



University of Liverpool GCP Laboratories

GCPLab

Processing of Samples for the LPRG Acute Pancreatitis Bioban

Reference GCLPTSS049

Version 9

Category GCPLab\Trial

Status Active

Issue date 24/07/2023

Author Latawiec, Diane

Owner Shaw, Victoria

This document is controlled using Q Pulse and made available to all relevant personnel.

Distribution Please note that the web version of this document is the only version that is maintained. This document is uncontrolled when printed and as such, may not necessarily contain the latest updates and amendments.

Next scheduled review date 24/07/2025

Last completed review <QPulse_DocLastReviewDate>

Review Process Prior to Sign Off

| Name of Group/Department/Specialist Committee | Date |
|-----------------------------------------------|------|
| | |
| | |
| | |

CONTENTS

| Section | Title |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. | WHO? |
| 2. | BACKGROUND |
| 3. | PURPOSE |
| 4. | SCOPE |
| 5. | PROCEDURE |
| 5.1 | RESPONSIBILITY |
| 5.2 | PROTOCOL |
| 5.2.1 | PROCEDURES TO BE FOLLOWED TO MINIMISE RISK ASSOCIATED WITH HANDLING BLOOD SAMPLES (E.G SARS-CoV2 VIRUS) FOR STAFF WHEN PROCESSING SAMPLES |
| 5.2.2 | CHECKLIST SHEET AND RECEIPTION OF THE BLOOD SAMPLES |
| 5.2.3 | PROCESSING OF SAMPLES |
| 5.2.3.1 | PROCESSING OF THE PAXGENE BLOOD RNA TUBE |
| 5.2.3.2 | PROCESSING OF THE EDTA TUBE (Extraction and storage of plasma and cell pellet) |
| 5.2.3.3 | PROCESSING OF THE SERUM TUBE |
| 5.2.3.4 | PROCESSING AND STORAGE OF URINE |
| 5.2.4 | LIMS PROCEDURE FOR SAMPLE PROCESSING AND STORAGE |
| 5.2.4.1 | PATIENT INFORMATION DATA IN LIMS |
| 5.2.4.2 | RECEIPT OF ALL SAMPLES |
| 5.2.4.3 | PROCESSING AND STORAGE OF PAXGENE TUBE |
| 5.2.4.4 | PROCESSING OF EDTA TUBES FOR PLASMA AND CELL PELLET PROCESSING |
| 5.2.4.5 | PROCESSING AND STORAGE OF SERUM |
| 5.2.4.6 | SAMPLE PROCESSING AND STORAGE OF URINE |
| 6. | WITHDRAWAL OF INFORMED CONSENT |
| 7. | ABBREVIATIONS |
| 8 | OTHER RELATED PROCEDURES AND DOCUMENTS |
| 9. | APPENDIX |
| 9.1 | Appendix 1: Example of Acute Pancreatitis Biobank Sample Processing Checklist |

1. WHO?

This Standard Operating Procedure (SOP) applies to all staff on the study delegation log for the Liverpool Pancreatitis research group (LPRG) Acute Pancreatitis Research Biobank who will process samples for storage in the ground floor GCP freezer facility, William Henry Duncan building (WHD).

2. BACKGROUND

Samples (blood and urine) for the Acute Pancreatitis Biobank will be obtained from patients presenting at Royal Liverpool University Hospital with the diagnosis of Acute Pancreatitis. Patients will be identified through the Biochemistry department who will generate a list of all patients admitted in the last 24 hours with an amylase of >450 U/l at the time of admission. These patients will then be approached for consent. If a patient is unable to give informed consent, a personal consultee or nominated consultee will be approached. Initial contact will be made by a member of staff on the study delegation log who is delegated to consent and recruit patients to the study. They will explain in detail to the patient or consultee, the purpose of the biobank, the process of recruitment and various issues surrounding it. Please refer to GCLPTSS116 for specific procedural details.

3. PURPOSE

The purpose of this SOP is to describe the procedure for the safe processing of samples for storage in the GCP facility room WHD.

4. SCOPE

This SOP applies to all staff involved in processing samples for the LPRG Acute Pancreatitis Research Biobank with long term storage in the GCP facility on the University of Liverpool campus (WHD).

