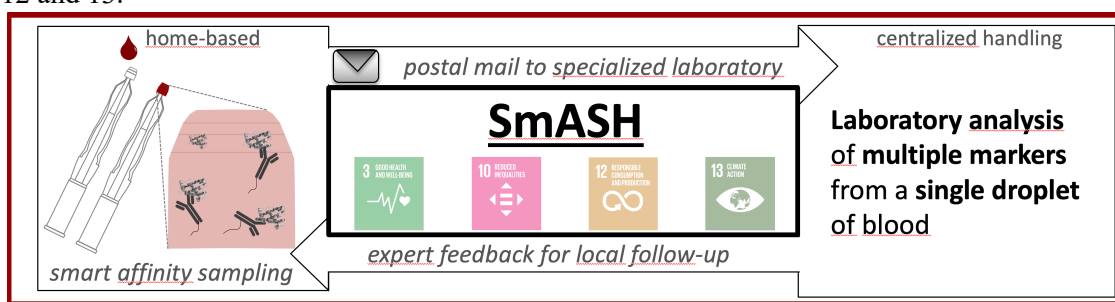


Abstract

This project description follows the application for one PhD position at the Department of Pharmacy. A combination of *cutting edge pharmaceutical analytical chemistry* and *innovative sampling strategies* will set a new standard for *highly sustainable home-based patient monitoring*. We are collaborating with leading researchers within *transplantation medicine at OUS* in order to demonstrate the clear clinical and sustainability potential of this proposal. Together we designed a proof-of-concept study for patient groups in need of intensive monitoring using pancreas transplanted patients as our suitable well defined model cohort. *The outcome of this project will contribute towards green, simplified, and affordable disease monitoring, while directly improving the quality of life for the model patient group.*

Our proposal is directly relevant to the call and several of the **UN Sustainable Development Goals** (3 – good health and well-being, 10 – reduce inequalities, 12 – responsible production, and 13 – climate action):

- Improved health and lower carbon footprint through reduced travel and hospital stays for vulnerable patients: UN Sustainable Development Goals 3, 10, and 13.
- Improved survival of transplanted pancreas patients through early detection of rejections resulting in improved health and patient safety, prolonged life, and organs available to more patients: UN Sustainable Development Goals 3, 10, and 13.
- Lowered carbon footprint through elimination of cold-chain transportation by sending samples through regular mail: UN Sustainable Development Goal 13.
- Improved use of resources through reduced use of reagents and solvents: UN Sustainable Development Goals 12 and 13.



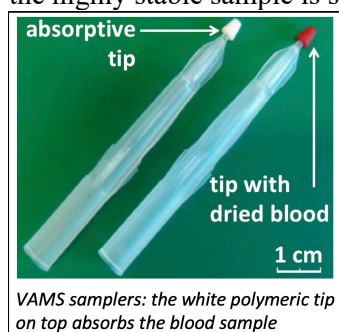
Project bullet-point description

- **Challenge:** Wet blood sampling is integral to contemporary patient diagnosis and monitoring. This is an environmentally stressing, expensive, and uncomfortable sampling technique. A shift to the more sustainable alternative of dried blood sampling requires proof-of-concept that multiple markers can be quantified from a small blood volume. C-peptide, fasting glucose, and tacrolimus are the minimal analytes required for routine monitoring of graft function in pancreas transplant patients. The project will develop an easy-to-use sampling technology where multiple markers can be quantified from a droplet of blood.
- **Proposed solution:** The introduction of patient convenient smart affinity sampling will allow for sample processing on its way to the clinical laboratory as the surface of the smart affinity samplers contain antibodies which start to capture target analytes at the moment of sampling. This is beneficial for analytes that are currently challenging to determine from home sampling devices, *e.g.*, low abundant peptides. Application of such intelligent extraction strategies will make multiple marker determination from a single droplet of blood possible.
- **Benefits and gain:** Lower carbon footprint, improved health, and accessible healthcare for all as well as dramatically reduced use of reagents contribute to a sustainable society. Home-based patient monitoring is advantageous reducing stressful traveling, improve patient involvement in their own treatment, and limit mobility-related infectious pressure. It will allow earlier detection of acute organ rejection episodes, which can potentially prolong graft survival. The follow-up can be centralized to the national transplant centre where the few experts in the field work.
- **Specific goal:** To integrate *smart affinity sampling* on micro-samplers providing a sustainable tool for tailor-made patient-centric sampling. It will be used in multiple marker detection from a single sample for clinical monitoring of pancreas-transplanted patients in a true home-hospital setting.

