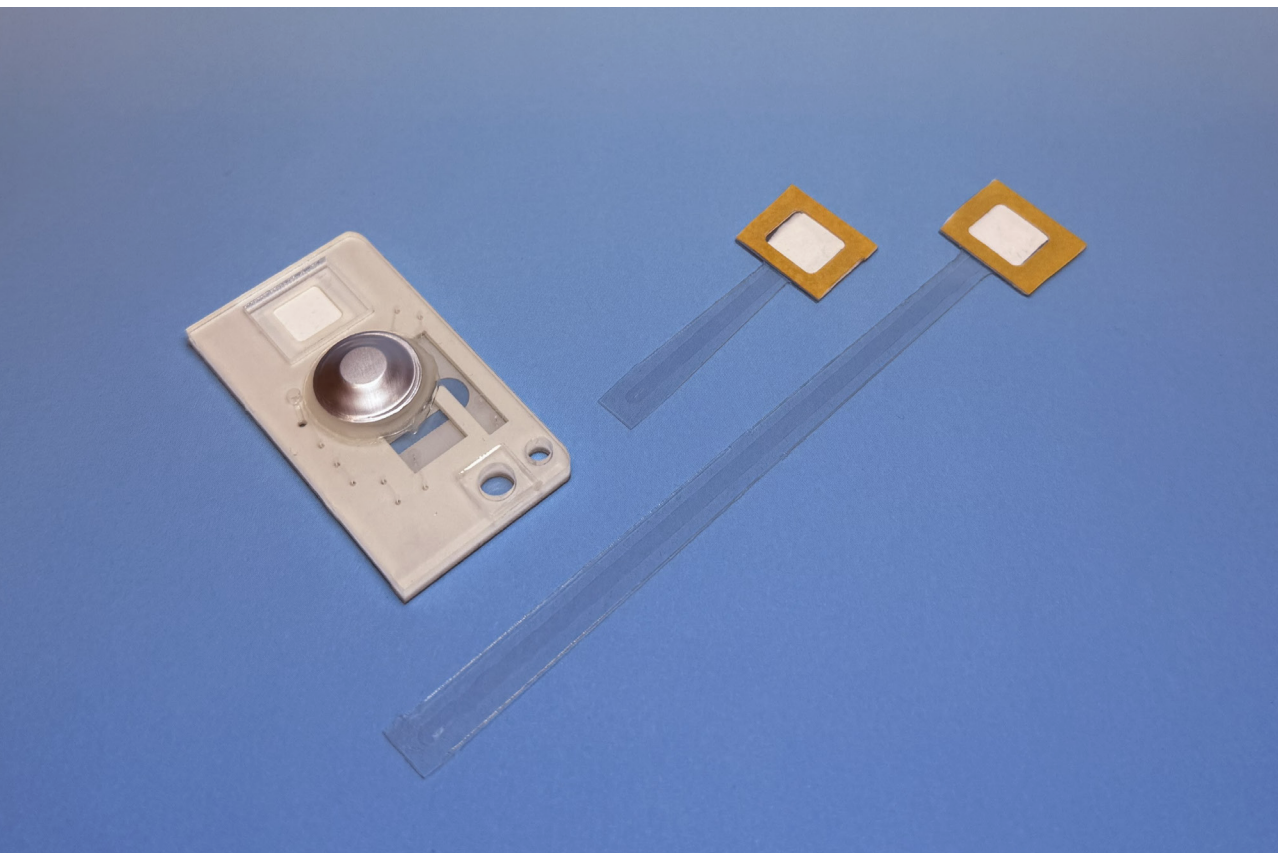
Doctoral Thesis in Electrical Engineering

Patient-centric devices for sample preparation and analysis using capillary microfluidics

CARL OLSSON

KTH ROYAL INSTITUTE OF TECHNOLOGY



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CARL OLSSON

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Abstract

Blood sampling is an essential component of modern healthcare. However, due to the centralized nature of blood processing and analysis, it typically requires patients to visit a hospital or clinical laboratory. Patient-centric testing (PCT), including patient-centric sampling (PCS) and point-of-care (POC) testing, seeks to reduce this burden by simplifying sample collection, processing, and analysis. By lowering the barriers associated with blood testing, these approaches have the potential to improve accessibility for patients while reducing workload within healthcare systems.

Blood is a complex biological fluid containing a wide range of cellular and molecular components, many of which require specific processing prior to analysis. Consequently, the analytical requirements vary considerably between different biomarkers, creating a challenge for the development of PCS and POC testing technologies.

This thesis presents novel approaches for therapeutic drug monitoring (TDM) of lithium, the first-line treatment for bipolar disorder. Lithium therapy requires regular monitoring due to its narrow therapeutic window and risk of toxicity, resulting in repeated hospital or clinic visits for blood sampling and serum lithium analysis. Two approaches are investigated to simplify monitoring. First, volumetric dried blood spots (DBS) are evaluated as a patient-centric home-sampling method through the development of an elution and analytical protocol for lithium determination from volumetric DBS samples, followed by a longitudinal clinical study comparing DBS-based measurements with conventional clinical analyses. Second, a POC device concept for lithium monitoring based on on-chip serum separation and colorimetric detection is developed and evaluated in patients to assess both the technical feasibility of the approach and patient interest in simplified testing methods.

In addition, this thesis evaluates a capillary-driven microfluidic device designed to process whole blood into a nanofiltrate. The device utilizes a dual-filtration strategy combined with wedge-induced filtration to extract nanoparticles from whole blood. Filtration performance was assessed using nanoparticles, liposomes, and

synthetic extracellular vesicles (EV), and the biological characteristics of the resulting nanofiltrate were compared with ultracentrifugation. The device was evaluated using samples from healthy controls and cancer patients. Nanofiltrates from healthy controls were characterised using EV protein profiling, whereas samples from cancer patients were evaluated for downstream analysis of circulating tumour DNA, cell-free DNA, and copy number variations.

Overall, this work presents novel approaches for lithium monitoring and blood sample processing. These technologies have the potential to reduce barriers to therapeutic drug monitoring, improve accessibility to lithium treatment, and facilitate downstream analysis of blood biomarkers.

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Sammanfattning

Blodprovstagning är en viktig del av dagens sjukvård men kräver nästan alltid ett besök på vårdcentral eller sjukhus på grund av centraliserad hantering och analys. Som svar på detta har patientcentrerade testmetoder, såsom hemprovtagning och patientnära analyser, syftar till att förenkla provtagning, provberedning och analys utanför den etablerade kliniska infrastrukturen. Genom att sänka trösklarna för provtagning har dessa strategier potential att öka tillgängligheten för patienter samtidigt som arbetsbördan inom sjukvården minskar.

Blod är en komplex biologisk vätska som innehåller ett stort antal cellulära och molekylära komponenter, varav många kräver specifika provberedningssteg före analys. Detta medför att de analytiska kraven varierar mellan olika biomarkörer, vilket utgör en betydande utmaning vid utvecklingen av patientcentrerade provtagningsmetoder och patientnära testteknologier.

I denna avhandling presenteras nya metoder för terapeutisk läkemedelsövervakning av litium, förstahandsbehandlingen vid bipolär sjukdom. Litiumbehandling kräver regelbunden provtagning på grund av dess smala terapeutiska intervall och risken för toxicitet, vilket innebär att patienter behöver lämna blodprover flera gånger per år för att mäta litiumkoncentrationen i serum. För att förenkla denna process utvärderas här två olika strategier. Den första bygger på volymetriska torkade blodprover (dried blood spots, DBS), där ett eluerings- och analysprotokoll för bestämning av litiumkoncentrationer från DBS-prover utvecklades, följt av en longitudinell klinisk studie där volymetrisk DBS-baserad provtagning jämfördes med konventionell klinisk analys. Den andra strategin är ett patientnära testkoncept för litiumbestämning. Metoden utvecklades och utvärderades i patientprover för att undersöka den tekniska genomförbarheten samt patienters intresse för förenklade provtagnings- och analysmetoder.

Vidare utvärderas ett kapillärdrivet mikrofluidiskt kort utvecklad för att processa helblod till ett nanofiltrat. Kortet använder en dubbel filtreringsstrategi i kombination

med filter i vinkel för att extrahera nanopartiklar från blod. Filtreringsprestandan utvärderades med hjälp av nanopartiklar, liposomer och syntetiska extracellulära vesiklar, medan de biologiska egenskaperna hos det resulterande nanofiltratet jämfördes med ultracentrifugering. Vidare utvärderades kortet med prover från friska kontrollpersoner och cancerpatienter. Nanofiltrat från friska kontrollpersoner karakteriserades genom proteinprofilering av extracellulära vesiklar, medan prover från cancerpatienter användes för analys av cirkulerande tumör-DNA, cellfritt DNA och kopienummervariationer.

Sammantaget presenterar denna avhandling nya metoder för litiumövervakning och blodprocessering. Dessa teknologier har potential att öka tillgängligheten till terapeutisk läkemedelsmonitorering, förbättra möjligheterna till litiumbehandling och möjliggöra analys av biomarkörer från blod.

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Dedicated to my family,
and to all the friends and family we lost along the way.

May you live on in our memories
– C. Olsson

Contents

Abstract iii

Sammanfattning v

List of Publications xiii

Abbreviations xvii

Thesis Aim and Structure xix

1 Introduction 1

1.1 Decentralised Sampling and Testing . . . 1
1.1.1 Laboratory vs Point-of-Care Testing . . . 2
1.1.2 Point-of-Care applications . . . 3
1.2 Blood as a sample . . . 5
1.2.1 Conventional Blood Analysis Workflow . . . 5
1.2.2 Challenges When Working with Blood . . . 6
1.2.3 Blood Home Sampling . . . 7

2 Physics and Fabrication 9

2.1 Microfluidics Theory . . . 9
2.1.1 Scaling and Dominant Forces . . . 9
2.1.2 Capillary Action and Wetting . . . 11
2.1.3 Capillary-Driven Transport . . . 13
2.2 Capillary-Driven Microfluidics . . . 15
2.2.1 Flow Regulation . . . 15
2.2.2 Capillary Valves . . . 17
2.2.3 Capillary Blood-to-Plasma Separation . . . 17
2.3 Microfluidic Prototyping and Fabrication . . . 18
2.3.1 Lamination Technology Platform . . . 19
2.3.2 Idea to Prototype . . . 19

3 Bipolar Disorder 21

3.1 Clinical Overview . . . 21
3.1.1 Prevalence and Disease Burden . . . 22
3.1.2 Clinical Management . . . 22
3.2 Lithium for Bipolar Disorder: Historical Summary . . . 23
3.2.1 The 1800s: Early Foundations . . . 23
3.2.2 1950s–1960s: The Road to Clinical Acceptance . . . 23
3.2.3 Establishing First-Line Treatment . . . 24
3.2.4 Present Day: The Enduring Benchmark . . . 24
3.3 Lithium and Therapeutic Drug Monitoring . . . 26

3.3.1 Current Monitoring Workflows . . . 26
3.3.2 Challenges in Modern Lithium Management . . . 26
3.4 Emerging Approaches in Lithium Monitoring . . . 27

4 Decentralised Approaches to Lithium Monitoring 29
4.1 Dried Blood and Plasma Spot Sampling . . . 29
4.1.1 Volumetric Dried Blood Spots . . . 31
4.1.2 Serum vs Red Blood Cell Fractions . . . 33
4.2 Point-of-care approaches . . . 37
4.3 On-chip Colorimetric Assay . . . 37
4.3.1 Colorimetric assays and absorption . . . 37
4.3.2 Design inspiration and approach . . . 38
4.3.3 The Device . . . 39
4.3.4 Results . . . 41
4.3.5 Patient interest in home monitoring . . . 44

5 Extracellular Vesicles 45
5.1 Biological origin and Categorization . . . 45
5.2 Isolation and Extraction . . . 46
5.2.1 Ultracentrifugation . . . 46
5.2.2 Size Exclusion Chromatography (SEC) . . . 47
5.2.3 Immunoaffinity Capture . . . 48
5.2.4 Precipitation . . . 48
5.2.5 Microfluidic Approaches . . . 48
5.3 The Role of Extracellular Vesicles in Cancer . . . 49
5.3.1 The Tumour Microenvironment . . . 50
5.3.2 The Role of EVs in Cancer Progression . . . 50
5.3.3 Liquid Biopsy and EV-based Diagnostics . . . 50

6 Autonomous nanofiltration 51
6.1 Microfluidic Nanofiltration of Whole Blood . . . 51
6.1.1 Design Inspiration and Approach . . . 51
6.1.2 The Device . . . 52
6.2 Device Evaluation . . . 53
6.2.1 Initial results and material selection . . . 53
6.2.2 Device Characterization . . . 54
6.2.3 Nanofiltrate Compared to Differential Ultracentrifugation . . . 55
6.2.4 Analysis of Cancer Patient Nanofiltrates . . . 57

7 Summary and future outlook 61
7.1 Concluding remarks . . . 61
7.2 Thesis contributions, limitations, and future directions . . . 63
7.2.1 Sustainability . . . 65
7.3 Future perspectives . . . 66

Acknowledgment 69

References 73

Paper Reprint 85

List of Publications

This thesis is based on the following papers published in *peer-reviewed international journals*, conferences and manuscripts (* indicates equal contribution):

I. "Determination of lithium concentration in capillary blood using volumetric dried blood spots"
Fredrik Wikström, **Carl Olsson**, Bonita Palm, Niclas Roxhed, Lena Backlund, Martin Schalling, and Olof Beck.
Journal of Pharmaceutical and Biomedical Analysis, 227, 115269, 2023
DOI: 10.1016/j.jpba.2023.115269

II. "Home lithium monitoring using dried blood spots"
Fredrik Wikström, **Carl Olsson**, Olof Beck, Niclas Roxhed, Lena Backlund, and Martin Schalling.
Submitted for Journal Publication, 2026

III. "On-chip colorimetric assay for determining serum lithium concentration from whole blood"
Carl Olsson, Janosch Hauser, Federico Ribet, Fredrik Wikström, André Görgens, Olof Beck, Martin Schalling, Lena Backlund, and Niclas Roxhed.
Lab on a Chip, 25, 2270–2277, 2025
DOI: 10.1039/d5lc00044k

IV. "Advancing precision psychiatry: clinical testing of a novel colorimetric point-of-care device for monitoring lithium treatment in bipolar patients"
Carl Olsson, Fredrik Wikström, Lena Backlund, Martin Schalling, and Niclas Roxhed.
In Review at Bipolar Disorders, 2026

V. "Passive filtration of blood drops enriches extracellular vesicles and tumor-derived DNA"
Ellinor Hedberg*, **Carl Olsson***, Mariano A. Molina, Clara Öh, Magnus Nilsson, Fredrik Klevebro, Stefan Gilg, Per Stål, Andrea Ponzetta, Samir EL Andaloussi, André Görgens, Niclas Roxhed, and Daniel W. Hagey.
Submitted for Journal Publication, 2026

Contribution overview of the author to each of the above publications, major (• • •), partial (••), minor (•):

	Design	Fabrication	Measurements	Analysis	Writing
I	•	•	•	•	•
II	••	•	•••	••	••
III	•••	•••	•••	•••	•••
IV	•••	•••	•••	•••	•••
V	•••	•••	•••	•••	•••

Detailed statement of contributions for the listed publications:

I. The author assisted with the sample **measurements** and contributed to the **writing** through review and feedback of the manuscript.

II. The author **designed** and conducted the dried blood spot sample preparation and **measurements** for the study. Additionally, the author **authored** the corresponding sections of the method, and initial evaluations of **analysis** approaches.

III. The author was the primary **designer** and the sole **fabricator** of the device. All laboratory **measurements** and **analysis** were conducted by the author, with the exception of the MACSPlex experiments. The author served as the **primary author** of the manuscript.

IV. The author was one of the primary **designers** of the clinical trial and the sole **fabricator** of the device used. All **measurements** and **analysis** were conducted by the author, with the exception of the clinical sample collection conducted by a certified nurse. The author served as the **primary author** of the manuscript.

V. The author was the primary **designer** of the device and the primary of the two **fabricators**. All device characterization **measurements** and **analysis** were conducted by the author, with the exception of cancer sample processing and sequencing-related analyses performed by collaborators. The author served as one of the two **lead authors** of the manuscript.

This work has also been presented at the following national and international conferences (* indicates presenting author)

VI. "On-chip colorimetric assay for point-of-care lithium blood level determination using finger-prick-blood"
Carl Olsson*, Janosch Hauser, Federico Ribet, Fredrik Wikström, Olof Beck, Martin Schalling, Lena Backlund, and Niclas Roxhed.
At Medicinteknikdagarna 2023 (MTD), Stockholm, Sweden, 2023
Oral Presentation

VII. "On-chip colorimetric assay for point-of-care lithium blood level determination using finger-prick-blood"
Carl Olsson*, Janosch Hauser, Federico Ribet, Fredrik Wikström, Olof Beck, Martin Schalling, Lena Backlund, and Niclas Roxhed.
At the 27th International Conference on Miniaturized Systems for Chemistry and Life Sciences - Micro-Total Analysis Systems (μTAS), Katowice, Poland, 2023
Poster

VIII. "Autonomous microfluidic device for extracellular vesicle extraction from whole blood"
Ellinor Hedberg *, **Carl Olsson**, Mattias Hällbrink, André Görgens, Niclas Roxhed, and Daniel W. Hagey.
At the 28th International Conference on Miniaturized Systems for Chemistry and Life Sciences - Micro-Total Analysis Systems (μTAS), Montréal, Canada, 2024
Poster

IX. "Autonomous Microfluidic Device for Extracellular Vesicle Extraction from Whole Blood"
Ellinor Hedberg*, **Carl Olsson**, Mattias Hällbrink, André Görgens, Niclas Roxhed, and Daniel W. Hagey.
At the 1st Swedish Extracellular Vesicles Network Meeting, Stockholm, Sweden, 2025
Oral Presentation

X. "Autonomous microfluidic device for extracellular vesicle extraction from whole blood"
Ellinor Hedberg *, **Carl Olsson**, Mattias Hällbrink, André Görgens, Niclas Roxhed, and Daniel W. Hagey.
At Medicinteknikdagarna 2025 (MTD), Uppsala, Sweden, 2025
Oral Presentation

XI. "Autonomous microfluidic blood drop filtration enriches tumour-derived extracellular vesicle DNA for improved cancer detection"
Ellinor Hedberg, **Carl Olsson**, Mariano A. Molina, Clara Öh, Magnus Nilsson, Fredrik Klevebro, Stefan Gilg, Per Stål, Andrea Ponzetta, Samir EL Andaloussi, André Görgens, Niclas Roxhed, and Daniel W. Hagey*.
At ctDNA Symposium 2026 - Circulating Tumor DNA in Cancer: From Biology to Clinical Practice and Beyond, Aarhus, Denmark, 2026
Poster

XII. "Clinical testing of a novel colorimetric device for point-of-care capillary serum lithium measurement in bipolar patients"
Carl Olsson, Fredrik Wikström, Martin Schalling, Lena Backlund, and Niclas Roxhed*.
At the 26th WPA World Congress of Psychiatry, Stockholm, Sweden, 2026
Oral Presentation

Other contributions by the author not included in the thesis:

XIII. "Potato Protein Nanofibrils Produced from a Starch Industry Sidestream"
Leila Josefsson, Xinchen Ye, Calvin J. Brett, Jonas Meijer, **Carl Olsson**, Amanda Sjögren, Josefin Sundlöf, Anton Davydok, Maud Langton, Åsa Emmer, and Christofer Lendel.
ACS Sustainable Chemistry & Engineering, 8(2), 1058–1067, 2020
DOI: 10.1021/acssuschemeng.9b05865

Abbreviations

AAS	Atomic absorption spectrometer
AB	Apoptotic bodies
CAD	Computer-aided design
CGM	Continuous glucose monitoring
cmDNA	Circulating microbial DNA
CNV	Copy number variation
CryoEM	Cryo-electron microscopy
ctDNA	Circulating tumour DNA
CV	Coefficient of variation
DBS	Dried blood spot
DGUC	Density gradient ultracentrifugation
DPS	Dried plasma spot
DUC	Differential ultracentrifugation
EDTA	Ethylenediaminetetraacetic acid
ESR	Erythrocyte sedimentation rate
EV	Extracellular vesicle
FITC	Fluorescein isothiocyanate
GCLP	Good clinical laboratory practice
HCT	Hematocrit
ISF	Interstitial fluid
LEV	Large EV
LFA	Lateral flow assay
LOD	Limit-of-detection
MCE	Mixed cellulose ester
NAAT	Nucleic acid amplification test
PCR	Polymerase chain reaction
PCS	Patient-centric sampling
PDMS	Polydimethylsiloxane
PEG	Polyethylene glycol
PES	Polyethersulfone
POC	Point-of-care
PP	Polypropylene
PVDF	Polyvinylidene fluoride
RBC	Red blood cell
SEC	Size exclusion chromatography

SEV	Small EV
SMBG	Self-monitoring of blood glucose
SP	Soluble proteins
STI	Sexually transmitted infection
TDM	Therapeutic drug monitoring
TTR	Time to result
UC	Ultracentrifugation
UMAP	Uniform manifold approximation and projection
UV	Ultraviolet
WBC	White blood cell
WHO	World Health Organization

Thesis Aim and Structure

This thesis presents interdisciplinary research covering microfluidics, therapeutic drug monitoring, clinical chemistry, liquid biopsy, and patient-centric approaches to blood sampling and analysis. The work can be divided into two themes, therapeutic drug monitoring and whole blood nanofiltration.

Aims

The overall aim of this thesis was to develop and evaluate patient-centric approaches for therapeutic drug monitoring of lithium and whole-blood nanofiltration, with the overarching goal of improving access to testing while reducing the logistical barriers associated with traditional centralised healthcare workflows.

Objectives for this thesis:

1. Develop and evaluate patient-centric approaches for blood collection, processing, and analysis.

Therapeutic Drug Monitoring of Lithium

Lithium is a first-line treatment for bipolar disorder. However, its therapeutic window is narrow and lies close to toxic concentrations [1]. As a result, clinical guidelines recommend regular therapeutic drug monitoring, with lithium concentrations typically measured every 3–6 months [1]. Achieving this level of monitoring can be challenging even in high-income countries [2]. The burden that therapeutic drug monitoring places on both patients and healthcare systems through the need for repeated blood sampling may limit treatment accessibility and contribute to underutilization of this effective therapy [3]. There is therefore a need for more patient-centric approaches to lithium monitoring.

Objectives for this thesis:

2. Evaluate dried blood spot sampling as a patient-centric approach for therapeutic drug monitoring of lithium in patients with bipolar disorder.
3. Develop and evaluate a cartridge-based point-of-care microfluidic device for therapeutic drug monitoring of lithium.
4. Evaluate patient interest in patient-centric sampling and point-of-care approaches for lithium monitoring.

Whole Blood Nanofiltration

Extracellular vesicles are promising biomarkers for liquid biopsy [4]. However, conventional extracellular vesicle isolation methods are largely restricted to laboratory environments, requiring specialised equipment and labour-intensive processing [5]. There is therefore a need for simple sample-processing approaches capable of generating analysis-ready samples without the need for specialised equipment.

Objectives for this thesis:

5. Develop a user-friendly device to collect and process micro-volumes of whole blood, yielding a ready-to-use nanofiltered sample.
6. Evaluate the nanofiltered sample to verify the presence of extracellular vesicles and characterise the operational performance of the microfluidic device.
7. Conduct an initial pilot study on oncology patient samples using proteomic and genomic profiling to demonstrate the capability to extract biological information relevant to the disease from the nanofiltrate.

Structure of the thesis

Chapter 1
Introduces decentralised sampling and testing, their advantages, limitations, and current applications, before discussing blood as a diagnostic sample and the challenges associated with its collection, handling, and processing.

Chapter 2
Introduces the fundamentals of microfluidics and the principles used in the design of the devices presented in **Papers III and V**, together with the fabrication and prototyping methods used in this thesis.

Chapter 3
Introduces bipolar disorder, the role of therapeutic drug monitoring, and the historical development of lithium as a treatment for bipolar disorder.

Chapter 4
Provides an overview of **Papers I–IV**, including their hypotheses, methodologies, and key findings.

Chapter 5
Introduces extracellular vesicles, their biological origins, isolation strategies, and their roles in cancer biology and liquid biopsy applications.

Chapter 6
Discusses the autonomous nanofiltration device presented in **Paper V**, including its development, evaluation, and potential for extracellular vesicle and liquid biopsy applications.

Chapter 7
Summarises the main findings of the thesis, discusses its limitations, and presents future perspectives for the technologies and approaches described.

Use of generative AI

For this thesis, generative AI tools were utilised to improve the structural flow and readability of the original text draft, as well as for code debugging in LaTeX (Google Gemini and Microsoft Copilot). All content was reviewed and verified by the author and AI did not add any sources to the work. Additionally, AI-assisted literature discovery tools were used during the literature review process to identify relevant primary sources (Google Scholar Labs and Undermind). All retrieved sources were independently evaluated and selected by the author.

CHAPTER 1
Introduction

This chapter introduces point-of-care testing and decentralised sampling in clinical healthcare and examines how these approaches differ from conventional clinical workflows. The chapter also discusses blood as a diagnostic sample and the challenges associated with its collection, handling, and processing.