5. PROCEDURE

5.1 RESPONSIBILITY

It is the responsibility of all staff on the delegation log to process samples for the Acute Pancreatitis Research Biobank to follow this SOP.

It is the responsibility of all staff on the delegation log to process samples, and ensuring all details are entered onto the checklist sheet GCLPTSS49/F1.

It is the responsibility of all staff on the delegation log who transfer the information on GCLPTSS049/F1 to the laboratory information management system (LIMS) follow this SOP to ensure an auditable track is maintained for the sample.

5.2 PROTOCOL

5.2.1 Procedures to be followed to minimise risk associated with handling blood samples (including SARS-CoV 2 virus) for staff when processing samples

Hazard: Biological contaminants in human blood/urine

Risk: Possible exposure to the above biological contaminants present in human blood/urine

Procedures to minimise risk:

- **ALL** sample processing must be performed in Class II cabinets, except where equipment is located outside the cabinets. Staff processing the samples should AT ALL TIMES wear personal protective equipment as appropriate. This should include Howie lab coat, mask and gloves and adhere to social distancing rules, in line with local guidance and policy, to help prevent spread of SARS-CoV 2 virus.
- All plastics should be soaked in 1% virkon (using buckets/waste pots) for at least 1 hour to remove biological contamination.
- Decontaminated plastics (from buckets) should be placed into yellow clinical waste bags for incineration.
- Biological waste from human blood/urine should be placed in autoclave bags and then autoclaved.
- Pipette tips and sharps (needles, etc.) should be placed into yellow sharps bins for incineration. (**NO RESHEATHING of SHARPS**).
- Any spills must be cleaned thoroughly first with 1% virkon, then 70% ethanol to remove the virkon.

5.2.2 Checklist sheet (GCLPTSS049/F1) and reception of the blood samples:

All blood tube samples collected in the Royal Liverpool University Hospital will be first brought to the 2nd floor Sherrington building, where the samples will be receipted into LIMS for the time of entry into the laboratory on the Acute Pancreatitis Sample Processing Checklist sheet (GCLPTSS049/F1). This sheet is provided with the sample collection part of the kit and **must** be handed to the person who is responsible for processing the sample at the time of receipt into the laboratory. **This sheet MUST be completed during processing, with all the LIMS numbers of all tubes used and storage location entered onto the checklist sheet. LIMS entry of the processing procedure is completed at any LIMS terminal located on the University campus.** **Any blood samples received from outside of the Royal Liverpool University Hospital must be processed immediately upon timed receipt into the laboratory..**

All checklist sheets arriving with the samples, must be first checked for which consent has been obtained. Consent has been ethically approved for the Acute Pancreatitis Research Biobank to allow prospective or patient regaining capacity to consent personal consultee and nominated consultee consent. All consultee consented samples must remain quarantined until consent from the patient regaining capacity to consent has been obtained.

If consent is withdrawn at any time, then any samples for that patient which are stored, must be destroyed. Any sample destroyed must be entered into the Q pulse management system following procedure as described in GCLPPP017. At the same

time, sample destruction should be updated in LIMS (as an adverse event) to ensure an auditable trail of the sample is maintained.

5.2.3 Processing of samples

All samples received into the laboratory should be processed as described below. At the same time the checklist sheet must be filled in with the information asked for on the sheet.

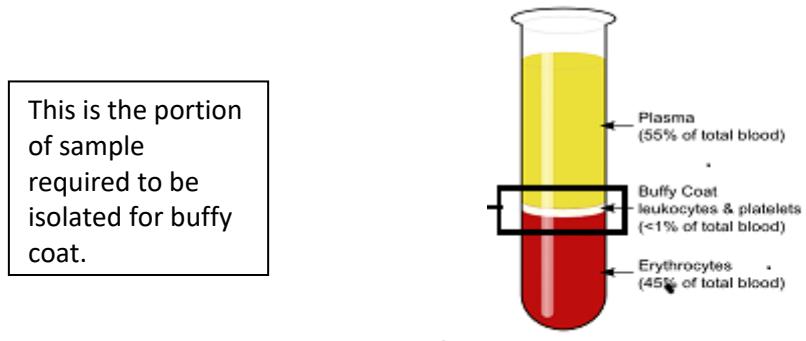
5.2.3.1 Processing of the PAXgene™ Blood RNA Tube:

1. The PAXgene™ Blood RNA tube (BRT) should be inverted 10 times. This is verified by checking the sampling processing checklist. If it hasn't been inverted, invert the tube 10 times and leave in an upright position.
2. Leave for 2 hours (from time of collection) in an upright position at room temperature. Store the PAXgene tube in the appropriate freezer in the GCP freezer facility (WHD). Record its location onto both the checklist sheet and into LIMS.

