# Study Protocol Needle-free Sensing of Blood Glucose

Coordinating Investigator (CI): Andrew Taberner

Co-investigators: Michael Hoffman, Jiali Xu, James McKeage, Bryan Ruddy, Bahareh Madadkhahsalmassi, Nandoun Abeysekera

## Background

Diabetes is diagnosed in 5.8% of the population of New Zealand, and disproportionately affects lower socioeconomic status groups, as well as Māori, Pacific, and Asian peoples [1]. Type 1 and insulin dependent type 2 diabetics require injections of insulin multiple times per day to manage the glucose concentration in their blood. The appropriate dose of insulin is determined using a measurement of blood glucose concentration that typically requires the fingertip be pricked with a lancet. The drop(s) of blood resulting from this prick are used to perform the glucose measurement. In an effort to make this process easier for diabetics, we are investigating whether this testing can be achieved with, and integrated into, a needle-free jet injection device. Performing this process without a needle/lancet will avoid the associated issues of sharps waste, accidental needle stick, and needle-phobia [2].

Needle-free jet injection is an established method of transdermal drug delivery. The technique involves the liquid drug being formed into a high speed jet which is capable of penetrating the skin, thus removing the need for a needle. Jet injection has been shown to be an effective delivery method for vaccines, insulin, hormones, and anaesthetics, among others [2], [3]. In many studies, the pain and discomfort associated with a jet injector has been shown to be similar to that experienced with the equivalent needle based injection [2].

Blood extraction with a jet injector was recently trialled in vivo for the first time. This study investigated the volume of blood released from the fingertip following jet injection and whether this blood was diluted with injectate. The unpublished results showed that an average of 1.7 µL of blood and injectate could be collected. This demonstrated the feasibility of jet injection for needle-free blood sampling but highlighted that the major challenge in performing a glucose measurement will be to quantify the extent to which the injected fluid dilutes the blood sample collected.

The application of vacuum has been established in a series of studies as a technique that can increase the volume of blood released following lancing [4], [5]. The results showed that the volume of blood released increased in proportion to the duration and pressure of suction applied. The range of interventions explored include continuous vacuum durations of up to 40 seconds, and vacuum pressures of up to 70 kPa, with no reported adverse effects. We wish to incorporate this technique as a strategy to increase the volume of blood released following jet injection. In doing so we expect to be able to draw up to 20 µL of blood from the fingertip. It is anticipated that the application of suction may increase the proportion of interstitial fluid or plasma in the blood collected. Our measurement system has been designed to be able to specifically distinguish the presence of injectate in the blood sample collected.

As part of this study, we wish to investigate the extent of this dilution issue and validate a novel method to quantify the dilution. We propose to measure the dilution by marking our injected fluid with indocyanine green (ICG), an FDA approved fluorescent marker. By illuminating the retrieved blood samples with 780 nm light, and measuring the resulting fluorescence we will be able to measure the dilution of the sample. ICG is commonly used for diagnostic purposes (e.g., cardiocirculatory, retinal and liver function diagnostic tests), typically at a dose of no more than 2 mg/kg and a concentration of 5 g/L [6]. ICG has been delivered transdermally into the subcutaneous tissue in multiple fluorescent lymphography studies, and into the dermal layer in a recent study performing fluorescent microscopy. Delivery of 20 µL of 5 g/L ICG into the dermis was described as producing a “short-time tolerable burning sensation” [7], but in this study we plan to use a concentration of only 0.2 % of this value: 10 mg/L.

## Research Aims

In this study we intend to use needle-free jet injection not to deliver a drug, but instead just to pierce the skin with a small volume of ICG solution, and then retrieve a capillary blood sample. Ultimately we believe a single jet injection device may be able to extract blood, perform a blood glucose measurement and then, based on this measurement, deliver the ideal volume of insulin. However, this study will focus only on establishing the ability of this technique to achieve a measurement of blood glucose concentration.

This work will test the ability of a needle-free jet injection device to release capillary blood when assisted by the application of vacuum to the wound post injection. We wish to evaluate how this technique compares to the current best practice where the skin is broken with a lancet. We will compare the volume, dilution, and glucose concentration of blood collected following a needle free injection and standard finger prick. We will also evaluate what level of suction is best suited to assist in the collection of a blood sample following jet injection and lancing. The comparisons will require measurement of the volume of blood released, blood sample dilution, blood glucose concentration, and participant discomfort.

### Principal Research Question

“Can a suction-assisted needle-free jet injection device obtain a capillary blood sample sufficient for a glucose concentration measurement?”

