The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	Dried Blood Spot Analysis of Heavy/Light and Free Light Chains to improve the patient pathway for Myeloma Patients

SUPERVISORY TEAM	
Primary Supervisor(s):	Professor Mitch Dowsett
Other supervisory team members:	Dr Martin Kaiser Dr Robyn Shea
Lead contact person for the project:	Dr Robyn Shea

DIVISIONAL AFFILIATION	
Primary Division:	Molecular Pathology
Primary Team:	Biochemistry

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Dried Blood Spots (DBS) have been used for sample collection and delivery to the laboratory since 1913 but became established with new-born screening in the 1960's[1]. DBS are now used in the pharmaceutical industry[2] for large scale epidemiological studies[3] and to help overcome the challenges of handling traditional phlebotomy samples[4].

Venous phlebotomy has inherent disadvantages, requiring a trained phlebotomist with patients often being inconvenienced in order to have their blood taken[5]. DBS can overcome this as patients can take samples themselves at a convenient time and place making blood sampling much more accessible[6]. DBS have improved the patient pathway for many areas e.g. therapeutic drug monitoring[7] and blood-borne virus testing[8].

Myeloma features the production of monoclonal immunoglobulin proteins and/or free light chains (FLC) and measurement of these markers are crucial for the diagnosis and monitoring of patients as they are a measure of disease activity[9]. Heavy/light chain assays (HLC) allow an alternative to traditional monoclonal protein electrophoresis[10]. Due to the high morbidity associated with disease relapse (lytic lesions, kidney impairment, hypercalcaemia), patients are regularly monitored throughout their care. However, remote monitoring e.g. telephone clinics, are under used due to the need for blood tests to monitor disease progress.

This project will aim to introduce methods to analyse HLC, FLC, calcium and CRP in DBS sent to the laboratory by patients prior to their myeloma clinic. Results would then be available in clinic; an improvement over current practice as results are not available during clinic as blood is only taken on the clinic day. Having results available, without making a separate journey to hospital for phlebotomy, will improve patient management, help improve patient satisfaction with their care and may help patient engagement. This would also be assessed as part of the project.

PROJECT AIMS

To investigate the use of alternative methods of collecting and analysing human blood in routine clinical analysis using DBS. This work will include the following key aspects:

- Consideration of all aspects of DBS technology to achieve robust sample collection and analysis.
- Develop methods in the laboratory suitable for dried blood spot analysis of FLC, HLC, calcium and CRP.
- Investigation of different methods of DBS collection – traditional filter paper, filter paper with capillary collection tube and Mitra collection – in order to ascertain which method produces the most reliable results but is also the most suitable for collection by the patient.
- Study of the benefit to the myeloma patient journey which can be achieved by appropriate use of DBS in routine clinical practice.
- Investigation into the feasibility of setting up a national service using DBS which could result in collaboration with the National Amyloidosis Centre and allow the potential for future large scale studies into premalignant conditions such as MGUS which require a blood test to be detected, and could help to improve early diagnosis of disease progressing into symptomatic disease.

RESEARCH PROPOSAL

Development of DBS methods

DBS are drops of capillary whole blood collected onto filter paper from a finger prick. When a disk is punched from a DBS, it can be considered a volumetric measurement, similar to that of a liquid measuring device. The disk can be any size that is suitable for the assay. The sample is then eluted from the disk and the DBS is effectively reconstituted as haemolysed liquid whole blood. Using a punched disk is a way of ensuring that for each sample analysed the same volume of blood is used in the assay regardless of how much blood has been absorbed onto the filter paper. Current routine methods in the laboratory can be modified to enable them to be suitable to analyse extracts from DBS disks.

FLC are run using the Binding Site assay and SPA+ analyser. This would be adapted for use with DBS through the use of DBS calibrators, quality control material and assay parameter modification. Proof of principle of lambda light chain analysis has already been achieved. Hevylite is an assay also offered by the Binding Site for HLC analysis and can be run on the SPA+ analyser. This would be set up for serum and for DBS following the same principles as for the FLC. Calcium and CRP are run on routine chemistry analytical platforms (currently Beckman DXc) and the assay would be adjusted for use with DBS following the same principles as for FLC. This will require considerable method development in some cases due to the lower levels of analyte to be detected in extracts from DBS.

Development of DBS methods would require investigation of appropriate calibration and quality control material, size of DBS, stability of DBS, location of punch and effect of haematocrit and haemolysis.

Method Validation

Once methods have been developed they will be assessed with regards to accuracy, precision, sensitivity, linearity, carryover and robustness. Method development will necessarily be performed on EDTA whole blood used to make DBS however method validation will require a three way comparison between serum (traditional sample type), EDTA (readily available whole blood) and capillary blood (patient DBS sample type) to understand the relationship between traditional serum results and the capillary DBS. Ideally the

same reference ranges will be able to be used as for serum samples; however it may be necessary to set up a DBS specific reference range.

