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Microsampling and Dried Blood Spot / Capillary Matrices in Routine Clinical Biochemistry: Analytical Validation, Reference Interval Challenges, and Implementation Barriers in Adult and Pediatric Practice

Adel Hamad Ahmed Awaji[(1)] , Mohammed Essa Maqbul Oraybi[(1)] , Joawid Mohammed Ali Alshogairan[(1)] , Hatim Mohammed Hamad Oraybi[(1)]

(1)Al Muwassam General Hospital, Jazan Health Cluster, Ministry of health, Saudi Arabia

Abstract

Background: Microsampling, including dried blood spots (DBS), volumetric absorptive microsampling (VAMS), and capillary collection devices, offers minimally invasive, remotely deployable alternatives to venipuncture in routine clinical biochemistry. **Aim:** To appraise analytical validation requirements, reference interval (RI) challenges, and implementation barriers in adult and pediatric practice. **Methods:** A narrative review of literature published between 2017 and 2024, supplemented by regulatory and standards documents, was synthesised thematically. **Results:** Hematocrit, volume, punch, extraction, and stability effects dominate analytical risk, while matrix differences between capillary whole blood and venous serum or plasma undermine direct use of established RIs. Pediatric populations gain most from low-volume sampling yet face sparse age-partitioned reference data. Barriers include traceability, accreditation, logistics, and clinician acceptance. **Conclusion:** Microsampling is fit for defined, validated applications; wider adoption requires matrix-specific validation, dedicated reference intervals, harmonised standards, and structured implementation.

Keywords: microsampling; dried blood spot; volumetric absorptive microsampling; reference intervals; clinical biochemistry.

Introduction

Venous blood collection by trained phlebotomists remains the default pre-analytical pathway for clinical biochemistry, and virtually all contemporary reference intervals, decision limits, and quality specifications presuppose serum or plasma obtained in this way. Yet this model carries recognised costs. Venipuncture requires a clinic or collection centre, a trained operator, a cold chain or rapid transport, and a patient able to attend. It is poorly suited to neonates, infants, frail older adults, people with difficult venous access, and populations living far from laboratory services. Microsampling has re-emerged as a credible response to these limitations, and the technological repertoire now extends well beyond the classical filter paper card to include volumetric devices and capillary collection systems that approach the analytical convenience of conventional samples (Lei & Prow, 2019; Thangavelu et al., 2023).

The history of dried blood collection is long, rooted in newborn screening, where a few drops of heel-prick blood on filter paper have supported population-scale detection of inherited metabolic disease for decades. The same principles now underpin applications in global health surveillance, therapeutic drug monitoring, serology, and chronic disease monitoring (Lim, 2018). The COVID-19 pandemic accelerated acceptance of self-collected capillary samples by demonstrating that large numbers of people could collect and post specimens that still yielded usable antibody results (Brown et al., 2021; Karp et al., 2020). Consequently, laboratory professionals are increasingly asked whether microsamples can serve not only specialist or research purposes but also the routine panels of electrolytes, enzymes, lipids, glucose, glycated hemoglobin, renal and liver markers, and endocrine analytes that dominate daily workload.

Answering that question is not straightforward. A dried blood spot is not simply a small serum sample. It is a whole blood matrix, dried on a cellulose or polymer substrate, extracted into a solvent, and then measured on platforms that were typically designed and calibrated for liquid serum or plasma. Each step from skin puncture to result introduces variability that differs from the venous pathway. The pre-analytical phase is already the dominant source of laboratory error, and microsampling shifts much of that phase from the controlled clinic to the uncontrolled home or community setting (Lippi et al., 2019; Simundic et al., 2024). Pediatric practice adds further complexity because reference values change rapidly with age, sex, and pubertal stage, and because the very population that benefits most from reduced blood volumes is the one with the most limited reference data (Adeli et al., 2017).

*Corresponding author aawaji@moh.gov.sa (Adel Hamad Ahmed Awaji).

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This narrative review therefore addresses three linked questions. First, what analytical validation is required before DBS, VAMS, and capillary matrices can be used for routine biochemistry, and how well do current guidance documents cover this need? Second, why do reference intervals present a particular challenge for microsamples, and what practical strategies exist for adults and children? Third, what organisational, regulatory, and behavioural barriers impede implementation, and how might they be overcome? The intention is to provide laboratory specialists, clinicians, and policymakers with an integrated, critical overview rather than an exhaustive systematic catalogue of individual assays.