This can be broken down into four further questions which relate directly to the measurements we will be making:

1. What is the relationship between suction pressure and volume of blood release?
2. What is the volume of injectate present in the collected samples? Does this change relative to the time since the intervention?
3. Is there a relationship between the suction pressure applied and the dilution of the blood collected?
4. Is the incorporation of suction into the blood collection process well tolerated?

## Research Methods

### Study Design Overview

This is a pilot study aiming to assess the feasibility of using a jet injector assisted by suction to retrieve a capillary blood sample. Each participant in the study will receive four interventions: the current best practice for capillary blood sampling (a lancet prick), a lancet prick with suction applied, a jet injection, and a jet injection with suction applied. This study is designed to be single-blinded and all participants will serve as their own controls as each participant will receive both the lancet based sampling and the jet injections. Participants will be aware they are receiving one of each intervention, but will be blinded to the order and to the identity of which intervention is occurring at which site. The study will be conducted in the ground floor laboratory space (G20) of the Auckland Bioengineering Institute (70 Symonds Street).

Participant groups

Each participant will be randomly assigned into one of three groups. Each group will contain 5 participants. The same intervention procedure will be followed across all groups, with the only exception being the suction pressure applied (group 1: 20 kPa, group 2: 40 kPa, group 3: 60 kPa). Participants will be blinded to the group they are in. They will be informed of which group they are in at the completion of their verbal questionnaire following the interventions.

### Population & Recruitment

In this study we intend to recruit at least 15 healthy participants divided into three groups of 5 participants. The difference between these groups relates to the suction pressure applied during blood collection. The blood samples collected will be analysed for volume and haematocrit, and tested for ICG concentration and glucose concentration. Participants will be aware that the study is structured in this way, and will be aware of the nature of the interventions to be performed.

With a minimum sample size of 5 in each group we predict we will be able to distinguish statistically significant differences in mean volume released as low as 1.4 µL (= 0.7 µL, *α*= 0.05, *β*= 0.1). We predict we will be able to observe differences in mean blood dilution as low as 6 % between groups with a sample size of at least 5 (= 3 % , *α*= 0.05, *β*= 0.1), and we predict we will be able to observe differences between groups whose mean glucose concentration differs by as little as 10 % (= 0.28 mmol/L, *α*= 0.05, *β*= 0.1).

The study will be advertised within the Auckland Bioengineering Institute and Department of Engineering Science at the University of Auckland. This will involve flyers posted within Auckland Bioengineering House (70 Symonds Street) and an email sent to the Auckland Bioengineering Institute. These advertising materials will invite anybody interested in participating to contact the researchers for more information. In response to expressions of interest the researchers will provide a copy of the participant information sheet (PIS) and consent form (CF) to give detailed information about the study. Participants will be asked to take their time reading these forms and, if they are interested in participating, to indicate times that might suit them to come in for the study. This will also allow potential participants adequate opportunity to ask questions and seek external advice regarding participation in the study.

Participants will be required to visit the Auckland Bioengineering Institute for one 45 minute session where the interventions will take place. The participants will be asked to complete a short questionnaire 24 hours after the interventions, and send a photograph of the fingertips to the researchers.

We will aim for gender and ethnic representation in approximate proportion with the greater New Zealand population. We imagine we will be able to get the required number of participants from within the Auckland Bioengineering Institute and Department of Engineering Science. However, if this proves more difficult than expected we will extend the recruitment to the wider University (UoA) using the same flyers and emails.

### Eligibility Criteria

Inclusion and exclusion criteria will be the same for all groups in this study.

#### Inclusion Criteria

* Aged >20 and <60 years old. (Human skin thickness is relatively constant for people between 20 and 60 years, and is reduced outside of this range [7])
* Able to communicate in English
* Able to give full informed consent (i.e. no neurological impairment)

#### Exclusion Criteria

* Insulin-dependent diabetes. (Due to regular finger-prick testing, these individuals may have scarring of their fingertips, which may influence blood release.)
* Haemophilia (or other bleeding/clotting disorders)
* Carrier of blood-borne infectious agent (e.g. HIV, HBV)
* Amputation affecting a number of fingertips
* Significant peripheral circulatory reduction (e.g. Raynauds disease or beta blocker use)
* History of allergy to iodides and/or indocyanine green
* History of kidney disease

### Interventions

Each participant will be subjected to four interventions: a standard lancet prick, a jet injection with a standard circular orifice, a standard lancet prick followed by suction and a jet injection followed by suction. These interventions will be performed on the side of the fingertip of the middle (3rd finger) and ring finger (4th finger) of either hand with suction applied directly over the intervention site. Which finger receives which intervention will be randomised, as will the order of the interventions. The participants will be blinded by an opaque barrier which will prevent them observing the procedure but allow them to see and communicate with the practitioner. After each intervention has been applied, and the associated blood samples collected, there will be a 2 minute ‘break’ before performing the next intervention. The participants will be shown the jet injector and lancing device following the procedures. The interventions will be identical for all groups with the exception of the suction pressure applied (group 1: 20 kPa, group 2: 40 kPa, group 3: 60 kPa).

