ND*	*NA*	*NA*	DIA SWATH-MS	30	*ND* LFQ

Both microsample and standard blood sample types were listed, and additional details were listed for only for microsamples, including the volume of blood used in the microsample if measured precisely, the punch size (DBS samples only), and the microsample collection device. Any sample preparation steps outside of reduction/alkylation, digestion and sample cleanup were reported. The acquisition strategy was reported. For untargeted studies, the effective gradient was described, defined as the length of time from 2 to 5% organic solvent to 20–45% organic solvent where peptides were actively eluting from the column during reverse-phase high-performance liquid chromatography. Throughput described the maximum number of proteins and/or peptides identified based on analysis of microsamples. Quantification strategies were specified and the LLOQ was reported if assessed

DBS: dried blood spot; DDA: data-dependent acquisition; DIA: data-independent acquisition; DPS: dried plasma spot; IT: ion trap analyzer; LLOQ: lower limit of quantification; LQF: label-free quantification; MRM: multiple reaction monitoring; MTBE: methyl tert-butyl ether; NA: not applicable; NBS: newborn dried blood spot; ND: not described; Orbi: Orbitrap analyzer; PAR: peak area ratio; PASEF: parallel accumulation—serial fragmentation; PRM: parallel reaction monitoring; q: quadrupole isolation; SIS: stable isotope-labeled standard; SISCAPA: stable isotope standards and capture by anti-peptide antibodies; SRM: single reaction monitoring; SWATH-MS: Sequential Windowed Acquisition of all Theoretical Mass Spectra; TOF: time-of-flight analyzer; VAMS: volumetric absorptive microsampling

[56]. One study compared DBS and VAMS samples, and found that although fewer proteins were detected with DBS compared to VAMS, high protein coverage could be achieved in both sample types by washing in lithium chloride buffer with pulse centrifugation to remove high-abundant proteins [57]. Sample collection methods were also highly heterogeneous for clinical research studies. Most studies exclusively analyzed capillary blood (16, 42%), while 13 papers (34%) studied venous blood, five (13%) studies included both, and four studies did not provide information. Clinical proteomics research with microsamples is still at an early stage where the standard blood draw is often included in the study design, either for comparison with serum or plasma or for production of microsamples in the laboratory. Of the 26 studies which used DBS samples, ten of the studies used finger pricks for sample collection while seven studies deposited venous blood onto the DBS paper. The remaining studies used a combination of collection methods or did not describe sample collection in detail. As was the case for proof-of-concept research, sampling practices for clinical research were varied and not always adequately described.

The experimental groups included in the 38 clinical research studies were highly diverse, demonstrating that microsamples were used for a wide range of applications within proteomics research. Many of the disease groups were relevant for microsamples specifically, either due to difficulties regarding sample collection or the disease itself. As an example, hemoglobinopathies, including

sickle cell disorder and thalassemias, were the most common disease group studied (Fig. 1D, Supplementary Table 6). Hemoglobin can be easily detected in whole blood microsamples without complex sample preparation procedures, since erythrocytes, primarily containing hemoglobin, comprise circa 55% of whole blood [58]. Microsampling was also used for applications where standard blood sampling was difficult. One study followed an athlete over 28 weeks, including an 8 week Antarctic trek, and could demonstrate an upregulation of immune system processes during the trek, including proteins associated with complement activation and leukocyte cells. Microsampling enabled the athlete to collect and store their own DBS samples while on the expedition, without the need for heavy or specialized equipment [59]. Many psychiatric diseases lack established biomarkers, and the stigma of these illnesses makes diagnosis difficult. Han et al. used home testing kits and a digital questionnaire to detect depression, resulting in a cohort of nearly 300 participants and prediction models with an average test AUC of 0.80 ± 0.01 [60]. Another study used NBS samples to identify prognostic proteins associated with the development of schizophrenia in adulthood [61]. Four studies were intervention studies, where the effects of an intervention on the blood proteome were explored, including an experiment where healthy women were exposed to three days of dry immersion to simulate the effects of microgravity [62].