1.1 Decentralised Sampling and Testing

Clinical research, and increasingly healthcare delivery, are undergoing a gradual shift toward models that place greater emphasis on accessibility, convenience, and patient participation [6]. In this context, decentralisation aims to lower the barrier to entry for both routine healthcare and clinical trials by removing the need to visit a formal healthcare environment. Decentralised sampling frameworks can rely on patient-centric sampling (PCS), which encompasses sampling approaches designed to reduce the burden of specimen collection and may enable self-collection at a time and location convenient for the patient [7]. This approach was seen during the COVID-19 pandemic through the widespread deployment of PCS together with point-of-care (POC) tests, which provide a diagnostic result [8]. This deployment became a cornerstone of the global pandemic response by identifying and isolating infected individuals.

To guide the design and implementation of these decentralised frameworks, the World Health Organization (WHO) published the **ASSURED** criteria in the mid-2000s defining the requirements for ideal diagnostic tests at every level of the healthcare system [9, 10]. Originally developed to guide treatment decisions for infectious

tropical diseases and sexually transmitted infections (STIs) in the developing world and other resource-limited settings, the **ASSURED** criteria represent a set of seven core attributes:

Affordable refers to the goal of enabling accessibility by minimising the overall costs.

Sensitive and Specific describe the analytical performance required to ensure clinical reliability and avoid false negative or positive results.

User-friendly implies that the system is intuitive, requiring minimal interaction or specialised training from the patient.

Rapid and Robust focuses on achieving a short time to result (TTR) while ensuring the test and or sample remains stable without refrigeration.

Equipment-free means that sample collection and stabilisation are independent of specialised laboratory equipment.

Deliverable ensures that the testing kits are easily transportable to and from the patient, including those in rural or remote locations.

This framework has since become widely used as a benchmark for the development and evaluation of POC tests. In 2019, an expansion of **ASSURED** into **REASSURED** was proposed by adding two new attributes **Real-time connectivity** and **Ease of specimen collection** [11]. These additions promote the use of portable readers for standardised result interpretation and encourage non- or minimally invasive sampling.

Crucially, the application of these criteria extends beyond overcoming logistical and geographical barriers in resource-limited settings [12]. The COVID-19 pandemic highlighted the utility of PCS and POC testing within high-resource settings, where they served to maintain diagnostic continuity while keeping patients out of clinics [8]. Ultimately, whether in a rural village or an advanced healthcare centre, PCS creates benefits by shifting the operational focus toward patient convenience and workflow efficiency [8, 12].

1.1.1 Laboratory vs Point-of-Care Testing

There are two primary pathways for processing PCS samples, centralised [13] or decentralised [14] analysis (Figure 1), both with their own advantages and disadvantages. Centralised analysis requires that the sample is collected and stable during transport so as not to interfere with the downstream analysis and the results [13]. However, the use of centralised labs with advanced and sensitive equipment under good clinical laboratory practice (GCLP) guidelines, alongside established protocols and institutional accreditation, ensures that high analytical quality is consistently maintained.

Decentralised analysis is a type of POC testing in which diagnostic results are obtained and delivered directly at the POC. These tests are usually not as accurate and precise as centralised tests, but they provide immediate information that can be acted upon. This definition includes larger tabletop and handheld systems in a clinic providing

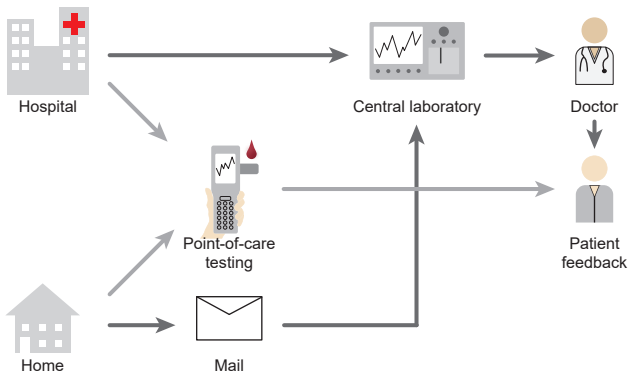

Figure 1: Comparison of centralised laboratory and decentralised POC testing pathways. Samples collected at home or in healthcare settings may either be analysed using POC devices, providing immediate patient feedback, or be transported to a centralised laboratory for analysis and clinical review before feedback is returned to the patient.

fast results and reduces sample logistics [15] as well as pregnancy tests and glucose monitors [14].

The benefit of both approaches were highlighted during the COVID-19 pandemic, where the sensitive polymerase chain reaction (PCR) test samples were collected and stabilised at home by individuals before being shipped to the laboratory. In contrast, the mass-produced and distributed antigen rapid tests, which emerged later in the pandemic, were not as accurate or sensitive but could give fast results directly to the patient making them useful for rapid, decentralised screening, alongside the heavily deployed centralised PCR infrastructure [8].

1.1.2 Point-of-Care applications

POC tests are well established in our society today and have, in some cases, become an invisible part of everyday life. At-home pregnancy tests were one of the first established POC tests for commercial use through the lateral flow assay (LFA) [16], the same technology used for the COVID-19 antigen rapid tests (Figure 2A) [17, 18]. Another success story is the handheld finger-prick glucose meters utilised in self-monitoring of blood glucose (SMBG) that revolutionised diabetes management and later transitioned into continuous glucose monitoring (CGM) (Figure 2B) [19, 20].

Although these are examples of accessible POC tests found almost everywhere today there are also several POC tests available in healthcare settings [21]. One of the main advantages POC tests have in healthcare settings is that they offload the sample logistics and provide faster results, reducing the need for scheduling sample collection and revisits. This immediate turnaround is particularly vital for time-critical diagnostics used to evaluate serious conditions requiring immediate intervention, such as cardiac troponin testing to evaluate suspected heart attacks [22].

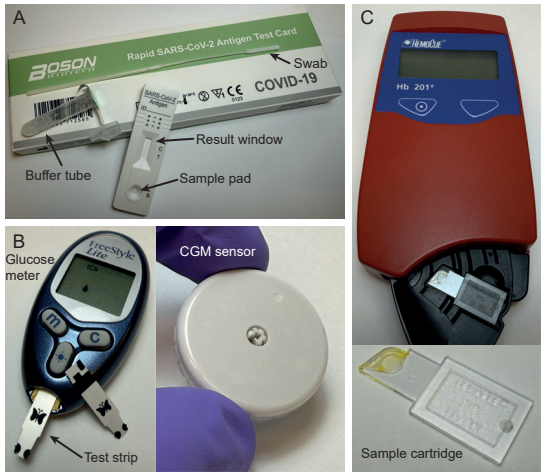

Figure 2: Examples of point-of-care diagnostic technologies. A) Lateral-flow antigen test showing the swab, buffer tube, sample pad, and result window. B) Glucose monitoring devices including a glucose meter, test strip, and continuous glucose monitoring (CGM) sensor. C) HemoCue haemoglobin analyser and disposable sample cartridge.

However, where POC shines is in low resource settings and to identify high-burden infectious diseases such as STIs and rare life threatening infections. There are several LFA POC tests available to detect STIs such as Syphilis [23] or HIV [24, 25]. LFAs for serious infectious tropical diseases such as Ebola are also available [26, 27], where tests can allow for fast large scale screening to quickly isolate infected individuals. In addition to LFAs, more modern bench top nucleic acid amplification tests (NAATs) POC systems are also available for several STI [23] and Ebola [28].

Even if these tests are invaluable in low-resource settings, they also play essential roles in the modern healthcare system. Handheld spectrophotometric systems, such as the HemoCue system (Figure 2C), provide instant haemoglobin quantification from a single drop of blood, serving as an early example of cartridge-based POC device and is still in use today [29, 30]. An example of a more advanced handheld system in use is the Abbott i-STAT system that can measure a multitude of parameters including electrolytes, blood gases, and coagulation profiles making them useful in emergency departments and other acute clinical settings [31]. Furthermore, both SMBG and CGM remain a hallmark of modern diabetes management, proving that everyday tracking is where decentralised diagnostics achieve their most routine clinical impact.

1.2 Blood as a sample

Blood holds a central role in routine clinical diagnostics and serves as one of the primary samples used in laboratory analysis, a status strongly reinforced by decades of extensive historical data and clinical experience. Whole blood consists of several distinct components suspended in a liquid plasma matrix containing solutes ranging from electrolytes and small molecules to proteins and macromolecules, as well as biological nanoparticles and cellular fractions (Figure 3). These cellular components comprise red blood cells (RBCs), also known as erythrocytes; white blood cells (WBCs), or leukocytes; and platelets, known as thrombocytes [32, 33]. Since whole blood comprises both soluble and insoluble fractions it can be described as a complex fluid [32].

The volumetric fraction of RBCs (Figure 3) in whole blood is called hematocrit (HCT) and normally ranges between 35 % and 55 % [34]. This volumetric variation can impact the concentration of some analytes in the blood. [35, 36]. Consequently, many clinical laboratory analyses are performed on plasma or serum rather than whole blood when appropriate for the analyte of interest.

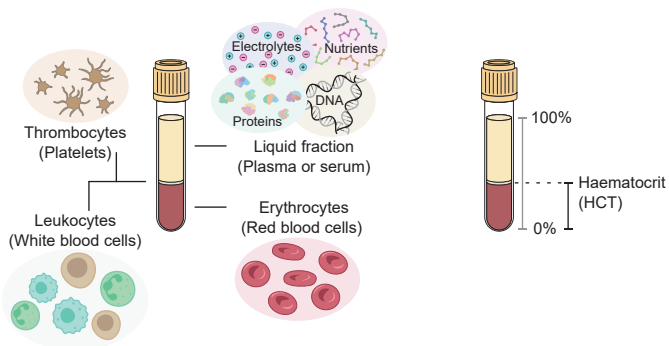

Figure 3: Main components of whole blood. HCT represents the volume fraction of RBC in a blood sample.

1.2.1 Conventional Blood Analysis Workflow

The art of blood collection is called phlebotomy and is generally divided into three sections, venous, arterial and capillary blood [37]. Venous sampling also known as venipuncture is conducted by a trained healthcare professional who inserts a needle into the vein to collect the blood in a vacuum tube. Vacuum tubes can have additives to inhibit or promote coagulation depending on the sample collected. Some tubes include buffers specially designed to preserve cells, DNA or RNA [38]. The order the tubes are collected in can also be important to prevent cross-contamination of additives [39]. Arterial blood is generally only used when there is a clinical need to and venous blood cannot provide the information needed [39]. Lastly, capillary blood is generally collected through finger-prick sampling (or heel-prick for infants), blood

is normally collected into a micro-tube, onto paper or applied directly onto a cartridge [13, 39].

When the sample arrives at a clinical lab it is logged and undergoes the appropriate pre-analytical processing required for downstream testing [37]. Depending on the analyte of interest, the blood is managed according to different protocols. Blood drawn into tubes is typically centrifuged within a predetermined timeframe, usually within 2 hours for plasma, to obtain plasma or serum [37]. There are notable exceptions, where tubes remain uncentrifuged, such as erythrocyte sedimentation rate (ESR) where the rate at which RBCs sediment to the bottom of the tube under the influence of gravity is measured [40]. Capillary blood, by contrast, is normally analysed immediately at the collection site, stored in specialised micro-tubes, or dried onto filter paper cards [13]. Collectively, these pre-analytical processes are to prepare the sample for analysis or function as a critical part of the analysis itself. Following processing, the samples are introduced into high-throughput automated analysers, such as Roche's cobas system, that feature integrated quality control [37].

1.2.2 Challenges When Working with Blood

Working with blood as a clinical sample introduces several analytical and operational challenges. While some challenges are universally obvious, such as the risk of infectious pathogens and sample contamination, a multitude of less obvious variables can also introduce significant pre-analytical errors. These include subtle physical alterations, localised physiological shifts during phlebotomy, strict volumetric constraints, and sample degradation [41, 42].

The analytical integrity of a blood sample is highly sensitive to the mechanics of the collection process itself. Several aspects of the collection procedure, including needle size and whether or not the tourniquet is left on the patient's arm for a prolonged period (typically more than one minute), impact the risk of haemolysis and can impact the concentration of certain analytes [43].

Once blood enters the collection system, volumetric precision becomes paramount. Commercial vacuum tubes are designed to collect a specific volume, if the tube is under filled the ratio of additives such as anticoagulants or stabilisers can be suboptimal. This can impact the downstream processing and influence assay results [41].

Furthermore, physiological variations in the patient can drastically alter the analytical matrix. Fluctuations in HCT influence the viscosity of the sample [33, 44]. This can complicate mechanical fluid handling and affect volumetric precision. When analysing whole blood, HCT can also influence the relationship between whole-blood and plasma or serum concentrations of analytes that partition differently between cellular and non-cellular compartments, including lithium [45]. Thus, the measured whole-blood concentration depends partly on the relative volumes of these compartments. Similarly, non-fasting sample collection can alter the concentrations of various analytes and thereby influence analytical results [46]. Blood contains living cells that metabolise nutrients within the sample over time. For instance, RBCs and WBCs continuously deplete glucose levels. This changes the concentration of metabolic analytes in the sample ex vivo and influence the results [47].

Specific analytes have strict processing requirements and some must be centrifuged and isolated within 2 hours of collection to ensure reliable clinical results [37]. For serum isolation, however, this time constraint must be balanced against the requirement for complete clot formation before centrifugation. Serum tubes cannot be centrifuged immediately since they must first stand undisturbed at room temperature for at least 30 minutes to permit complete coagulation [48]. This ensures that fibrinogen (a blood-clotting protein) has polymerized into a stable fibrin clot [37]. If the sample is centrifuged before complete clot formation, residual fibrin may remain in the serum and potentially interfere with downstream analysis or instrumentation [37].

Blood cells are also susceptible to damage during the subsequent transport and storage [41]. Damage to RBCs can result in haemolysis with the subsequent release of haemoglobin, intracellular ions, and cytoplasmic enzymes into the plasma [41]. Haemolysis can interfere with optical readout, inflate the concentration of intracellular components and interact with reagents used, rendering the analytical results highly unreliable [37, 41].

The composition of small-volume capillary samples can vary between successive samples obtained from a single skin puncture [49]. Collection practices such as excessive squeezing or milking of the finger to facilitate blood flow may further alter sample composition by affecting the HCT and analyte distribution, thereby potentially influencing measurement results.

The pre-analytical variables detailed above represent some of the challenges encountered when handling and processing blood. They illustrate how easily a sample can be altered or damaged before it ever reaches an instrument. Mitigating these specific vulnerabilities requires operational standardisation, which underscores the clinical complexity in handling large volumes of blood samples and the need for integrated quality control and automated monitoring in the workflow.

1.2.3 Blood Home Sampling

The centralization of blood sampling has traditionally been regarded as an important strategy for minimising pre-analytical variation and ensuring standardised sample collection and handling [50]. However, emerging technologies have enabled decentralised, patient-led home blood sampling for a growing range of compatible analytes [13]. Traditional dried blood spot (DBS) sampling has a well-established history in this field and has long been used for newborn screening of inherited metabolic and genetic disorders, including phenylketonuria [51]. Building upon this foundation, modern blood sampling technologies, including automated upper-arm capillary blood collection devices and advanced DBS systems, have complemented conventional finger-prick microtubes. In combination with plasma and serum separation technologies, these platforms enable patients to collect both liquid blood samples and dried plasma spot (DPS) samples without assistance from healthcare professionals [13]. These innovations, together with blood stabilisation strategies, have created new opportunities for large-scale screening and longitudinal home monitoring of patient cohorts. Rather than requiring patients or study participants

to travel to healthcare facilities for sample collection, samples can be collected at home and mailed or shipped to centralised laboratories for analysis [13].

Upper-arm blood collection devices, such as Tasso, together with DBS-based sampling systems, have already been used to reach patients in rural and geographically dispersed regions [52, 53]. These approaches have also been evaluated for population-scale screening of infectious diseases and other biomarkers [54, 55]. By reducing dependence on in-person visits, decentralised sampling may facilitate the recruitment of larger and more diverse study populations while enabling repeated sampling over extended periods. This could support both clinical care and research applications where longitudinal monitoring is desirable. However, successful decentralisation requires simplified sample handling and analytical technologies, with microfluidics providing one approach to achieving this.

CHAPTER 2
Physics and Fabrication

This chapter introduces the fundamentals of microfluidics, including capillary action, fluid transport, and flow control within microfluidic systems. It also discusses capillary blood-to-plasma separation and the fabrication and prototyping methods used for laminated microfluidic devices.

2.1 Microfluidics Theory

2.1.1 Scaling and Dominant Forces

In contrast to many macroscale flows, where inertial effects can be significant and turbulent flow may occur, microfluidic flows are typically dominated by viscous and interfacial effects [56]. This creates an environment where laminar flow conditions and material boundary properties exert a much larger impact on the behaviour of the flow.

As the characteristic length scale decreases, viscous forces become increasingly important relative to inertial forces. This balance between inertial and viscous forces is captured mathematically by the dimensionless Reynolds number (Re)

$$Re = \frac{Inertial\ Forces}{Viscous\ Forces} = \frac{\rho v L}{\mu} \tag{1}$$

where ρ is the fluid density, v is the mean velocity of the flow, μ is the dynamic viscosity of the fluid and L the characteristic length of the system. In conventional

macro-scale plumbing, the transition between laminar and turbulent flows (Figure 4) is generally associated with a critical threshold of $Re \approx 2300$, below which viscous forces suppress turbulent instabilities and maintain ordered laminar flow, although the reported threshold varies [57, 58].

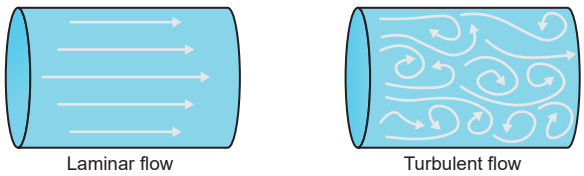

Figure 4: Illustration of laminar ($Re < 2300$) and turbulent ($Re > 2300$) flow.

In turbulent flows, rapid mixing is achieved through chaotic advection and the continual rearrangement of fluid elements. However, under laminar flow conditions, the flow remains highly ordered, and transport across streamlines occurs primarily through molecular diffusion. The diffusion time t_d over a distance L is given by

$$t_d \approx \frac{L^2}{D} \tag{2}$$

where D is the diffusion coefficient. Because diffusion time scales with the square of distance, transport over micrometer length scales can occur rapidly, whereas diffusion becomes slower as the length scale increases.

At these small scales, surface tension becomes increasingly important relative to gravity [59]. A familiar, real-world demonstration of this phenomenon occurs when water gathers and eventually starts dripping, or when small droplets bead up into near-perfect spheres on a hydrophobic surface. In the macro-world, large volumes of water puddle flat because gravity forces the fluid downward. However, when the fluid volume is sufficiently small, interfacial forces overpower gravity, and the system dictates its own shape based on the minimization of surface free energy (ΔG).

Thermodynamically, every interface possesses an inherent energy cost proportional to its surface area, expressed as:

$$\Delta G = \gamma \Delta A \tag{3}$$

where γ is the surface tension (or interfacial energy) and A is the surface area. Because nature spontaneously drives systems toward lower-energy states, with the Gibbs free energy reaching a minimum at equilibrium, a droplet will naturally alter its shape to minimise its exposed surface area. Geometrically, a sphere possesses the minimum surface-area-to-volume ratio (S/V) for a given volume. In microfluidics, this tendency to minimise free energy is utilised to create and manipulate flows, by controlling the surface properties of the channel walls and the interfacial tension between different phases.

2.1.2 Capillary Action and Wetting

Depending on the properties of the surface that water touches, it might behave very differently. If the water spreads out on the surface, the surface behaves as hydrophilic (water-loving), whereas if the water forms beads, the surface behaves as hydrophobic (water-fearing) (Figure 5). This behaviour can be quantified by the contact angle (θ) between the liquid and the surface. If the contact angle is smaller than 90°, the surface is hydrophilic, whereas if it is larger than 90°, it is hydrophobic. This behaviour is governed by the free energy in the system, with the liquid tending towards a lower-energy state. If it achieves this by spreading out, it will do so; if not, it will avoid the surface. This energy balance is described in Young's Equation, where the interfacial energies between the different interfaces determine the contact angle [59].

$$\gamma_{sg} = \gamma_{sl} + \gamma_{lg} \cos\theta \tag{4}$$

where γ_{sg}, γ_{sl}, and γ_{lg} represent the interfacial tensions between the solid–gas (sg), solid–liquid (sl), and liquid–gas (lg) phases, respectively.

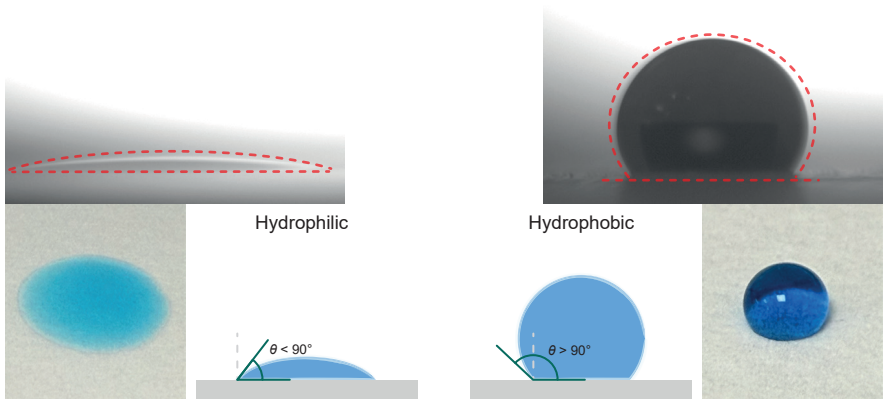

Figure 5: Examples of hydrophilic and hydrophobic droplets. A hydrophilic surface is characterised by a contact angle ($\theta < 90^\circ$), whereas a hydrophobic surface exhibits a contact angle ($\theta > 90^\circ$).

The contact angle also plays a central role in the spontaneous movement of liquids within microfluidic systems. This spontaneous movement arises from a pressure difference across the liquid–gas meniscus, described by the Young–Laplace equation [59]:

$$\Delta P = \gamma_{lg} \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \tag{5}$$

In a cylindrical channel (Figure 6), the principal radii of curvature R of the meniscus are identical ($R_1 = R_2 = \frac{r}{\cos\theta}$), and the Young–Laplace equation reduces to:

$$\Delta P = \frac{2\gamma_{lg} \cos\theta}{r} \tag{6}$$

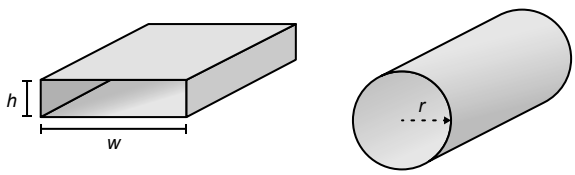

Figure 6: Geometrical parameters used to describe capillary pressure according to the Young–Laplace equation in rectangular and cylindrical channels.

In a rectangular channel (Figure 6), the channel walls are not necessarily composed of the same material. If different materials are present, the contact angle of each surface must be taken into account. The capillary pressure can then be expressed as [60]

$$\Delta P = \gamma_{lg} \left(\frac{\cos\theta_{left} + \cos\theta_{right}}{w} + \frac{\cos\theta_{top} + \cos\theta_{bottom}}{h} \right) \tag{7}$$

where w and h are the width and height of the channel, respectively. However, if all channel walls have the same contact angle the expression reduces to

$$\Delta P = 2\gamma_{lg} \cos\theta \left(\frac{1}{w} + \frac{1}{h} \right) \tag{8}$$

When $\theta < 90^\circ$, ΔP is positive and provides a natural driving force that spontaneously pulls the fluid through the channel network, without the need for external pumps or other active components.

2.1.3 Capillary-Driven Transport

In a capillary channel, the pressure difference generated by surface interactions can overcome gravitational forces, resulting in capillary rise (Figure 7). The height h a liquid can rise in a vertical cylindrical capillary of radius r is described by Jurin's law [59]

$$h = \frac{2\gamma \cos\theta}{\rho g r} \tag{9}$$

where ρ is the fluid density and g is the gravitational acceleration. A hydrophobic surface ($\theta > 90°$), on the other hand, results in a negative capillary pressure and a corresponding capillary depression rather than capillary rise.

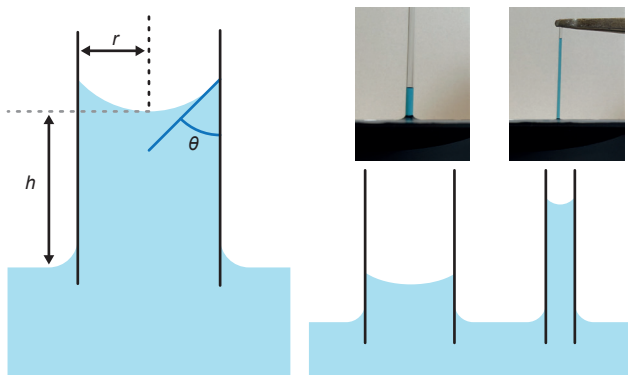

Figure 7: Capillary rise in cylindrical capillaries. The capillary radius r, rise height h, and contact angle θ are shown. Decreasing the capillary radius increases the capillary rise height, as illustrated by the photographs and schematic comparisons on the right.

The capillary rise effect is one of the reasons porous materials such as paper, fabrics, and sponges can spontaneously absorb and transport liquids. However, the rate at which a liquid propagates through a porous material depends on both the properties of the liquid and the structure of the material. The penetration distance of a liquid front (L) into a porous medium over time (t) is described in its idealised form by the Lucas–Washburn equation [61]:

$$L(t) = \sqrt{\frac{\gamma_{lg} r \cos\theta}{2\mu} t} \tag{10}$$

where μ is the dynamic viscosity of the fluid and r is the effective radius of the channel or porous structure. For porous materials, the Lucas–Washburn relation provides an idealised description in which the pore network is represented by an effective capillary radius. The resulting square-root dependence of penetration distance on time means that the velocity of the fluid front decreases as the liquid penetrates further into the

material. This characteristic behaviour (Figure 8) is an important consideration in capillary-driven microfluidics, explaining why entirely passive flows naturally slow down as they travel deeper into a porous material.