#### Lancet

Capillary blood will be sampled in accordance with the “WHO guidelines on drawing blood” [8]. This will involve a lancet piercing the skin to a depth of 2.3 mm. The collection of the blood into three sequentially-collected samples is not specified in the WHO guidelines, but is necessary to the study (see section 3.5), and adds no extra risk to the participant.

#### Jet Injection

A jet injection of less than 30 µL of sterile isotonic saline will be performed to pierce the skin. ICG will be added to the injected saline to a concentration of (10 mg/L). An ICG concentration of 10 mg/L is well below the dose used for other diagnostic procedures (5 g/L) approved by the FDA.

The jet will target the same depth in the dermis as the lancet (2.3 mm). Similarly to the lancet, the jet-injector based blood sampling will be performed as closely as possible to the WHO guidelines. However, the use of a jet injector itself is not currently within these guidelines, as this is a novel element of this study.

#### Suction

The surface of the finger will be wiped clean with a tissue following the intervention. A partial vacuum will then be applied within 1 kPa of the specified value (group 1: 20 kPa, group 2: 40 kPa, group 3: 60 kPa) for a 10 second period. This suction will be applied to the wound produced by lancing or jet injection by placing a suction cup in direct contact with the fingertip. The surface of the finger will be wiped clean again prior to any reapplication of suction.

### Blood collection

A suction pressure will be applied to the intervention site to encourage blood release in accordance with the participant group (group 1: 20 kPa, group 2: 40 kPa, group 3: 60 kPa). The suction will be applied for 10 s intervals following the intervention (0 s – 10 s, 20 s – 30 s, 40 s – 50 s). Blood collection and cleaning of the fingertip taking place between these intervals (10 s – 20 s, 30 s – 40 s, 50 s - 60 s). The collected blood will be separated into different sample containers based on these intervals. Hence, each intervention will be associated with three blood samples (a total of 12 blood samples per participant). We are collecting blood in this way to allow us to observe the time-related behaviour of blood release and glucose concentration following breaking the skin.

Where suction is not applied, standard capillary blood collection techniques will be used in accordance with WHO guidelines. This will include a gentle pressure or milking of the finger to encourage bleeding between each collection step. The extent of pressure/milking will be consistent across all collections, and independent of the amount of bleeding.

### Measurements

#### Injectate Concentration (Fluorescence)

We will measure the ICG concentration in the sample using a benchtop fluorimeter that has been developed at the Auckland Bioengineering Institute specifically for this purpose [8]. The capillary tube containing the blood sample will be placed into the fluorimeter, which will excite the sample with 780 nm light. The resulting fluorescence will be measured, indicating the concentration of ICG and hence the proportion of injectate in the blood sample. The blood volume as a fraction of the fluid collected volume will be used to scale the measured glucose concentration.

#### Volume and Haematocrit Measurement

The blood will be collected from the skin using capillary tubes. As the dimensions of these capillary tubes are known the sample volume will be measured by the length of tube that is filled with blood. This will be imaged with a USB desktop microscope.

This image of the sample will also be used to measure the haematocrit of the sample. The pixel intensities of each colour of the image will be used to estimate the haematocrit. This measurement will inform the calculation of dilution.

#### Glucose Concentration

Following volume and haematocrit measurement, we will measure the glucose concentration of each blood sample using a CareSens N point of care glucometer. This approved (and Pharmac funded) device involves the blood sample being collected onto a single use test strip inserted into the glucometer. In the case that the volume of blood dispensed is insufficient for a glucose measurement this will be recorded as a failure of blood release. The test strip will be disposed of as medical waste following this glucose measurement.

#### Perceived Pain

After each intervention and its associated blood collection, the participant will be asked to score the pain associated with the intervention on a standard verbal numeric rating scale. Each participant will be asked to rate the pain experienced from 0-10, with zero representing no pain, and 10 representing extremely severe pain. After 24 hours following the interventions, the participants will be asked to complete a questionnaire to re-assess the level of pain and any swelling, redness, bruising or soreness they have perceived at each intervention site.

#### Acceptability of intervention

After each intervention and its associated blood collection, the participant will be asked how acceptable they find the intervention on a standard verbal numeric rating scale. Each participant will be asked to rate how willing they are to have the intervention repeated from 0-10, with zero representing completely unacceptable, and 10 representing without hesitation.