Background

Patient-centric sampling – the sustainable alternative

Blood sampling is an integral part of patient diagnosis and monitoring in modern healthcare, requiring in-person visits for venous blood draws by trained medical personnel. Blood sample analysis is essential for optimal short- and long-term patient outcomes, such as avoiding graft loss after organ transplantation and optimizing treatment for infections or cancer. Traditional blood draws are labour-intensive, require cold-chain transportation and storage, and biohazard material handling. Additionally, patients must travel to and from a medical facility, which can be costly, time-consuming, stressful, and contribute to pollution. The development of blood sampling on filter paper, known as dried blood spots (DBS), offers a substantially more sustainable and affordable alternative to the venous blood draw. The patients collect their own blood sample at home through a finger prick followed by dripping a droplet of blood onto a filter card. Once dry, the highly stable sample is sent to the laboratory with regular mail, eliminating the need for cold chain and



biohazard handling. This collection method, termed “patient-centric sampling”, has become increasingly popular, especially as a result of the COVID-19 pandemic.^{1,2}

Mitra® tips by Neoteryx, LLC (Trajan) is an alternative to filter paper for patient-centric sampling³ that ensures high-quality sampling by employing volumetric absorptive micro-sampling (VAMS) technology⁴ (see figure). VAMS technology has been successfully used in patient-centric sampling for therapeutic drug monitoring and in clinical studies.^{5,6} Compared to conventional sampling which typically requires 5-10 mL of blood, only 10-30 µL is sampled from a finger prick. All in all, patient-centric sampling using

filter-paper sampling or VAMS sampling *improves the patient's quality of life through a reduction in the number of in-person medical visits, saves on health care costs, and is highly sustainable.*

Need for efficient and selective sample clean-up from volume controlled micro-samples

Shifting from a hospital-/lab-centric sampling setting to the more sustainable patient-centric sampling requires the current analytical methods to be revisited and the sample preparation strategies to be reevaluated. The move from large sample volumes (5-10 mL) of *wet* blood to small volumes (10-30 µL) of *dried* hemolyzed blood requires approaches that can handle the complexity of dried blood as well as the lower amounts of available sample. Inclusion of a highly efficient and selective sample clean-up step utilizing reagent antibodies prior to the conventional analytical workflow is one way of circumventing the more complex matrix. This will allow for efficient clean-up and removal of matrix components to assure accurate measurements also in the restricted volume. The advantage is that only minute amounts of organic solvents are needed when downscaling the sample size with a factor of approx. 500. The disadvantage is the dramatic increase in sample pretreatment time, resulting in reduced sample throughput.

Introducing smart affinity micro-samplers – the tailor-made patient-centric sampling tool

Using multifunctional, easy to use micro-samplers that can collect a set sample volume and perform sample pre-treatment will reduce intensified sample handling. In addition to targeted biomarker capture, such micro-samplers collect specimens for generic analysis, making it possible to combine a variety of analytical techniques while assuring high sample throughput. This approach will enable us to take a *major step* towards *tailor-made patient-centric sampling for personalized treatment from one single droplet of blood.*

We will combine our established expertise in dried micro-sampling⁷⁻¹¹ with our knowledge of and experience in modifying DBS and VAMS samplers for integration of sample preparation in the sampling step¹²⁻¹⁵ in order to obtain a modified volumetric micro-sampler capable of instant affinity capture of a biomarker target from a predefined sample volume. *This will contribute to the applicability of patient-centric sampling making healthcare more affordable, comfortable, and sustainable.* Our mutual confidentiality and non-disclosure agreement and our collaboration agreement with Neoteryx (owned by Trajan Medical and Scientific) provides us with insight into the chemistry of the VAMS technology. We have previous experience using VAMS in protein analysis as well as knowledge of modifying paper-based surfaces with antibodies¹⁴ and VAMS with trypsin¹⁷. This will enable us to set up antibody binding through VAMS-surface modification by tosylation.

Showcasing the use of sustainable smart samplers – early detection of acute pancreas rejection using remote sampling

Early detection of acute pancreas rejection in pancreas transplanted patients is challenging. When fasting blood glucose is elevated, it is often too late to save the transplanted pancreas. Changes in the C-

peptide:glucose ratio is an early marker for potential rejection. Timely monitoring, *e.g.*, bi-weekly, will allow early detection of changes in this ratio, enabling anti-rejection interventions during the immune activation phase, *i.e.*, prior to irreversible damage. This may dramatically improve long-term outcomes.