5.2.3.2 Processing of the EDTA Tube Extraction and storage of plasma and cell pellet)

- The EDTA tubes must be processed between **20-25** minutes of blood collection from the patient.
 - All other tubes of samples collected from outside of the Royal Liverpool University Hospital must begin processing immediately upon receipt into the laboratory.
 - Prior to processing, remove red blood cell lysis buffer aliquot from the fridge and place in water bath set at 25°C for 10 minutes.
 - All references to labelled tubes, are part of the processing kit.
1. Invert EDTA tube (purple top) containing blood 10 times.
 2. Pool both EDTA tubes into one 15ml Falcon tube (labelled **B1**) and centrifuge the Falcon tube at 600 x g at 24°C for 30 minutes in the centrifuge.. Ensure that the centrifuge is properly balanced.
 3. Upon completion of the centrifugation run, take tube **B1**, out of the centrifuge and carefully remove as much of the top plasma layer (Figure 1) without disturbing the white blood cell layer (buffy coat), with a 3ml Pasteur pipette and pipette into the Falcon tube labelled **B2**, as supplied in the processing kit. Return this tube back to the centrifuge and centrifuge for a further 10 minutes at 1500 x g at 24°C for 10 minutes. Ensure the centrifuge is balanced before proceeding.
 4. Upon completion of centrifugation of the plasma layer, remove the falcon tube (**B1**) from the centrifuge and aliquot the plasma into the cryovial tubes with red top (provided in each processing kit) using a 1ml pipette. Any pellet obtained must be discarded into autoclave bags in accordance with the GCLPRPS024. Store the plasma tubes in the appropriate -80 freezer in the GCP facility

(WHD). Complete the checklist sheet for the processing of plasma for LIMS data entry.



i.
Figure 1: Blood separation after the 30 minute centrifugation (Falcon Tube B1)

Process the buffy coat as follows:

5. Remove the 'buffy coat' from the Falcon tube (B1) (Figure 1) with the same sterile 3ml Pasteur pipette used to remove the plasma and transfer it into the 7ml labelled **Bijou** tube as supplied in the kit Mix by inverting 3 times. The buffy coat is sometimes difficult to visualise, being just a smear of white on top of the red blood cells. To ensure capture of all leukocytes remove the top layer of blood taking up to a volume of 2.5ml of the blood.
6. Using a 1ml pipette transfer the buffy coat cells into 500 µl aliquots into the Eppendorf tubes provided with the laboratory processing part of the kit. These are labelled **Epp-Ery** and there is a maximum of 5 tubes per kit.
7. Using a 1ml pipette, add 1ml of red blood cell lysis buffer to each Eppendorf tube containing the buffy coat (a 2:1 volume – 1ml lysis buffer/500µl of buffy coat).
8. Mix by inverting 3 times.
9. Allow the tubes to incubate on a rocker for 10 minutes at room temperature at 100 rpm.
10. Once the buffy coat aliquots have incubated for 10 minutes on the rocker in Lysis buffer, centrifuge the tubes at 600 x g for 5 minutes at room temperature in the micro-centrifuge.
11. Using a 1ml pipette, carefully remove and dispose of the clear, red supernatant (indicative of complete red blood cell lysis), into 1% virkon waste pot.
12. Add another 1ml red blood cell lysis buffer, cap the tubes and mix by 'flicking' (tap the tube sharply) the tubes until the pellets are re-suspended.
13. Centrifuge the Eppendorfs at 600 x g for 3 minutes at room temperature in the

micro-centrifuge.