2. Review Approach

This work was designed as a narrative review, an approach suited to heterogeneous topics that span analytical chemistry, laboratory medicine, pediatrics, and health services, where a quantitative synthesis is neither feasible nor especially informative. Peer-reviewed articles, guidelines, and standards published predominantly between 2017 and 2024 were identified through targeted searches of biomedical databases using combinations of terms such as dried blood spot, volumetric absorptive microsampling, capillary blood, hematocrit effect, reference interval, pediatric, and clinical chemistry. Reference lists of key papers were examined to identify additional relevant sources. Priority was given to official guidelines, systematic or narrative reviews, method validation studies, multicentre reference interval initiatives, and implementation reports.

Because no formal risk-of-bias appraisal was undertaken, the conclusions should be read as a structured expert interpretation rather than a pooled estimate of effect. Evidence was organised thematically into matrix and device characteristics, analytical validation, analyte-specific performance, reference interval considerations, adult and pediatric implementation, and system-level barriers. Where evidence originates mainly from therapeutic drug monitoring or newborn screening, this is stated, since transfer of conclusions to routine biochemistry analytes is not automatic. Studies were weighed according to design, sample size, use of paired venous comparators, and transparency of validation procedures. Because the field changes quickly, the findings represent a snapshot, and readers should consult current standards and manufacturer documentation before designing validation protocols.

3. Microsampling Matrices and Devices

3.1 Classical dried blood spots

The classical DBS is created by applying a drop of capillary blood, usually obtained by fingertip or heel puncture, to a cellulose-based card and allowing it to dry. The simplicity of this approach underlies its enduring appeal: cards are inexpensive, can be shipped at ambient temperature in many circumstances, and generate small biohazard risk once dried. Its principal analytical weakness is that the spread of blood on paper depends on viscosity and therefore hematocrit, so that a fixed punch diameter does not correspond to a fixed blood volume. This is the origin of the well-known hematocrit effect, as well as concerns about spot homogeneity, chromatographic effects, and variability introduced by operators who apply too much or too little blood (Nys et al., 2017; Londhe & Rajadhyaksha, 2020).

3.2 Volumetric approaches

Volumetric absorptive microsampling addresses the volume problem by using a calibrated polymeric tip that absorbs a fixed volume of blood irrespective of hematocrit within a defined range. The tip is then dried and extracted in its entirety. Tutorial and review articles describe improved volumetric accuracy and the elimination of punching, although the hematocrit may still influence extraction recovery, drying behaviour, and analyte distribution between cells and plasma (Kok & Fillet, 2018; Protti et al., 2019). Comparative work with analytes such as miltefosine and antiepileptic drugs indicates that VAMS can reduce, but does not necessarily abolish, hematocrit-related bias compared with standard DBS (Kip et al., 2017; Velghe & Stove, 2018). Other devices pursue volumetric precision through microfluidic channels, capillary-fed cartridges, or user-independent collection mechanisms designed to deliver reproducible spots even when operators are inexperienced (Neto et al., 2018).

3.3 Capillary collection devices and liquid microsamples

A further family of technologies collects capillary blood in liquid form rather than drying it immediately. Microneedle-based systems can obtain a few hundred microlitres of blood from the upper arm with minimal pain and without the need for a fingerstick, and have been used for remote collection in both research and clinical contexts (Blicharz et al., 2018). Metabolomic comparisons suggest that blood obtained by such devices can closely resemble venous blood in many measured analytes, although differences remain that must be considered analyte by analyte (Catala et al., 2018). Some devices incorporate serum separator gel or plasma separation membranes, which allows measurement on conventional analysers with modest modification and addresses part of the matrix mismatch problem. Evidence for antibody testing with self-collected capillary serum devices illustrates this potential (Hendelman et al., 2021). Reviews of alternative sampling devices emphasise that each design involves trade-offs among volume, user burden, stability, cost, and compatibility with existing analytical platforms (Delahaye et al., 2021).