#### Site Reactions

After blood samples are collected for each intervention, a sterile cotton-wool ball will be applied until bleeding stops. A microscope image of the intervention site will be taken immediately after the bleeding has stopped. This will reveal whether each intervention site results in a visible wound. The participants will be asked to take a photograph of each of the intervention sites, and answer some questions regarding their fingertips, 24 hours after receiving the interventions. The questions will reveal any persisting discomfort or reactions at the intervention sites, and the pictures will give the researchers an indication of any swelling and/or redness.

### Materials

Please refer to our “Summary of Device Safety” document for a more thorough analysis of the potential hazards associated with the materials used in this trial.

#### Lancet

The lancing will be performed using a standard, commercially available tool (Accu-Check, Safe-T-Pro-Plus). This will be set to penetrate to a depth of 2.3 mm. This device shields the lancet following penetration, making accidental stick injury extremely unlikely.

#### Jet Injector

The injection system has been designed in accordance with medical electrical equipment safety standard IEC 60601-1. The jet injector includes an electric motor which is momentarily (<0.1 s) supplied with a voltage to perform the injection. All elements of the injector that are exposed to the user are made from plastic and as such, in the very unlikely event of a short circuit within the injector, it is not possible for the participant or researcher to be exposed to the driving voltage. It would require a minimum of three simultaneous faults to expose a participant or researcher to any electrical voltage. During use all external surfaces of the device remain well below the maximum temperature (40 °C) dictated by the IEC 60601-1 standard. The ampoules with the standard circular orifices come in sterile packaging and will be single use.

*Injectate*

The jet injections will be performed using a volume of less than 50 µL of sterile isotonic saline. This will include ICG at concentration of 10 mg/L. ICG is an FDA approved, fluorescent marker which is commonly used at a concentration of 5 g/L. We will deliver ICG at a concentration five hundred times smaller than this. The ICG solution will be used as a marker that will allow us to measure the dilution of blood samples.

*Suction system cup*

A 12 V DC vacuum pump will be controlled by a motor controller. This module will receive power 12 VDC 1 A supply from a mains adapter. The motor control module will disable power supply to the vacuum pump in the event of a surge. The inlet to the vacuum pump will be connected by plastic tubing to a reservoir that contains an inline pressure sensor. This pressure sensor is used for feedback control of the vacuum pump through a LabVIEW virtual instrument interface designed to regulate the pressure applied. A solenoid relief valve is included in series to automatically vent the loop in the case that the applied suction pressure is greater than specified, or the duration of the application of suction has elapsed.

Suction will be applied through a PTFE (‘Teflon’) suction cup that is placed over the intervention site to make a seal with the fingertip. The suction cup is connected to a small vacuum pump with pressure sensors in series to control the pump to achieve a desired level of suction.

*Blood collection/containment*

The blood released at the intervention sites will be collected using disposable capillary tubes. The capillary tubes containing the blood will be placed directly into the fluorimeter. The blood will then be ejected from the capillary tubes onto a glucose test strip. All samples and capillary tubes will be disposed as medical waste following collection and measurement.

### Personnel

Our research team includes experts in needle-free drug delivery (bioengineers) and Dr Nandoun Abeysekera, who provides clinical oversight. In addition, a healthcare professional trained in phlebotomy (e.g. nurse) will be employed to oversee the blood sampling procedures.

### Safety Monitoring

An internal safety monitoring committee will review the data collected during the trial looking for evidence of adverse effects or unexpected risks to participants or researchers. The committee will be particularly interested in any evidence of unexpected behaviour from the jet injections, or lasting symptoms at the intervention sites. The trial will be terminated at the discretion of the committee if evidence of these issues were to arise. The committee will consist of three members of the research team: Dr Nandoun Abeysekera, Dr Bryan Ruddy, Dr James Mckeage, as well as the health care professional hired to oversee the blood sampling procedures. This committee will meet every fortnight, assuming around 3 participants per week complete the trial, or more regularly as necessary.

Elevated pain scores, bruising, and excessive bleeding have all been identified as signs of unexpected behaviour from the jet injections. Extreme levels of pain will be measured as an average pain score of >7.5 after at least 5 participants. If evidence of unexpected adverse effects (e.g. infection, nerve damage, etc.) are observed in the participant feedback and “day after” photos the study will be terminated.

If the average glucose measurement in the control samples (lancet) suggest the participant had an extreme glucose concentration (less than 4.0 mmol/L or greater than 11.1 mmol/L) at the time of sampling, the participant will be contacted to describe this measurement, and encourage the participant to talk to their GP.

### Timeline

This protocol is planned over a 12 month period (01/10/2021 – 01/10/2022). As the involvement of each individual participant is primarily just a single site visit (and a follow up questionnaire), most recruitment and data collection will occur in parallel. Data collection will likely be completed within 6 months, with the remaining 6 months set aside for data processing, reporting and publication.

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