The implementation of proteomics into established newborn screening programs was also investigated. Screening programs already use triple quadrupole mass spectrometers to detect small molecules associated with inborn errors of metabolism, therefore additional proteomics assays would not require the purchase of new, expensive equipment and can be integrated into already-established workflows [63, 64]. Several studies included in this review developed and validated methods which could potentially be incorporated these programs, such as assays for Duchenne muscular dystrophy [65], Pompe disease [66], primary immunodeficiency disorders [67, 68], Wilson disease [55, 69], and β-thalassemia [54]. This included a significant use case of microsample proteomics in current clinical practice, where multiple reaction monitoring was used to screen over 100,000 NBS samples for hemoglobin variants for detection of sickle cell disorder, resulting in the detection of ten cases [70]. In another study, over 30,000 NBS samples were screened for Wilson disease and three inborn errors of immunity, demonstrating that a single assay can easily be multiplexed to detect multiple diseases [71]. The ease of microsampling also makes these sample types ideal for longitudinal disease monitoring, where baseline samples from participants can function as control samples. One relatively straightforward instance is the detection of acute phase proteins, which are well-characterized and increase tremendously in abundance during inflammatory events. One study quantified inflammatory and nutritional markers in an elderly cohort, a population where frequent travel to a hospital or clinic for testing can be prohibitive [72, 73]. Razavi et al. also demonstrated that a panel of 22 relevant biomarkers, including C-reactive protein and serum amyloid A-1, could be used to detect inflammatory events such as pneumonia, colds, and respiratory infections [33]. Longitudinal sampling was also used in the context of monitoring training distress in professional athletes [74, 75].

New advances within clinical research were defined as papers published in 2020 or later and included 28 papers (Table 4), comprising 74% of all clinical research described in this review. This indicates the large growth of microsample use for proteomics in recent years, especially within a clinical context. This increase in interest could indicate that proteomics biomarkers based on microsamples will soon appear more broadly in the clinic, however, presumed biomarkers must be well-characterized and carefully evaluated before use [23]. Among the 28 studies, several described biomarker assays in various stages of development, mostly within the context of disease screening. For instance, primary immunodeficiency disorders are an obvious choice for screening, since early detection allows relevant treatment and prevents serious infections. Collins et al. designed a multiplex single reaction monitoring assay for eight peptides to test for five primary immunodeficiency disorders. The assay was designed with screening in mind, with a run time of only 2.5 min, and importantly, diagnostic results were in agreement when analyzed by two separate laboratories [68]. One study investigated the ability of single reaction monitoring to replace genotyping of apolipoprotein L1 protein variants, and optimized an assay for both liquid and dry plasma which demonstrated full agreement with Sanger sequencing [76]. Two recent studies focused on hereditary angioedema, a disease where delayed diagnosis and a lack of treatment can worsen symptoms and increase morbidity. The targeted assays were developed to quantify complement components, which are dysregulated due to deficient or defective C1 esterase inhibitor. The multiplexed format allowed multiple complement components to be measured simultaneously, in contrast with the current standard for diagnosis, enzyme-linked immunosorbent assay. Correlation to enzyme-linked immunosorbent assay measurements in plasma were high, although absolute measured concentrations differed, since the presence of erythrocytes diluted the analytes. The assay in combination with genetic sequencing for confirmation

could be used for two-tier screening [77, 78]. An important aspect of emerging biomarkers in ongoing validation. Four clinical studies included validation cohorts, with two of the studies was published in 2020 or later [61, 67, 79]. Therefore, although an abundance of new research within this field has been produced, independent validation cohorts were almost completely absent.

Discussion

This scoping review found that microsamples were used for a myriad of proteomics applications based on 64 included papers. Most of the studies were clinical proteomics research, although a wide range of methods and purposes were seen. The review also found that the use of microsamples within proteomics has grown quickly, and over half of the included studies were published in 2020 or later. Despite this increase in use, issues surrounding sampling methodology, standardization, and validation have not yet been resolved, which remains the main barrier for implementation of microsamples as a mainstay of clinical proteomics. For proof-of-concept research, sample procedures and assay characteristics were described, including validation of these new sampling methods. Comparison to standard blood samples such as plasma or serum, microsample stability, and assay precision were assessed by many of the studies. Within clinical research, sample procedures and experimental groups were described to provide an overview of current clinical applications within microsampling proteomics. The earliest published papers which combined microsampling and proteomics were targeted assays for diagnosis of β-thalassemia and Wilson disease, indicating a strong foundation for these sampling methods within clinical proteomics, especially for disease screening [54, 55]. The broad range in study aims and disease groups revealed that microsamples were widely applied for both targeted and untargeted experiments and in a diverse array of clinical contexts, often in disease groups or circumstances where standard blood sampling was challenging, or where longitudinal sampling was necessary.