3.4 Comparative characteristics

Table 1 summarises the principal platforms and their strengths and limitations in the context of clinical biochemistry. Although classification is useful, it should not obscure the fact that performance

depends on the combination of device, analyte, extraction protocol, and instrument rather than on the device alone.

Table 1. Principal microsampling and capillary platforms relevant to clinical biochemistry

Platform	Typical sample and format	Main strengths	Main limitations	Representative applications
Classical DBS (filter paper card)	Capillary whole blood dried on cellulose; punched disc extracted	Low cost; ambient transport; long experience in newborn screening	Hematocrit-dependent spot size; punch variability; limited volume per punch (Nys et al., 2017; Londhe & Rajadhyaksha, 2020)	Newborn screening; metabolic monitoring; serosurveys (Lim, 2018; Moat et al., 2020)
VAMS	Fixed-volume absorbent tip dried and extracted whole	Volumetric accuracy; no punching; reduced hematocrit bias (Protti et al., 2019; Kok & Fillet, 2018)	Residual hematocrit effect on recovery; device cost; drying time	Therapeutic drug monitoring; metabolomics (Kip et al., 2017; Velghe & Stove, 2018)
Volumetric or user-independent DBS devices	Controlled-volume spot generated by device mechanism	Reduced operator dependence (Neto et al., 2018)	Limited commercial availability; validation burden	Remote monitoring research
Microneedle capillary collection	Liquid capillary blood or serum from upper arm in a collection tube	Lower pain; larger volume; compatibility with liquid assays (Blicharz et al., 2018; Hendelman et al., 2021)	Higher unit cost; incomplete filling; matrix differences from venous samples (Catala et al., 2018)	Remote serology; research biochemistry
Capillary tubes with gel or separation membranes	Liquid capillary blood or plasma/serum	Closest to conventional analyser workflows (Delahaye et al., 2021)	Haemolysis; contamination; need for centrifugation	Point-of-care and clinic capillary testing

4. Analytical Validation

4.1 Validation frameworks and their limits

No single document currently provides a complete validation pathway for microsampling in routine clinical biochemistry. The most developed frameworks derive from bioanalysis and therapeutic drug monitoring. The international guideline for DBS-based therapeutic drug monitoring offers detailed recommendations on hematocrit, spot homogeneity, sample volume, stability, and cross-validation against venous samples (Capiau et al., 2019). Regulatory documents for bioanalytical method validation, notably the United States Food and Drug Administration guidance and the harmonised ICH M10 guideline, define parameters such as selectivity, calibration, accuracy, precision, carry-over, matrix effect, and stability, but were conceived for pharmaceutical bioanalysis (Food, 2018; Guideline, 2022). Clinical laboratories accredited under ISO 15189 must in addition demonstrate that the examination procedure is fit for its intended clinical use, including verification of pre-examination processes and sample suitability (International Organization for Standardization [ISO], 2022). Neither pharmaceutical nor accreditation frameworks specify how to treat the unique matrix, collection, and drying issues of microsamples used with clinical chemistry analysers and reference intervals. Laboratories therefore need to combine elements of these documents and justify their choices explicitly.

4.2 The hematocrit effect and related matrix issues

Hematocrit is the best-known confounder. In classical DBS, higher hematocrit produces a smaller spot with greater analyte concentration per unit area, whereas lower hematocrit yields larger and more diffuse spots, so that a fixed-diameter punch contains a variable blood volume. The resulting bias can be material for analytes with high precision requirements. However, hematocrit also affects viscosity, drying kinetics, recovery from the substrate, and, importantly, the partitioning of analytes between plasma and cells. A review focused on whether hematocrit remains a problem concluded that it is still relevant, even though its magnitude varies by analyte, device, and clinical population, and that its impact must be quantified rather than assumed negligible (Velghe et al., 2019).

Several mitigation strategies have been developed. Volumetric devices reduce volume-related bias, but extraction conditions may still need to be optimised to limit differential recovery across hematocrit levels (Ye & Gao, 2017). Non-destructive measurement of hematocrit from the dried spot, for instance by reflectance-based prediction, enables numerical correction and has shown promise in

research settings (Capiau et al., 2018). Whole-spot analysis, internal standards that migrate with the analyte, and calibrators prepared in blood of matched hematocrit provide additional safeguards. For analytes confined largely to plasma, such as sodium, many proteins, and creatinine, the conversion from whole blood concentration to a serum-equivalent value requires knowledge of hematocrit, which complicates routine reporting. The hematocrit range of the intended population is therefore a critical design parameter: neonates, who typically have high values, patients with anaemia, pregnant women, and individuals with chronic kidney disease may fall outside the range covered by validation experiments.

