SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Clinical Chemistry Sample Analysis

INTRODUCTION:

This document outlines the general procedures involved in setting up and running plasma chemistry samples with an Olympus AU400 or Beckman Coulter AU480.

ABBREVIATIONS:

CHSS: Campus Health and Safety Service
DCF: Data capture form
HR: Human Resources
ISE: Ion selective electrode
MSDS: Material Safety Data Sheet
PPE: Personal protective equipment
QC: Quality control
Rcf: relative centrifugal force
SD: Standard deviation
SOP: Standard operating procedure

HEALTH & SAFETY:

This procedure is covered by the following risk assessment WTSI_1191

- Appropriate personal protective equipment (PPE) is to be worn at all times when using the anlayser and handling samples – Laboratory coat, gloves (cold resistant where indicated).
- See **Appendix** for full list of reagents and associated risks.
- Lone working permitted by fully competent staff members.
- Working outside of “normal” working hours is not permitted.
- There is an increased level of risk for physical hazards to young persons, therefore, before starting, a risk assessment for the procedures planned to be performed in to be undertaken in conjunction with HR and CHSS.
- New employees, or those returning from a significant period of absence, must be supervised until deemed competent at performing this procedure.
- Ensure the cover of the analyser is closed when it is running.
- Ergonomic pipettes should be used to minimize the risk of repetitive strain injury as this process involves multiple pipetting steps.
- Users should correctly organize their workspace to minimize stretching and other ergonomic hazards.
- There should not be any increased risk for new or expectant mothers for the running of the analyzer as all chemicals are kept on board within a closed system.

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying risk assessment has been read and understood. All staff should be trained and competent to perform the procedure.

RESOURCES:

Equipment:

1. Refrigerated centrifuge (5415R Eppendorf/equivalent that can take 2.0ml tubes)
2. Computer and attached barcode scanner
3. Pipettes (1000µl, 200 µl, and 20 µl) and filter tips
4. Microtube racks
5. Sharps bin
6. Hitachi cups (Sarstedt, Catalogue number 73.666)
7. Barcode labels (CILS, part number: 44C-9143)
8. Analyser + QC, calibration and sample racks.
9. ISE calibrators
10. Reagents (see Appendix 4)
11. Parafilm
12. 0.5ml colourless Eppendorf tubes
13. 0.5ml green microtubes (Thistle Scientific, Catalogue number: MCT-060-G)
14. Plastic grid microtube storage boxes (StarLab, Catalogue number: E2350-5009)

Staff: This procedure can be performed by one member of staff.

PROCEDURE A: Analyser set up – reagent change, calibration, daily maintenance and quality control (QC) analysis

1. Change reagents as required to ensure sufficient volume for the samples required that day, plus any calibration and QC runs that may be needed.

2. Remove old reagents and replace with new reagents, in the same positions. Old reagents should be poured away into a specific, labelled waste bottle.

3. Perform a reagent scan or manually reset details for any reagents that have been changed.
   3.1. Check through the reagent list again after running the reagent check in case the analyser didn't detect any of the new bottles correctly. If this occurs, check there are no bubbles in the bottle and run the regent check again for that specified position.

4. Calibrate reagents
   4.1. This may be for any reagents that have just been changed, or full calibration/reagent blank for those reagents that were not changed but require this
   4.2. Collect calibrator solutions required from the fridge/freezer and pipette into cups in the specified positions
   4.3. Load the racks on to the rack feeder of the analyser (rack barcode facing away from you). For calibration, always place the blue rack with dH_2O in position 1 on the analyser first, followed by the yellow calibrator rack/s.
   4.4. Run the calibrations and check they have run and passed. If any were missed or failed, repeat the calibration for these.

5. Reconstitute new vials of System Calibrator, Control Serum 1 & Control Serum 2
   5.1. Add **5ml** of **dH_2O** to each vial.
   5.2. Invert vials 3 times gently, leave standing for 10 min, then place on the roller for **30 min**.
      5.2.1. The vials should be stored at 4ºC and can be used for two weeks before discarding
      5.2.2. If the lot number changes, enter the new QC or CAL values onto the analyser.

6. Reset the index on the analyser so that today's samples are labelled starting with sample #1

7. Perform daily maintenance on the analyser:

   7.1. Inspect the syringes for leaks
   7.2. Inspect the wash solution roller pump for leaks
   7.3. Inspect the wash solution, ISE buffers and change if required
   7.4. Inspect, clean and prime the sample and reagent probe and mix bars
      7.4.1. Clean using alcohol wipes
      7.4.2. Clean the probe by wiping from the top to the bottom
      7.4.3. If probes are removed, ensure the silver connectors are tightly screwed back on
      7.4.4. When priming, watch the probes as they eject water to verify that a thin straight stream of water is dispensed and it doesn't spray or go at an angle (this would indicate that there is a blockage).
   7.5. Inspect the printer and paper