Most studies used either DBS or VAMS microsampling, which results in dry samples that require an additional extraction step to solubilize proteins, thereby increasing the length of time for sample preparation. The extraction step was often performed in ammonium bicarbonate buffer, since this buffer is compatible with trypsin digestion. Proper extraction is crucial to ensure that the full proteome is represented in the sample without bias, and can be affected by several factors, including the type of filter paper [20]. Many different types of DBS filter paper exist, and the studies included here mostly used 903 Protein Saver cards (Whatman), although other DBS samples, such as FTA cards (Qiagen) and 226 Spot Saver cards (PerkinElmer), were also applied (Tables 2 and 4). DBS samples are continuously evolving, and new sample types could prove useful for proteomics. For example, HemaSpot SE cards (Spot On Sciences) automatically separate the cellular and soluble blood components [80]. However, these newer DBS samples were investigated in studies outside the scope of this review, for example metabolomics studies, and were therefore not included here. Overall, the included studies were very heterogeneous regarding sample collection procedures. Many factors regarding sample collection can be safely assumed to influence final assay precision and accuracy, including whether blood is spotted volumetrically or dripped without measurement, whether the sample is of capillary or venous origin, or whether samples are collected by the participant or trained personnel [6]. Some studies were far removed from microsampling in a clinical context, for example where venous blood was volumetrically spotted onto filter paper [41, 47, 49]. Other studies were on the opposite end of the spectrum, where remote sampling kits were sent to participant's homes [33, 59, 60]. This meant it was difficult to generalize some results to a clinical situation, and also reduced comparability between studies. Comparison between studies was also rendered difficult due to a lack of information regarding sample collection in some studies, where the sampling procedure, microsampling device, or other crucial information was omitted. This lack of sampling standardization is not unexpected in a new field, and the most suitable sampling procedure can also depend on the experimental goal. However, the field currently lacks best practice guidelines for reliable and reproducible sample collection, similar to guidelines which exist for newborn screening [81]. These guidelines should be designed specifically for MS-based proteomics and include reporting recommendations to make sure all sample collection steps are well documented.

The hematocrit bias was addressed using different strategies and to varying degrees by the included studies. A common practice was to use a pipette to spot a known volume of blood onto a DBS sample and subsequently extract protein from the entire blood spot, ensuring that identical blood volumes were analyzed despite varying hematocrit. Some studies also used a punch to remove subsamples from DBS samples, but they did not address the issue of unknown blood volumes, even when adding external standards for absolute quantification. Two studies developed internal normalization methods to

take the hematocrit bias into account [33, 34]. However, the degree to which the hematocrit bias affects protein abundances specifically has not been systematically studied and this is a clear topic for future research. The hematocrit bias has consequences for both absolute and relative quantification if not properly accounted for, since varying hematocrit means that proteins from the cellular and soluble components of whole blood will be present in varying proportions. Unknown blood volumes can be addressed through volumetric microsampling such as VAMS devices, which sample a precise volume of blood and are therefore well-suited for studies where high precision and accuracy are required. These sample types were the second most common sampling method in the studies included in this review. For studies where semi-quantitative, relative, or qualitative results are sufficient, the cheaper DBS samples could suffice. This includes diseases resulting in protein deficiency or absence, which only requires a confident lack of detection, or variant-specific peptide sequencing, which only requires confident identification of peptides. For studies which included quantification, untargeted studies generally used a form of label-free quantification while targeted studies generally used standard heavy isotope-labeled standards to calculate PARs. No example was seen where chemical labeling, such as tandem mass tags, was used to multiplex samples. For untargeted discovery studies, increasing proteome coverage was a priority, often through sample preparation steps to remove high-abundant proteins, such as washing with lithium chloride or sodium carbonate precipitation [42, 57]. In contrast, studies which described targeted assays were more focused on sensitivity, accuracy and precision to detect disease-causing proteins or known protein biomarkers, and occasionally used antibodies to purify peptides or proteins of interest. So far, only one published study described post-translational modifications in DBS samples [82].