4.3 Volume, homogeneity, extraction, and carry-over

Beyond hematocrit, quantitative performance depends on the volume of blood applied, the uniformity of the spot, the extraction procedure, and the sensitivity of the measurement system. Under-filled or layered spots, contamination from alcohol hand gels or cosmetics, and squeezing of the fingertip, which dilutes blood with interstitial fluid, are plausible sources of error in self-collection. Because routine biochemistry analysers are normally optimised for microlitre-to-millilitre volumes of serum, the eluate from a 3 mm punch may be too dilute for some immunoassays or require adaptations of reagent volume and calibration. Recovery experiments should include multiple concentration levels, multiple hematocrits, and multiple lots of cards or tips, and extraction time, solvent composition, and agitation must be fixed in the standard operating procedure. In mass spectrometric workflows, matrix effects from haemoglobin, phospholipids, and the substrate itself must also be assessed; metabolomic evaluations of volumetric devices show that pre-analytical variables such as drying time and storage can alter the measured profile (Volani et al., 2017).

4.4 Stability and transport

Stability is often cited as an advantage of dried matrices, since enzymatic degradation and microbial growth are reduced. This is true for many small molecules, but not universally. Some labile analytes, certain proteins, and enzyme activities may degrade or change during drying and storage, and humidity, temperature, and light exposure can differ substantially between a clinic and a postal route. The stability evaluation should therefore mimic real transport conditions, including elevated temperature and humidity for defined periods, and should use desiccant-containing packaging where appropriate. Samples should be stored and shipped with clear requirements for minimum drying time. For routine biochemistry, an analyte-specific stability matrix, rather than a generic statement, is the appropriate evidence base.

4.5 Method comparison, bias, and commutability

The most informative validation experiment is a paired comparison of microsamples and venous samples obtained from the same individuals at about the same time, covering the clinically relevant concentration range and the intended patient groups. Measurement procedure comparison should follow recognised statistical principles, including appropriate regression approaches, difference plots, and bias estimation with confidence intervals (Clinical and Laboratory Standards Institute [CLSI], 2018). Agreement should be interpreted against performance specifications that reflect clinical need, such as allowable bias or measurement uncertainty derived from outcome-based, biological variation, or state-of-the-art models (Braga & Panteghini, 2021). A method with a statistically significant but clinically trivial bias may be acceptable, whereas a modest systematic bias on a tightly regulated analyte such as potassium may not be.

A further subtlety is commutability. Calibrators and quality control materials made of serum or lyophilised material may not behave like dried whole blood, so standard internal quality control and proficiency testing schemes may not reflect true performance on microsamples. Laboratories frequently have to prepare in-house controls from spiked whole blood and cross-validate regularly against venous reference procedures. Because the metrological traceability of results on whole blood matrix is not always defined, the laboratory must state clearly whether results are reported as whole blood concentrations or converted to serum-equivalent values, and must justify the conversion.

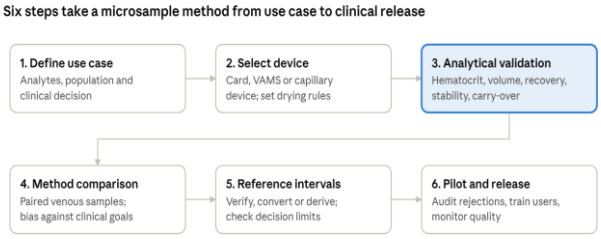


Figure 1. Validation pathway for microsampling methods in routine clinical biochemistry, from collection to clinical release.

5. Analyte-Specific Performance in Routine Clinical Biochemistry

The evidence base is uneven across analyte groups. It is strongest for newborn screening metabolites and therapeutic drugs, moderate for serological targets, and thinnest for the high-volume electrolytes, enzymes, and lipids that make up most routine workload. Table 2 summarises the position, and the following paragraphs discuss the most relevant groups.