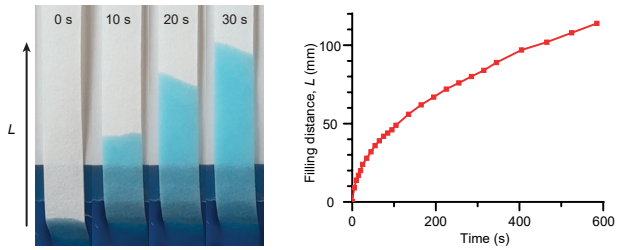

Figure 8: Illustration of capillary-driven liquid imbibition in porous materials. The left panel shows the progression of the liquid front over time, while the right panel shows the corresponding wicking distance (L) as a function of time. The liquid transport follows the behaviour described by the Lucas–Washburn equation.

Physically, this behaviour arises from the balance between the capillary pressure driving the flow and the viscous resistance opposing it. As the liquid penetrates deeper into a porous material or microchannel, the capillary pressure remains approximately constant. However, the wetted flow path becomes longer, increasing the viscous resistance experienced by the liquid and consequently reducing the flow velocity.

Once the material is fully wetted, the continuous flow through the porous matrix is governed by Darcy's law [62]:

$$Q = \frac{kA}{\mu}\frac{\Delta P}{L} \tag{11}$$

where Q is the volumetric flow rate, A is the cross-sectional area normal to the flow direction, L is the length of the flow path, μ is the dynamic viscosity of the fluid, and k is the intrinsic permeability of the material.

By rearranging Darcy's law, the pressure drop required to drive a given flow rate can be expressed as a fluidic analogue of Ohm's law ($U = RI$). This yields the fluidic resistance (R_f) of the porous material [62]:

$$R_f = \frac{\Delta P}{Q} = \frac{\mu L}{kA} \tag{12}$$

By changing the system geometry and knowing its fluidic resistance and the desired flow rate, the pressure required to drive the flow rate can be estimated. This pressure can then be compared with the available capillary pressure or provided by an external driving force to control fluid flow. This framework provides a mathematical basis for predicting and controlling fluid transport through both porous materials and enclosed microfluidic channels.

2.2 Capillary-Driven Microfluidics

Exploiting the power of capillaries and hydrophilic surfaces allows for high fluid control in microfluidic devices. Changes to channel geometry provide additional control over how fluids are transported and distributed through the device.

2.2.1 Flow Regulation

When designing microfluidic devices, controlling fluid flow is one of the most important aspects of the design process. We have already discussed how the material surface impacts fluid movement in the section 2.1.2. However, the dimensions of the channels also impact the flow resistance and the flow in the channels. In microfluidic systems, where the flow is typically laminar, the Hagen–Poiseuille equation can be used to determine the hydraulic resistance of channels with known geometries. For common channel shapes, the fluidic resistance R_f is given by:

Cylindrical channel [63]

$$R_f = \frac{8\mu L}{\pi r^4} \tag{13}$$

and the corresponding approximation of a rectangular channel [64] (Figure 9A)

$$R_f \approx \frac{12\mu L}{wh^3(1 - 0.63\frac{h}{w})} \tag{14}$$

where L is the channel length, r is the channel radius, w is the channel width, h is the channel height ($h \leq w$), and μ is the dynamic viscosity of the fluid.

From these equations, it can be seen that reducing the channel dimensions increases the fluidic resistance. Consequently, maintaining the same flow rate through a smaller channel requires a larger pressure drop across the channel.

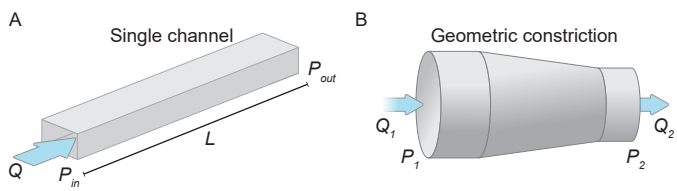

Figure 9: Examples of hydraulic flow restrictors A) Straight microchannel. B) Geometric constriction.

This relationship can be used to manipulate flow splitting and merging, providing control over fluid routing within microfluidic networks. When channels change shape or size, such as through a geometric constriction (Figure 9B), the hydraulic resistance changes accordingly. For channels connected in series the total resistance can be calculated from the total pressure drop, since the volumetric flow rate remains constant throughout the flow path.

Figure 10: Hydraulic resistances connected in series and in parallel.

$$Q = Q_1 = Q_2, \qquad \Delta P_1 = QR_1, \qquad \Delta P_2 = QR_2 \Rightarrow$$
$$\Delta P = \Delta P_1 + \Delta P_2 = QR_1 + QR_2 \Rightarrow$$
$$\Delta P = Q(R_1 + R_2)$$

giving a total resistance for channels connected in series (Figure 10):

$$R_f = R_1 + R_2 \tag{15}$$

Similarly, other channel geometries or microfluidic elements can be represented by their corresponding hydraulic resistance. Depending on the network configuration, either the flow rate or the pressure drop is shared between the elements. The total resistance for diverging parallel channels (Figure 10) that reconnect is given by

$$\frac{1}{R_f} = \frac{1}{R_1} + \frac{1}{R_2} \tag{16}$$

and can also be written as

$$R_f = \frac{R_1 R_2}{R_1 + R_2} \tag{17}$$

Knowing the hydraulic resistance of different channel geometries allows the flow to be manipulated and distributed throughout a microfluidic network. By adjusting channel dimensions and thereby resistances, more or less flow can be directed into specific pathways. This control can be achieved using either passive capillary forces or active pumping methods.

In capillary-driven systems, capillary pumps provide the driving force required to transport liquids through the device. Porous materials such as paper, fabrics, and absorbent membranes can act as capillary pumps by continuously wicking liquid into their structure until saturated (seen in Figure 8). More controlled capillary pumps can also be created through engineered microfluidic geometries or porous structures with tailored hydraulic resistances [60]. By controlling the flow rate, these passive pumping strategies can be used to regulate incubation and reaction times within a device.

Additional control over fluid movement can be achieved through venting. Air vents allow trapped air to escape during filling, while restricted ventilation can be used to generate pressure differences that influence the flow. Active approaches may also be employed, including external pumps or centrifugation, where pressure generated by centrifugal forces can be used to transfer liquids between different chambers of a microfluidic device [60].

2.2.2 Capillary Valves

There is often a need to temporarily stop or delay fluid flow within microfluidic networks. This may be required to store pre-loaded reagents, allow sufficient reaction or incubation time, release wash buffers at specific stages of an assay, or prime parts of the microfluidic network before further operation. Several valve concepts have been developed to provide this functionality. Broadly, microfluidic valves can be divided into time-delay valves, pressure-actuated valves, and trigger-actuated valves, although some designs combine multiple mechanisms [60].

Dissolvable delay valves use water-soluble materials, typically polymer films of varying thicknesses, which dissolve when wetted and open a flow path after a predetermined delay [65]. Geometric stop valves and capillary burst valves utilise abrupt changes in channel geometry to pin the liquid meniscus, preventing further flow until a predefined pressure threshold is exceeded, after which they remain open [66, 67]. Trigger valves extend this concept by using a secondary fluid front to release a previously halted flow [68, 69]. By careful placement within a microfluidic network, these valves can be used to retain liquids in specific regions, control reaction times, and coordinate multi-step assay workflows [60].

The placement of vents is also critical in valve design, since trapped air can interfere with valve actuation and channel filling. Proper venting helps maintain predictable pressure conditions and reliable fluid control throughout the device.

In addition to controlling fluid transport, microfluidic assays may require sample preparation before downstream on-chip processing. A common requirement is the separation of plasma or serum from whole blood.

2.2.3 Capillary Blood-to-Plasma Separation

There are several ways to separate plasma or serum from whole blood in a microfluidic device. Lateral flow tests achieve this through transport in a porous medium, where the plasma or serum fraction moves more readily than the larger cellular components of blood. Other approaches utilise capillary microstructures that selectively retain RBCs and other blood cells while allowing the plasma or serum fraction to continue through the device (Figure 11).

A more recent approach is a wedge configuration, where a filter is placed on top of a hydrophilic surface such that an acute angle is formed between them. This geometry enables transfer of the filtrate from the porous filter to the underlying hydrophilic surface, allowing the liquid to transition from the porous filter back into a continuous liquid phase. The liquid can subsequently be guided into a capillary channel and

integrated into a microfluidic network. Combining this configuration with a gradient flow-through filter, where the filter gradually goes from 35 µm to 2.5 µm pores, allows whole blood to be filtered, yielding a plasma or serum sample [70].

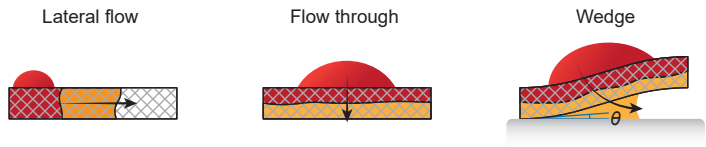

Figure 11: Illustration of membrane-based microfluidic flow configurations. From left to right: lateral flow, flow-through, and wedge geometries.

2.3 Microfluidic Prototyping and Fabrication

Microfluidic devices can be fabricated using a wide range of manufacturing techniques, including photolithography, etching, injection moulding, hot embossing, and other replication-based processes [71]. High-volume manufacturing methods can offer excellent reproducibility and throughput, but tooling requirements and development lead times may limit their suitability for rapid iterative prototyping. Rapid prototyping technologies such as polydimethylsiloxane (PDMS) soft lithography, additive manufacturing, and lamination provide greater flexibility during early-stage device development [71].

PDMS is a transparent silicone elastomer widely used in microfluidics because it is readily moulded and can be bonded to glass or PDMS following surface activation, enabling sealing of microfluidic channels. When initially mixed with a curing agent, it is liquid, allowing for easy casting on top of a mould. The mould can be milled or fabricated using photolithography, allowing for small, high-fidelity structures to be imprinted into the PDMS, which is thermally cured before being ready to use. When cured, the PDMS can be removed from the mould, retaining the imprinted structures. This process is referred to as soft lithography [71]. While the gas permeability of PDMS can be advantageous for certain applications, it also has several well-known limitations, including the absorption of hydrophobic molecules, water vapor permeability, and deformation under high pressures [71]. Additionally, PDMS is intrinsically hydrophobic, although surface treatments or chemical additives can be used to temporarily render it hydrophilic.

Another manufacturing technique is additive manufacturing, also known as 3D printing, which can be used to fabricate complex, three-dimensional components or entire microfluidic devices. In the context of microfluidics, photopolymer resins are commonly employed, with structures built up layer-by-layer using material-specific ultraviolet (UV) or visible light wavelengths to polymerize and cure each successive layer. Other engineering materials, such as thermoplastics and metals, can also be printed. However, depending on the printing orientation, material, and printing method, the surface smoothness may vary, and the printed parts may also require post-processing or surface treatments to achieve the desired properties. Another limitation is the resolution and dimensional accuracy, as small or closely spaced

features may not be accurately reproduced as distinct structures. Despite these limitations, additive manufacturing is well suited to early-stage prototyping, allowing for rapid design iterations and complex geometries [71, 72].

2.3.1 Lamination Technology Platform

The lamination technology platform offers a compelling middle ground between PDMS and 3D printing. It relies on the sequential stacking of hydrophilic polymer sheets, functional films, double-sided adhesive tapes, and integrated porous matrices (such as paper or membrane filters) to define enclosed microfluidic flow paths. The geometric features of the channels, inlets, and vents are patterned into individual layers using methods ranging from mechanical stamping and cutting plotters to CO_2 lasers. Crucially, the intrinsic thickness of the selected sheet, film, or adhesive tape dictates the final channel height, allowing for the engineering of complex, multi-layer fluidic structures simply by varying layer selection [71].

The primary advantage of lamination is its rapid turnaround time compared to 3D printing and PDMS. Depending on the design complexity, multiple design iterations can be fabricated, assembled, and tested within a single day using manual or guide-pin-assisted alignment. The constituent layers can be selected according to the required material properties, reducing the need for post-processing during early-stage prototyping.

Although this technology can be assembled manually, the workflow can also be adapted to automated pick-and-place assembly or high-throughput roll-to-roll manufacturing, providing a pathway toward large-scale production [71].

Despite these advantages, lamination also introduces several design and fabrication constraints. Alignment during manual or pin-assisted assembly can introduce geometric tolerances between layers, while the available layer thicknesses and surface properties are constrained by the selected sheet, film, and adhesive materials. The design must also provide sufficient material between adjacent features to maintain structural integrity. Furthermore, the layer-by-layer architecture can produce rough channel boundaries and stepped vertical interconnects, which may influence capillary flow, bubble trapping, and dead volume [71]. These considerations therefore need to be incorporated into the device design and material selection. Nevertheless, the combination of rapid prototyping, flexible material selection, and scalability makes lamination particularly suitable for the iterative development of the capillary-driven devices investigated in this thesis.

2.3.2 Idea to Prototype

To illustrate the design process of a laminated microfluidic device, a simplified version of the nanofiltration device developed in **Paper V** of this thesis is presented in Figure 12. The first step is to identify the available materials and their relevant properties, particularly their thicknesses. In this design, the adhesive thickness is the primary constraint and should be kept as small as possible due to the acute wedge angle required between the nanofilter and the hydrophilic collection layer. This wedge geometry is

essential for extracting the nanofiltrate from the filter and transferring it onto the hydrophilic surface.

The next step is to design the required geometries in a computer-aided design (CAD) program. These geometries are cut into the adhesive layers to define the microfluidic channels, while openings in the top hydrophilic layer form the sample inlet and venting structures. The dimensions of the channels determine the volume of liquid that can be collected and therefore provide a means of controlling the sampled volume. To simplify assembly and improve reproducibility, alignment holes can also be incorporated into each layer, allowing the device to be assembled using alignment pins.

Once the design is finalised, the individual layers are fabricated using either a CO_2 laser cutter or a cutting plotter. The layers are then aligned and assembled into the final device. The final device design contains additional features used to secure the filter material and improve device performance, but the process described above captures the general workflow used for the design and fabrication of laminated microfluidic devices.

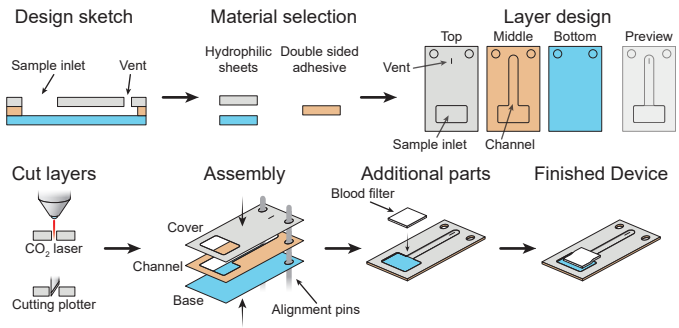

Figure 12: Schematic representation of the laminated microfluidic device fabrication workflow from **Papers III and V**. Starting from a design sketch and material selection, the individual layers are designed, cut, assembled, and combined with functional components such as the blood filter to create the final device.

CHAPTER 3
Bipolar Disorder

This chapter introduces bipolar disorder and the history of lithium therapy. It also describes the principles and challenges of lithium therapeutic drug monitoring and reviews emerging decentralised approaches for patient-centric lithium monitoring.

3.1 Clinical Overview

Bipolar disorder is a chronic mood disorder characterised by recurring episodes of mania and depression, resulting in substantial fluctuations in mood, energy levels, and activity [1]. These episodes typically last from several days to many months and can significantly affect daily functioning and quality of life (Figure 13) [1, 73, 74]. Manic episodes are characterised by elevated or irritable mood, increased energy, reduced need for sleep, and impulsive behaviour. A milder form, known as hypomania, presents with similar symptoms but is generally less severe and is not associated with psychotic symptoms or severe functional impairment [1]. Depressive episodes are characterised by persistent low mood and a loss of interest or pleasure, often accompanied by changes in energy, sleep, cognition, and daily functioning.

Bipolar disorder is commonly classified into bipolar disorder type I and type II. Type I bipolar disorder is defined by the occurrence of at least one manic episode, whereas type II bipolar disorder is characterised by at least one hypomanic episode and at least one major depressive episode, without a history of a manic episode. Depressive episodes occur in both forms of the disorder, although type II bipolar disorder requires the presence of at least one major depressive episode for diagnosis [1].

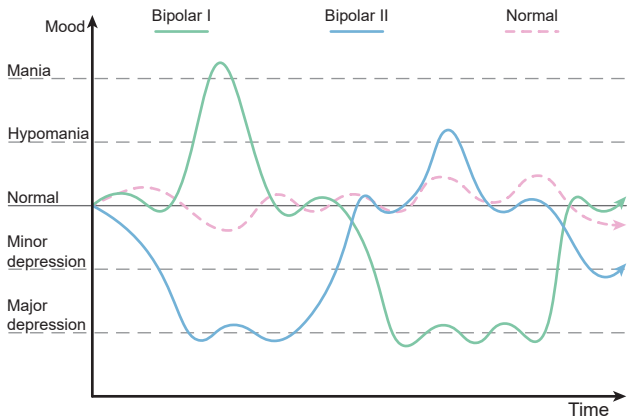

Figure 13: Schematic representation of mood fluctuations in bipolar disorder type I and type II compared with a normal mood trajectory.

3.1.1 Prevalence and Disease Burden

Bipolar disorder is estimated to affect approximately 1–2 % of the global population [1, 75]. Historically, the prevalence has been considered relatively similar between males and females, although a recent study has reported a slightly higher prevalence among females. It remains unclear whether this reflects a true difference in prevalence or whether females are more likely to receive a diagnosis [76]. Regardless of these differences, bipolar disorder is associated with a substantial personal and societal burden. The consequences may include impaired employment, strained social relationships, financial difficulties arising from impulsive behaviour during manic episodes, and social withdrawal or reduced motivation during depressive episodes. Bipolar disorder is also associated with an increased risk of suicide compared with the general population, making effective long-term treatment particularly important [1].

3.1.2 Clinical Management

Acute mood episodes may require medical intervention, including pharmacological treatment and, in severe cases, hospitalisation. However, bipolar disorder is primarily managed through maintenance therapy, with the aim of reducing symptom burden, preventing relapse, and restoring functioning and quality of life. As bipolar disorder is a chronic condition, treatment is often lifelong. Management typically includes condition specific education, therapy, medication, and the establishment of a social support network to facilitate the early recognition of mood episodes and timely intervention. In addition, lifestyle modifications, including regular exercise, healthy dietary habits, and maintaining regular daily routines, are recommended to support long-term mood stability and overall well-being. Current treatment recommendations are informed by international clinical guidelines and evidence updates, including the 2018 CANMAT/ISBD guidelines and their 2023 update [1, 77]. While specific

recommendations may differ slightly between countries, the overarching treatment strategies are largely consistent across modern clinical guidelines.

3.2 Lithium for Bipolar Disorder: Historical Summary

3.2.1 The 1800s: Early Foundations

Lithium was first discovered in 1817 by Johan August Arfwedson during his analysis of petalite from Utö, Sweden [78]. The element was not isolated in its pure metallic form until a few years later, when William Thomas Brande successfully utilised electrolysis [78, 79].

Lithium has a long history of medical use [78]. One of its earliest documented medical applications was the treatment of gout in the 1840s, based on the hypothesis that it could dissolve urate deposits [78, 80]. Over the subsequent decades, physicians worldwide began observing and documenting its broader therapeutic effects, noting its utility as an anticonvulsant, and as a treatment for general nervousness, acute mania, and depression [79, 81].

3.2.2 1950s–1960s: The Road to Clinical Acceptance

In 1949, John F. Cade reported the successful treatment of manic patients with lithium and concluded that lithium medication

"... would be much preferred to leucotomy" (lobotomy) John F. Cade 1949 [82].

The 1949 paper laid the foundation for the 1952 randomised controlled trial published in 1954, which provided some of the first clinical evidence supporting the efficacy of lithium in the treatment of manic-depressive illness, today known as bipolar disorder [83]. The authors noted that lithium offered a useful alternative to electroconvulsive therapy while also allowing some patients to be maintained in a normal state through continued maintenance treatment. Several later studies confirmed the efficacy of lithium in the treatment and maintenance management of manic-depressive illness [78].

Despite its clinical benefits, lithium dosing remained largely a matter of clinical judgment informed by observations. Advances in flame photometric measurement during the 1950s enabled routine monitoring of lithium concentrations in blood and serum, providing a more reliable basis for dose adjustment and contributing to the widespread clinical adoption of lithium therapy [79, 81]. During the 1960s lithium was approved for the treatment of bipolar disorder in several countries, including France, the United Kingdom and Germany while others followed later [79, 81].

The timing of regulatory approval varied between countries, and clinical adoption was not determined solely by regulatory approval dates. In several countries, including Denmark, lithium became widely established in clinical practice and research, contributing substantially to the evidence base supporting its use in bipolar disorder.

3.2.3 Establishing First-Line Treatment

The standardisation of lithium therapy continued during the 1970s. A 1976 study reported adjusting lithium dosage to maintain 12-hour post-dose serum concentrations between 0.7 and 1.3 mmol/L [84]. Such target concentrations enabled more consistent treatment and remain an important component of modern clinical guidelines for lithium therapy. Together with an expanding clinical evidence base, these developments helped establish lithium as a cornerstone of long-term maintenance therapy [81].

Lithium was subsequently incorporated into national and international treatment guidelines [85, 86]. Accumulating evidence for a significant reduction in suicide risk among treated patients further strengthened its position as a first-line treatment for bipolar disorder [87, 88].

3.2.4 Present Day: The Enduring Benchmark

Today, lithium is a first-line treatment for long-term maintenance therapy of bipolar disorder, with well-documented efficacy and suicide-preventive effects [1, 77]. Despite its age and the introduction of numerous alternative treatments since lithium first entered clinical practice, lithium has retained its position as the recommended first-line maintenance treatment for bipolar disorder and remains the benchmark against which newer therapies are compared (Figure 14) [1].

Despite its established efficacy, lithium has acquired a reputation for being difficult to manage owing to its narrow therapeutic window, potential toxicity, and requirement for regular monitoring. These practical challenges, together with the availability of newer treatment options, contributed to a decline in lithium use in some regions despite its continued recommendation as a first-line treatment in clinical guidelines [89, 90].

In recent years, lithium has also attracted renewed research interest beyond its role as a mood stabiliser, including its potential anti-ageing and neuroprotective effects, as well as a possible role in reducing the risk of neurodegenerative disorders [91, 92]. While these findings remain an active area of research, they further highlight the continued clinical and scientific relevance of lithium and ongoing interest in its potential applications beyond the treatment of bipolar disorder.

	Level of evidence by phase of treatment					Considerations for treatment selection			
	Maintenance			Acute		Acute		Maintenance	
	Prevention of any mood episode	Prevention of depression	Prevention of mania	Depression	Mania	Safety concerns	Tolerability concerns	Safety concerns	Tolerability concerns
First-line treatments									
Lithium	●	●	●	◔	●	+	+	++	++
Quetiapine	●	●	●	●	●	+	++	++	++
Divalproex	●	◔	◑	◔	●	–	+	++[c]	+
Lamotrigine	●	●	◔	●	■	++	–	–	–
Asenapine	◔	◔	◔	n.d	●	–	+	–	+
Quetiapine + Li/DVP	●	●	●	◔	●	+	++	+++[c]	++
Aripiprazole + Li/DVP	◔	n.d.[a]	◔	◔	◔	+	+	++[c]	++
Aripiprazole	◔	n.d.[a]	◔	■	●	–	+	–	+
Aripiprazole OM	◔	n.d.[a]	◔	n.d.	n.d.	–	+	–	+
Second-line treatments									
Olanzapine	●	●	●	●[b]	●	+	++	+++	++
Risperidone LAI	●	n.d.[a]	●	n.d.	n.d.	–	+	+	++
Risperidone LAI (adj)	◔	◔	◔	n.d.	n.d.	+	++	+++	++
Carbamazepine	◔	◔	◔	◑	●	++	++	+[c]	++
Paliperidone (>6 mg)	◔	n.d.[a]	◔	n.d.	●	–	+	+	++
Lurasidone + Li/DVP	◑[d]	◑[e]	◔	◔	n.d.	+	++	++[c]	++/–
Ziprasidone + Li/DVP	◔	n.d.[a]	◔	◧	■	++	++	++[c]	+

DVP, divalproex; LAI, long-acting injectable; Li, lithium, OM, once monthly.
●, level 1 evidence; ◔, level 2 evidence; ◑, level 3 evidence; ◴, level 4 evidence; ■, level 1 negative evidence; ◪, level 2 negative evidence; ◧, level 3 negative evidence; ◱, level 4 negative evidence; n.d., no data; – limited impact on treatment selection; +, minor impact on treatment selection; ++, moderate impact on treatment selection; +++, significant impact on treatment selection.
[a]Did not separate from placebo in those with index mania; no studies available in index depression.
[b]Did not separate on core symptoms of depression.
[c]Divalproex and carbamazepine should be used with caution in women of child bearing age.
[d]Trend for superiority on the primary efficacy measure, hence the lower rating.
[e]Effective in those with an index episode of depression.