8. Perform ISE cleaning and calibration:
   8.1. In the CLEAN position of the STAT table fill the sample cup with Beckman Coulter Cleaning Solution (concentrated bleach).
   8.2. Check W1 (wash 1) position, fill up if necessary.
   8.3. To make more: pour 20ml of Cleaning Solution and 30ml dH_2O into a 50ml falcon tube and store in fridge.
   8.4. Dispense approx. 500µl (~ 7 drops) of serum high (red lid) in a new Hitachi cup and place in the S-H position on the STAT table.
   8.5. Dispense approx. 500µl (~7 drops) of serum low (yellow lid) in a new Hitachi cup and place in the S-L position on the STAT table.
   8.6. Dispense approx. 500µl (~7 drops) of Urine High (black lid) in a new Hitachi cup and place in the U-H position on the STAT table.
   8.7. Dispense approx. 500µl (~7 drops) of Urine Low (white lid) in a new Hitachi cup and place in the U-L position on the STAT table.
   8.8. Run the ISE cleaning cycle (~5 mins)
   8.9. Perform a total prime to clear the lines of cleaning solution.
   8.10. Run the ISE calibration cycle (~5 mins)
   8.11. After calibration check the results in the table, verify they are in blue. Invalid results will appear in yellow

9. Run QC for all tests that are to be performed that day
   9.1. Collect required QCs and allow to thaw or come to room temperature
   9.2. Pipette into cups in the specified positions
   9.3. Load the green QC racks on to the rack feeder of the analyser (rack barcode facing away from you).

9.4. Run the QCs and check they have run and passed (within ±2SD of target). If any have failed, try the following steps:
  9.4.1. Check that the aliquot being used is the same lot number as the values that are in the machine. If not, update the values.
  9.4.2. Check lot number and expiry date and re-run the QCs (use new aliquots)
  9.4.3. If they fail a second time, recalibrate the tests involved, then re-run QCs
  9.4.4. Try running a W1 Clean then re-run QCs
  9.4.5. If values are within 3SD and consistent and you have tried all the troubleshooting techniques, then the QC result can be accepted
  9.4.6. If they still fail, replace reagent, re-calibrate and re-run QCs
  9.4.7. If they still fail, and it is not just the odd one, the QC vials could have been defective or prepared incorrectly, so prepare new vials and re-run QCs.

PROCEDURE B: Specimen Analysis:

1. Samples are received in lithium heparin tubes (orange cap), each labelled with an individual tube and mouse bar code.

2. Centrifuge samples for 10 minutes at 5,000 g, at 8°C.

3. Use the database to assign tests to be performed to the samples depending on the pipeline requirements:
   3.1. Pipeline 1: Gluc, Trigs, Chol, HDL, LDL, NEFAC, Glyc, Frct
   3.2. Pipeline 2/Mouse GP/MGP Select: Na, K, Cl, Gluc, Trigs, Chol, Amy, NEFAC, TP, Alb, Ca, Phos, Iron, AST, ALP, ALT, Creat, Urea, TBilC, TBilB, CK, Mg, HDL, LDL, Glyc, Frct, THYRX, LIH

4. Scan the blood tube barcode and the barcode of a labelled Hitachi cup to link them together.

5. Remove the required volume of plasma from sample carefully with a pipette and filter tip and transfer to corresponding Hitachi cup. Take care not to disturb the buffy coat or red cell layer below.
   5.1. Pipeline 1: 100 µl
   5.2. Pipeline 2/Mouse GP/MGP Select: 240 µl
   Note: If there is insufficient plasma then delete the sample, go back to step 3 and select appropriate tests for the volume available.
6. Place the sample in the Hitachi cup in order in a grey rack. Make sure the barcode on the cup can be seen through the gap at the front of the rack, as if the analyser cannot read it an error will be generated.
7. Make any observations about a particular sample by selecting from the drop down list (e.g. degree of haemolysis, lipaemic and/or icteric). Alternatively, if an issue has arisen and the samples need to be frozen, a comment must be added to the DCF.

8. Press Save on the database (this should be done after each rack of 10). Wait until the batch has saved before starting the analysis.
   Note: Do not load all racks at once. This prevents sample deterioration due to temperature. If multiple grey racks are used, keep racks in order (use rack barcode to keep track) covered in Parafilm in the under bench fridge until ready to load for analysis.
9. Place the first grey rack onto the feeder rack
   Note: ensure the rack barcode is facing away from you
10. Press the Play button on the touch screen to start the run
    Ensure the cover of the analyser is closed when it is running due to the risk from moving parts.
11. When the analyser has generated results for sample 6 of the rack, the next rack can be loaded. Place the next grey rack onto the feeder rack and press Play on the touch screen. Do this for each subsequent rack until all samples have been analysed.
12. Once the samples have finished running and all data have been checked, the cups should be discarded into a sweetie jar.