5.1 Glycaemic and hematological markers

Capillary testing for glycated hemoglobin has been evaluated extensively at the point of care, where whole blood is analysed directly. A systematic review and meta-analysis of point-of-care HbA1c devices found that performance varies markedly

among devices and that only some meet commonly cited analytical criteria, demonstrating that suitability for capillary matrices is a property of the specific method rather than of the analyte (Hirst et al., 2017). The same principle applies to dried whole blood: an HbA1c method that is fit for venous EDTA samples cannot be assumed to be fit for a dried spot without independent verification. For hemoglobin, a narrative review of measurement in clinical and field settings highlights that capillary values may differ from venous values depending on the collection site, the technique, and the device, and that interpretation against venous-derived cut-offs requires awareness of these differences (Karakochuk et al., 2019).

5.2 Electrolytes, renal markers, and enzymes

For electrolytes, urea, creatinine, and enzymes, the principal obstacles are the whole blood matrix and the fraction of analyte located in cells. Potassium, lactate dehydrogenase, aspartate aminotransferase, and phosphate are released or altered by haemolysis, which is hard to detect in a dried spot, whereas sodium and chloride are distributed predominantly in plasma water and are sensitive to hematocrit and interstitial fluid contamination. Creatinine is more tractable, as demonstrated by work combining remote immunosuppressant and kidney function monitoring from volumetric microsamples in transplant recipients, which suggests that selected renal markers can be integrated with drug monitoring in specific, well-controlled pathways (Zwart et al., 2018). Extrapolating this success to all electrolytes and enzymes would be premature.

5.3 Inherited metabolic disease and newborn screening

Dried blood spots are the foundation of newborn screening, and the associated standards address collection, drying, transport, and quality assurance in detail (CLSI, 2021). Beyond screening, DBS are used to monitor patients with inherited metabolic disorders at home, reducing the burden of clinic attendance and allowing more frequent measurement of amino acids, acylcarnitines, and other metabolites (Moat et al., 2020). Phenylketonuria is a paradigm: contemporary European guidelines emphasise regular monitoring of blood phenylalanine as a cornerstone of management, an objective that microsampling can support when methods are appropriately validated and results are communicated promptly (van Spronsen et al., 2017). These experiences show how a mature, standardised infrastructure can make DBS reliable, but they also show that such reliability depends on tightly controlled methods and population-specific cut-offs.

5.4 Serology and immunoassays

The pandemic generated a large body of evidence on antibody detection from self-collected capillary samples. Home-collected finger-prick DBS have been shown to support serological assays for SARS-CoV-2 antibodies (Karp et al., 2020), and sensitive detection from DBS has been reported using established laboratory formats (Morley et al., 2020). Evaluation of DBS on a high-throughput commercial immunoassay platform indicated that integration with existing analysers is feasible when elution and cut-offs are validated (Mulchandani et al., 2021). Self-collected capillary samples from several approaches have likewise proved acceptable in large community studies (Brown et al., 2021), and microneedle-based serum collection has been used for antibody testing (Hendelman et al., 2021). Qualitative or semi-quantitative serology, however, is more tolerant of modest bias than quantitative clinical chemistry, so these results cannot simply be generalised to analytes with narrow biological variation.

5.5 Therapeutic drug monitoring as a methodological template

Therapeutic drug monitoring offers the most mature template for validation because decades of work have produced consensus guidance, cross-validation practices, and published comparisons between devices (Capiau et al., 2019; Delahaye et al., 2021). Studies of VAMS for antiepileptics and miltefosine show how hematocrit, extraction, and stability can be systematically examined (Kip et al., 2017; Velghe & Stove, 2018). Routine biochemistry laboratories can adopt these designs but must remember that endogenous analytes, unlike drugs, have no blank matrix, so calibration and recovery experiments require surrogate matrices or standard addition and are more difficult to interpret.