The Norwegian pancreas transplant patient population is currently monitored through hospital visits at a minimum of every three months. Due to the limited number of pancreas transplant specialists in Norway, centralized monitoring through patient-centric sampling would be an advantage in this population. Pancreas transplant recipients are the patient group of choice for this project as they 1) are used to self-monitoring using finger-prick samples (blood glucose) and 2) need life-long monitoring of graft function (C-peptide) and correct level of immunosuppression (tacrolimus). In addition to the positive effects on this patient group, hard endpoint results of this monitoring intervention will be achievable in a relatively short time-period of one year. In other words, this is an excellent pilot group for this project and the results can be extrapolated to other patient groups that will need more than the three-year project period to generate hard endpoint data. Introducing VAMS-based smart affinity samplers will save time and cost for patients and the healthcare system. In addition, this approach enables targeted use of limited resources in a small country such as Norway. Local endocrinologists usually have limited experience in pancreas graft monitoring. Because the samples will be analyzed by a central, university hospital lab, results interpretation by pancreas transplant experts can be incorporated into the workflow. This will enable local standard patient care and central transplant-specific follow-up at the transplant center.

C-peptide, fasting glucose, and tacrolimus must be monitored in this patient group. Only tacrolimus is currently validated for VAMS technology.⁶ Analytical limitations prevent C-peptide measurement from the VAMS. Using selective antibody extraction of C-peptide will resolve these analytical limitations, making VAMS a suitable sampling technology where the C-peptide is measured *on-tip*, while tacrolimus and glucose is measured in the eluate using existing methods. *This will enable monitoring of all biomarkers from a single droplet of blood.*

Overall project objectives

The overarching aim is to establish smart affinity samplers as a sustainable, affordable, and convenient method for patient-centric blood sampling. Pancreas transplant recipients throughout Norway will be used to model the approach. Using smart affinity samplers in this healthcare context will to UN sustainability goals 3, 10, 12, and 13.

Hypotheses, research questions, work-packages, and milestones

A fundamental understanding and evaluation of the modified micro-sampler is essential for developing a smart affinity-capture micro-sampler based on VAMS technology that can sample an accurate volume while selectively capturing selected biomarkers and keeping the remaining sample available for analysis of additional markers.

Hypotheses:

- The VAMS sampler can replace the conventional venous blood sampling in disease monitoring.
- The VAMS sampler can be translated into a micro-sampler for affinity capture of C-peptide without significantly altering the physicochemical properties of the device (smart affinity sampler).
- Smart affinity micro-sampling can aid in determination of low-abundant biomarkers.
- The smart affinity micro-sampler can be used both as a conventional sampler and a C-peptide capture sampler.

Research questions (RQ):

- RQ1:** Which functionalization and immobilization conditions make the best smart affinity micro-sampler?
RQ2: Do smart affinity micro-samplers exhibit properties different from conventional samplers?
RQ3: Can smart affinity micro-samplers selectively capture targeted biomarkers from blood samples?
RQ4: Can we quantify multiple markers in the remaining whole blood collected by a single smart affinity micro-sampler?
RQ5: Can we make smart affinity sampling the standard for affordable and sustainable disease monitoring?

Work-packages:

Work package 1 – Optimizing and evaluating antibody immobilization on the micro-sampler (RQ1, 2)

Goal Identify the antibody immobilization strategy with the best performance

- Sub-goals**
- a) Optimize the parameters influencing preparation of smart affinity micro-samplers
 - b) Evaluate the physicochemical properties of the smart affinity micro-samplers
 - c) Investigate the stability of the smart affinity micro-samplers

d) Investigate the capture performance of the smart affinity micro-samplers

In this WP we will evaluate antibody immobilization on the VAMS tips. We will use the degree of capture of the low-abundance biomarker human chorionic gonadotropin (hCG) as a model system because we have a selection of available antibodies for immobilization and extensive experience in mass spectrometric analysis from various biological matrices, including dried sampling using conventional DBS⁷ and paper based immunoreactors.¹⁴ Parameter optimization and capture performance will be obtained through recovery measurement of hCG (*sub-goals a*) and *d*). Stability measurements are performed by making smart affinity samplers, storing them at various conditions (*e.g.*, room temperature, 4 °C) for up to 3 months. The performance will be measured through hCG recovery measurement (*sub-goal c*). The effect of immobilization on the physicochemical properties is evaluated through the ability to absorb an exact volume (10, 20, or 30 µL), absorption speed, drying speed, and visual examination, *e.g.*, microscopy (*sub-goal b*)).

