Sub Study:

SNAPPER: SNAP prospective evaluation of renal effects

Staphylococcus aureus Network Adaptive Platform (SNAP) trial

Sub Study: SNAPPER Version 1.0 dated 31 August 2023

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1. SUB STUDY SYNOPSIS

The *Staphylococcus aureus* Network Adaptive Platform (SNAP) trial is a multicentre, international adaptive platform trial aimed to identify the most effective treatment for *S. aureus* bacteraemia. Within the SNAP trial, we plan to undertake a sub study to look at the release pattern of a panel of urinary biomarkers in patients with *S. aureus* bacteraemia, and the utility of these urinary biomarkers to signal acute kidney injury (AKI).

As the optimal urinary biomarker for early detection AKI in *S. aureus* bacteraemia in adults has not been defined, we will study a panel of urinary biomarkers that are supported by available evidence. Our approach aims to better understand the relationship between AKI and *S. aureus* bacteraemia and document the patterns of release of urinary biomarkers in this population.

Aims:

- Determine the temporal profile of urinary biomarker release in patients with *S. aureus* bacteraemia (stratified by AKI [and KDIGO-defined grade] and no AKI).
- Determine the temporal profile of urinary biomarker release in AKI relative to current diagnostic criteria (ie serum creatinine).
- Assess usefulness of each urinary biomarker in the diagnosis of AKI.

Key eligibility criteria:

- Adults (aged 18 years and older) enrolled in the SNAP randomised platform
- At least one of the following risk factors for AKI:
    - concurrent administration (within the 48 hours before study enrolment) of vancomycin with flucloxacillin or piperacillin+tazobactam for at least 24 hours
    - receipt of vasopressors or inotropes in the 48 hours before study enrolment
    - pre-existing chronic kidney disease (not requiring dialysis)
    - admission to HDU or ICU at study enrolment.
- Able to collect first urine sample before the end of the calendar day of platform entry (platform day 1).

Planned sample size: we aim for recruitment of at least 50 patients. This is a pilot study, and thus the sample size is largely based on feasibility (the number of patients we can reasonably expect to enrol with available resources and funding).

2. ABBREVIATIONS

aHR	Adjusted hazard ratio
AKI	Acute Kidney Injury
DDDs	Defined daily doses
DSA	Domain-Specific Appendix
DSWG	Domain-Specific Working Group
DSMC	Data and Safety Monitoring Committee
GTSC	Global Trial Steering Committee
HITH	Hospital in the home
IE	Infective Endocarditis
ICU	Intensive Care Unit
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MIC	Minimum Inhibitory Concentration
OPAT	Outpatient Antimicrobial Therapy
PSSA	Penicillin-susceptible *Staphylococcus aureus*
RAR	Response Adaptive Randomization
RCT	Randomized Controlled Trial
SAE	Serious Adverse Event
SNAP	*Staphylococcus aureus* Network Adaptive Platform trial

3. SUB STUDY GOVERNANCE

3.1. Sub Study Members

Sub Study Lead: Amy Legg

Members: Joshua Davis, Steven Tong, Rinaldo Bellomo, Jason Roberts, Alan Cass, Marc Scheetz, Jane Davies, Marjoree Sehu, Owen Robinson, Kylie Alcorn, Kate Garnham, Bridget Barber

Proposed 1st Author: Amy Legg

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3.3. Participating sites

Sites participating in the SNAP trial within Australia will be able to participate.

4. BACKGROUND AND RATIONALE

4.1. Sub study aims

1. Determine the temporal profile of urinary biomarker release in patients with *S. aureus* bacteraemia (stratified by AKI [and KDIGO-defined grade] and no AKI).
2. Determine the temporal profile of urinary biomarker release in AKI relative to current diagnostic criteria (ie serum creatinine).
3. Assess usefulness of each urinary biomarker in the diagnosis of AKI (using biomarker sensitivity and specificity, and timing of biomarker change relative to serum creatinine).

4.2. Sub study background

Serum creatinine (SCr) and urine output are predominantly used in current published criteria for diagnosis and staging of acute renal injury, however, these have significant limitations (1). Urine output is not specific for a decrease in GFR, and AKI can occur despite patients producing a 'normal' amount of urine (2). Creatinine is delayed in its elevation, only occurring after significant damage to the kidney has already occurred (3). Additionally, creatinine does not solely reflect kidney damage, it can be altered due to blockade of active secretion pathways, changes in diet, or changes in muscle mass (2).