Table 2. Summary of evidence and readiness of selected analyte groups for microsample-based routine use

Analyte group	Main evidence base	Principal analytical concerns	Overall readiness for routine use
Newborn screening metabolites	Decades of population use; standardised practices (CLSI, 2021)	Cut-offs specific to matrix and method; spot quality	High within established programmes
Metabolic disease monitoring (e.g., phenylalanine)	Home DBS monitoring and guideline support (Moat et al., 2020; van Spronsen et al., 2017)	Hematocrit; calibration; timely feedback	High to moderate with local validation
Therapeutic drugs	International guideline and device comparisons (Capiau et al., 2019; Delahaye et al., 2021)	Hematocrit; cross-validation with plasma	Moderate to high for selected drugs

Serology and antibodies	Pandemic-era DBS and capillary studies (Karp et al., 2020; Mulchandani et al., 2021)	Elution; cut-off definition; analyser compatibility	Moderate; assay-specific
HbA1c and hemoglobin	Point-of-care and capillary reviews (Hirst et al., 2017; Karakochuk et al., 2019)	Device-dependent performance; sampling technique	Moderate; device-specific
Creatinine and selected renal markers	Combined remote monitoring in transplant care (Zwart et al., 2018)	Hematocrit; conversion to serum-equivalent values	Moderate in defined pathways
Electrolytes, enzymes, lipids, proteins	Limited, mainly method-specific reports	Haemolysis; plasma–cell partitioning; interstitial fluid	Low to moderate; requires extensive validation

6. Reference Interval Challenges

6.1 Why venous reference intervals do not transfer

A reference interval describes the central 95% of values in a defined reference population measured by a defined procedure on a defined sample type. Each element of that definition changes when a microsample replaces a venous serum or plasma specimen. The biological matrix is different, since capillary blood is a mixture of arterial, venous, and interstitial fluid components whose proportions depend on temperature, technique, and perfusion. The analytical matrix is different, since dried whole blood is extracted and diluted. The measurement procedure is often different, since calibration may be performed with modified calibrators or conversion factors. Finally, the pre-analytical procedure is different, since collection is often performed by the patient. A reference interval transferred from a venous serum method to a microsample method without evaluation therefore violates the conditions under which the interval was derived.

Guidance on verification of reference intervals in routine laboratories stresses that transference is acceptable only when the new procedure is sufficiently comparable and when a modest number of local reference individuals confirm that the interval fits the population (Ozarda et al., 2018a). Where a microsample matrix is not equivalent, verification is likely to fail, and the laboratory must either derive new intervals or apply a validated conversion to serum-equivalent values and retain the original interval. Both options carry costs and uncertainties that should be explicitly documented.

It is also important to distinguish reference intervals from clinical decision limits. Decision limits for conditions such as diabetes, dyslipidaemia, or iron deficiency are derived from outcome and diagnostic studies using specific matrices and methods and are not statistical descriptions of health (Ozarda et al., 2018b). For these analytes the key question is not whether a new interval can be defined but whether the microsample result is equivalent to the matrix on which the threshold was validated. A systematic bias that would be tolerable for a population-level interval can lead to misclassification at a threshold, and this risk is magnified when results are used for diagnosis or treatment initiation.

6.2 Direct reference interval studies

The direct approach recruits healthy individuals, collects specimens under standardised conditions, and calculates percentile limits with confidence intervals. It is the gold standard but is resource-intensive, particularly for microsamples that require the same individuals to provide both matrices if bias is to be characterised. Multicentre initiatives have shown how harmonised protocols, exclusion criteria, and statistical handling, including partitioning by age and sex and treatment of latent abnormal values, can improve comparability across populations and laboratories (Ichihara et al., 2017). Applying this model to microsamples would require harmonised collection protocols for finger-prick technique, spot quality, drying, and shipping, which do not currently exist for routine biochemistry. The conventional requirement of about 120 individuals per partition becomes burdensome when partitions multiply for age, sex, and perhaps hematocrit.

6.3 Indirect approaches and real-world data

Indirect methods derive reference intervals from routinely generated patient results by statistically separating the non-pathological distribution from the mixed population. Recommendations from an international working group describe the assumptions, data requirements, and limitations of these approaches (Jones et al., 2018). Newer algorithms, such as refineR, estimate reference intervals from real-world data without requiring explicit knowledge of the pathological component and have been shown to perform well in validation studies (Ammer et al., 2023). A broader review of the field notes the growth of indirect approaches alongside continuing debate about when they are acceptable (Doyle & Bunch, 2023). The potential for microsamples is attractive, because the data would reflect actual users and sampling conditions. At present, however, volumes of routine microsample results are small, are concentrated in selected clinical pathways, and include a high proportion of patients with known disease, which makes the non-pathological fraction difficult to isolate.