DBS collection methodology

DBS can be collected by patients simply by pricking their finger, allowing a drop of blood to form and then collecting this onto filter paper. This can result in different sized DBS being collected depending on the DBS collection device and the patient. Investigation into whether or not patients can collect samples of adequate size and quality will need to be undertaken and it may be necessary to investigate the feasibility of using capillary collection tubes in the collection process in order to collect a DBS with a known volume of blood. An alternative way of collecting capillary blood samples is via Phenomenex's Mitra collection device which behaves like a sponge on a stick collecting exactly 10µL of blood from a finger prick. This method would be compared with the filter paper method to see which provided better analytical results and useable samples from patients.

A DBS collection kit, including robust instructions, will need to be put together to enable patients to take samples at home and post them into the laboratory.

Patient Samples

Excess EDTA samples, where a paired serum sample has already been tested and reported, will be anonymised and used in the method development stages to create DBS. Paired samples of serum, EDTA and DBS will be required for method validation. Ethical committee approval will be sought to take DBS samples after informed consent from patients who will already be having a serum and EDTA sample taken after clinic. Ethical committee approval will also be sought to create DBS from excess EDTA samples and store the DBS for method validation purposes.

Use in routine Myeloma clinics

Once appropriate DBS methods have been established and a DBS collection kit has been created using the appropriate sampling methodology, DBS can be put into routine clinical use. Work will be done with clinicians to find the most appropriate way of doing this. Returned DBS will be assessed for quality to ensure that patients are able to collect quality DBS and that DBS are a viable way of returning samples to the laboratory.

The project will include an assessment of the impact of the use of DBS in routine clinical care of myeloma patients. Evidence will be gathered to show any improvement in patient care that can be given including improved quality, efficiency and overall benefit to the patient's experience.

Equipment and reagents

Filter paper and DBS collection devices can be obtained from SWBH NHS Trust which has a history of using DBS for direct to the patient DBS testing. Their collection device has been designed for use in patient's homes. DBS disc cutters can be obtained from GE Healthcare. Mitra collection devices can be obtained from Phenomenex.

Further analytical equipment will not be required as the SPA+ and analysers for calcium and CRP analysis are already available for use in the routine clinical biochemistry department in The Royal Marsden. There is spare capacity for this work on the analysers as currently the SPA+ is only used 1-2 days a week and there are four routine chemistry analysers available across both sites which are open access allowing scope for the analysis of additional samples. Additional reagents for FLC, HLC, CRP and calcium analysis will be required.

Expected Project Outcomes

- Development of robust DBS methods for FLC, HLC, calcium and CRP
- Establish if myeloma patients are able to provide quality DBS

- Establish best methodology for collection of DBS by patients
- Development of DBS service for myeloma patients
- Assessment of impact DBS service has on clinical practice and myeloma patients
- Potential development of a national/international service for analysis of FLC and/or HLC by DBS

LITERATURE REFERENCES

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2. Emmons, G. and Rowland, M. (2010). Pharmacokinetic considerations as to when to use dried blood spot sampling. *Bioanalysis,* 2(11), pp. 1791-1796.
3. McDade, T., Williams, S. and Snodgrass, J. (2007). What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. *Demography*, 44(4), pp. 899-925.
4. Johannessen, A. (2010). Dried blood spots in HIV monitoring: applications in resource-limited settings. *Bioanalysis*, 2(11), pp. 1893-1908.
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9. Bird, J., Owen, R., D'Sa, S., Snowden, J., Pratt, G., Ashcroft, J., Yong, K., Cook, G., Feyler, S., Davies, F., Morgan, G., Cavenagh, J., Low, E. and Behrens, J. (2014). Haemato-oncology Task Force of British Committee for Standards in Haematology (BCSH) and UK Myeloma Forum. Guidelines for the diagnosis and management of multiple myeloma 2014. *Published online (www.bcshguidelines.com).*
10. Ludwig, H., Milosavljevic, D., Zojer, N., Faint, J., Bradwell, A., Hubl, W. and Harding, S. (2013). Immunoglobulin heavy/light chain ratios improve paraprotein detection and monitoring, identify residual disease and correlate with survival in multiple myeloma patients. *Leukemia*, 27, pp. 213-219.

CANDIDATE PROFILE

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

Pre-requisite qualifications of applicants:	BSc or equivalent in: Biochemistry Biology Biological Sciences Natural Sciences Biomedical Sciences Relevant science/medical subject

Intended learning outcomes:

- Skills in scientific writing
- Skills in developing and validating clinical laboratory methods
- In depth knowledge of the use of DBS
- Skills in obtaining samples from the public
- Ability to set up a novel clinical service involving the public, clinicians and laboratory
- Knowledge of ethics procedures
- In depth understanding of the role of clinical biochemistry services and their impact on patient care