Figure 14: Hierarchical ranking of first- and second-line treatments recommended for maintenance treatment in bipolar disorder from the 2018 CANMAT/ISBD guidelines. Reprinted from Table 17 in Yatham et al. [1] under the CC BY-NC-ND license.

3.3 Lithium and Therapeutic Drug Monitoring

The primary challenge associated with lithium therapy is the need for regular therapeutic drug monitoring (TDM). This requirement stems from the narrow therapeutic range of lithium (0.4–1.2 mmol/L; for lithium, a monovalent ion, this is equivalent to 0.4–1.2 mEq/L) [1] that lies close to the toxic range (>1.5 mmol/L) [93]. Consequently, maintaining serum lithium concentrations within the therapeutic window is essential to ensure therapeutic efficacy while minimising the risk of adverse effects and toxicity.

Furthermore, the serum lithium concentrations vary over time as it is excreted from the body. To ensure consistent and clinically meaningful measurements, blood samples are typically collected 12-hours after the last dose and after at least five days on a stable dosing regimen to ensure steady-state conditions [1]. This standardised sampling strategy provides a consistent basis for comparison between measurements and allows reliable interpretation of lithium concentrations during long-term therapy.

Another reason why TDM is important is that optimal lithium doses and serum concentrations vary between individuals. Furthermore, lithium concentrations vary over time and can be influenced by factors such as renal function, age, hydration status, sodium balance, and drug interactions [94, 95]. Consequently, regular monitoring of serum lithium concentrations is required throughout treatment to ensure therapeutic efficacy while minimising the risk of toxicity.

3.3.1 Current Monitoring Workflows

TDM of lithium typically requires serum concentration measurements every 3–6 months, in addition to monitoring of thyroid and renal function and plasma calcium at least annually [1]. During treatment initiation, monitoring is performed more frequently to establish therapeutic efficacy and ensure safe treatment. This process includes dose titration until a therapeutic serum concentration is achieved that provides clinical benefit while minimising adverse effects.

Monitoring typically involves venous blood sampling, with samples collected approximately 12-hours after the last dose. Patients must therefore carefully coordinate medication intake and attend a clinic or hospital for blood collection multiple times each year. Results are reported to the treating physician and interpreted alongside clinical response and reported adverse effects. These findings are then used to guide dose adjustments and support safe and effective treatment.

3.3.2 Challenges in Modern Lithium Management

While this monitoring strategy is effective, it requires repeated blood sampling, clinical follow-up, and active patient engagement throughout treatment, placing a considerable monitoring burden on both patients and healthcare systems [96].

For patients, this burden includes the need to attend clinics or hospitals for blood collection multiple times per year. In rural and remote areas, access to these facilities may require substantial travel, potentially affecting adherence to recommended

monitoring schedules through delayed or missed appointments [96]. Furthermore, lithium monitoring depends on the availability of laboratory infrastructure capable of accurately measuring serum lithium concentrations[97].

Adherence to recommended monitoring schedules remains challenging. Suboptimal adherence to guideline-recommended lithium monitoring has been reported in both resource-limited and highly developed healthcare systems [2, 97]. These findings suggest that factors beyond healthcare infrastructure may contribute to poor adherence to lithium monitoring guidelines.

In addition to adherence to monitoring schedules, effective lithium therapy relies on long-term medication adherence. A Swedish study found that 54 % of patients treated with lithium experienced at least one treatment discontinuation, with common reasons including perceived lack of effectiveness, non-adherence, and treatment-related adverse effects [98].

3.4 Emerging Approaches in Lithium Monitoring

One approach to reducing the monitoring burden associated with lithium therapy is to decentralise sample collection or analysis. Two broad strategies have emerged: PCS and POC testing.

PCS is primarily based on microsampling techniques such as DBS and DPS, enabling patients to collect samples at home and mail them to a laboratory for analysis [99]. While this reduces the need for travel and venous blood sampling, a delay remains between sample collection and the availability of results.

Alternatively, POC testing aims to perform lithium measurements at or near the POC, potentially providing results directly to patients and healthcare providers within a short period of time [100]. Such systems could enable more rapid dose adjustments and clinical decision-making.

Both approaches aim to reduce the burden associated with conventional lithium monitoring by minimising the need for hospital and clinic visits while improving access to testing. However, neither approach eliminates the need for periodic clinical follow-up, as monitoring of renal function, thyroid function, and plasma calcium remains necessary throughout treatment.

CHAPTER 4

Decentralised Approaches to Lithium Monitoring

*This chapter discusses the background and results of **Papers I–IV**, covering patient-centric microsampling approaches based on dried blood and plasma spots, as well as the development and evaluation of a microfluidic POC device for lithium monitoring.*

4.1 Dried Blood and Plasma Spot Sampling

DBS and DPS are microsampling techniques in which small volumes of blood or plasma are collected and dried on a paper-based substrate. Samples may be applied as droplets, pipetted directly, or delivered through a microfluidic channel (Figure 15) [13]. Following drying, the samples can be stored and transported to a laboratory for analysis.

When applying a blood sample directly from a finger onto paper, several factors can influence the analytical results obtained from the sample (Figure 16). Upon contact with the paper, both the deposited sample volume and the HCT influence the size and shape of the resulting blood spot. In punch-based DBS analysis, the HCT and punch location may affect the amount of analyte collected [101]. Chromatographic migration and drying-related redistribution can lead to a non-uniform analyte distribution across the spot, causing some regions to contain more or less analyte than others [102]. While this is less important for qualitative analysis (detecting the presence of an analyte), it can become important when quantifying concentrations, as the punched disk may

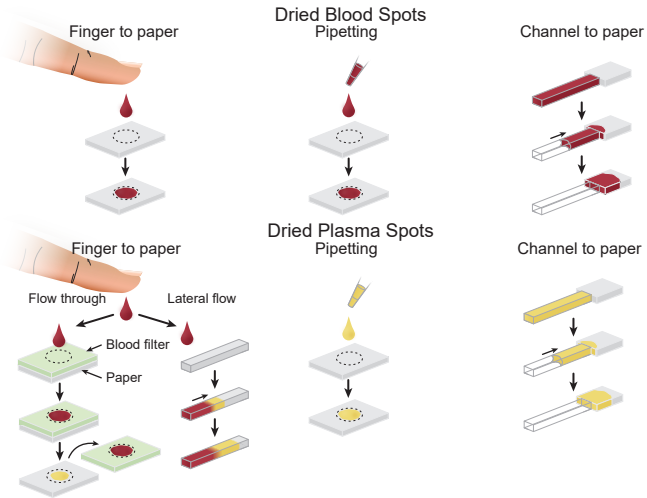

Figure 15: Examples of blood and plasma sampling approaches in paper-based microfluidic devices. Whole blood can be applied directly from a finger-prick, transferred by pipetting, or delivered through microfluidic channels. Plasma may be obtained by blood filtration prior to collection on paper substrates.

not accurately represent the composition of the entire blood spot. This effect can be reduced by analysing the entire spot, thereby averaging potential analyte gradients across the sample [103]. Following collection, the analyte is typically eluted into a liquid. The proportion of the analyte present in the dried blood spot that is transferred to the elution solution is referred to as the recovery. Most of the factors discussed for DBS are also relevant to DPS sampling. However, since DPS contains plasma rather than whole blood, HCT-dependent effects on spot formation are largely mitigated when comparing analyte concentrations with those measured in serum or plasma samples [103]. Although these factors can contribute to analytical variability, DBS and DPS remain attractive approaches for decentralised sampling due to their simplicity, stability, and ease of transport [13].

Both DBS and DPS have been investigated as sampling approaches for decentralised lithium monitoring. Manfro et al. demonstrated that lithium could be quantified from patient-collected DBS and DPS samples, although the clinical evaluation showed greater variability than the analytical validation performed using laboratory-prepared samples [99]. More recently, the non-volumetric HealthID PSD device demonstrated the continued feasibility of DPS-based lithium monitoring, although endogenous chloride was used as an internal reference to compensate for unknown plasma volumes in individual samples [105].

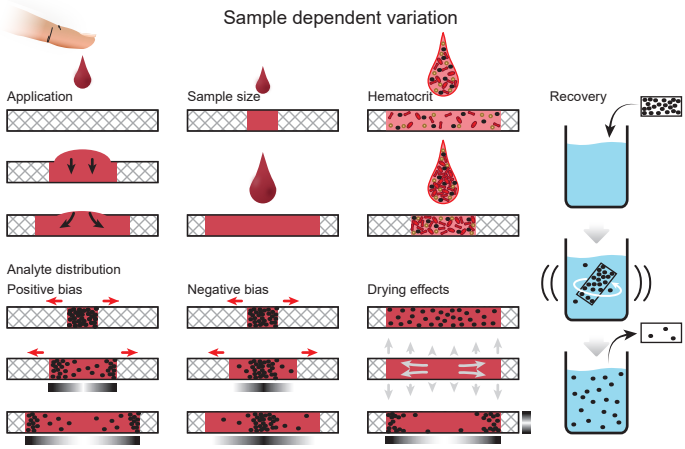

Figure 16: Effects influencing the quantitative use of DBS, including sample application, sample variability, analyte distribution within the paper matrix, and analyte recovery during extraction. Inspired by Figure 2.2 in [104].

4.1.1 Volumetric Dried Blood Spots

To reduce uncertainty in sample volume associated with non-volumetric dried spot sampling, a volumetric approach was used in **Paper I**. The DBS device, developed by Capitainer AB (Figure 17), collects a predefined blood volume into a pre-cut paper disc, allowing the entire sampled volume to be analysed and thereby addressing several of the sources of variation illustrated in Figure 16. We developed and validated an atomic absorption spectrometer (AAS)-based analytical workflow for lithium quantification from volumetric DBS samples, illustrated in Figure 18A.

Figure 17: Volumetric dried blood spot sampling using the Capitainer® DBS device employed in **Papers I and II**.

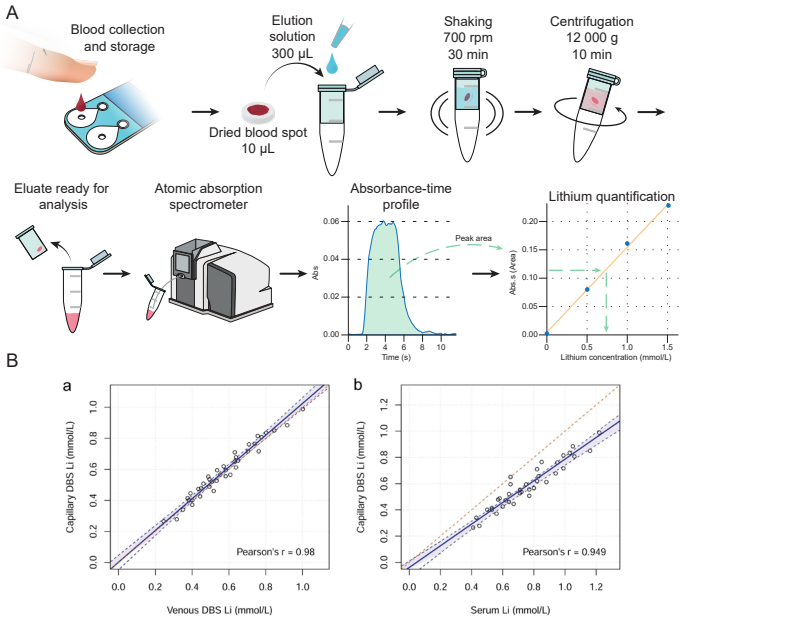

Figure 18: A) Illustration of the analytical workflow for lithium quantification from volumetric DBS samples. B) Passing–Bablok regression analyses of lithium concentrations measured in capillary and venous DBS samples (a), and in capillary DBS and serum samples (b). Reproduced from Figure 4 in Wikström et al. [106] under CC BY 4.0.

Furthermore, we demonstrated excellent agreement between capillary and venous DBS samples (Figure 18Ba) and showed that DBS measurements exhibited a linear relationship with conventional serum lithium concentrations, enabling the use of a correction factor to estimate serum-equivalent lithium concentrations (Figure 18Bb) [106]. Together, these findings support the use of volumetric DBS microsampling for decentralised lithium TDM.

4.1.2 Serum vs Red Blood Cell Fractions

Following **Paper I** and the observed linear relationship between DBS and serum lithium concentrations (Figure 18Bb), we investigated whether an individual-specific relationship existed between DBS and serum concentrations that could be used for personalised serum-to-DBS estimations. To evaluate this hypothesis, **Paper II** followed patients longitudinally over four visits separated by at least two weeks. The aim was to determine whether the serum-to-DBS ratio obtained during the first visit could be used to estimate serum concentrations from DBS measurements collected during subsequent visits (Figure 19) and to assess patient acceptability of the method using self-rating scales.

While several individuals showed good agreement between serum concentrations estimated from DBS measurements and corresponding measured serum concentrations, others exhibited one or more measurements that deviated from the expected values (Figure 20). The estimated and measured serum concentrations were strongly correlated (Pearson's r=0.88), while Passing-Bablok regression and Bland-Altman analysis indicated no systematic bias, with a mean deviation of 0.02 mmol/L (Figure 21A and C).

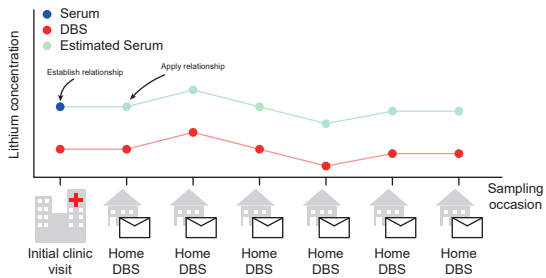

Figure 19: Conceptual illustration of the hypothesis evaluated in **Paper II**. An individual serum-to-DBS ratio is established during an initial clinic visit where both DBS and serum samples are collected. This relationship is subsequently applied to DBS samples allowing estimation of serum-equivalent lithium concentrations from DBS measurements alone.

Using the individual average serum-to-DBS ratio from visits 1 and 2 to predict serum concentrations at subsequent visits improved the correlation (Pearson's r = 0.92) and reduced the variability; however, the mean deviation remained unchanged (Figure 21B and D).

The mean error was 8.3 %, which was within the <10 % error margin of the reference colorimetric method used in this study. Furthermore, HCT had a measurable but minor impact on the results (**Paper II**).

Some of the participants in **Paper II** had also participated in **Paper I**. This allowed us to evaluate whether the serum-to-DBS ratio obtained in 2021 (**Paper I**) remained stable over time when applied to the longitudinal data collected in **Paper II**.

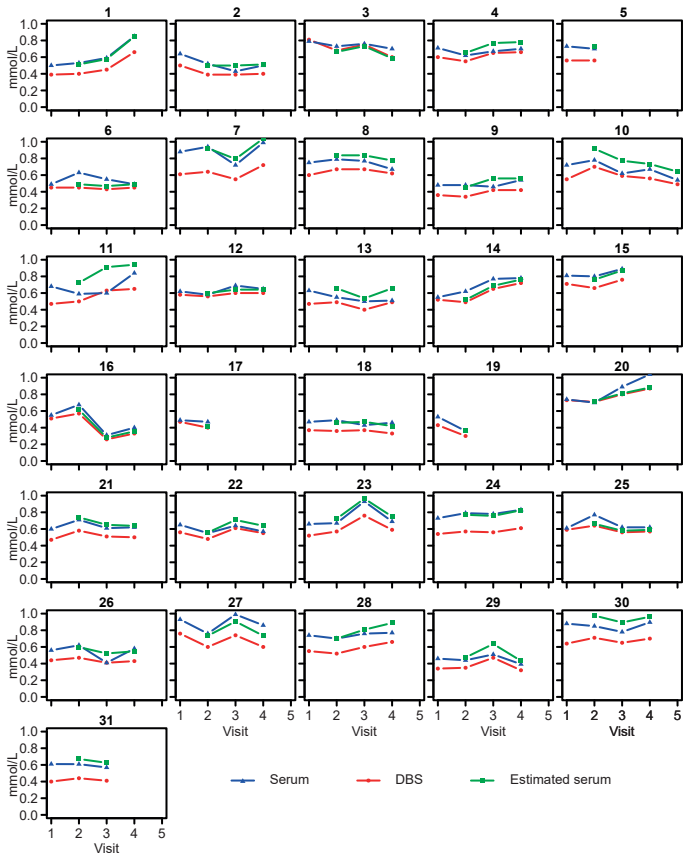

Figure 20: Individual longitudinal trajectories of measured and estimated serum lithium concentrations. Estimated serum lithium concentrations were derived from volumetric DBS measurements using calibration based on the serum/DBS ratio obtained at visit 1.

It is worth noting that the 2021 study did not follow the standard 12-hour post-dose sampling procedure, as samples were intentionally collected across a wider concentration range to validate the method over both low and high lithium concentrations. Nevertheless, applying the individual ratio obtained from the first visit to the 2021 measurements produced values that were comparable with those observed in the longitudinal study (Figure 22A). Similarly, applying the 2021 ratio to the longitudinal measurements resulted in good agreement, with a Pearson's r of 0.92 (Figure 22B). Furthermore, individual longitudinal trajectories generated using the 2021 ratio (Figure 22C) showed trends similar to those observed in the longitudinal study itself (Figure 20).

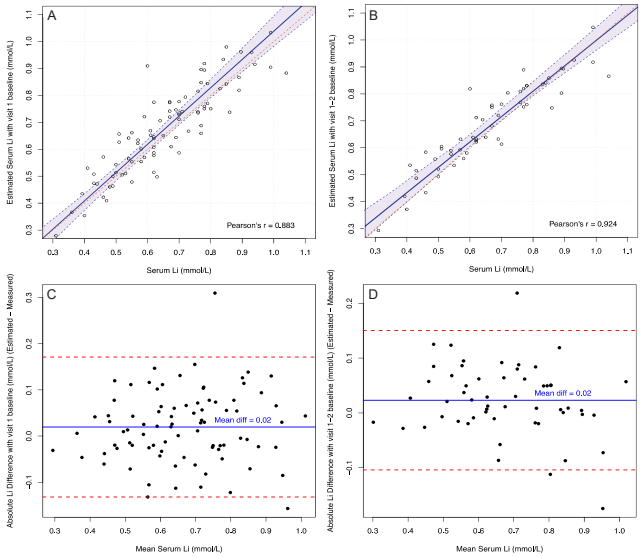

Figure 21: Evaluation of serum lithium estimation using subject-specific serum/DBS calibration. A) Passing–Bablok regression using the visit 1 serum/DBS ratio to estimate serum lithium concentrations at visits 2–4. B) Passing–Bablok regression using the mean serum/DBS ratio from visits 1–2 to estimate serum lithium concentrations at visits 3–4. C) Bland–Altman analysis for the visit 1 calibration approach. D) Bland–Altman analysis for the visit 1–2 calibration approach.

These findings suggest that volumetric DBS sampling combined with ratio correction may be suitable for decentralised TDM of lithium. The observed accuracy appears acceptable for clinical practice, where dosing decisions are generally guided by serum lithium concentrations in conjunction with clinical response and adverse effects. Importantly, participants reported high acceptance of the self-sampling procedure, indicating that the method is well suited for repeated long-term monitoring. Periodic recalibration could be performed alongside the routinely recommended laboratory assessments, while unexpected or clinically suspicious results could be confirmed using conventional venous sampling. Ultimately, such an approach has the potential to reduce the burden associated with lithium monitoring and improve adherence to recommended testing schedules by reducing the need for frequent clinic or hospital visits.

While these results demonstrate the potential of ratio-corrected DBS sampling for decentralised lithium monitoring, understanding the source of the remaining variation is important for further improving the method. Although the underlying cause of this variation remains uncertain, we hypothesise that it is primarily biological in origin, as the comparison between venous and capillary DBS samples in **Paper I** showed excellent agreement (Figure 18Ba), suggesting that differences between capillary and venous sampling are unlikely to be the dominant source of variability. Notably, the observed deviations could not be explained by differences in HCT, suggesting

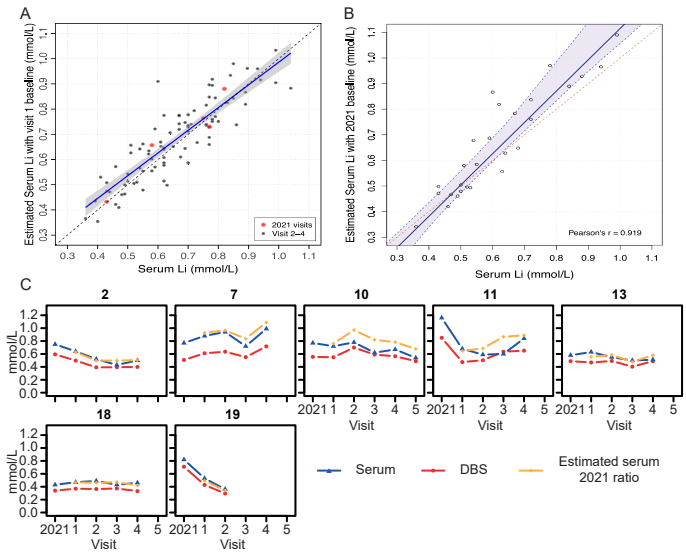

Figure 22: Long-term stability of individualised serum/DBS ratios over a three-year interval. A) Serum Estimation of 2021 (**Paper I**) serum lithium concentrations using serum/DBS ratios determined during the first 2024 (**Paper II**) study visit. B) Estimation of 2024 serum lithium concentrations using serum/DBS ratios determined in 2021. C) Representative longitudinal trajectories of measured and estimated serum lithium concentrations of the participants in both studies. These findings indicate that individualised serum/DBS ratios remain relatively stable over several years.

that additional biological factors may influence the distribution of lithium between blood compartments. We therefore hypothesise that the partitioning of lithium between serum and RBCs contributes to the observed variability in the serum-to-DBS relationship. Variation in erythrocyte-serum lithium relationships has previously been reported [107, 108, 109] and could alter concentrations measured in whole-blood samples while leaving serum concentrations unaffected.

These observations suggest that volumetric DPS may provide a more consistent sampling matrix for decentralised lithium monitoring by removing the direct contribution of RBC-associated lithium to the measured sample. However, this hypothesis remains to be experimentally validated, and further studies are required to determine whether volumetric DPS sampling can improve the long-term agreement between microsamples and conventional serum measurements.

4.2 Point-of-care approaches

The approaches investigated in **Paper I and II** decentralise sample collection while retaining laboratory-based analysis. Although this can reduce the need for conventional venous sampling, obtaining a lithium result still requires sample transport and laboratory analysis. A complementary strategy is therefore to decentralise both sampling and analysis through POC testing, enabling lithium concentrations to be determined directly at or near the patient.

There has long been interest in developing a POC device for lithium monitoring [110], and more recently patients have expressed a desire for portable POC solutions for the TDM of lithium [96]. Several approaches have been investigated over the years [100], including optical methods such as fluorimetric [111] and colorimetric [112, 113, 114, 115] techniques, ion-selective electrodes [116, 117, 118], organic electrochemical transistors [119], and electrophoretic methods [110, 120]. These approaches have been evaluated in a range of biological and simulated matrices, including whole blood [110, 116, 120], plasma or serum [112, 114, 115], sweat [117, 119], urine[118], saliva [111, 118], and interstitial fluid (ISF) [113].

Although a wide range of alternative sample matrices have been investigated for lithium POC testing, serum and plasma remain the reference matrices for clinical lithium monitoring. This is largely because therapeutic ranges have been established for these matrices, and there are decades of clinical experience with their use. Consequently, measurements in alternative matrices need to be related to established serum or plasma concentrations or require matrix-specific therapeutic ranges. This challenge was also encountered in **Paper I**, and we sought to address it in **Paper II**.

4.3 On-chip Colorimetric Assay

Papers III and IV explore the feasibility of a cartridge-based POC platform that brings colorimetric lithium quantification, an analytical approach also used in clinical laboratories, closer to the patient. By combining whole-blood sampling with on-chip serum separation, the approach maintains compatibility with established serum-based lithium monitoring and circumvents the need for matrix translation associated with alternative biological fluids.

4.3.1 Colorimetric assays and absorption

In **Papers III and IV**, the colorimetric response is generated by F28 tetraphenylporphyrin (Figure 23), which contains a central cavity that interacts with lithium ions. Lithium interaction alters the absorption spectrum of the molecule, resulting in a concentration-dependent colour change.

Colorimetric assays rely on analyte-dependent changes in light absorption, resulting in a change in the observed colour (Figure 24). The change in transmitted light at a specific wavelength can be quantified as absorbance according to the Beer-Lambert law:

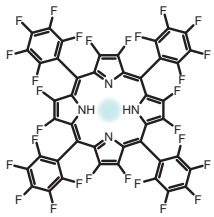

Figure 23: Chemical structure of F28 tetraphenylporphyrin (2,3,7,8,12,13,17,18-octafluoro-5,10,15,20-tetrakis(pentafluorophenyl)porphyrin), the sensing molecule used in the colorimetric lithium assay. The highlighted central cavity indicates the region that interacts with lithium ions.

$$A = \log_{10}\left(\frac{I_0}{I}\right) = \varepsilon l c \tag{18}$$

where A is the absorbance, I_0 is the intensity of the incident (incoming) light, I is the intensity of the transmitted light, ε is the molar absorptivity coefficient, l is the optical path length, and c is the concentration of the absorbing molecule. In the lithium assay, lithium interaction with F28 tetraphenylporphyrin modifies its absorption spectrum, allowing the lithium concentration to be determined by measuring the absorbance at 546 nm relative to a reference wavelength at 600 nm (Figure 24).