14. Using a 1ml pipette, carefully remove and properly dispose of the clear red supernatant (liquid above the cell pellet) into a 1% virkon waste pot. Remove any remaining supernatant using a 200 µl pipette. Re-suspend each cell pellet in 1ml sterile PBS by gently pipetting up and down using a 1ml pipette.
15. Mix together all re-suspensions into one 15ml falcon tube, (labelled **Falcon-P**) using a 1ml pipette.
16. Double the amount of PBS (add an extra 1ml of PBS for every pellet added).
17. Perform cell count in accordance with SOP GCLPTSS161, and record the cell count value on the worksheet. If cell count is shown to be **out of range** on the cell counter, the pellet needs to be further diluted in PBS. Do this until a value is obtained within the range. **Record all values in the worksheet and complete the calculations on the worksheet to achieve a total cell count.**
18. Centrifuge the falcon tube at 600 x g at 24°C for 10 minutes, in the Heraeus Megafuge 16R Refrigerated Bench Top Centrifuge.
19. After centrifugation, remove the supernatant (liquid above the cell pellet) by inverting the Falcon tube above the virkon pot and dispose of according to the GCLPRPS004 Disposal of Hazardous waste.
20. Re-suspend the cell pellet with 1ml of sterile PBS, using a 1ml pipette and gently pipetting up and down.
21. Add a maximum additional 4ml of sterile PBS using a 1ml pipette to the cell suspension and aliquot into 1ml aliquots in the Eppendorfs supplied (labelled **EppP**) using a 1ml pipette. Centrifuge the Eppendorfs at 600 x g for 5 minutes at room temperature in the micro-centrifuge.
22. Using a 1ml pipette, carefully remove and properly dispose of the supernatant. Store tubes containing cell pellet in the appropriate -80 freezer in the GCP facility (WHD). Record all processing steps on the GCLPTSS049/F1

5.2.3.3 Processing of the serum tube

1. The clot activation should have been carried out at the time the blood was taken. Refer to Checklist sheet GCLPTSS049/F1 to determine whether this has occurred. If not, invert the tube 10 times and allow the blood to stand in a vertical position for a minimum of 30 minutes.
2. Centrifuge the serum tube at 1500 x g at 24°C for 10 minutes. Ensure centrifuge is balanced.
3. Return the tube to the Class II safety cabinet and perform the next step in the Class II safety cabinet C.
4. Carefully remove the layer of serum above the dense gel using a 1ml pipette and transfer into 2 sterile cryovial tubes with white top (provided with kit). Store the tubes in the appropriate -80 freezer in the GCP facility (WHD). Complete the checklist sheet.

5.2.3.4 Processing and Storage of Urine:

If urine has been collected, the following steps must be taken using a pH meter.

1. Transfer 10ml of urine from the 50ml falcon tube, and place in a fresh tube near the pH meter.
2. Determine the pH of the urine sample using the pH meter.
3. **Record this initial pH reading on the Checklist sheet GCLPTSS049/F1**
4. Add 1ml of Tris (pH 7) to adjust the pH to 7.
5. **Record pH reading after addition of 1ml of Tris buffer.**
6. If the pH reading is still under pH 7, add additional Tris buffer in aliquots of 250 µl until the reading is pH 7. **Record final pH and volume of Tris buffer used on the checklist.**
7. One tablet of protease cocktail inhibitor should be added for each 10mls. 1ml of this urine should be transferred to each of the 5 pre-labelled cryovials (green top) provided with the kit and stored in the appropriate -80 freezer GCP facility (WHD building).

5.2.4. LIMS entry of sample receipt, processing and storage

All information from the Checklist sheet (GCLPTSS049/F1) must be transcribed into LIMS as soon as possible after processing of the sample. LIMS terminals are available in the University's WHD building GCP facility and Sherrington building

5.2.4.1 Patient Information: (AP number and Timepoint)

Data for samples collected must be entered into LIMS individually

1. **To Log into LIMS.** Select Matrix Plus Icon. Select Access and select Login. Complete login process and select 'Live' to generate the 'Live' screen (Figure 2). Select '**Trials and Studies**' from the dropdown menu and select '**Panc Studies**'. Select '**PBRU**' and then select '**AP Biobank**'. The selection of '**AP Biobank**', results in the '**PBRU Workflow**' screen (Figure 3). This screen allows for entering of both the kit code used to collect the sample and the Patient ID. This is an Acute Pancreatitis (AP) number, which is predetermined by the person collecting the sample in accordance with SOP GCLPTSS116.