This review represents the first scoping review of microsampling proteomics. Recent narrative reviews have also described an increase in the use of microsamples within proteomics, but have not been systematic in nature [19, 20, 83]. The PRISMA guidelines were followed to ensure that all relevant literature was discussed without bias, which allowed this review to describe the current state of microsampling as pertains to proteomics, including relevant clinical applications. Forward and backward literature searches were performed in addition to the database search to confirm that relevant literature was not missed, which resulted in the inclusion of an additional 18 studies. The number of studies included in this review was high due to the broad inclusion criteria and purpose. This was intentional to allow a full characterization of this new field of research, however as the field evolves more focused reviews will be appropriate. The division of studies into proof-of-concept and clinical research allowed studies to be categorized according to their overall purpose. This subgroup analysis resulted in a more comprehensible and focused text, although some studies contained multiple experiments or a complicated study design. To address this, we defined clear criteria for whether a study should be proof-of-concept or clinical: if a study included any experiments involving an experimental or disease group, the study was classified as clinical research. This meant that some studies with a clear clinical application were assigned to proof-of-concept research, as long as all experiments were performed using healthy samples. One notable example of this was Whelan et al. which described a health surveillance panel to quantify 60 known biomarkers to allow longitudinal health monitoring [30]. Therefore, although this categorization allowed a meaningful division of included studies for subgroup analysis, studies included as proof-of-concept research did not necessarily lack relevance to real-world scenarios.

More research is needed before microsamples can be readily implemented into clinical proteomics. New innovations within microsampling stand to simplify blood collection further while maintaining a precise blood volume, for example the TAP device, which was designed for self-sampling and only requires the push of a button for activation [84]. Volumetric alternatives to VAMS, such as Capitainer B, HemaPEN, and HemaXis devices are available for commercial use but have not yet been employed in the context of MS-based proteomics [6]. Recent advances within proteomics, including improvements in instrument sensitivity and speed, are also expected to provide a benefit for clinical proteomics overall [85, 86]. Antibody-based affinity proteomics has also been used in combination with microsamples, demonstrating that these sample types are compatible with other proteomics methods, not just MS-based proteomics [87]. Microsamples could provide an important benefit for clinical proteomics by making sample collection cheaper and easier, which would result in increased cohort sizes and more statistical power. This is especially relevant for applications such as disease screening, where it is necessary to test a large cohort—potentially a whole population—to detect disease cases. Another important area is longitudinal sampling, which can be applied in a general aspect to monitor overall health or in more specific cases, such as ongoing risk assessment for individuals already diagnosed with a disease, where personal baseline samples become important for assessing prognosis. One aspect of precision medicine includes regular self-sampling by individuals as part of routine screening for early

detection of disease, which necessitates the use of easy and reliable microsampling. Home-based kits ensure that population subsections which do not typically participate in screening have an easy option for inclusion. Microsamples which do not require specialized personnel or cold-chain transport are also the only option for blood sampling in remote areas of the world. Within the studies included in this review, microsamples were already been used for a variety of purposes, although these studies were a small minority within proteomics as a whole. As awareness of these sampling alternatives grows and clinical proteomics develops further, use of microsamples is expected to increase, especially for routine analysis.

Conclusion

This review has established the current practices regarding microsampling within the proteomics field. The majority of published research was clinical proteomics, fitting with the intended use of microsamples for simple and low-cost sample collection. Exploratory research using microsamples existed as well. Although a multitude of use cases for microsamples exist within proteomics, as established by the wide variety in the publications included in this review, no standard exists within proteomics for sample collection or normalization of the hematocrit bias. Further experimentation is necessary to clarify how the potential bias in many microsampling methods can affect quantitative precision before microsamples can become a mainstream option for proteomics.

Abbreviations

CV	Coefficient of variation
DBS	Dried blood spot
Hct	Hematocrit
LLOQ	Lower limit of quantification
LOD	Limit of detection
MALDI-TOF	Matrix-assisted laser desorption/ionization-time-of-flight analyzer
MS	Mass spectrometry
NBS	Newborn dried blood spot
PAR	Peak area ratio
PRISMA-ScR	Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews
VAMS	Volumetric absorptive microsampling

Supplementary Information

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Supplementary Material 1.

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Availability of data and materials

This manuscript contains no original experimental data. All data analyzed within this study were sourced from previously published studies, and relevant data from these studies are included in the manuscript, its tables, or in the supplementary information. The review protocol can be accessed at Open Science Framework (https://osf.io/2tz8h/).

Declarations

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Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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