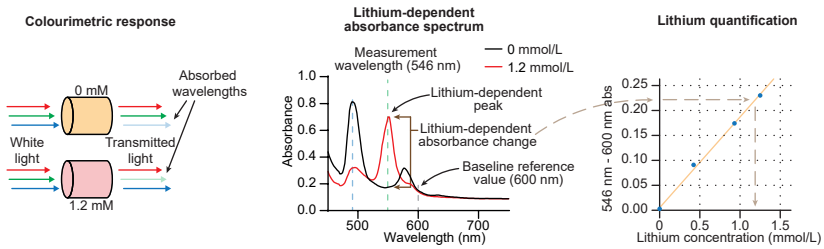

Figure 24: Overview of the colorimetric detection principle used in **Papers III and IV**. Lithium interaction with F28 tetraphenylporphyrin alters the absorbance spectrum of the molecule, leading to a concentration-dependent colour change. The lithium concentration is determined from the absorbance at 546 nm relative to a reference wavelength at 600 nm.

4.3.2 Design inspiration and approach

The proposed device was inspired by conventional laboratory colorimetric lithium assays. Rather than changing the analytical principle, the goal was to translate an established serum-based colorimetric measurement into a decentralised POC format.

Initial experiments explored a lateral flow paper-based implementation of the assay. However, analyte transport through the paper was found to be dependent on HCT.

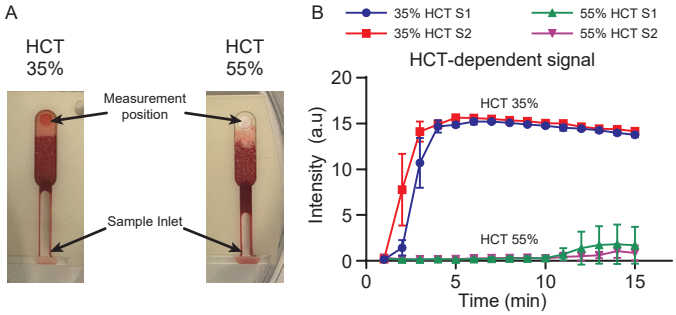

Figure 25: Effect of HCT on the fluorescence signal in a paper-based device. Fluorescein isothiocyanate (FITC) was used as a fluorescent tracer and added to two blood samples prior to adjustment to 35 % and 55 % HCT. A) Representative devices showing the sample inlet and measurement position are shown. B) Time-dependent fluorescence intensity measured at the same position is shown in (n = 5). A clear difference in signal intensity was observed between samples with 35 % and 55 % HCT.

Using FITC as a fluorescent tracer, differences in signal intensity were observed between samples with different HCT at a fixed measurement position in a lateral flow setup, despite identical tracer concentrations (Figure 25). These observations suggested that HCT-dependent transport effects could introduce an additional source of analytical variability in quantitative lithium measurements.

To avoid the HCT-dependent transport effects observed in the lateral-flow format, we built on previous experience with liquid serum and plasma analysis and developed a liquid-to-liquid workflow in which serum separated from fresh capillary blood could be analysed directly using a colorimetric assay.

4.3.3 The Device

The device developed in **Paper III** (Figure 26A) consists of a microfluidic cartridge containing a sample inlet with an integrated serum separation membrane, a serum metering channel, a readout chamber, and a reagent blister pouch. Following application of whole blood, serum is separated and fills the metering channel with a predefined volume (Figure 26B I–III). The reagent is then released from the blister pouch, displacing the metered serum volume into the readout chamber where mixing occurs (Figure 26B IV–V). After a 10 minutes incubation period, specified by the reagent manufacturer, absorbance is measured through the readout chamber.

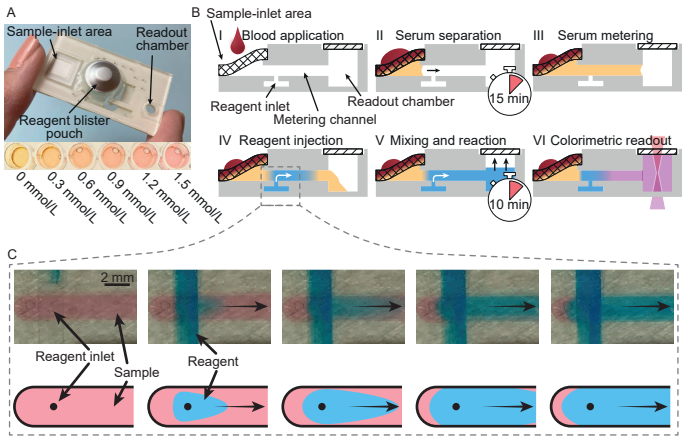

Figure 26: A) The developed device cartridge with an inset showing representative reagent colours. B) Working principle of the device, showing the serum pathway in cross-section. C) Representative images and an illustration of reagent-driven displacement in a liquid microfluidic channel. Following injection, the reagent partitions the sample into two fractions and displaces one fraction ahead of the advancing reagent front. This mechanism can be used to control fluid movement and sample routing within the device. Panels A and B are from Olsson et al. **Paper III** [121] under CC BY 4.0.

Initial manually controlled prototypes successfully demonstrated the feasibility of combining serum separation with liquid-phase colorimetric analysis (Figure 26C). However, subsequent device development was largely driven by the need to improve fluid-handling reliability, particularly by controlling fluid flow and minimising air bubble formation, which could interfere with optical measurements. Addressing these challenges was essential for reliable assay operation and accurate lithium quantification.

The device can be divided into three pathways (Figure 27); the serum pathway, where serum fills the metering channel and is connected to the readout chamber; the reagent pathway, where the reagent is introduced and bubbles are removed before priming the system and entering the serum pathway through the transfer junction; and the air pathway, through which air escapes during priming. The air pathway also contains flow restrictors that direct flow towards the serum pathway and the readout chamber, as well as a chamber that collects any reagent escaping through the restrictors.

The reagent pathway links the reagent blister pouch to the serum pathway. When the reagent blister pouch is pressed and punctured, the reagent is introduced into the microfluidic network and fills the system upstream of the flow restrictor, where a sacrificial volume is located to trap bubbles (Figure 28 II). As the reagent is pushed through the flow restrictor, the pressure increases (Figure 28 III), causing the pressure regulator to fill with reagent. Once the microfluidic network is primed, the flow is redirected into the metering channel and pushes the sample into the readout chamber (Figure 28 IV–VI). When the readout chamber is filled, pressure on the blister is released and the pressure regulator returns the excess reagent volume towards the

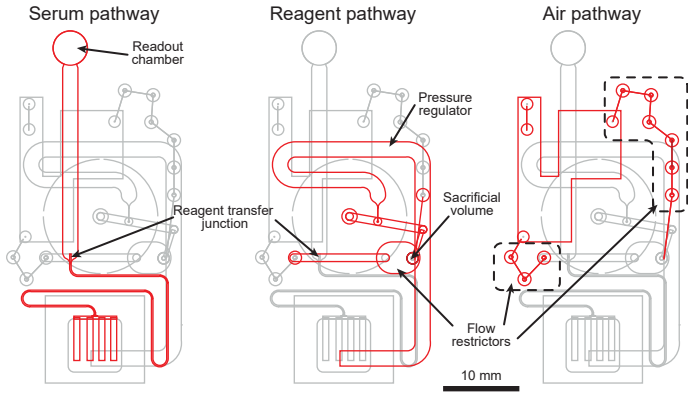

Figure 27: Schematic of the device, with the three pathways serum, reagent, and air being highlighted. Adapted from Figure 2 in Olsson et al. **Paper III** [121] under CC BY 4.0.

blister, equalizing the pressure and preventing air from flowing back into the readout chamber (Figure 28 VII–VIII).

4.3.4 Results

In **Paper III**, plasma samples separated from anticoagulated whole blood were used experimentally, whereas serum refers to the intended finger-prick samples collected without anticoagulants. The reagent functions in both matrices.

The device was originally evaluated using whole blood collected in citrate tubes from five different healthy donors spiked with different concentrations of lithium to cover the full therapeutic range (0.4 mmol/L – 1.2 mmol/L) and volumes ranging from 50 µL to 100 µL. The reference lithium concentrations in the spiked whole blood samples were established by AAS analysis of the plasma from each spiked sample and compared with the device measurements to obtain a calibration curve (Figure 29A) resulting in a limit-of-detection (LOD) of 0.3 mmol/L. Of 33 devices 25 (76 %) operated successfully and Passing Bablok regression showed good linearity Pearson's r 0.96 (R^2 = 0.92) and a slope of 0.98 (Figure 29B). The Bland–Altman plot showed that most samples were within 0.1 mmol/L with larger deviations occurring at the higher end of the concentration range (Figure 29C). However, the average absolute deviation was 0.05 mmol/L (6 %) indicating that the sampling concept could be used to estimate plasma lithium concentrations directly from whole blood, with performance within the range reported for other lithium monitoring devices [112, 116, 120].

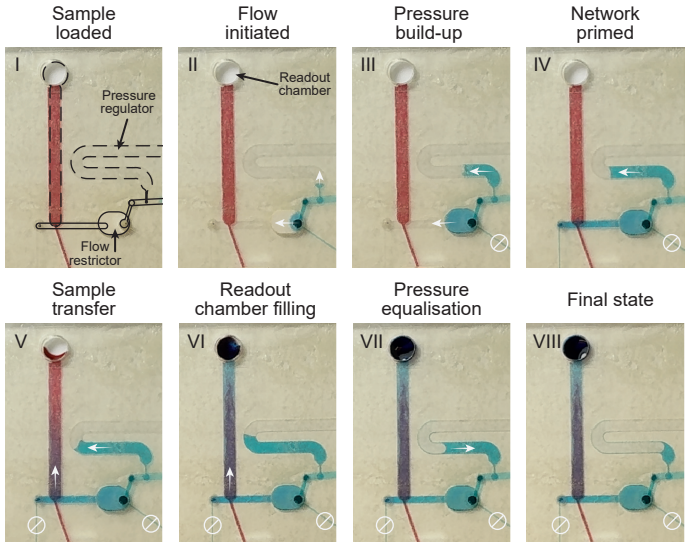

Figure 28: Spread out transparent device prototype of the reagent pathway with step-by-step images of the reagent flow (blue) and the sample (red) being pushed into the readout chamber.

In **Paper IV**, we evaluated the device using patient samples and collected duplicate samples from 16 individuals over a 9-week period, resulting in 78 finger-prick samples applied directly to the device. A nurse performed the sampling during the first visit, while patients self-sampled during subsequent visits. We also evaluated patient interest in a POC system for monitoring blood lithium concentrations.

When evaluating the device in patients, we were limited to 12-hours post-dose sampling, which resulted in most measurements being clustered within the therapeutic range. We did, however, successfully identify one patient with subtherapeutic lithium concentrations, while all remaining samples were correctly classified as being between 0.5 and 1.1 mmol/L, consistent with the AAS reference measurements. To evaluate performance across a broader concentration range, the clinical data were combined with samples from healthy donor blood spiked with lithium to concentrations extending to a plasma concentration of 2.7 mmol/L (Figure 30A).

Most variation was within $\pm$0.3 mmol/L; however, larger deviations occurred primarily above 1.5 mmol/L, reaching up to 0.7 mmol/L (Figure 30B). Unsurprisingly, coefficient of variations (CVs) showed the opposite trend, with values of 10-15 % at concentrations of 1.1 mmol/L and above, and 27-47 % at concentrations of 0.7 mmol/L and below. However, CVs are known to be sensitive to small fluctuations at low concentrations. As similar variability was observed in both clinical and spiked samples, the variation is likely attributable to inherent device factors, potentially related to the assembly process rather than sample type. One important finding was that patients were able to self-sample and successfully apply their own finger-prick blood samples to the cartridges.

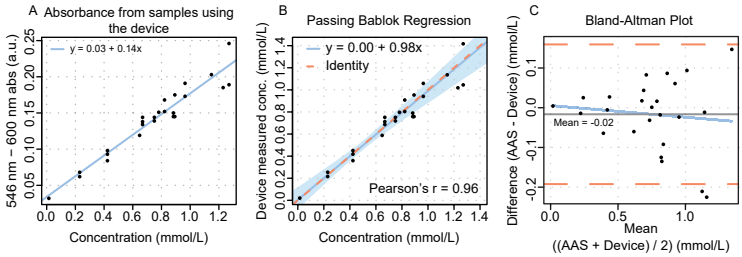

Figure 29: A) Calibration plot comparing the absorbance from 25 devices, with lithium spiked whole blood, and the sample concentration. B) Passing Bablok regression plot with a 95 % confidence interval comparing the device measured concentration to the sample concentration determined by AAS. C) Bland–Altman plot comparing the absolute difference between the device determined concentration and the sample concentration. From Figure 4 in Olsson et al. **Paper III** [121] under CC BY 4.0.

We did not observe any significant effect of user experience on device performance, and results obtained by patients were comparable to those obtained by the nurse during the initial visit. Furthermore, 69 % of devices from the patient study exhibited complete filling of the serum channel within 5 minutes. Overall, 69 % of devices were fully functional and produced a usable result, with most device failures attributed to assembly inconsistencies, primarily leakage. While sufficient to demonstrate proof-of-concept operation, this failure rate highlights the need for improved fabrication consistency before practical implementation, while supporting the feasibility of the underlying concept.

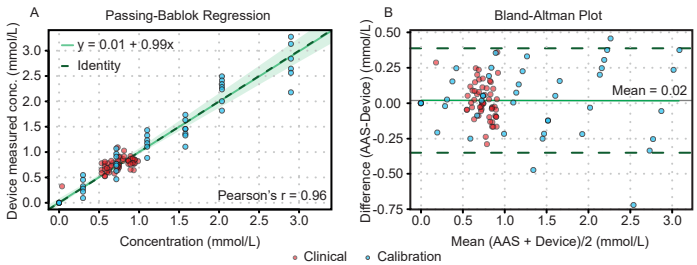

Figure 30: Fig. 3 Correlation and agreement between the cartridge-based and the reference method. A) Passing-Bablok regression analysis for clinical and spiked samples, comparing the cartridge device to AAS. B) Bland-Altman plot for clinical and spiked samples, illustrating the mean bias and limits of agreement between the two methods. From **Paper IV**

Additionally, the results from **Papers III and IV** were compared to investigate differences in device sensitivity, indicating a potential effect of the anticoagulant used during sample collection. Citrate- and ethylenediaminetetraacetic acid (EDTA)-treated samples exhibited different sensitivities, with citrate-treated samples (**Paper III**) producing a higher response per unit lithium concentration. The finger-prick samples collected in **Paper IV** appeared to be more consistent with the EDTA-based measurements, although the limited concentration range of the clinical samples

prevents firm conclusions. **Paper IV** also demonstrated the feasibility of a low-cost portable reader and showed that patients could successfully self-sample using the device.

4.3.5 Patient interest in home monitoring

Beyond technical feasibility, the practical value of home-based lithium monitoring also depends on patient acceptance and willingness to use such systems. Accordingly, a preliminary assessment of patient interest in a PCS or POC system for lithium monitoring was conducted in **Papers II and IV**. In **Paper II**, the 31 participating patients generally expressed a positive attitude towards using the volumetric DBS device. The sampling procedure was perceived as practical, although some patients reported that it required an initial period of familiarisation. Participants also indicated that the device could improve adherence to lithium treatment and increase motivation for monitoring. Several patients expressed an interest in adopting volumetric DBS sampling as part of their routine care due to its potential to simplify the monitoring process.

Similar findings were obtained in **Paper IV**, where 22 patients with bipolar disorder completed a questionnaire examining their experiences and preferences regarding lithium monitoring. Around 50 % of respondents indicated that they would like to know their lithium levels more frequently, with interest increasing further if testing could be performed at home using finger-prick sampling. Patients generally preferred the idea of home sampling and expressed a willingness to perform finger-prick blood collection themselves. Interestingly, respondents also reported being more diligent with lithium dosing before scheduled testing, suggesting that the prospect of monitoring may itself influence medication-taking behaviour, with potential implications for adherence. This potential monitoring effect is further discussed in **Paper IV**.

The relatively small number of participants limits the conclusions that can be drawn from these findings. In addition, patients enrolled in the clinical trial in **Paper II** were informed about the study method as part of the consent process, which may have introduced selection bias towards individuals more receptive to alternative sampling approaches. Nevertheless, the findings suggest patient interest in simplifying the sampling procedure and increasing the frequency of lithium monitoring, underscoring the clinical relevance of developing alternative sampling and POC testing strategies.

Together, **Papers I–IV** demonstrate complementary strategies for bringing lithium monitoring closer to the patient through volumetric DBS sampling and POC testing. The positive patient response to simplified sampling and home-based monitoring further supports the relevance of these approaches from a patient perspective. Although further development and larger clinical evaluations are required, these approaches demonstrate the potential to reduce the practical burden of lithium monitoring while maintaining compatibility with established serum-based therapeutic monitoring.

CHAPTER 5
Extracellular Vesicles

This chapter introduces extracellular vesicles, their biogenesis, and current isolation strategies. It further discusses their role in cancer progression and their potential as biomarkers in liquid biopsy applications.

Extracellular vesicles (EVs) are nano- to micrometer-sized extracellular particles that contain proteins, lipids, and nucleic acids derived from their cell of origin. By transferring this cargo between cells, they mediate local and systemic intercellular communication and contribute to numerous physiological and pathological processes, including inflammation and immune regulation. As EVs carry molecular information from their cells of origin and are present in most biological fluids, they have attracted considerable interest as potential biomarkers for disease detection and monitoring [122].

5.1 Biological origin and Categorization

EVs can originate through ectocytosis, exocytosis, or apoptosis [122]. Ectocytosis occurs through outward budding of the plasma membrane, exocytosis through the release of intracellular vesicles, and apoptosis through vesicle formation during programmed cell death (Figure 31A). Ideally, EVs would be classified according to their biogenesis [5]. However, since the identification and isolation of EVs based on biogenesis is technically challenging, operational classifications are commonly used instead. In this framework, small EVs (SEVs) are typically defined as vesicles smaller than 200 nm, whereas large EVs (LEVs) are generally larger than 200 nm [5]. Apoptotic bodies (AB) are typically larger than 500 nm and can extend up to several

micrometers in diameter [123]. These size ranges are not strict or mutually exclusive, and vesicles with different biogenetic origins may overlap substantially in size (Figure 31B) [5].

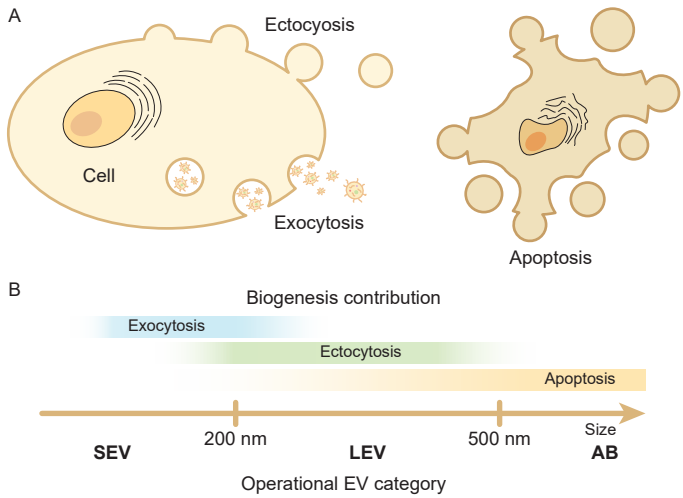

Figure 31: Biogenesis pathways and operational classification of EVs. A) EV release through exocytosis, ectocytosis, and apoptosis. B) Relative contributions of the different biogenesis pathways to SEVs, LEVs, and AB, shown according to their typical size ranges.

Additionally, several classes of non-vesicular extracellular particles have been described, including exomeres [124], which are typically smaller than 50 nm, and supermeres [125], which are typically 20–30 nm in diameter. However, these particles are generally considered distinct from classical EV populations and are therefore not discussed further in this thesis.

5.2 Isolation and Extraction

Since EVs are biologically important and present in complex biological fluids, several approaches for extracting and isolating them have been developed. These conventional approaches can generally be divided into ultracentrifugation (UC), chromatography, affinity capture, and precipitation, and can be used individually or combined in different ways [5]. The processing times associated with these methods can vary considerably, with examples given in Table 1.

5.2.1 Ultracentrifugation

UC is one of the most established methods for EV isolation and is commonly divided into differential ultracentrifugation (DUC) and density gradient ultracentrifugation (DGUC) [132]. Ultracentrifugation is centrifugation at high g-forces, generally exceeding 100,000 g. Since particle sedimentation depends on factors such as particle size, density, applied centrifugal force, and time, changes in the protocol can influence

Table 1: Approximate processing times for common EV isolation methods. The reported times refer to the method-specific isolation step. If multiple isolation methods were combined, only the duration of the specific step is considered.

Method	Approximate time
Differential ultracentrifugation (DUC)	2–6 h[a]
Density gradient ultracentrifugation (DGUC)	15–16 h[b]
Size exclusion chromatography (SEC)	1–5 h[c]
Immunoaffinity capture	1–19 h[d]
Precipitation	1 h[e]

[a] [126, 127, 128, 129, 130, 131] [b] [126, 129, 132] [c] [129, 131] [d] [130, 133] [e] [131, 134]

the composition of the resulting sample [5, 135]. UC has historically been used as a reference method against which newer EV isolation methods are compared.

DUC is based on centrifuging a sample, such as a biological fluid (e.g., blood or urine) or cell culture medium, multiple times at increasing centrifugal forces, with the supernatant transferred between centrifugation steps. Each centrifugation step generates a different fraction containing distinct sample components. Although protocols vary, the initial centrifugation steps typically remove cells and cellular debris, intermediate steps enrich for LEVs, and later ultracentrifugation steps pellet SEVs [5, 132]. Steps may be combined and additional washing steps may be included to remove soluble proteins (SP) from the SEV fraction depending on the intended application. Furthermore, particle aggregation may cause EVs to sediment during earlier centrifugation steps, thereby depleting subsequent fractions [136].

Another method is DGUC, which uses density gradients to separate particles based on their buoyant density into different fractions that can subsequently be collected [132]. Although this process produces higher-purity samples compared to conventional DUC, its processing times can be several hours, and overlapping density distributions may still result in contamination from particles with densities similar to those of the desired EVs fraction [137].

5.2.2 Size Exclusion Chromatography (SEC)

In chromatography, a mobile phase, typically a liquid, passes through a chromatography column packed with a stationary phase. Depending on the chromatographic technique, differences in the interactions between sample components and the stationary phase result in their separation. In size exclusion chromatography (SEC), separation of EVs occurs primarily based on particle size, with larger particles eluting before smaller particles because smaller particles can enter the pores of the stationary phase and therefore require a longer time to pass through the column.

A key advantage of SEC is that it is a relatively gentle separation method that preserves EV integrity while reducing contamination from soluble proteins. However, it dilutes the sample and due to the overlapping size distributions and limited resolution of

the column, complete separation is rarely achieved, resulting in a risk of co-isolation between fractions. In addition, the sequential processing of samples can limit throughput [138].

5.2.3 Immunoaffinity Capture

EVs carry membrane-associated proteins that can be targeted for antibody-based isolation. Commonly targeted markers are EV-associated proteins including CD9, CD63, and CD81, and antibodies targeting these proteins are typically immobilised on magnetic beads or microfluidic channels [139]. Immunoaffinity capture offers high selectivity by enriching EVs expressing specific surface markers. However, specific markers for EV subpopulations remain poorly established, making selective isolation of specific EVs populations challenging [5]. Additionally, targeting specific markers can bias what is captured and subsequently influence the results.

5.2.4 Precipitation

Another isolation method is precipitation, in which a chemical is added to the sample to promote the formation of a pellet. This is commonly achieved using polyethylene glycol (PEG), protamine, or a combination of the two. PEG promotes particle aggregation by reducing particle solubility, whereas protamine exploits the negative surface charge of EVs to induce precipitation [140].

Although precipitation is simple to perform and does not require specialised instrumentation, the main drawback of this isolation method is its limited specificity, as it promotes the co-precipitation of a wide range of particles and macromolecules present in the sample. As a result, the recovered pellet often contains contaminants such as protein aggregates, lipoproteins, and other non-EV particles [140].

5.2.5 Microfluidic Approaches

In addition to these conventional approaches, microfluidic approaches have also been developed for EV isolation [141]. These approaches can generally be divided into active and passive methods. Active methods use external forces, such as electric fields or acoustic waves, to manipulate and isolate EVs [133, 142, 143, 144]. Passive methods rely on channel geometry and fluid dynamics, including filtration, hydrodynamic focusing, and deterministic lateral displacement. Filtration separates particles using size-dependent retention, whereas hydrodynamic focusing and deterministic lateral displacement direct particles into different flow paths according to their physical properties, typically size. Notably, most microfluidic approaches require externally driven flow, typically provided by syringe pumps, pressure-driven systems, or centrifugation [141]. Microfluidic platforms can also integrate downstream processing steps such as labeling, detection, and molecular profiling [145]. Furthermore, magnetic bead-based systems using immunocapture have been integrated into microfluidic platforms to improve the selective isolation of EVs [133, 139].

A major drawback of most microfluidic isolation platforms is their reliance on specialised laboratory equipment, such as pumps and external control systems, increasing system complexity and limiting their use outside specialised laboratory environments. Furthermore, the wide range of available isolation strategies and the differences in the EV populations they recover remain important challenges for the standardisation of EV isolation workflows. Recognising these challenges, MISEV2023 acknowledges the need for improved standardisation of EV research workflows [5].