6.4 Pragmatic strategies

Four pragmatic strategies emerge from the literature. First, laboratories can establish equivalence between microsample and venous results through paired studies and report a serum-equivalent value, interpreted against existing intervals, provided that bias and imprecision are quantified and the conversion is validated across the hematocrit range. Second, laboratories can verify transferred intervals with local reference individuals using microsamples. Third, for analytes and populations in which equivalence cannot be demonstrated, method-specific intervals can be derived, preferably through multicentre collaboration. Fourth, for longitudinal monitoring, interpretation can rely on within-individual comparison, reference change values, and trend analysis rather than population intervals, because each patient serves as their own control provided that the sample type remains constant. In every case the report should state the sample type and the interpretive framework so that clinicians do not unknowingly compare microsample results with venous-based intervals.

7. Pediatric Practice

7.1 Where the benefit is greatest

Children stand to benefit most from microsampling. Blood volume in neonates and small infants is limited, repeated venipuncture is distressing, and cumulative sampling in hospitalised neonates contributes to iatrogenic blood loss. Heel-prick DBS have already transformed newborn screening, and extensions to monitoring of metabolic disease, drug therapy, and chronic conditions are logical (Moat et al., 2020; Nys et al., 2017). Home sampling by parents can reduce hospital visits, school absence, and anxiety, and in low-resource settings DBS enable access to testing where cold chain and laboratory infrastructure are limited (Lim, 2018).

7.2 Distinct analytical and reference challenges

Pediatric samples pose specific challenges. Hematocrit is high in neonates and falls during the first months of life, so the hematocrit effect and any correction factor change with age. Capillary blood from heel puncture may be contaminated by tissue fluid, and warming and technique influence results. Higher fetal hemoglobin content affects some assays, including methods for glycated hemoglobin. Parent-collected samples introduce variability in application technique and drying. Crucially, reference values for many analytes, especially enzymes, hormones, and bone markers, change rapidly with age, sex, and pubertal stage, requiring finely partitioned or continuous intervals.

The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has demonstrated both the scale of the effort needed and the value of a comprehensive, healthy-child database to support age- and sex-specific intervals across analytes and platforms (Adeli et al., 2017). Yet such intervals were derived from serum or plasma, not from microsamples. For newborn screening, population-based percentile cut-offs for DBS are established locally and are continually revised, as standards for collection and programme performance recommend (CLSI, 2021). Outside screening, there is little age-partitioned reference information for microsamples in routine clinical chemistry, which means that clinicians who use them for children often interpret results using intervals derived from venous samples without formal verification.

8. Adult Practice

In adults, the attraction of microsampling lies in chronic disease monitoring, remote or rural care, frailty, and difficult venous access. Diabetes, kidney disease, transplantation, and dyslipidaemia require repeated measurement, and self-collection could reduce visits and support telehealth models. Evidence that large groups of adults can collect usable capillary samples at home, and that remote monitoring can be embedded in specialist care, is encouraging (Brown et al., 2021; Hendelman et al., 2021; Zwart et al., 2018). Nonetheless, adult populations are heterogeneous in precisely the variables that influence microsample performance. Anaemia, polycythaemia, pregnancy, and chronic kidney disease alter hematocrit, while older adults may have cold peripheries, reduced dexterity, or skin conditions that hinder adequate capillary collection. Many adult decision thresholds are tied to venous plasma or serum, so any bias in a converted result may shift a patient across a diagnostic or treatment boundary. Existing recommendations for venous sampling provide a model of structured pre-analytical guidance but do not address capillary self-collection, so parallel recommendations are needed (Simundic et al., 2024).

9. Implementation Barriers

Technical performance is necessary but insufficient for adoption. Figure 2 organises the barriers along the testing pathway, from collection to clinical action, and identifies enabling responses.