This investigation is expected to result in two papers, Paper #1 and #2.

Milestones Start-up: August 2023
Submission Paper #1: July 2024
Submission Paper #2: December 2024

Work package 2 – Immobilizing anti-C-peptide mAbs on the micro-sampler (RQ3)

Goal To develop smart affinity micro-samplers allowing detection of C-peptide

Sub-goals *e) Develop and validate an analytical method, using whole-blood volumes relevant to the smart micro-samplers, for selective measurement of C-peptide concentrations in clinically relevant levels by LC-MS.*

f) Identify an antibody capable of selectively capturing C-peptide which can be coupled to the VAMS.

In this WP we aim to develop a micro-sampler for the selective capture of C-peptide. The first step is to implement and evaluate an LC-MS method (*sub-goal e*) followed by screening of mAbs (anti-C-peptide) capable of selective capture of C-peptide using the optimal functionalization and immobilization conditions established WP1. Immobilization of at least five mAbs will be evaluated with respect to C-peptide recovery (*sub-goal f*). The mAb with the best recovery will be used in the full validation of the whole analytical strategy to determine C-peptide in whole blood (sampling, washing, elution, digestion, and LC-MS analysis). For this purpose, spiked whole blood from volunteers and clinical samples will be used (*sub-goal e*)).

This investigation is expected to result in two papers, Paper #3 and #4.

Milestones Start-up: January 2025
Submission Paper #3: October 2025
Submission Paper #4: April 2026

Work package 3 – Smart affinity micro-samplers to determine multiple biomarkers (RQ4, RQ5)

Goal Introduce the Anti-C-peptide mAb micro-sampler as a smart affinity micro-sampler for multiple biomarker determination

Sub-goals *g) Evaluate the first non-specific elution step as a sample for determination of tacrolimus and glucose by already existing methods*

h) Benchmark analytical results from smart affinity sampler approach against existing methods

In this WP, the micro-samplers modified with anti-C-peptide mAbs will be used to quantify C-peptide, glucose, and tacrolimus in blood samples. After sampling and drying, the smart affinity micro-sampler will undergo a washing step to release all blood components, except the C-peptide bound to the mAb. The levels of glucose and tacrolimus can be determined in the eluate (*sub-goal g*) by established analytical methods. After this, C-peptide is eluted from the smart affinity micro-sampler tip and determined using the method developed in WP2. Using spiked whole blood from volunteers and clinical samples from patients, the performance of this strategy will be benchmarked against the methods currently in clinical use (*sub-goal h*)). WP3 will therefore make a crucial contribution to sustainable healthcare for patients at risk for acute pancreas rejection.

This investigation is expected to result in at least one paper, Paper #5.

Milestones Start-up: May 2026
Submission Paper #5: April 2027

Milestones for the PhD candidate: Start: 15/8/23. Submission PhD application to the faculty: 15/10/23. Start practical work 1/10/23. Submission paper #1: 1/7/24, paper #2: 1/12/24, paper #3: 1/10/25, paper #4: 1/4/26, paper #5 1/4/27. Submission thesis: 14/5/27. Defense: 14/8/28.

Human subjects research

Biological matrices will be used in all WPs. Samples from healthy donors will be obtained from the Blood Bank of OUS. De-identified clinical samples will be obtained under the existing approval from the “Regional komité for medisinsk og helsefaglig forskningsetikk” (REK number: 2018/1328).

Impact

Sustainability: Patient-centric sampling reduces traveling to/from hospitals, the risk of community acquired infections, and eliminates the need for cold-chain transport and storage. The analytical workflow has an Eco-Scale score >75, representing *excellent green analysis*¹⁶. The project fits well within UN Sustainable Development Goals 3, 10, 12, and 13.

Healthcare: Frequent, targeted monitoring of pancreas-transplanted patients will improve early detection of acute graft rejection. Patient-centric sampling will substantially reduce the need for expensive and stressful travel to hospitals, enabling a strong, specialized centre supporting local endocrinologists. The results from this research can be extrapolated and used in many other patient groups.

Academic: This research will generate new information about how to advance the sustainable field of patient-centric sampling. This work will specifically address the analytical challenge of combined multiple analytes measurement (C-peptide, glucose, and tacrolimus) in minute amounts of dried blood. The resulting strategy/tool has applicability to all areas of research and analysis within the field of patient-centric bioanalysis, e.g., biomarker analysis, drug analysis, forensic analysis, and doping analysis.