5.3 The Role of Extracellular Vesicles in Cancer

Cancer begins when a cell acquires genetic alterations that allow it to proliferate uncontrollably. In solid tissues, the continued proliferation of cancer cells generally results in the formation of a tumour. Over time, the tumour grows and may invade the surrounding tissue, stimulate the formation of new blood vessels, and eventually spread to distant parts of the body through metastasis (Figure 32) [146].

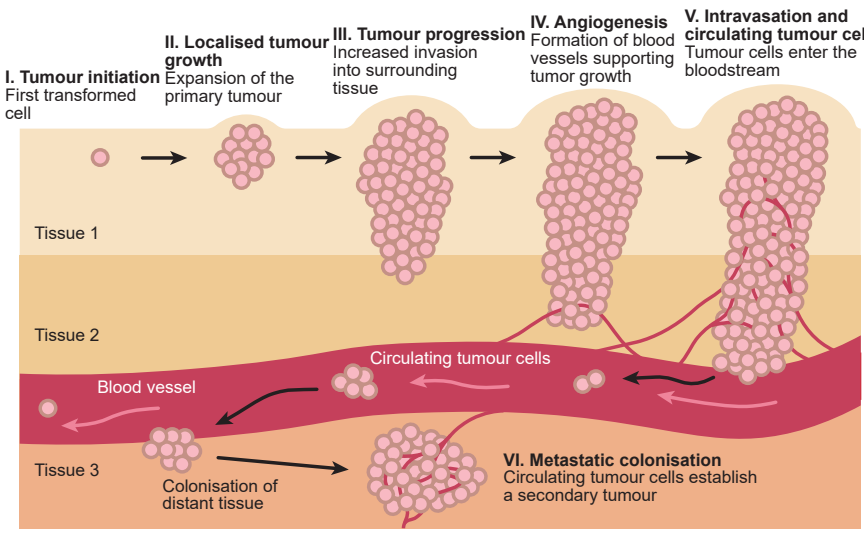

Figure 32: Schematic overview of cancer progression. (I) Tumour initiation occurs when a transformed cell begins uncontrolled proliferation. (II) Localised tumour growth leads to expansion of the primary tumour. (III) The tumour invades surrounding tissue. (IV) Angiogenesis supplies the growing tumour with oxygen and nutrients through the formation of new blood vessels. (V) Cancer cells enter the circulation (intravasation) and become circulating tumour cells. (VI) Circulating tumour cells colonise distant tissues and form secondary tumours through metastasis.

5.3.1 The Tumour Microenvironment

As tumours grow and interact with surrounding tissues, they establish a complex tumour microenvironment that plays a critical role in cancer progression. This microenvironment can promote tumour growth, angiogenesis, tissue invasion, and metastasis, while also contributing to the formation of pre-metastatic niches in distant tissues. These changes create conditions that support the survival and colonisation of disseminated cancer cells [146].

5.3.2 The Role of EVs in Cancer Progression

EVs play an important role in communication within the tumour microenvironment and have been implicated in many processes involved in tumour progression [147, 148]. Cancer cells often exhibit increased EV secretion and release EVs containing proteins, nucleic acids, and other molecules into the surrounding microenvironment. Tumour-derived EVs contribute to immune evasion by modulating immune-cell activity and suppressing anti-tumour immune responses, thereby promoting tumour survival and progression [149]. Numerous studies have reported elevated concentrations of circulating EVs in cancer patients compared with healthy individuals [148]. In addition, the molecular cargo carried by cancer-derived EVs reflects the biological state of the tumour. Consequently, EVs have emerged as promising biomarkers for cancer detection, disease monitoring, and liquid biopsy applications [4, 150].

5.3.3 Liquid Biopsy and EV-based Diagnostics

Given their ability to reflect the molecular characteristics of the tumour, EVs have attracted considerable interest as liquid biopsy biomarkers [4, 150]. Currently, tissue biopsies remain the gold standard for the diagnosis and characterization of solid tumours [150]. During a tissue biopsy, a portion of the suspected tissue is removed and subsequently analysed. However, depending on the location of the tumour, obtaining a tissue sample may require invasive procedures and may be associated with procedure-related risks.

Liquid biopsies utilise blood and other body fluids, such as urine, to provide minimally invasive alternatives for cancer screening and disease monitoring [4]. A wide range of biomarkers can be analysed using liquid biopsies, and there has been growing interest in the use of EVs [4]. Tumour-derived DNA has been reported to be enriched in SEVs compared with other blood fractions, highlighting their potential for monitoring disease progression [151]. However, as discussed previously, the isolation of EVs typically relies on time-consuming protocols involving multiple manual processing steps and specialised laboratory equipment.

These time-consuming processes can introduce pre-analytical variability, as EV populations may change during the interval between venepuncture and sample processing. The magnitude and direction of these changes depend on pre-analytical conditions, including temperature and anticoagulant choice, with platelet-derived EVs being particularly sensitive to handling delays [152, 153]. This highlights the need for standardisation of blood collection and processing procedures.

CHAPTER 6
Autonomous nanofiltration

*This chapter discusses the autonomous nanofiltration device presented in **Paper V**, its development, evaluation, and potential for extracellular vesicle and liquid biopsy applications.*

6.1 Microfluidic Nanofiltration of Whole Blood

As discussed in the previous section, filtration has been utilised as a strategy for isolating EVs. However, filtration-based approaches typically require an external driving force, such as centrifugation or pump-driven flow, to transport the sample through the filter.

6.1.1 Design Inspiration and Approach

The original question motivating the design presented in **Paper V** was whether a filter stack could be developed that received whole blood and separated particles of different pore sizes into distinct filter layers, allowing different particle sizes to be recovered through subsequent elution of the appropriate layer. Such an approach would require the filters to be separated after use and additional processing steps to recover the captured material.

However, early prototypes showed signs of haemolysis, likely caused by the high capillary forces generated as blood was drawn through increasingly smaller pores, resulting in RBCs rupturing and contamination of the sample.

As described previously, filters using the wedge configuration can be used to extract liquid from a filtration membrane; however, this approach had primarily been demonstrated using micrometer-scale filters. Initial experiments showed that the same principle could be applied to nanoporous membranes with pore sizes of 100 nm and 200 nm. Combining this concept with a simplified filter stack consisting of a blood filter and a single nanoporous membrane, rather than multiple nanoporous layers, enabled the development of a device capable of producing a liquid nanofiltrate.

6.1.2 The Device

The device from **Paper V** (Figure 33A) consists of a dual-filter stack arranged in a wedge configuration. The first layer is a gradient blood filter, which is placed in contact with a nanoporous filter whose pore size can be selected depending on the desired particle sizes. The nanoporous membrane is in contact with a hydrophilic layer that facilitates wetting and guides the filtrate into a microfluidic channel for storage.

When blood (70 µL) is applied to the device, the gradient blood filter retains RBCs and other components larger than 2.5 µm before the remaining fluid enters the nanoporous membrane (Figure 33B I–II). As the nanoporous membrane becomes saturated, liquid is continuously extracted from the membrane and transported into the microfluidic channel (Figure 33B III–V). Once the microfluidic channel has been filled, the collection channel can be cut from the device and placed in a microcentrifuge tube (1.5 mL). Brief centrifugation (1–2 s) transfers the collected nanofiltrate from the channel into the tube for subsequent analysis.

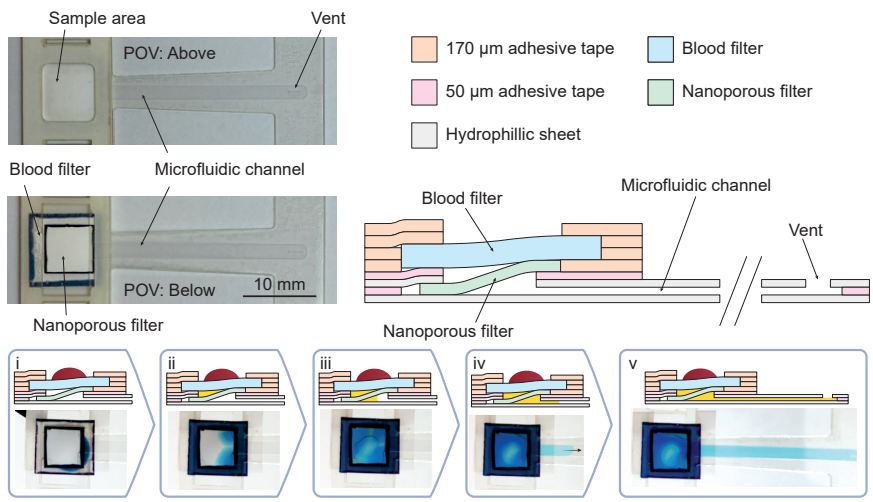

Figure 33: A) Images of and a layer schematic of the device with the layers visible. B) Sequential operation of device filling with illustrations and images of the different stages. Adapted from Figure 1 in **Paper V**.

The device was designed so that the sample channel with the filtrate can be stored in a 1.5 mL microcentrifuge tube. In its current configuration, the collected volume was limited to 3 µL, although the theoretical maximum volume was approximately 10 µL with the gradient blood filter producing approximately 20 µL of filtrate and the nanofilters saturating at around 10 µL.

6.2 Device Evaluation

6.2.1 Initial results and material selection

Following initial filtration experiments evaluating the removal and retention of fluorescent beads, synthetic EVs were filtered through a 200 nm membrane, and a luciferase assay was used to confirm their transport through the filter stack (Figure 34A). Following these validation experiments, six filter materials were evaluated using flow cytometry, a method that enables the detection and quantification of individual particles, to optimise device performance, mixed cellulose ester (MCE), nitrocellulose, nylon, polyethersulfone (PES), polypropylene (PP), and polyvinylidene fluoride (PVDF). Performance was assessed using different filter pore sizes and a beadmix of 500 nm and 1000 nm beads together with synthetic EVs. Among the tested materials, nitrocellulose, PES, and PVDF most consistently produced a nanofiltrate (Figure 34B).

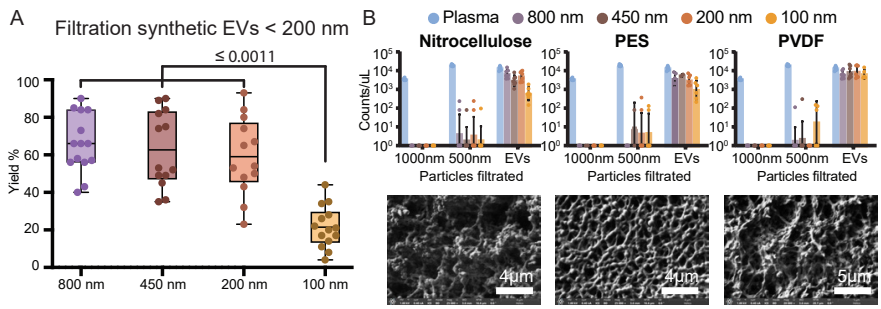

Figure 34: A) A Luciferase assay results using whole blood from six healthy donors spiked with synthetic EVs (< 200 nm) and processed using nitrocellulose devices. Measurements were performed in duplicate, with additional devices analysed when incomplete filling or device failure was suspected. B) Flow cytometry fluorescent bead mix results of device performance for nitrocellulose, PES and PVDF filters of different pore sizes using blood from two healthy donors, with SEM images of the filter morphology. Adapted from Figure 1 in **Paper V**.

Nitrocellulose and PVDF achieved filling success rates of 91 % and 84 %, respectively, with filtration typically completed within 5–20 minutes. Notably, nitrocellulose consistently completed filling within 15 minutes. Although PVDF demonstrated a higher overall EV recovery than nitrocellulose, the reduction in EV recovery observed with decreasing pore size was more pronounced for nitrocellulose, indicating effective filtration (Figure 34B). In contrast, the lack of a clear filtration trend based on pore size for PVDF raised concerns about whether the membrane provided meaningful filtration. The stronger size-dependent filtration, combined with the shorter processing time,

led to the selection of nitrocellulose for further development. Nevertheless, PVDF remained a material of interest for future optimisation.

6.2.2 Device Characterization

The nitrocellulose device was further characterised using flow cytometry by spiking whole blood with individual particle populations or a mixture of fluorescent particles comprising 70 nm, 500 nm, and 1000 nm beads, synthetic EVs (< 200 nm), and liposomes (< 400 nm). Liposomes are spherical vesicles enclosed by a phospholipid bilayer and were included as a simplified model of membrane-bound particles.

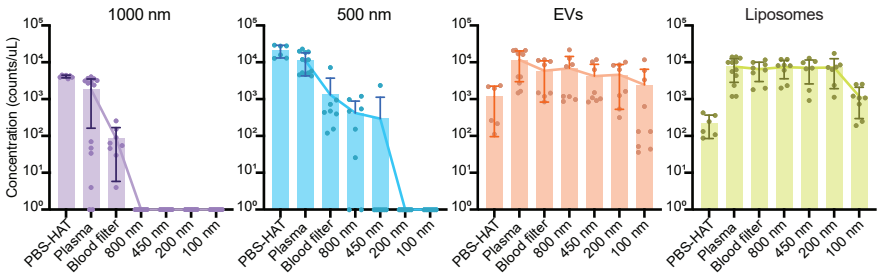

Figure 35: Nanofiltrates from a single particle type (500 nm, and 1000 nm beads, synthetic EVs < 200 nm, or liposomes < 400 nm) in spiked whole blood samples using nitrocellulose devices of different pore sizes. PBS-HAT spiked with the same particle type, centrifuged plasma, and blood-filter-only devices were included as controls and reference samples. Adapted from Figure 2 in **Paper V**.

Nanofiltrates from whole blood spiked with the same particle population demonstrated good filtration characteristics across all tested filter pore sizes, with particles larger than the filter pore size absent from the filtrate, while smaller particles were consistently detected (Figure 35). Whole blood was also spiked with mixtures comprising different combinations of 70 nm, 500 nm, and 1000 nm beads and synthetic EVs (< 200 nm). Similar filtration characteristics were observed as in the single particle type experiments, although minor breakthrough of larger particles was detected (Figure 36).

While spiked blood samples demonstrate that introduced particles can be recovered in the filtrate, they do not establish whether naturally occurring EVs are present. To address this, image flow cytometry was performed on whole blood samples from five healthy donors to determine the presence and concentration of EVs in nanofiltrates generated using nitrocellulose membranes of different pore sizes (Figure 37A). The presence of naturally occurring EVs was further corroborated using cryo-electron microscopy (CryoEM) (Figure 37B).

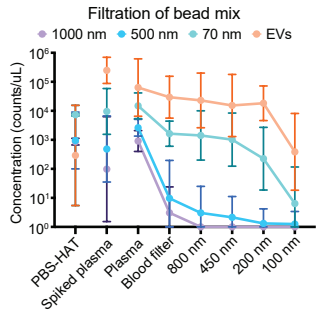

Figure 36: Nanofiltrates from whole blood spiked with bead mixtures containing 70 nm, 500 nm, and 1000 nm beads together with synthetic EVs (< 200 nm). PBS-HAT and plasma spiked with the same bead mix, centrifuged plasma, and blood-filter-only devices as controls and reference samples. Adapted from Figure 2 in **Paper V**.

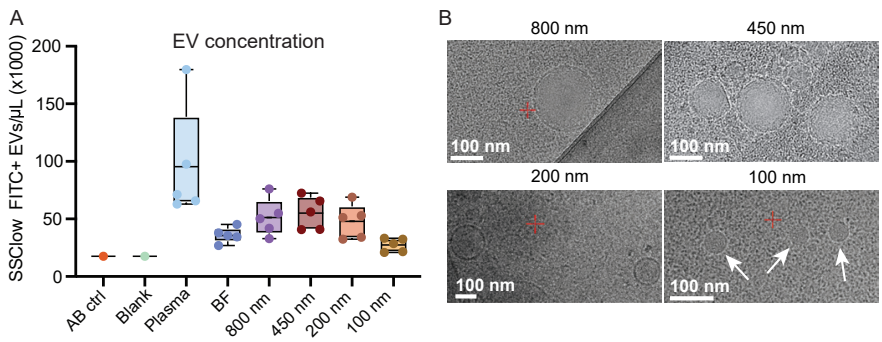

Figure 37: A) Box plot of EV concentrations measured by image flow cytometry in centrifuged plasma, blood-filter (BF) controls, and nanofiltrates obtained using nitrocellulose devices with different filter pore sizes. B) CryoEM images showing EVs in nanofiltrates obtained from devices with different filter pore sizes. Adapted from Figure 3 in **Paper V**.

6.2.3 Nanofiltrate Compared to Differential Ultracentrifugation

To evaluate the similarity of the nanofiltrates to DUC fractions (Figure 38A), see **Paper V** for times, a multiplex bead-based EV surface antigen profiling panel was used comparing blood samples from five healthy donors. An initial analysis was performed by normalizing the expression of CD63 (a general EV marker), CD42a (a platelet-associated EV marker), and CD62P (an activated platelet EV marker) to CD81 (a marker commonly associated with SEVs) (Figure 38B). The platelet-associated markers were particularly relevant in this context, as platelets are sensitive to pre-analytical handling [152, 153] and release EVs upon activation, making platelet-derived EVs useful indicators when evaluating new methods against established methods.

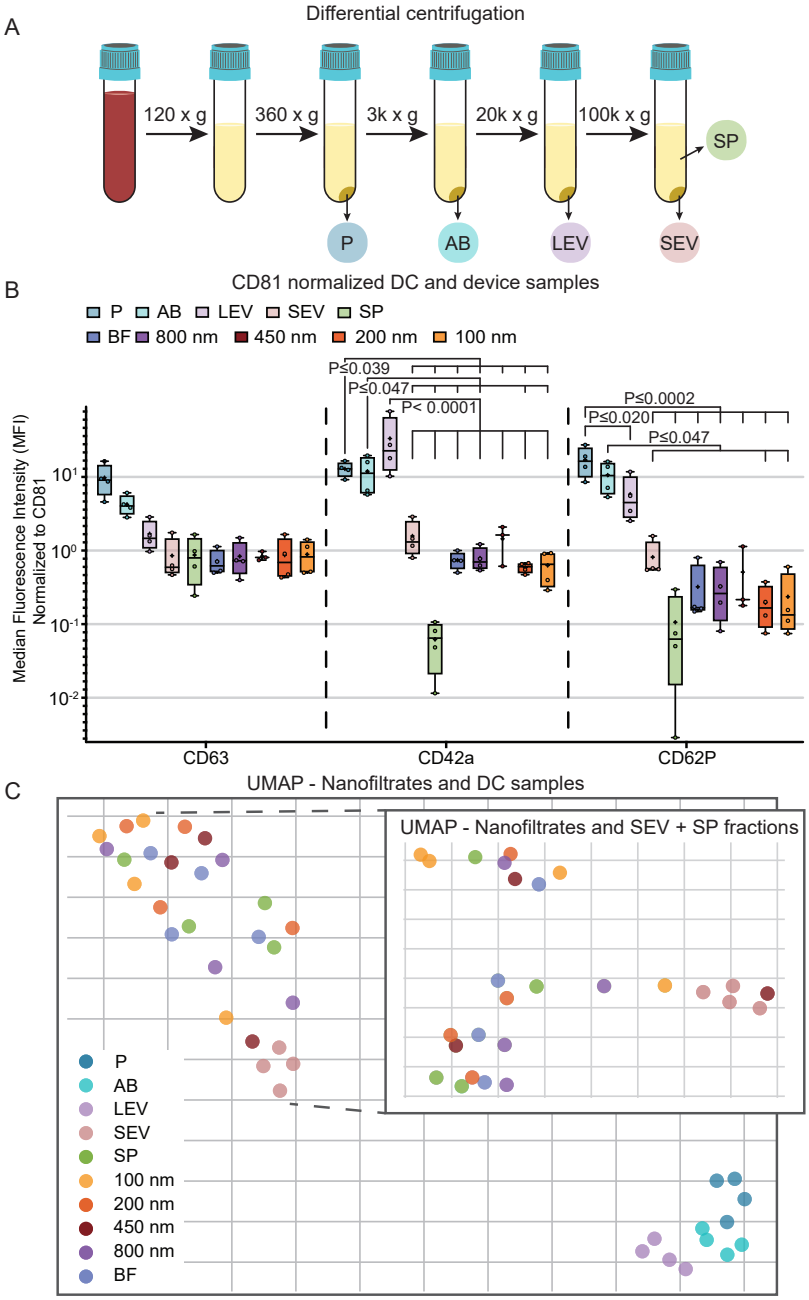

Figure 38: A) DUC process with fractions platelets (P), apoptotic bodies (AB), large EVs (LEV), small EVs (SEV) and soluble proteins (SP) marked. B) Boxplots of CD81-normalised multiplex results for CD63, CD42a and CD62P. C) Uniform manifold approximation and projection (UMAP) analysis of the multiplex panel results. Adapted from Figure 3 in **Paper V**.

The multiplex analysis (Figure 38B) showed that the overall abundance of CD63 surface markers normalised to CD81 in the filtrates (BF, 800, 450, 200, and 100 nm) was comparable to that of the SEV and SP fractions, suggesting a similar abundance of EVs. In contrast, the levels of CD42a and CD62P were lower in the filtrates than in the SEV. Furthermore, platelets, AB, and LEVs exhibited higher levels of CD63, CD42a, and CD62P, further supporting that the device removes larger EVs from the filtrate. The slightly lower levels of CD42a and CD62P in the nanofiltrates compared with the SEV fraction may be related to the short processing time of the device (5–15 minutes), limiting platelet activation during processing. Together, these findings suggest that the nanofiltrates contain a similar abundance of EVs to the SEV fraction while exhibiting limited evidence of platelet activation or degradation during processing of citrate-treated blood samples.

These findings were further supported by analysis of the multiplex panel results (Figure 38C), in which all nanofiltrates clustered together with the SEV and SP fractions. Notably, the nanofiltrates generally clustered closer to the SP fraction than to the SEV fraction. However, the limited number of samples makes it difficult to determine whether the observed clustering reflects donor-to-donor variability or differences introduced by the filtration and fractionation methods. Nevertheless, the clustering pattern suggests greater similarity between the nanofiltrates and the SEV and SP fractions than between the nanofiltrates and the other DUC fractions. It is also worth noting that the nanofiltrate was produced within 15 minutes, whereas DUC involves multiple sequential centrifugation and handling steps, with the initial centrifugation step alone requiring 20 minutes (see Methods, **Paper V**).

6.2.4 Analysis of Cancer Patient Nanofiltrates

Nanofiltrates from cancer patient blood samples were analysed using the multiplex panel together with finger-prick samples from healthy donors. The data were normalised by dividing each marker value by the average signal of the corresponding sample and subsequently analysed using uniform manifold approximation and projection (UMAP) (Figure 39). The UMAP analysis showed that the finger-prick samples clustered together with most of the healthy control samples. One cancer sample was also located within this cluster. However, this may be related to the exceptionally high average signal intensity of that sample compared with that of all other samples, which resulted in lower normalised marker values and may have contributed to its position in the UMAP projection.

Additional nanofiltrates from whole blood samples of patients with esophageal lesions, Barrett's esophagus, and gastroesophageal cancer were analysed for circulating tumour DNA (ctDNA). The tagmentation protocol targeted DNA fragments longer than 400 bp and was included to evaluate whether the enrichment of tumour-derived DNA observed in the SEV fraction [151] could also be detected in the nanofiltrates.

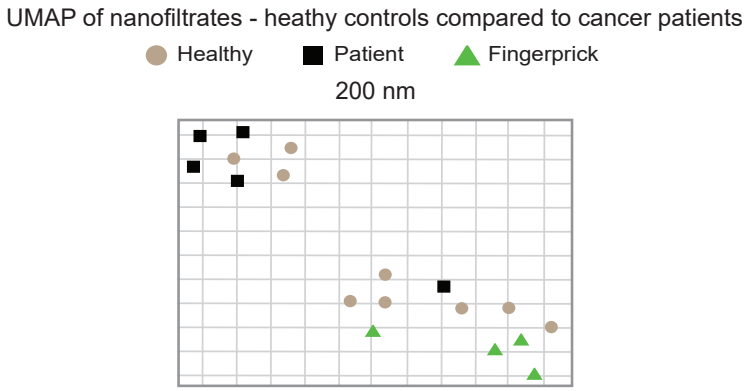

Figure 39: UMAP projection comparing 200 nm filtrate samples from healthy volunteer venous blood, healthy volunteer finger-prick blood, and patient samples. Adapted from Figure 4 in **Paper V**.

A copy number variation (CNV) analysis revealed a similar trend in the nanofiltrates, with larger filter pore sizes generally exhibiting lower CNV values and smaller filter pore sizes exhibiting higher CNV values (Figure 40A). This suggests that tumour-derived material may be enriched in filtrates generated using smaller pore sizes through the removal of larger particles containing a greater proportion of non-ctDNA, potentially increasing the relative abundance of tumour-associated EVs, consistent with previous reports showing enrichment of tumour-derived DNA in SEVs [151].

In addition to ctDNA, circulating microbial DNA (cmDNA) has recently emerged as a potential biomarker in cancer [154]. One proposed mechanism is that tumour-associated increases in tissue permeability may facilitate the release of bacteria and bacterial-derived material into the bloodstream. As a result, microbial signatures may reflect the tissue environment surrounding a tumour and could potentially aid in identifying its origin [155]. Consistent with this hypothesis, a similar trend was observed in the cmDNA analysis, where increasing numbers of cmDNA-positive samples and greater species enrichment were detected with decreasing filter pore size (Figure 40B and C). Notably, several of the identified species were associated with the oral microbiome, which is of particular interest given that the cohort primarily consisted of patients with esophageal lesions and Barrett's esophagus, both of which affect the esophagus and may therefore be anatomically relevant to the observed oral microbial signatures.