Improved biomarkers for AKI diagnosis are desperately needed. Biomarkers can overcome limitations with urinary output and creatinine as markers of AKI, facilitating early diagnosis and providing information about pathophysiology of injury (4, 5). Proposed biomarkers are molecules released directly from the nephron at the time of kidney injury and provide information on kidney function, tubule function or kidney damage (6).

We aim to undertake a pilot study looking at the release of urinary biomarkers in a subset of SNAP patients, enriched for likelihood of AKI. This will provide information on feasibility of recruitment, collection and urinary biomarker processing for research purposes, the proportion of patients undergoing AKI of the sub study cohort, and patterns of urinary biomarker release.

5. SUB STUDY DESIGN

This will be a prospective pilot sub study, nested within the SNAP trial. Urine samples will be collected daily for the first 5 days from platform entry for biomarker analysis. Additional patient specific data will be collected for the SNAPPER sub study.

5.1. Sample size

We aim to recruit 50 to 100 patients with an increased risk of developing AKI (i.e., an enriched sample), to assess patterns of urinary biomarker release. This is a pilot study, and thus the sample size is largely based on feasibility (the number of patients we can reasonably expect to enrol with available resources and funding). Pilot studies do not need to be based on sample size analyses, and should actively avoid formal hypothesis testing. Their key aim should be "road testing" measurement tools and refining assumptions in order to inform the design of a subsequent definitive trial.

5.2. Eligibility criteria

Patients are eligible for this sub study if they meet all of the platform-level inclusion and none of the platform-level exclusion criteria AND all of the sub study inclusion and none of the sub study exclusion criteria.

5.2.1. Sub study inclusion criteria

- At least one of the following risk factors for AKI:
    - concurrent administration (within the 48 hours before platform entry) of vancomycin PLUS one or both of i) flucloxacillin or ii) piperacillin + tazobactam, with this combination received for at least 24 hours within this 48-hour period.
    - receipt of vasopressors or inotropes in the 48 hours before platform entry
    - pre-existing chronic kidney disease (defined as stable baseline eGFR<50ml/min, but not requiring dialysis)
    - admission to HDU or ICU at platform entry.
- Patient able to have first urine sample collected by the end of the SNAP platform day 1

**Data supporting the above enrichment criteria:*

- *Piperacillin+tazobactam with vancomycin: from a meta-analysis the aOR for development of increased serum creatinine as an indicator of AKI is 1.57-4.18 compared to vancomycin alone or vancomycin plus another beta-lactam (7).*
- *Flucloxacillin plus vancomycin: 4.5-fold increased risk of AKI compared to vancomycin for MRSA (8).*
- *Pre-existing CKD: aOR increases with the degree of CKD, from 2.0 for those with baseline eGFR 45–59 mL/min/1.73m^2 up to 40.1 for those with baseline eGFR <15 ml/min/1.73m^2, compared to patients with baseline eGFR ≥ 60 ml/min/1.73m^2 (9).*
- *Admission to ICU/HDU or need for vasopressors or inotropes: rates vary, but ANZICS data has shown 42% of critically ill patients experience sepsis-induced AKI (10)*
- *A post-hoc analysis of CAMERA2 patients (unpublished) showed 37% (82/217) of patients were having AKI either at study enrolment or during the trial. In this sub study, using an enriched sample of patients with* S. aureus *bacteraemia, we expect as many as 45% of patients may experience AKI, allowing for comparison between AKI and no AKI groups.*

5.2.2. Sub study exclusion criteria

- < 18 years of age
- Previous kidney transplant (kidney transplant patients can often have elevated concentrations of urinary biomarkers due to graft fibrosis or AIN in the setting of decreased renal function)
- Has an ileal conduit
- Unable to produce urine for sample collection (e.g., anuric on platform day 1)
- Receiving maintenance dialysis or acute renal replacement therapy for AKI at time of SNAP trial enrolment

5.3. Interventions

This sub-study does not involve therapeutic or diagnostic interventions.

5.4. Concomitant care

As per SNAP Platform

5.5. Endpoints

5.5.1. Primary sub study endpoint

This is a pilot sub study to assess feasibility of recruitment and sample preparation and analysis. We aim to recruit between 50 and 100 patients and we expect around 45% of patients to experience AKI because we are using an enriched cohort of patients at high risk of AKI. Results will be hypothesis generating.