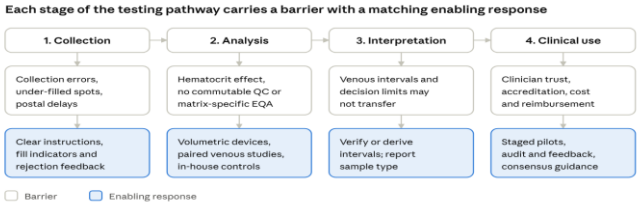


Figure 2. Implementation barriers and enabling responses along the microsampling testing pathway.

9.1 Analytical standardisation and quality assurance

The absence of harmonised validation standards, commutable quality control materials, and external quality assessment schemes designed for dried or capillary matrices leaves laboratories to build their own frameworks. Without proficiency testing on the actual matrix, it is difficult to demonstrate ongoing comparability. Device-specific differences in volume, extraction, and drying also mean that a validation is

not transferable between products, and changes in card or device lots may require revalidation (Delahaye et al., 2021).

9.2 Regulatory and accreditation barriers

Laboratories seeking accreditation must show that the entire examination process, including pre-examination activities performed outside the laboratory, is controlled (ISO, 2022). Self-collection devices raise questions about whether they are regulated as in vitro diagnostic devices, whether the laboratory-developed method is adequately validated, and who is responsible for training and instruction. Where bioanalytical guidance is used as a template, laboratories must justify its application to endogenous analytes (FDA, 2018; ICH, 2022).

9.3 Pre-analytical and logistical barriers

Pre-analytical errors remain the largest source of laboratory error, and decentralised collection widens the exposure (Lippi et al., 2019). Inadequate spot volume, contamination, insufficient drying, mislabelling, and postal delay produce rejections or misleading results. Clear illustrated instructions, device designs that signal adequate filling, and rejection criteria with feedback to the patient are essential. Laboratory information systems must also capture sample type, collection time, and rejection reason, and links between home collection, courier services, and result delivery require organisational investment.

9.4 Clinical, economic, and behavioural barriers

Clinicians may distrust results that look different from the familiar serum report, and uncertainty about reference intervals compounds this hesitation. Reimbursement frameworks may not recognise microsampling, and the unit cost of volumetric devices may exceed that of standard tubes, although savings from avoided visits and travel can offset this in suitable pathways. Patients and parents vary in confidence and preference, and digital exclusion or limited health literacy can create inequity if home sampling becomes the default.

9.5 A staged implementation approach

A practical approach begins by defining a specific clinical use case in which the benefit is clear, then selecting a device and analytes, completing analytical validation including hematocrit and stability studies, performing paired venous comparisons in the intended population, verifying or deriving reference intervals, piloting with audit of rejection rates and clinician feedback, and finally scaling with continuous quality monitoring. Restricting early implementation to analytes with the strongest evidence (Table 2) limits risk.

10. Future Directions

Several developments could accelerate progress. Collaborative multicentre studies comparing microsamples with venous samples across age groups would generate the paired data needed for conversion factors and intervals. Indirect methods applied to accumulating microsample datasets may eventually provide real-world intervals (Ammer et al., 2023). Improved devices that guarantee volume, separate plasma, and detect inadequate filling could reduce the hematocrit and operator problems that currently dominate validation (Thangavelu et al., 2023). Finally, consensus documents specific to clinical biochemistry, covering validation, quality assurance, and reporting, would give laboratories and regulators a common reference point.

11. Limitations of This Review

This narrative review did not follow a registered protocol or formal risk-of-bias assessment, and the search was targeted rather than exhaustive, so relevant studies may have been missed. Much of the evidence comes from drug monitoring, newborn screening, and serology rather than from the electrolytes, enzymes, and lipids central to routine biochemistry, and conclusions for these analytes are therefore partly inferential. Technology, standards, and device availability are evolving rapidly.

12. Conclusion

Microsampling and capillary matrices are credible tools for specific, well-defined applications in clinical biochemistry, particularly where venipuncture is burdensome, as in neonates and remote or frail patients. They are not yet universal replacements for venous serum or plasma. Analytical validation must address hematocrit, volume, extraction, stability, and commutability; reference intervals and decision limits cannot be assumed to transfer; and implementation depends as much on logistics, regulation, and clinical trust as on assay performance. Laboratories that adopt a staged, evidence-based pathway, focus on analytes with the strongest evidence, and collaborate on shared reference data will be best placed to extend microsampling safely from specialist niches into routine adult and pediatric practice.

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