To summarise, the autonomous nanofiltration device produces a nanofiltrate that closely resembles the SEV and SP fractions obtained through DUC within 5–15 minutes. The device requires minimal user interaction: 70 µL of whole blood is applied from either a finger-prick sample or a collection tube, the collection channel is cut from the device once filled, and the nanofiltrate is recovered by brief centrifugation into a microcentrifuge tube.

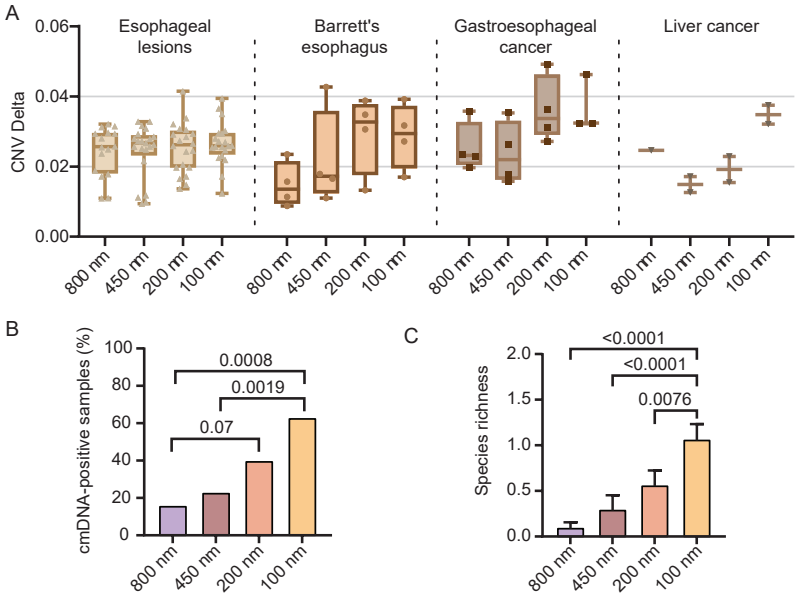

Figure 40: A) CNV Delta results from tagmented data from devices with filters of different pore sizes and patient groups. B) Percentage of cmDNA-positive samples detected across filtration fractions (100, 200, 450, and 800 nm). Statistical comparisons were performed using a Fisher's exact test. C) Species richness across filtration fractions, calculated as the number of taxa detected per sample in the curated cmDNA dataset. Statistical comparisons were performed using a Kruskal-Wallis test, followed by FDR correction Adapted from Figure 5 in **Paper V**.

The resulting nanofiltrate shows promise as a versatile, analysis-ready sample for downstream applications. Further development of both the device and analytical workflows may enable low-volume liquid biopsies, facilitating large-scale screening and longitudinal sampling in clinical settings and, potentially, in the home.

CHAPTER 7
Summary and future outlook

This chapter summarises the work presented in this thesis and evaluates it in relation to the stated objectives. It also discusses the limitations of the work and presents future perspectives for the technologies and approaches developed.

7.1 Concluding remarks

The overarching aim of this thesis was to develop and evaluate patient-centric technologies capable of reducing the barriers associated with conventional centralised healthcare workflows. To achieve this, the thesis explored both decentralised therapeutic drug monitoring of lithium and autonomous blood sample preparation for liquid biopsy applications through the development and evaluation of microfluidic devices and patient-centric sampling approaches.

A brief summary of the presented work:

Paper I: The development and evaluation of a volumetric DBS protocol for therapeutic drug monitoring of lithium in patients with bipolar disorder. The results demonstrated good agreement between capillary and venous DBS samples and a strong linear relationship between DBS and serum lithium concentrations.

Paper II: Evaluation of the longitudinal relationship between DBS and serum lithium concentrations in patients. The results indicated that individualised serum-to-DBS ratios remained sufficiently stable over time to permit estimation of serum lithium concentrations from volumetric DBS samples.

Paper III: The development and evaluation of a prototype POC cartridge device combining on-chip plasma separation with a colorimetric assay for lithium quantification from small volumes of whole blood. Measurements showed good agreement with the reference method and a linear response across the therapeutic range in spiked blood samples.

Paper IV: Evaluation of the prototype POC cartridge device in patients receiving lithium treatment. The results demonstrated the feasibility of cartridge-based lithium monitoring and indicated patient interest in POC systems for therapeutic drug monitoring.

Paper V: The development and evaluation of an autonomous whole-blood nanofiltration device using nanoparticles and clinical cancer samples. The results demonstrated rapid particle filtration from whole blood, generation of a nanofiltrate resembling the SEV and SP fractions obtained by DUC, and potential for liquid biopsy applications.

The following sections summarise how the individual papers contributed to the objectives of this thesis.

1. Develop and evaluate patient-centric approaches for blood collection, processing, and analysis, (**Paper I, II, III, IV, V**).
2. Evaluate dried blood spot sampling as a patient-centric approach for therapeutic drug monitoring of lithium in patients with bipolar disorder, (**Paper I, II**).
3. Develop and evaluate a cartridge-based point-of-care microfluidic device for therapeutic drug monitoring of lithium, (**Paper III, IV**).
4. Evaluate patient interest in patient-centric sampling and point-of-care approaches for lithium monitoring, (**Paper II, IV**).
5. Develop a user-friendly device to collect and process micro-volumes of whole blood, yielding a ready-to-use nanofiltered sample, (**Paper V**).
6. Evaluate the nanofiltered sample to verify the presence of extracellular vesicles and characterise the operational performance of the microfluidic device, (**Paper V**).
7. Conduct an initial pilot study on oncology patient samples using proteomic and genomic profiling to demonstrate the capability to extract biological information relevant to the disease from the nanofiltrate, (**Paper V**).

In summary, the objectives of this thesis were largely achieved. The work demonstrated the feasibility of patient-centric lithium monitoring through both microsampling and point-of-care approaches, while also establishing a novel autonomous nanofiltration platform for liquid biopsy sample preparation. Together, these findings highlight the potential of capillary microfluidic technologies to simplify clinical workflows and bring sampling, sample preparation, and analysis closer to the patient.

7.2 Thesis contributions, limitations, and future directions

This thesis investigated how capillary microfluidic technologies can support patient-centric sample collection, preparation, and analysis, thereby fulfilling **Objective 1**. The principal contributions, limitations, and future directions of the work are summarised below.

Dried blood spots for therapeutic drug monitoring of lithium

Papers I and II demonstrated that volumetric DBS sampling can be used for longitudinal lithium monitoring and that individual serum-to-DBS relationships remained sufficiently stable to permit the estimation of serum concentrations, thereby fulfilling **Objective 2**. In addition, **Paper II** explored patient perspectives on patient-centric monitoring among the participants and demonstrated a favourable attitude towards the use of such devices, thereby addressing **Objective 4**. These findings indicate that volumetric DBS sampling represents a viable patient-centric approach for decentralised lithium monitoring and may reduce reliance on conventional venous blood sampling.

The main limitation of lithium therapeutic drug monitoring using DBS is the need to convert whole-blood measurements into serum-equivalent concentrations. As demonstrated in **Paper II**, the individual serum-to-DBS ratio exhibited variability over time, although the magnitude of this variation differed between participants. We hypothesise that this variability is primarily biological in origin and may be related to differences in the partitioning of lithium between serum and RBCs. Consequently, whole-blood measurements may be affected by changes in the distribution of lithium between the cellular and liquid fractions, while serum concentrations remain unaffected.

One possible approach to reducing this source of variation is to replace DBS with volumetric DPS sampling. By analysing the liquid fraction directly, the influence of RBCs would largely be removed, potentially eliminating the need for individualised conversion ratios. Future work should therefore evaluate volumetric DPS sampling in a longitudinal patient cohort to determine whether serum-equivalent lithium concentrations can be obtained with improved accuracy and reduced inter- and intra-individual variation.

On-chip colorimetric assay for therapeutic drug monitoring of lithium

Papers III and IV demonstrated and evaluated a capillary microfluidic platform capable of autonomously separating and metering a defined volume of serum from whole blood, combining it with a colorimetric reagent, and delivering the fluids without bubble formation. The platform enabled quantitative lithium measurements within the therapeutic range and thereby fulfilled **Objective 3**. Furthermore, **Paper IV** showed interest among patients in at-home testing using finger-prick sampling, further addressing **Objective 4**. These findings demonstrate the feasibility of integrating serum metering, reagent handling, and quantitative lithium analysis within a single

disposable capillary microfluidic cartridge, supporting the potential of this approach for decentralised therapeutic drug monitoring.

Although the device demonstrated quantitative lithium measurements within the therapeutic range, the current manufacturing approach limits the precision and reproducibility required for further analytical optimisation. While lamination technology proved well suited for rapid prototyping and proof-of-concept studies, alternative manufacturing approaches should be considered for future development. Such approaches could enable alternative optical geometries, increasing the optical path length and thereby improving sensitivity and potentially analytical accuracy.

Furthermore, the current device requires manual mixing due to density differences between the reagent and serum. A custom-formulated reagent could potentially be adjusted to more closely match the density of serum, thereby promoting passive mixing within the readout chamber. The development of a dedicated reader would also be advantageous, as it could be specifically designed to accommodate alternative optical readout configurations.

Overall, the underlying concept has been successfully demonstrated. However, the tolerances achievable through manual assembly are unlikely to be sufficient for further optimisation and product development.

Nanofiltration of whole blood

Paper V demonstrated the development and evaluation of autonomous whole-blood nanofiltration using a capillary-driven microfluidic device and showed that the resulting nanofiltrate contained extracellular vesicles, tumour-derived DNA, and microbial DNA, thereby fulfilling **Objectives 5, 6, and 7**. These findings demonstrate the potential of this novel platform as a patient-centric sample-preparation technology for liquid biopsy applications.

One limitation of the nanofiltration device is the relatively small output volume. This restricts the types of downstream analyses that can be performed on a single sample. However, the small output volume is also a consequence of the intended application, as the device was designed to operate using finger-prick blood samples. If larger sample volumes are required, multiple filtrates could potentially be pooled prior to analysis.

Further characterization of the nanofiltrate is also warranted. Future studies should compare CNV profiles obtained from nanofiltrates with those obtained from conventional DUC fractions and evaluate whether other biomolecules, such as mRNA, can be reliably recovered and analysed. Such studies would provide a more comprehensive understanding of the information retained in the nanofiltrate.

Additional development opportunities also exist. The current workflow could potentially be extended by integrating paper-based storage, enabling shipping and storage of the collected sample in a dried format. Alternatively, the material retained on the filters could be eluted and analysed separately, allowing different filtration fractions to be recovered and characterised.

More broadly, the device was conceived as a platform for parallel processing of small blood volumes. In the future, it may be possible for users to collect samples at home and return the devices to a central laboratory either frozen or in a stabilised dried format. Such an approach could facilitate decentralised liquid biopsy sampling and enable longitudinal monitoring and screening of larger patient populations.

7.2.1 Sustainability

From a sustainability perspective, the technologies presented in this thesis were not primarily developed to reduce environmental impact through reduced plastic consumption. The devices rely on disposable components, and while some material use may be shifted away from healthcare facilities, it is largely transferred to the sampling device itself. Consequently, the environmental benefits of these approaches are not immediately obvious and would require a dedicated life-cycle assessment to evaluate.

However, sustainability can also be considered from social and healthcare-system perspectives. From a patient perspective, reducing the burden associated with sampling, monitoring, and follow-up may support long-term adherence to recommended treatment and monitoring schedules. This is particularly relevant for lithium therapy, where regular therapeutic drug monitoring is required throughout treatment. Patient-centric sampling and testing approaches may therefore improve the long-term sustainability of lithium therapy by reducing barriers to monitoring and supporting continued access to treatment.

The long-term sustainability of a healthcare intervention depends not only on analytical performance but also on accessibility. Technologies that reduce logistical barriers may support more sustainable models of care by improving patient participation, adherence to monitoring schedules, and access to healthcare services. Improved accessibility to monitoring may also facilitate earlier clinical intervention and more proactive disease management.

From a healthcare-system perspective, patient-centric sampling and testing approaches may contribute to more sustainable healthcare for patients by reducing the need for repeated in-person visits, alleviating demands on healthcare personnel, and enabling more efficient monitoring workflows. By shifting parts of the analytical workflow closer to the patient, these technologies may improve the utilisation of healthcare resources while increasing the capacity to monitor larger patient populations.

7.3 Future perspectives

The development of patient-centric approaches for sampling, sample preparation, and testing has the potential to reduce barriers to healthcare and improve access to effective treatment and monitoring. By shifting parts of the analytical workflow closer to the patient, these approaches may reduce the logistical challenges associated with conventional centralised testing while supporting more frequent monitoring and follow-up.

Therapeutic drug monitoring of lithium is a particularly relevant example. Current monitoring relies on repeated blood sampling and careful coordination between medication intake, sample collection, and laboratory analysis. Patient-centric sampling approaches, such as DBSs and DPSs, together with POC technologies, have the potential to reduce this burden. Although regular contact with healthcare providers remains necessary, enabling patients to collect samples themselves introduces greater flexibility and reduces dependence on clinic visits and time-sensitive sampling appointments.

More broadly, patient-centric sampling and testing technologies may facilitate longitudinal monitoring by making repeated measurements easier and more accessible. This could improve adherence to recommended monitoring schedules, increase patient engagement, and support more individualised healthcare. Importantly, the value of these approaches extends beyond lithium monitoring and may also benefit other applications that rely on repeated sampling or long-term follow-up.

The ability to process small volumes of whole blood and generate analysis-ready samples without the need for specialised laboratory equipment may increase the accessibility of liquid biopsy testing. By reducing sample volume requirements, the device could facilitate finger-prick sampling and potentially support large-scale screening programs while reducing the burden associated with conventional venous blood collection. The low sample volume requirements may also enable frequent longitudinal sampling, allowing disease progression, treatment response, or biomarker dynamics to be monitored more closely over time.

As a novel sample preparation approach, further studies are required to determine which downstream applications are compatible with the generated nanofiltrate and to establish when sample volume becomes a limiting factor. While the current work demonstrated similarities between the nanofiltrate and DUC-derived SEV and SP fractions, additional characterization is needed. In particular, although recovery of tumour-derived and microbial DNA was demonstrated, evaluation of other biomolecules, such as mRNA, proteins, and metabolites, would further define the broader applicability of the platform.

The most significant advantages of the presented device are its rapid processing time, low sample volume requirements, and ability to generate a liquid nanofiltrate directly from whole blood. Unlike conventional isolation approaches, the platform may also be well suited for parallel processing of large numbers of samples. In the future, this could enable decentralised liquid biopsy workflows, including home-based sample collection followed by shipment to centralised laboratories for analysis. While the future directions discussed above focus on specific technological developments, the broader impact of these approaches extends beyond their analytical performance.

Taken together, the technologies presented in this thesis represent small but meaningful steps toward a more patient-centric healthcare system. By reducing barriers associated with sampling, sample preparation, and analysis, they have the potential to support more accessible monitoring, facilitate earlier intervention, and promote long-term patient engagement. While significant challenges remain, the continued development of such approaches may help shape a future in which clinically relevant tests, where the analyte and analytical requirements permit, are increasingly performed closer to the patient rather than the laboratory.

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Thank you for being part of it.

Sincerely,

Carl Olsson

References

[1] Lakshmi N Yatham, Sidney H Kennedy, Sagar V Parikh, et al. “Canadian Network for Mood and Anxiety Treatments (CANMAT) and International Society for Bipolar Disorders (ISBD) 2018 guidelines for the management of patients with bipolar disorder”. In: *Bipolar Disorders* 20.2 (2018), 97–170.

[2] Alessandro Bosi, Laura Ceriani, Carl-Gustaf Elinder, et al. “Quality of laboratory biomarker monitoring during treatment with lithium in patients with bipolar disorder”. In: *Bipolar Disorders* 25.6 (2023), 499–506.

[3] Lilly Josephine Bindel and Roland Seifert. “Evidence of lithium underuse in bipolar disorder: analysis of lithium and antipsychotic consumption, prediction of future trends, regional disparities and indicators of rational and inappropriate use in Europe”. In: *Naunyn-Schmiedeberg’s archives of pharmacology* 398.12 (2025), 18049–18070.

[4] Liwei Ma, Huiling Guo, Yunxiang Zhao, et al. “Liquid biopsy in cancer: current status, challenges and future prospects”. In: *Signal Transduction and Targeted Therapy* 9.1 (2024), 336.

[5] Joshua A Welsh, Deborah CI Goberdhan, Lorraine O’Driscoll, et al. “Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches”. In: *Journal of Extracellular Vesicles* 13.2 (2024), e12404.

[6] Girardin Jean-Louis and Azizi A. Seixas. “The value of decentralized clinical trials: Inclusion, accessibility, and innovation”. eng. In: *Science* 385.6711 (2024), eadq4994–. ISSN: 0036-8075.

[7] Christopher A James, Matthew D Barfield, Katie F Maass, et al. “Will patient-centric sampling become the norm for clinical trials after COVID-19?” In: *Nature Medicine* 26.12 (2020), 1810–1810.

[8] Rosanna W Peeling, David L Heymann, Yik-Ying Teo, et al. “Diagnostics for COVID-19: moving from pandemic response to control”. In: *The Lancet* 399.10326 (2022), 757–768.

[9] Hannah E. Kettler, K. White, and S. Hawkes. *Mapping the landscape of diagnostics for sexually transmitted infections*. Tech. rep. WHO/TDR/SDI/04.1. Geneva, Switzerland: World Health Organization (WHO) Special Programme for Research and Training in Tropical Diseases (TDR), 2004.

[10] David Mabey, Rosanna W Peeling, Andrew Ustianowski, et al. “Diagnostics for the developing world”. In: *Nature Reviews Microbiology* 2.3 (2004), 231–240. DOI: 10.1038/nrmicro841.

[11] Kevin J. Land, Debrah I. Boeras, Xiang-Sheng Chen, et al. “REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes”. eng. In: *Nature Microbiology* 4.1 (2019), 46–54. ISSN: 2058-5276.

[12] Paul K Drain, Emily P Hyle, Farzad Noubary, et al. “Diagnostic point-of-care tests in resource-limited settings”. In: *The Lancet Infectious Diseases* 14.3 (2014), 239–249.

[13] Neil Spooner, Daniel Baker, Rachel S Carling, et al. “Patient centric blood sampling and analysis for diagnostics and laboratory medicine”. In: *Bioanalysis* 17.20 (2025), 1283–1293.

[14] Peter B. Luppa, Carolin Müller, Alice Schlichtiger, et al. “Point-of-care testing (POCT): Current techniques and future perspectives”. In: *TrAC Trends in Analytical Chemistry* 30.6 (2011), 887–898. ISSN: 0165-9936. DOI: 10.1016/j.trac.2011.01.019.

[15] Belinda Hengel, Louise Causer, Susan Matthews, et al. “A decentralised point-of-care testing model to address inequities in the COVID-19 response”. In: *The Lancet Infectious Diseases* 21.7 (2021), e183–e190.

[16] Nathan K Khosla, Andrew J Demello, and Daniel A Richards. “On paper diagnostics: a brief history and future perspectives”. In: *Chimia* 79.1-2 (2025), 29–35.

[17] Boris G Andryukov. “Six decades of lateral flow immunoassay: from determining metabolic markers to diagnosing COVID-19”. In: *AIMS Microbiology* 6.3 (2020), 280.

[18] Hans R Boehringer and Brendan J O’Farrell. “Lateral flow assays in infectious disease diagnosis”. In: *Clinical Chemistry* 68.1 (2022), 52–58.

[19] SF Clarke and JR Foster. “A history of blood glucose meters and their role in self-monitoring of diabetes mellitus”. In: *British Journal of Biomedical Science* 69.2 (2012), 83–93.

[20] So Yoon Kwon and Jun Sung Moon. “Advances in continuous glucose monitoring: clinical applications”. In: *Endocrinology and Metabolism* 40.2 (2025), 161–173.

[21] Deon Lingervelder, Hendrik Koffijberg, Ron Kusters, et al. “Point-of-care testing in primary care: a systematic review on implementation aspects ad-

dressed in test evaluations". In: *International Journal of Clinical Practice* 73.10 (2019), e13392.

[22] Cyril Camaro, Goaris WA Aarts, Eddy MM Adang, et al. "Rule-out of non-ST-segment elevation acute coronary syndrome by a single, pre-hospital troponin measurement: a randomized trial". In: *European Heart Journal* 44.19 (2023), 1705–1714.

[23] Paul C Adamson, Michael J Loeffelholz, and Jeffrey D Klausner. "Point-of-care testing for sexually transmitted infections: a review of recent developments". In: *Archives of Pathology & Laboratory Medicine* 144.11 (2020), 1344–1351.

[24] Nitika Pant Pai, Bhairavi Balram, Sushmita Shivkumar, et al. "Head-to-head comparison of accuracy of a rapid point-of-care HIV test with oral versus whole-blood specimens: a systematic review and meta-analysis". In: *The Lancet Infectious Diseases* 12.5 (2012), 373–380.

[25] Ronald M Galiwango, Richard Musoke, Lawrence Lubyayi, et al. "Evaluation of current rapid HIV test algorithms in Rakai, Uganda". In: *Journal of Virological Methods* 192.1-2 (2013), 25–27.

[26] Mara Jana Broadhurst, John Daniel Kelly, Ann Miller, et al. "ReEBOV Antigen Rapid Test kit for point-of-care and laboratory-based testing for Ebola virus disease: a field validation study". In: *The Lancet* 386.9996 (2015), 867–874. ISSN: 0140-6736. DOI: 10.1016/s0140-6736(15)61042-x.

[27] Zheng Wang, Richard S Bennett, Michele Roehler, et al. "Development and clinical evaluation of a rapid point of care test for ebola virus infection in humans". In: *Viruses* 15.2 (2023), 336.

[28] Aurora Bettini, Daniele Lapa, and Anna Rosa Garbuglia. "Diagnostics of Ebola virus". In: *Frontiers in Public Health* Volume 11 - 2023 (2023). ISSN: 2296-2565. DOI: 10.3389/fpubh.2023.1123024.

[29] H Von Schenck, M Falkensson, and B Lundberg. "Evaluation of" HemoCue," a new device for determining hemoglobin." In: *Clinical Chemistry* 32.3 (1986), 526–529.

[30] Pritam Halder, Shashi Kant, Archana Singh, et al. "Comparison of measurement of hemoglobin concentration by HemoCue 201 and HemoCue 301 with Sysmex automated hematology analyzer among nonpregnant women of reproductive age group (15–49 years) in a rural area of Haryana". In: *Indian Journal of Public Health* 69.3 (2025), 273–279.

[31] Romaric Larcher, Maxence Lottelier, Stephanie Badiou, et al. "Analytical performances of the novel i-STAT alinity point-of-care analyzer". In: *Diagnostics* 13.2 (2023), 297.

[32] Aleksander S Popel and Paul C Johnson. "Microcirculation and hemorheology". In: *Annu. Rev. Fluid Mech.* 37.1 (2005), 43–69.

[33] C.M. Rodkiewicz and International Centre for Mechanical Sciences. *Arteries and Arterial Blood Flow*. CISM International Centre for Mechanical Sciences. Springer Vienna, 1983. ISBN: 9780387816357. URL: https://books.google.se/books?id=W9dFAAAAYAAJ.

[34] Ralph Giorno, John H Clifford, Shirley Beverly, et al. “Hematology reference values analysis by different statistical technics and variations with age and sex”. In: *American Journal of Clinical Pathology* 74.6 (1980), 765–770.

[35] Edmond Magner. “Detection of ferricyanide as a probe for the effect of hematocrit in whole blood biosensors”. In: *Analyst* 126.6 (2001), 861–865.

[36] Mariëtte T Ackermans, Vera de Kleijne, Frans Martens, et al. “Hematocrit and standardization in DBS analysis: a practical approach for hormones mainly present in the plasma fraction”. In: *Clinica Chimica Acta* 520 (2021), 179–185.

[37] Nader Rifai, Andrea R. Horvath, and Carl T. Wittwer. *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*. 8th. St. Louis, MO: Elsevier, 2019.

[38] Hongwei Peng, Ming Pan, Zongning Zhou, et al. “The impact of preanalytical variables on the analysis of cell-free DNA from blood and urine samples”. In: *Frontiers in Cell and Developmental Biology* 12 (2024), 1385041.

[39] World Health Organization. *WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy*. Geneva: World Health Organization, 2010. ISBN: 9789241599221. URL: https : / / www . who . int / publications / i / item / 9789241599221.

[40] JM Jou, SM Lewis, C Briggs, et al. “ICSH review of the measurement of the erythocyte sedimentation rate”. In: *International Journal of Laboratory Hematology* 33.2 (2011), 125–132.

[41] Giuseppe Lippi, Alexander Von Meyer, Janne Cadamuro, et al. “Blood sample quality”. In: *Diagnosis* 6.1 (2019), 25–31.

[42] Christina Ellervik and Jim Vaught. “Preanalytical variables affecting the integrity of human biospecimens in biobanking”. In: *Clinical Chemistry* 61.7 (2015), 914–934.

[43] Michael P Phelan, Edmunds Z Reineks, Jesse D Schold, et al. “Preanalytic factors associated with hemolysis in emergency department blood samples”. In: *Archives of Pathology & Laboratory Medicine* 142.2 (2018), 229–235.