The primary endpoint is the proportion of patients who experience AKI (at trial enrolment and during the first 5 days of the trial).

In a recent cohort study, Miano et al included 192 patients with sepsis aiming to determine if elevated serum cystatin C (a kidney function biomarker) occurs in concert with serum creatinine (to confirm true kidney injury) for patients on combination antibiotic therapy (11). AKI (based on serum creatinine elevation) occurred in almost 20% of patients on vancomycin and piperacillin + tazobactam combination therapy, which was higher than other antibiotic combinations, yet elevations with cystatin C were not different between the antibiotic regimens. This highlights the deficit in using serum creatinine to diagnose acute kidney injury and the significant proportion of AKIs occurring based on serum creatinine.

5.5.2. Secondary sub study endpoints

The secondary endpoints include urinary biomarker concentrations per day stratified by patients that do and do not experience AKI (and broken down into grade of AKI)

6. SUB STUDY CONDUCT

6.1. Sub study-specific data collection

6.1.1. Clinical data and sample collection

Additional sub study-specific data or samples will be collected as follows:

- Urine sample daily for 5 days from day of platform entry (collection can be via midstream urine or indwelling catheter, detailed in Appendix 1 and 2).
- Serum creatinine and urea for 5 days from day of platform entry – creatinine concentrations and serum urea concentrations as ordered by the treating team will be recorded, additional blood samples will not be taken.

The following data will be collected to aid interpretation of urinary biomarker profiles:

- age
- weight
- sex
- pre-morbid baseline creatinine concentrations (0-7 and 8-365 days before index blood culture collection, if >1 creatinine concentration available using the lowest creatinine the 7 days before index blood culture collection and the most recent value in the 8-365 days before index blood culture collection, while patient was not acutely unwell)
- administration of other drugs that can affect renal function (eg loop diuretics, NSAIDs, aminoglycosides, ACE/ARB, calcineurin inhibitors, contrast dye, amphotericin B) from 48 hours before platform entry to platform Day 5
- relevant comorbidities: diabetes, cardiovascular disease, chronic kidney disease
- admission to intensive care unit (from 48 hours before platform entry until Day 5)
- creatinine and urea concentrations as available for each day from 48 hours before enrolment to platform day 5.
- time from index blood culture collection to first urine collection will be recorded.

NSAID use will be broken into 3 groups from 48 hours before platform entry until Day 5, no NSAID use, 1-2 doses or more than 3 doses. This is based on the finding that 7 defined daily doses (DDDs) of NSAID over a month is associated with acute kidney injury (aHR, 1.2; 95% CI, 1.1-1.4) and chronic kidney disease (aHR, 1.2; 95% CI, 1.0-1.3) (12).

For this sub study, a baseline creatinine is obtained ideally between 7- and 365-days pre-hospitalisation or before the acute episode (i.e., for long-stay hospital admissions that develop

nosocomial *S. aureus* bacteraemia) from a time when the patient was not acutely unwell. For some patients, more than one result in this time-period may be available. Using the lowest creatinine value can overestimate AKI diagnosis (especially for long-stay hospital inpatients). The preferred option is to use the most recent outpatient creatinine concentration within the specified time-period. If this pre-morbid baseline creatinine is unavailable, baseline creatinine will be used per SNAP core protocol (platform day 1 or in the 24 hours prior). In a recent *post hoc* analysis of patients from the CAMERA2 trial, baseline creatinine in the year before trial enrolment was available for 88% of patients (unpublished). In another analysis using a panel of urinary biomarkers to predict AKI in patients with COVID-19, only 17% of participants didn't have a baseline creatinine from 7-365 days before admission (13).

6.1.2. Sub study-specific study timeline

Table 1: Sub study-specific schedule of visits and follow-up

Platform Day	Day 1	Day 2	Day 3	Day 4	Day 5
Check sub study eligibility and obtain sub study consent[1]	X				
Blood sample for creatinine and urea (per treating team)	X	X	X	X	X
Urine sample for biomarkers	X	X	X	X	X
Data entry into REDCap database	X[2]				X[2]

[1] Eligibility to be assessed and consent obtained at the same time as the SNAP platform.