Communication strategy: We aim to publish five papers in high-ranked peer-reviewed international journals, e.g., Analytical Chemistry, Analyst, Analytica Chimica Acta, American Journal of Transplantation, Clinical Chemistry. The publications will be made available either as open-access articles or by use of public repositories and preprint servers such as BioRxiv and ChemRxiv. Results will be disseminated through participation and oral presentations at national and international conferences. To reach the broader audience we will use the Department of Pharmacy's well established channels of popular scientific outlets (titan.uio.no, forskning.no), as well as LinkedIn, Twitter, and Instagram, and we aim for participation at “Forskningsdagene.”

Implementation (project group and management)

The *project team* will consist of **Professor Léon Reubsæet** (LR; Department of Pharmacy, UiO, 117 scientific papers, h-index 38), **Professor Trine Grønhaug Halvorsen** (TGH; same dept. as LR), and **Professor Anders Åsberg** (AA; Head of Laboratory for Renal Physiology, OUS). LR will head the project, and lead the scientific work in WP1 and WP2. TGH will be involved in all WPs and lead WP3. AA will be involved in WP2 and 3. Other members: **PhD Karsten Midtvedt** (KM; senior consultant, Department of Transplantation Medicine, OUS, WP 3). **PhD candidate** (this application) and master students.

What are we applying for and how will the position be used?

We are applying for funding for one PhD position (4 years). The PhD position will be used for research (75 %: as described in WP1-3) and teaching obligations (25 %: where needed at the Department of Pharmacy).

References

1. James, C. A., et al., Will patient-centric sampling become the norm for clinical trials after COVID-19? *Nat. Med.* **2020**, *26* (12), 1810.
2. Koster, R. A., Have we got ‘patient-centric sampling’ right? *Bioanalysis* **2020**, *12* (13), 869.
3. Mei, J., Dried Blood Spot Sample Collection, Storage, and Transportation. In *Dried Blood Spots*, Li, W.; Lee, M. S., Eds. Wiley: 2014; pp 21-31.
4. Denniff, P.; Spooner, N., VAMS: a dried sample collection technique for quantitative bioanalysis. *Anal. Chem.* **2014**, *86* (16), 8489.
5. Roadcap, B., et al., Clinical application of VAMS to the gefapixant development program. *Bioanalysis* **2020**, *12* (13), 893.
6. Vethe, N. T., et al., Tacrolimus Can Be Reliably Measured With Volumetric Absorptive Capillary Microsampling Throughout the Dose Interval in Renal Transplant Recipients. *Ther. Drug Monit.* **2019**, *41* (5), 607.
7. Rosting, C., et al., Determination of the low-abundant protein biomarker hCG from dried matrix spots using immunocapture and nano liquid chromatography mass spectrometry. *J. Chromatogr. B* **2018**, *1077–1078*, 44.
8. Andersen, I. K. L., et al., VAMS vs. other blood sampling materials in LC–MS-based protein analysis – preliminary investigations. *J. Pharm. Biomed. Anal.* **2018**, *156*, 239.
9. Rosting, C., et al., Water-Soluble Dried Blood Spot in Protein Analysis: A Proof-of-Concept Study. *Anal. Chem.* **2015**, *87* (15), 7918.
10. Rosting, C., et al., Expanding the knowledge on dried blood spots and LC-MS-based protein analysis: two different sampling materials and six protein targets. *Anal. Bioanal. Chem.* **2017**, *409* (13), 3383.
11. Rosting, C., et al., Evaluation of water-soluble DBS for small proteins: a conceptual study using insulin as model. *Bioanalysis* **2016**, *8*, 1051.
12. Skjærø, O., et al., Smart blood spots for whole blood protein analysis. *Analyst* **2018**, *143* (13), 3184.
13. Skjærø, Ø., et al., All-in-one paper-based sampling chip for targeted protein analysis. *Anal. Chim. Acta* **2019**, *1089*, 56.
14. Skjærø, Ø., et al., Pre-lab proteolysis for dried serum spots – a paper-based sampling concept targeting low abundant biomarkers. *Anal. Meth.* **2020**, *12* (1), 97.
15. Skjærø, Ø., et al., Paper-based immunocapture for targeted protein analysis. *Talanta* **2019**, *195*, 764.
16. Galuszka, A., et al., Eco-Scale for assessing the greenness of analytical procedures. *TrAC*, **2012**, *37*, 61.
17. Reubsæet, J.L.E. et al., Next generation VAMS®–Trypsin immobilization for instant proteolysis in bottom-up protein determination. *Adv Sample Preparations* **2022**, *3*, 100027.