[44] Otwin Linderkamp, Achim A Stadler, and Eugen P Zilow. “Blood viscosity and optimal hematocrit in preterm and full-term neonates in 50–to 500–μm tubes”. In: *Pediatric Research* 32.1 (1992), 97–102.

[45] GH Hisayasu, Jordan L Cohen, and Richard W Nelson. “Determination of plasma and erythrocyte lithium concentrations by atomic absorption spectrophotometry.” In: *Clinical Chemistry* 23.1 (1977), 41–45.

[46] Ana-Maria Simundic, Michael Cornes, Kjell Grankvist, et al. “Standardization of collection requirements for fasting samples: for the Working Group on Preanalytical Phase (WG-PA) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)”. In: *Clinica Chimica Acta* 432 (2014), 33–37.

[47] David E Bruns and William C Knowler. *Stabilization of glucose in blood samples: why it matters*. 2009.

[48] Becton, Dickinson and Company. *BD Vacutainer® Blood Collection Tube Guide (Product Reference Leaflet)*. Ref: 2013_EN_LAB_1551. Available at: https://www.fishersci.co.uk/webfiles/uk/web-docs/2013_EN_LAB_1551.PDF. Distributed by Fisher Scientific UK. 2013.

[49] Meaghan M Bond and Rebecca R Richards-Kortum. "Drop-to-drop variation in the cellular components of fingerprick blood: implications for point-of-care diagnostic development". In: *American Journal of Clinical Pathology* 144.6 (2015), 885–894.

[50] Ana-Maria Simundic and Giuseppe Lippi. "Preanalytical phase–a continuous challenge for laboratory professionals". In: *Biochemia medica* 22.2 (2012), 145–149.

[51] Xingyu Guo, Lina Zhou, Yi Wang, et al. "Development of a fast and robust liquid chromatography-mass spectrometry-based metabolomics analysis method for neonatal dried blood spots". In: *Journal of Pharmaceutical and Biomedical Analysis* 230 (2023), 115383.

[52] Julia Wigren Byström, Linnea Vikström, Ebba Rosendal, et al. "At-home sampling to meet geographical challenges for serological assessment of SARS-CoV-2 exposure in a rural region of northern Sweden, March to May 2021: a retrospective cohort study". In: *Eurosurveillance* 28.13 (2023), 2200432.

[53] AJ Haack, LG Brown, Y Zeng, et al. "A Flexible and Responsive Remote Study Design to Assess Gene Expression Changes During Wildfire Smoke Exposure with homeRNA, an At-home Blood Sampling Kit". In: *medRxiv* (2025), 2025–10.

[54] Niclas Roxhed, Annika Bendes, Matilda Dale, et al. "Multianalyte serology in home-sampled blood enables an unbiased assessment of the immune response against SARS-CoV-2". In: *Nature Communications* 12.1 (2021), 3695.

[55] Hanna Huber, Laia Montoliu-Gaya, Wagner S Brum, et al. "A minimally invasive dried blood spot biomarker test for the detection of Alzheimer's disease pathology". In: *Nature Medicine* (2026), 1–10.

[56] Todd M Squires and Stephen R Quake. "Microfluidics: Fluid physics at the nanoliter scale". In: *Reviews of Modern Physics* 77.3 (2005), 977–1026.

[57] David J Beebe, Glennys A Mensing, and Glenn M Walker. "Physics and applications of microfluidics in biology". In: *Annual Review of Biomedical Engineering* 4.1 (2002), 261–286.

[58] Kerstin Avila, David Moxey, Alberto De Lozar, et al. "The onset of turbulence in pipe flow". In: *Science* 333.6039 (2011), 192–196.

[59] Pierre-Gilles Gennes, Françoise Brochard-Wyart, David Quéré, et al. *Capillarity and wetting phenomena: drops, bubbles, pearls, waves*. Springer, 2004.

[60] Ayokunle Olanrewaju, Maïwenn Beaugrand, Mohamed Yafia, et al. "Capillary microfluidics in microchannels: from microfluidic networks to capillaric circuits". In: *Lab on a Chip* 18.16 (2018), 2323–2347.

[61] Edward W Washburn. "The dynamics of capillary flow". In: *Physical Review* 17.3 (1921), 273.

[62] Shantanu Bhattacharya, Sanjay Kumar, and Avinash K Agarwal. *Paper microfluidics*. Springer, 2019.

[63] Behrouz Aghajanloo, Wil Losereewanich, Christopher J Pastras, et al. "Wicking pumps for microfluidics". In: *Biomicrofluidics* 18.6 (2024).

[64] Valerio Francesco Annese and Chunxiao Hu. "Integrating microfluidics and electronics in point-of-care diagnostics: Current and future challenges". In: *Micromachines* 13.11 (2022), 1923.

[65] Gabriel Arne Lenk, Göran Stemme, and Niclas Roxhed. "Delay valving in capillary driven devices based on dissolvable thin films". In: *18th International Conference on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS 2014, 26 October 2014 through 30 October 2014*. Chemical and Biological Microsystems Society. 2014, 216–218.

[66] Hansang Cho, Ho-Young Kim, Ji Yoon Kang, et al. "How the capillary burst microvalve works". In: *Journal of Colloid and Interface Science* 306.2 (2007), 379–385.

[67] Alain Glière and Cyril Delattre. "Modeling and fabrication of capillary stop valves for planar microfluidic systems". In: *Sensors and Actuators A: Physical* 130 (2006), 601–608.

[68] Jessica Melin, Niclas Roxhed, Guillem Gimenez, et al. "A liquid-triggered liquid microvalve for on-chip flow control". In: *Sensors and Actuators B: Chemical* 100.3 (2004), 463–468.

[69] Roozbeh Safavieh and David Juncker. "Capillarics: pre-programmed, self-powered microfluidic circuits built from capillary elements". In: *Lab on a Chip* 13.21 (2013), 4180–4189.

[70] Janosch Hauser, Gabriel Lenk, Jonas Hansson, et al. "High-yield passive plasma filtration from human finger prick blood". In: *Analytical Chemistry* 90.22 (2018), 13393–13399.

[71] Bruce K Gale, Alexander R Jafek, Christopher J Lambert, et al. "A review of current methods in microfluidic device fabrication and future commercialization prospects". In: *Inventions* 3.3 (2018), 60.

[72] Chengpeng Chen, Benjamin T Mehl, Akash S Munshi, et al. "3D-printed microfluidic devices: fabrication, advantages and limitations—a mini review". In: *Analytical Methods* 8.31 (2016), 6005–6012.

[73] Vincenzo Oliva, Giovanna Fico, Michele De Prisco, et al. "Bipolar disorders: an update on critical aspects". In: *The Lancet Regional Health–Europe* 48 (2025).

[74] David A Solomon, Andrew C Leon, William H Coryell, et al. "Longitudinal course of bipolar I disorder: duration of mood episodes". In: *Archives of General Psychiatry* 67.4 (2010), 339–347.

[75] Kathleen R Merikangas, Robert Jin, Jian-Ping He, et al. "Prevalence and correlates of bipolar spectrum disorder in the world mental health survey initiative". In: *Archives of General Psychiatry* 68.3 (2011), 241–251.

[76] Bernardo Dell'Osso, Rita Cafaro, and Terence A Ketter. "Has bipolar disorder become a predominantly female gender related condition? Analysis of recently published large sample studies". In: *International Journal of Bipolar Disorders* 9.1 (2021), 3.

[77] Kamyar Keramatian, Nellai K Chithra, and Lakshmi N Yatham. "The CANMAT and ISBD guidelines for the treatment of bipolar disorder: summary and a 2023 update of evidence". In: *Focus* 21.4 (2023), 344–353.

[78] Frederick Neil Johnson. *The history of lithium therapy*. Springer, 1984.

[79] Konstantinos N Fountoulakis. "The rise of a legend: Lithium and the extraordinary story of its discovery". In: *Pharmaceuticals* 18.8 (2025), 1230.

[80] Alfred Baring Garrod. *The nature and treatment of gout, and rheumatic gout*. Longmans&Company, 1876.

[81] Edward Shorter. "The history of lithium therapy". In: *Bipolar Disorders* 11 (2009), 4–9.

[82] John FJ Cade. "Lithium salts in the treatment of psychotic excitement." In: *Medical Journal of Australia* (1949).

[83] Mogens Schou, Niels Juel-Nielsen, Erik Strömgren, et al. "The treatment of manic psychoses by the administration of lithium salts". In: *Journal of neurology, neurosurgery, and psychiatry* 17.4 (1954), 250.

[84] P Bech, PB Vendsborg, and OJ Rafaelsen. "Lithium maintenance treatment of manic-melancholic patients: Its role in the daily routine". In: *Acta Psychiatrica Scandinavica* 53.1 (1976), 70–81.

[85] American Psychiatric Association. "Practice Guideline for the Treatment of Patients With Bipolar Disorder (Revision)". In: *American Journal of Psychiatry* 159.4 Suppl (2002), 1–50. URL: https://pubmed.ncbi.nlm.nih.gov/11958165/.

[86] GM Goodwin. "Evidence-based guidelines for treating bipolar disorder: recommendations from the British Association for Psychopharmacology". In: *Journal of Psychopharmacology* 17.2 (2003), 149–173.

[87] Ross J Baldessarini, Leonardo Tondo, John Hennen, et al. "Effects of lithium treatment and its discontinuation on suicidal behavior in bipolar manic-depressive disorders". In: *Journal of Clinical Psychiatry* 60.2 (1999), 77–84.

[88] Leonardo Tondo, Kay R Jamison, and Ross J Baldessarini. "Effect of Lithium Maintenance on. Suicidal Behavior in Major Mood Disorders a". In: *Annals of the New York Academy of Sciences* 836.1 (1997), 339–351.

[89] Allan H Young and Judith M Hammond. "Lithium in mood disorders: increasing evidence base, declining use?" In: *The British Journal of Psychiatry* 191.6 (2007), 474–476.

[90] Lars Vedel Kessing. "Why is lithium [not] the drug of choice for bipolar disorder? a controversy between science and clinical practice". In: *International Journal of Bipolar Disorders* 12.1 (2024), 3.

[91] Gregory J Moore, Niranjan Bose, Ioline D Henter, et al. “The 25-Year Evolution of Lithium as a Disease-Modifying Agent in Dementia: A Narrative Review”. In: *JAMA Psychiatry* (2026).

[92] Erika M Salarda, Ning O Zhao, Camila NNC Lima, et al. “Mini-review: The anti-aging effects of lithium in bipolar disorder”. In: *Neuroscience letters* 759 (2021), 136051.

[93] Michael Ott, Bernd Stegmayr, Ellinor Salander Renberg, et al. “Lithium intoxication: incidence, clinical course and renal function–a population-based retrospective cohort study”. In: *Journal of Psychopharmacology* 30.10 (2016), 1008–1019.

[94] Seena George, Rituparna Maiti, Biswa Ranjan Mishra, et al. “Effect of regulated add-on sodium chloride intake on stabilization of serum lithium concentration in bipolar disorder: A randomized controlled trial”. In: *Bipolar Disorders* 25.1 (2023), 66–75.

[95] Gin S Malhi, Erica Bell, Tim Outhred, et al. “Lithium therapy and its interactions”. In: *Australian Prescriber* 43.3 (2020), 91.

[96] Santiago Pedraza-Sanabria, Seetal Dodd, Angela Marianne Paredes Castro, et al. “Will We Soon See a Low Cost, Small Portable Device for the Therapeutic Monitoring of Lithium? An Implementation Science Investigation”. In: *Bipolar Disorders* 27.4 (2025), 334.

[97] Uthpali Mannapperuma, Priyadarshani Galappatthy, Raveendra Laal Jayakody, et al. “Safety monitoring of treatment in bipolar disorder in a tertiary care setting in Sri Lanka and recommendations for improved monitoring in resource limited settings”. In: *BMC Psychiatry* 19.1 (2019), 194.

[98] Louise Öhlund, Michael Ott, Sofia Oja, et al. “Reasons for lithium discontinuation in men and women with bipolar disorder: a retrospective cohort study”. In: *BMC Psychiatry* 18.1 (2018), 37.

[99] Iuri Dias Manfro, Mariane Tegner, Maria Eduarda Krutzmann, et al. “Determination of lithium in dried blood spots and dried plasma spots by graphite furnace atomic absorption spectrometry: method development, validation and clinical application”. In: *Talanta* 216 (2020), 120907.

[100] Mahsa Sheikh, Meha Qassem, Iasonas F Triantis, et al. “Advances in therapeutic monitoring of lithium in the management of bipolar disorder”. In: *Sensors* 22.3 (2022), 736.

[101] Margareta Holub, Karin Tuschl, Rene Ratschmann, et al. “Influence of hematocrit and localisation of punch in dried blood spots on levels of amino acids and acylcarnitines measured by tandem mass spectrometry”. In: *Clinica Chimica Acta* 373.1-2 (2006), 27–31.

[102] Gabriel Lenk, Jonas Hansson, Olof Beck, et al. “The effect of drying on the homogeneity of DBS”. In: *Bioanalysis* 7.16 (2015), 1977–1985.

[103] Pieter MM De Kesel, Sara Capiau, Willy E Lambert, et al. *Current strategies for coping with the hematocrit problem in dried blood spot analysis*. 2014.

[104] Gabriel Lenk. “Capillary driven devices for patient-centric diagnostics”. PhD thesis. Kungliga Tekniska högskolan, 2018.

[105] Eduarda Milena Reichert, Carolina Weber Ferrareze, Mayara Bernardes, et al. “Simple lithium measurement in capillary dried plasma microsamples collected with the HealthID PSD device”. In: *Clinical Biochemistry* (2025), 111069.

[106] Fredrik Wikström, Carl Olsson, Bonita Palm, et al. “Determination of lithium concentration in capillary blood using volumetric dried blood spots”. In: *Journal of Pharmaceutical and Biomedical Analysis* 227 (2023), 115269. ISSN: 0731-7085. DOI: 10.1016/j.jpba.2023.115269.

[107] CR Lee, SE Hill, M Dimitrakoudi, et al. “The relationship of plasma to erythrocyte lithium levels in patients taking lithium carbonate”. In: *The British Journal of Psychiatry* 127.6 (1975), 596–598.

[108] Alan C Swann, Nancy Berman, Alan Frazer, et al. “Lithium distribution in mania: plasma and red blood cell lithium, clinical state, and monoamine metabolites during lithium treatment”. In: *Psychiatry Research* 20.1 (1987), 1–12.

[109] M Camus, G Hennere, G Baron, et al. “Comparison of lithium concentrations in red blood cells and plasma in samples collected for TDM, acute toxicity, or acute-on-chronic toxicity”. In: *European journal of clinical pharmacology* 59.8 (2003), 583–587.

[110] Elwin X Vrouwe, Regina Luttge, and Albert van den Berg. “Direct measurement of lithium in whole blood using microchip capillary electrophoresis with integrated conductivity detection”. In: *Electrophoresis* 25.10-11 (2004), 1660–1667.

[111] Jung Ho Kim, Dermot Diamond, and King Tong Lau. “Development of non-invasive biochemical device for monitoring the lithium level from saliva for bipolar disorder patients”. In: *SENSORS, 2011 IEEE*. IEEE. 2011, 1744–1747.

[112] Takeshi Komatsu, Masatoshi Maeki, Akihiko Ishida, et al. “Based device for the facile colorimetric determination of lithium ions in human whole blood”. In: *ACS sensors* 5.5 (2020), 1287–1294.

[113] Mahsa Sheikh, Meha Qassem, and Panicos A Kyriacou. “A paper-based colorimetric method for monitoring of lithium therapeutic levels”. In: *Analytical Methods* 15.7 (2023), 979–986.

[114] Zitong Ye, Ziwei Huang, Jie Zhou, et al. “A portable optical device for quantitative detection of lithium in blood plasma”. In: *Analytical Methods* 17.26 (2025), 5355–5361.

[115] Takeshi Komatsu, Manabu Tokeshi, and Shih-Kang Fan. “Determination of blood lithium-ion concentration using digital microfluidic whole-blood separation and preloaded paper sensors”. In: *Biosensors and Bioelectronics* 195 (2022), 113631.

[116] Marta Novell, Tomàs Guinovart, Pascal Blondeau, et al. “A paper-based potentiometric cell for decentralized monitoring of Li levels in whole blood”. In: *Lab on a Chip* 14.7 (2014), 1308–1314.

[117] Francesca Criscuolo, Ivan Ny Hanitra, Simone Aiassa, et al. “Wearable multifunctional sweat-sensing system for efficient healthcare monitoring”. In: *Sensors and Actuators B: Chemical* 328 (2021), 129017.

[118] Farbod Amirghasemi, Sina Khazaee Nejad, Ruitong Chen, et al. “LiFT (a Lithium Fiber-Based Test): An At-Home Companion Diagnostics for a Safer Lithium Therapy in Bipolar Disorder”. In: *Advanced Healthcare Materials* 13.18 (2024), 2304122.

[119] Mohammad Shafiqul Islam, Brince Paul Kunnel, Munia Ferdoushi, et al. “Wearable organic-electrochemical-transistor-based lithium sensor for precision mental health”. In: *Device* 3.11 (2025).

[120] Arjan Floris, Steven Staal, Stefan Lenk, et al. “A prefilled, ready-to-use electrophoresis based lab-on-a-chip device for monitoring lithium in blood”. In: *Lab on a Chip* 10.14 (2010), 1799–1806.

[121] Carl Olsson, Janosch Hauser, Federico Ribet, et al. “On-chip colorimetric assay for determining serum lithium concentration from whole blood”. In: *Lab on a Chip* 25 (9 2025), 2270–2277. DOI: 10.1039/d5lc00044k.

[122] Raghu Kalluri and Valerie S LeBleu. “The biology, function, and biomedical applications of exosomes”. In: *Science* 367.6478 (2020), eaau6977.

[123] Samir El Andaloussi, Imre Mäger, Xandra O Breakefield, et al. “Extracellular vesicles: biology and emerging therapeutic opportunities”. In: *Nature Reviews Drug Discovery* 12.5 (2013), 347–357.

[124] Haiying Zhang, Daniela Freitas, Han Sang Kim, et al. “Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation”. In: *Nature Cell Biology* 20.3 (2018), 332–343.

[125] Qin Zhang, Dennis K Jeppesen, James N Higginbotham, et al. “Supermeres are functional extracellular nanoparticles replete with disease biomarkers and therapeutic targets”. In: *Nature Cell Biology* 23.12 (2021), 1240–1254.

[126] Jussara Rios de los Rios Resendiz, Freya Herrmann-Sim, Liliana Wilkesmann, et al. “A translational protocol optimizes the isolation of plasma-derived extracellular vesicle proteomics”. In: *Scientific Reports* 15.1 (2025), 24292.

[127] Scott M Langevin, Damaris Kuhnell, Melissa A Orr-Asman, et al. “Balancing yield, purity and practicality: a modified differential ultracentrifugation protocol for efficient isolation of small extracellular vesicles from human serum”. In: *RNA biology* 16.1 (2019), 5–12.

[128] Linda Bojmar, Han Sang Kim, Gabriel C Tobias, et al. “Extracellular vesicle and particle isolation from human and murine cell lines, tissues, and bodily fluids”. In: *STAR protocols* 2.1 (2021).

[129] Kshipra S Kapoor, Kristen Harris, Kent A Arian, et al. “High throughput and rapid isolation of extracellular vesicles and exosomes with purity using size exclusion liquid chromatography”. In: *Bioactive Materials* 40 (2024), 683–695.

[130] Shi Chen, Shu-Chu Shiesh, Gwo-Bin Lee, et al. “Two-step magnetic bead-based (2MBB) techniques for immunocapture of extracellular vesicles and

quantification of microRNAs for cardiovascular diseases: A pilot study". In: *PLoS One* 15.2 (2020), e0229610.

[131] Stephen David Robinson, Mark Samuels, William Jones, et al. "Confirming size-exclusion chromatography as a clinically relevant extracellular vesicles separation method from 1mL plasma through a comprehensive comparison of methods". In: *BMC Methods* 1.1 (2024), 7.

[132] Qin Zhang, Dennis K Jeppesen, James N Higginbotham, et al. "Comprehensive isolation of extracellular vesicles and nanoparticles". In: *Nature Protocols* 18.5 (2023), 1462–1487.

[133] Axel Broman, Megan Havers, Roman Sattarov, et al. "Immuno-acoustic trapping for extracellular vesicle subpopulations". In: *Scientific Reports* 15.1 (2025), 45805.

[134] Zoraida Andreu, Eva Rivas, Aitana Sanguino-Pascual, et al. "Comparative analysis of EV isolation procedures for miRNAs detection in serum samples". In: *Journal of Extracellular Vesicles* 5.1 (2016), 31655.

[135] Mikhail A Livshits, Elena Khomyakova, Evgeniy G Evtushenko, et al. "Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol". In: *Scientific Reports* 5.1 (2015), 17319.

[136] Romain Linares, Sisareuth Tan, Céline Gounou, et al. "High-speed centrifugation induces aggregation of extracellular vesicles". In: *Journal of Extracellular Vesicles* 4.1 (2015), 29509.

[137] Rebecca E Lane, Darren Korbie, Matt Trau, et al. "Purification protocols for extracellular vesicles". In: *Extracellular vesicles: methods and protocols*. Springer, 2017, pp. 111–130.

[138] Karim Sidhom, Patience O Obi, and Ayesha Saleem. "A review of exosomal isolation methods: is size exclusion chromatography the best option?" In: *International Journal of Molecular Sciences* 21.18 (2020), 6466.

[139] Xinghao Lan, Danyu Li, Yancong Yu, et al. "Extracellular vesicles isolation: a focus on magnetic beads-assisted platforms". In: *Frontiers in Bioengineering and Biotechnology* 13 (2025), 1646385.

[140] Maria Chiara Deregibus, Federico Figliolini, Sergio D'antico, et al. "Charge-based precipitation of extracellular vesicles". In: *International Journal of Molecular Medicine* 38.5 (2016), 1359–1366.

[141] Megan Havers, Axel Broman, Andreas Lenshof, et al. "Advancement and obstacles in microfluidics-based isolation of extracellular vesicles: Havers M. et al." In: *Analytical and Bioanalytical Chemistry* 415.7 (2023), 1265–1285.

[142] Anson Ku, Hooi Ching Lim, Mikael Evander, et al. "Acoustic enrichment of extracellular vesicles from biological fluids". In: *Analytical Chemistry* 90.13 (2018), 8011.

[143] Michael S Gerlt and Thomas Laurell. "Acoustofluidic chromatography for extracellular vesicle enrichment from 4 μL blood plasma samples". In: *Analytical Chemistry* 97.11 (2025), 6049–6058.

[144] Sergio Ayala-Mar, Victor H Perez-Gonzalez, Marco A Mata-Gómez, et al. “Electrokinetically driven exosome separation and concentration using dielectrophoretic-enhanced PDMS-based microfluidics”. In: *Analytical Chemistry* 91.23 (2019), 14975–14982.

[145] Hyun-Kyung Woo, Changhyun Kim, Yoonjeong Choi, et al. “Automated disc device for multiplexed extracellular vesicle isolation and labelling from liquid biopsies in cancer diagnostics”. In: *Nature Biomedical Engineering* 10.5 (2026), 882–896.

[146] Douglas Hanahan and Robert A Weinberg. “Hallmarks of cancer: the next generation”. In: *Cell* 144.5 (2011), 646–674.

[147] Kassandra Lopez, Seigmund Wai Tsuen Lai, Edwin De Jesus Lopez Gonzalez, et al. “Extracellular vesicles: A dive into their role in the tumor microenvironment and cancer progression”. In: *Frontiers in Cell and Developmental Biology* 11 (2023), 1154576.

[148] Raghu Kalluri and Kathleen M McAndrews. “The role of extracellular vesicles in cancer”. In: *Cell* 186.8 (2023), 1610–1626.

[149] Liwen Kuang, Lei Wu, and Yongsheng Li. “Extracellular vesicles in tumor immunity: mechanisms and novel insights”. In: *Molecular Cancer* 24.1 (2025), 45.

[150] W Yu, J Hurley, D Roberts, et al. “Exosome-based liquid biopsies in cancer: opportunities and challenges”. In: *Annals of Oncology* 32.4 (2021), 466–477.

[151] Daniel W. Hagey, Maximilian Kordes, André Görgens, et al. “Extracellular vesicles are the primary source of blood-borne tumour-derived mutant KRAS DNA early in pancreatic cancer”. In: *Journal of Extracellular Vesicles* 10.12 (2021), e12142. DOI: 10.1002/jev2.12142.

[152] Bert Dhondt, Cláudio Pinheiro, Edward Geeurickx, et al. “Benchmarking blood collection tubes and processing intervals for extracellular vesicle performance metrics”. In: *Journal of Extracellular Vesicles* 12.5 (2023), e12315.

[153] Naomi C Buntsma, Aleksandra Gąsecka, Yvo BWEM Roos, et al. “EDTA stabilizes the concentration of platelet-derived extracellular vesicles during blood collection and handling”. In: *Platelets* 33.5 (2022), 764–771.

[154] Haiming Chen, Yi Ma, Juqing Xu, et al. “Circulating microbiome DNA as biomarkers for early diagnosis and recurrence of lung cancer”. In: *Cell Reports Medicine* 5.4 (2024).

[155] Bernadeta Pietrzak, Iwona Kawacka, Agnieszka Olejnik-Schmidt, et al. “Circulating microbial cell-free DNA in health and disease”. In: *International Journal of Molecular Sciences* 24.3 (2023), 3051.

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