[2] A SNAPPER ID will be created in REDCap on Day 1 for sample labelling, other data collection can occur between platform days 8-10 to align with SNAP core data entry.

6.2. Blinding

Not blinded

6.3. Urine sample collection

Urinary samples will be collected daily for all patients enrolled in the sub study. The process for urine sample collection and processing is:

1. Collect urine per SOP (Appendix 1) using instructions for collecting a urine sample (Appendix 2a, 2b and 2C)

2. All urine specimens will be centrifuged and frozen (stored at -80 degrees) at the study site within 48 hours of collection and shipped to a central study laboratory at the end of the pilot phase, with subsequent batched sample analysis to occur at specified timepoints, e.g., 6-monthly or when 100 samples have been received.

3. **Proposed biomarker panel:**

    1. KIM-1 (ELISA)
    2. NGAL (Multiplex Millipore)
    3. clusterin (Multiplex Millipore)
    4. glycocalyx (DMMB - Menzies)
    5. albumin (to creatinine ratio) (Multiplex Millipore)
    6. urinary creatinine (Menzies - alkaline picrate method)

4. Absolute urinary biomarker concentrations and urinary biomarker concentrations normalised to urinary creatinine concentration will be used. A single normalised urinary biomarker measurement from a patient is a point estimate of a range of probable values for the actual excretion rate. Biomarkers excreted from the proximal tubules (eg KIM-1) can have associations with kidney outcomes strengthened by indexing to urinary creatinine, whereas biomarkers filtered (ie urinary albumin) or released from distal nephron (eg NGAL) show less significant changes in correlation with health outcomes based on indexing to urinary creatinine (13).

5. Each urine sample will be aliquoted into 2 vials at the study site, with one aliquot to be stored for use in future research projects that are an extension of this research project or research that is closely related to the original research project or as a control sample, provided consent is obtained.

7. Data Analysis Plan

1. On each of Days 1 to 5, to compare concentrations of each urinary biomarker for patients stratified into those with acute kidney injury stage 2-3, stage 1, or no acute kidney injury. Urinary biomarker concentrations (both normalized to urinary creatinine and absolute concentrations) will be plotted according either grouping of no AKI, AKI stage 1, 2 or 3. Between group differences will be compared using Wilcoxon rank sum test to identify differences between groups. As an exploratory study, we do not intend to adjust for multiplicity of testing.

2. To determine the potential for biomarkers to detect AKI (of any KDIGO stage), the area under

the receiver operating characteristic (ROC) curve will be calculated for each biomarker for each day, and combinations of biomarkers. Cut off values for AKI diagnosis are not yet known for these biomarkers and so will be determined.

3. For those with an AKI, time from platform entry to first significant biomarker elevation (compared to no AKI) will be compared with time to AKI diagnosis (based on serum creatinine) for each biomarker.

7.1. Definition of AKI in SNAPPER sub study

1. (If available) pre-trial creatinine increase of 1.5 times or more at trial entry ('AKI at enrolment')
   OR
2. From day 1 to day 5 of platform enrolment:
   a. an increase in serum creatinine of 26.5 micromol/L (or 0.3 mg/dL) within 48 hours
   b. an increase in serum creatinine to 1.5 times trial baseline, or more.

7.2. Definition of AKI in SNAP core protocol:

- Modified KDIGO stage 1 defined as an increase in serum creatinine of 26.5 mmol/L or more from platform entry to day 5 (in KDIGO this increase would occur in a 48-hour window)

  OR

- Increase in serum creatinine by 1.5 times or more the value at platform entry, within 14 days of platform entry (staged per KDIGO criteria).

7.2.1. KDIGO staging criteria:

Stage 1: serum creatinine increase 1.5 –1.9 times baseline (7 days) or SCr increase by 0.3 mg/dL (26.5 µmol/L) (48 hours)

Stage 2: serum creatinine increase 2 to 2.9 times baseline

Stage 3: serum creatinine increase more than 3 times baseline, or serum creatinine of 4.0 mg/dL (354 µmol/L) or more, with an acute increase of at least 0.5 mg/dL (44 µmol/L), or patient receiving kidney replacement therapy.

8. STATISTICAL CONSIDERATIONS

8.1. Statistical modelling

No specific additional statistical modelling is required.