PROCEDURE C: Preparing and storing frozen plasma aliquots

1. Print barcode labels for all aliquots required

2. Transfer all remaining plasma from the sample tube to a labelled 0.5 ml clear Eppendorf tube (biobank tube).

3. From this tube, remove aliquots for any other collaborations required into labelled tubes

4. Remaining volume to be left in the biobank tube and all tubes to be stored in labelled plastic grid boxes in a -80°C freezer.

Appendix: Reagents

Reagent	Supplier	Catalogue no.	Health hazard	Route of contamination
Albumin	Beckman Coulter	OSR6102	Toxic; harmful; irritant; skin sensitizer; Causes damage to organs	Inhalation; ingestion; eye contact; skin contact
Alkaline phosphatase (ALP)	Beckman Coulter	OSR6004	Harmful; irritant; skin sensitizer; causes damage to organs	Ingestion; eye contact; skin contact
Alanine aminotransferase (ALT)	Beckman Coulter	OSR6007	-	-

Alpha amylase	Beckman Coulter	OSR6106	-	-
Aspartate aminotransferase (AST)	Beckman Coulter	OSR6009	-	-
Calcium Arsenazo	Beckman Coulter	OSR60117	Harmful	Inhalation; ingestion
Cholesterol	Beckman Coulter	OSR6116	-	-
Creatine Kinase (NAC)	Beckman Coulter	OSR6179	Harmful; irritant; skin sensitizer; causes damage to organs	Inhalation; ingestion; eye contact; skin contact
Creatinine (Enzymatic)	Beckman Coulter	OSR61204	-	-
Glucose	Beckman Coulter	OSR6121	-	-
Glycerol	Randox	GY105	Harmful	Ingestion
HDL-Cholesterol	Beckman Coulter	OSR6187	Irritant; skin sensitizer	Inhalation; skin contact
Iron	Beckman Coulter	OSR6186	-	-
LDL-Cholesterol	Beckman Coulter	OSR6183	Harmful	Inhalation; ingestion
LIH	Beckman Coulter	OSR62166	Irritant; skin sensitizer	Inhalation; skin contact
Magnesium	Beckman Coulter	OSR6189	-	-
NEFA R1	Alphalabs	434-91795	Harmful	Ingestion; skin contact
NEFA R2	Alphalabs	436-91995	Harmful	Ingestion; skin contact
Inorganic Phosphorus	Beckman Coulter	OSR6122	Toxic; corrosive; irritant	Inhalation; ingestion; eye contact; skin contact
Thyroxine (DRI)	Thermo	454	Irritant	Ingestion; eye contact; skin contact
Total bilirubin	Beckman Coulter	OSR6112	-	-
Total protein	Beckman Coulter	OSR6132	Corrosive; irritant	Inhalation; ingestion; eye contact; skin contact
Triglycerides	Beckman Coulter	OSR61118	-	-
Urea	Beckman Coulter	OSR6134	-	-
Calibrators				
System Calibrator	Beckman Coulter	OE66300	-	-
HDL-Cholesterol Calibrator	Beckman Coulter	ODC0011	Irritant; skin sensitizer	Inhalation; ingestion; eye contact; skin contact

LDL-Cholesterol Calibrator	Beckman Coulter	ODC0012	Irritant; skin sensitizer	Inhalation; ingestion; eye contact; skin contact
NEFA Standard	Alphalabs	270-77000	-	-
Glyc. Cal	Randox	GY105 (included in reagent box)	-	-
Thyroxine DRI calibrator set	Microgenics	476	-	-
Quality Controls (QCs)				
Control Serum 1	Beckman Coulter	ODC0003	-	-
Control Serum 2	Beckman Coulter	ODC0004	-	-
HDL/LDL Cholesterol Control Serum	Beckman Coulter	ODC0005	-	-
NEFA Control Serum 1	Alphalabs	410-00102	-	-
NEFA Control Serum 2	Alphalabs	416-00202	-	-
Glycerol Control	Randox	GY1369	-	-
Thyroxine QC (Immunoassay Plus trilevel)	BioRad	40260	-	-
ISE				
ISE Na+/K+ Selectivity Check	Beckman Coulter	OE66313	-	-
ISE High Serum Standard	Beckman Coulter	OE66316	-	-
ISE Low Serum Standard	Beckman Coulter	OE66317	-	-
ISE Reference	Beckman Coulter	OE66318	-	-
ISE Mid Standard	Beckman Coulter	OE66319	Fatal/very toxic; carcinogen; irritant; skin sensitizer; causes damage to organs	Inhalation; ingestion; eye contact; skin contact
ISE Buffer	Beckman Coulter	OE66320	Fatal/very toxic; carcinogen; irritant; skin sensitizer; causes damage to organs	Inhalation; ingestion; eye contact; skin contact
Other				
Wash Solution	Beckman Coulter	OSR0001	Corrosive; irritant; causes damage to organs	Inhalation; ingestion; eye contact; skin contact
Cleaning Solution	Beckman Coulter	OE66039	-	-