8.2. Interactions with interventions in the SNAP trial domains

Nil

8.3. Potential impact on trial integrity if findings released prior to overall platform conclusions are reached

Due to a potential risk of revealing patient allocations and outcome data, any sub study publications using outcome data from the SNAP core protocol can only be submitted for publication after primary publications have been released. Please refer to the SNAP Authorship and Publications Policy for further information.

9. ETHICAL CONSIDERATIONS

9.1. Potential sub study-specific adverse events

An amendment to the existing ethics application for the SNAP trial will be undertaken to the lead ethics committee within Australia and international participating sites as necessary.

Collection of urinary samples is of negligible risk to participants. Urinary catheters will not be placed to specifically collect samples for this study, though they can be used if already *in situ*. No additional blood samples will be taken for the sub study, serum creatinine and urea will be recorded as ordered by patient's treating team.

9.2. Sub study-specific consent issues

Patient consent and medical treatment decision maker consent will be accepted for the sub study. Consent to the sub study will be undertaken at the time as consent to the SNAP platform. At the time of consent, patients will be informed that samples will remain in Australia but will travel to a central laboratory located at Menzies School of Health Research for processing. The patient will be informed that their samples will be assigned a unique identifying code and will be stored in a deidentified manner.

The patient will be provided with the opportunity to consent to future research.

- If the patient does not consent to the use of their samples for future research, any remaining samples will be destroyed at the end of the sub study.
- If the patient consents to the use of their samples for future research, their samples will be stored at Menzies School of Research indefinitely so they can be used for future research relating to extension of this sub study or closely related projects.

Any future use of these samples will be subject to Human Research Ethics Committee (HREC) review and approval.

10. GOVERNANCE ISSUES

10.1. Proposed budget

The substudy budget is subject to change as price may be altered by discounts, changes in duration of activities etc.

Item	**Total**	**Funds with**
Payment to sites per patient	$20,000.00	Uni of Melb
NGAL/CLU/Alb assay	$25,547.80	Menzies
KIM-1 ELISA assay	$7,650.00	Menzies
Glycocalyx/creatinine assays	$2,000.00	Menzies
Courier costs	$10,000.00	Menzies
Lab work (staff)	$5,000.00	Menzies
Tubes (falcon tubes, cryovials, eppendorf tubes)	$2,000.00	Menzies
Labels and printing	$1,000.00	Menzies
Statistical support	$1,500	Uni of Melb
Total	**$74 697.80**	

10.2. Funding of sub study

Sufficient funds are available from supervisor accounts to initiate the study. Further funds are being sought through grant applications.

10.3. Proposed timeline

July 2023 – June 2024

10.4. Sub study-specific declarations of conflicts of interest

All investigators involved in SNAP maintain a registry of potential interests. These are updated periodically and publicly accessible on the study website.

11. Appendices

11.1. Appendix 1: Urine collection and processing SOP

1. Nurse on ward or research staff: collect urine daily from platform day 1 to 5 in urine specimen jar and label with study number and time of collection and refrigerate at 4°C (samples cannot be left at room temperature).
2. Time from collection until freezing should ideally be within 24 hours of urine sample collection and must not be longer than 48 hours.

   For the weekend – if the lab can't centrifuge and freeze on Saturday or Sunday, ensure samples collected on Friday are centrifuged and frozen by the end of the day, samples from Saturday (collect around lunch time or early afternoon) and Sunday can be refrigerated, then and spun and frozen on Monday morning.
3. In the lab, spin 10 mL urine at 3000 rpm (or 1000 g) for 10 mins.
4. Into 2 cryovials, aliquot 1 - 2 ml of supernatant (do not overfill), if using a pipette, supernatant should be aspirated slowly and evenly to avoid resuspending the pellet.

   Label cryovials with the following convention:

   [SNAPPER ID].[DAY OF SNAPPER].[CRYOVIAL 1 OR 2]

   For the patient with SNAPPER ID 1, on Day 1 the 2 cryovials will be S1.D1.C1 and S1.D1.C2, then same patient on day 2 of SNAPPER will have S1.D2.C1 and S1.D2.C2, day 3: S1.D3.C1 and S1.D3.C2 etc.
5. Store all cryovials at -80°C.
6. Record number of vials and time collected and time frozen in the SNAP study logbook.
7. In discussion with central laboratory and study PI, send frozen samples to central laboratory.

11.2. Appendix 2a: Instructions for urine sample collection from patients with an indwelling catheter (using sampling port)

Collecting from a sampling port:

1. Ensure the patient is comfortable and that their privacy and dignity is maintained throughout the procedure.
2. Perform hand hygiene and put on an apron.
3. If taking a specimen from a sampling port check first whether there is urine in the catheter tubing. If the tubing is empty apply a clamp below the level of the sampling port. This allows urine to collect above the clamp so that a sample can be obtained.
4. Perform hand hygiene and apply non-sterile gloves. Clean the sampling port with an alcohol-impregnated swab according to local policy and allow to dry
5. Stabilise the tubing by holding it below the level of the sampling port.
6. Insert the syringe tip into the sampling port (following manufacturer's instructions. Be careful to protect the sterile syringe tip and disinfected sample port from contamination.
7. Aspirate at least 10mL of urine and withdraw the syringe.
8. Put the urine into a sterile urine specimen jar, avoiding contact between the syringe and the pot. Ensure the top of the specimen container is secured to prevent leakage and contamination of the specimen.
9. Wipe the sampling port with an alcohol-impregnated swab and allow to dry.
10. If a clamp was used, release it to allow urine drainage freely. Failure to do this will cause the bladder to fill and can result in discomfort and bypassing of urine around the catheter, which can be distressing for the patient.

11.3. Appendix 2b: Instructions for urine sample collection from patients with an indwelling catheter (using catheter valve)

Collecting from a catheter valve:

1. Ensure the patient is comfortable and that their privacy and dignity is maintained throughout the procedure.
2. Perform hand hygiene and put on an apron.
3. Ensure the patient has a full bladder.
4. Apply non-sterile gloves and clean the catheter valve port with an alcohol-impregnated swab according to local policy and allow to dry. This reduces the risk of cross infection.
5. Open the valve and release a small amount of urine to flush the valve.
6. Open the valve again and empty the remaining urine into a sterile jug, ensuring the valve does not come into direct contact with the jug.
7. Put a sample of urine in a sterile urine specimen jar. Ensure the top of the specimen container is secured to prevent leakage and any contamination of the specimen.
8. Close off the valve and wipe the port with an alcohol-impregnated swab.
9. Dispose of any remaining urine according to local policy.

Adapted from Shepherd E (2017) Specimen collection 2: obtaining a catheter specimen of urine. Nursing Times [online]; 113, 8, 29-31.

11.4. Appendix 2c: Instructions for midstream urine collection

Instruct patients to:

1. Wash hands thoroughly.
2. Men—gently retract foreskin (if present) as far as possible. Women—gently separate labia (skin flaps covering vagina). If the woman has significant vaginal discharge or is menstruating, insert a fresh tampon.
3. Pass a small amount of urine directly into the toilet, then catch a small volume (approximately 30mL, roughly half fill the container) of urine into the clean specimen container provided.
4. Finish by voiding the remaining urine directly into the toilet.
5. Replace the lid of the container—please make sure the lid is screwed on straight and tightly to avoid leakage and give to nursing staff immediately.

11.5. SNAPPER Urine Sample Log

Site_____

SNAPPER ID (from REDCap)	Study day	Participant date of birth	Date and time urine sample collected	Date and Time centrifuged	Date and Time frozen	Cryovial label details*	Date and time sent to Menzies
(example row) 1	*1*	*18/04/1956*	*14/10/23 2pm*	*14/10/23 5pm*	*14/10/23 6pm*	*S1 .D1 .C 1* *S1 .D1 .C2*	*31/01/24*
	1					S____. D1 . C1 S____. D1 . C2	
	2					S____. D2 . C1 S____. D2 . C2	
	3					S____. D3 . C1 S____. D3. C2	
	4					S____. D4 . C1 S____. D4 . C2	
	5					S____. D5 . C1 S____. D5 . C1	

11.6. Storage Matrix for 9x9 Freezer Box

Box 1: Storage position of cryovials and their corresponding sample number (eg Sx.Dx.Cx) to be completed by sites – see example below:

1	2	3	4	5	6	7	8	9
10	11	12	13	14	15	16	17	18
19	20	21	22	23	24	25	26	27
28	29	30	31	32	33	34	35	36
37	38	39	40	41	42	43	44	45
46	47	48	49	50	51	52	53	54
55	56	57	58	59	60	61	62	63
64	65	66	67	68	69	70	71	72
73	74	75	76	77	78	79	80	81

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