Figure 2: Live Screen

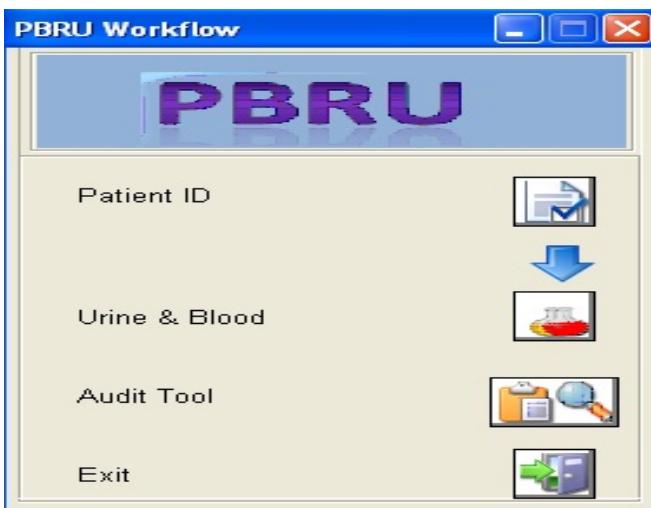


Figure 3: PBRU Workflow

2. Select the **Patient ID** icon in the PBRU workflow (Figure 3) on the **Patient ID** screen will appear (Figure 4). Highlight the kit code used and select '**Set patient ID and confirm consent**' button This will bring up the **Patient ID Code** screen (Figure 5). .
3. Enter the appropriate information into each box, as written on the checklist sheet. Select the '**Ok**' icon when complete. This will return you back to the **Patient ID** screen (Figure 4). Click '**Exit**' to return to the **PBRU workflow** screen (Figure 2). Click the '**Urine & Blood**' icon, which will bring you to the '**Sample Life Cycle**' screen (Figure 6).

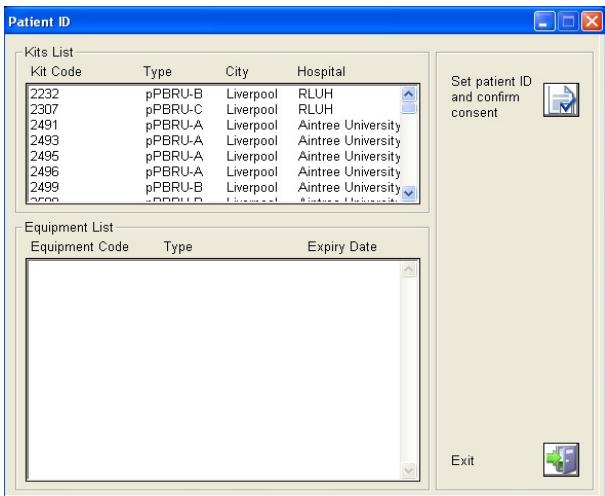


Figure 4: Patient ID screen

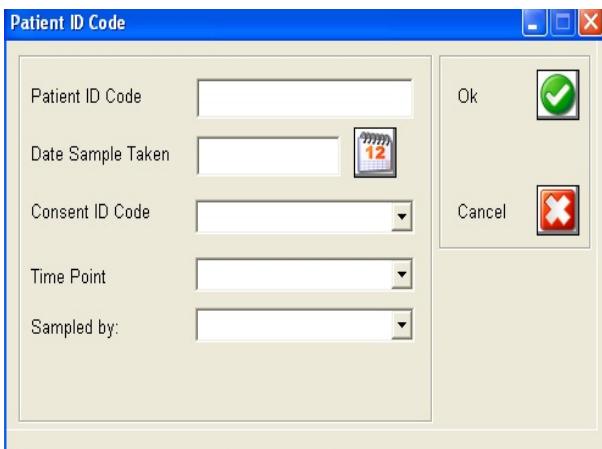


Figure 5: Patient ID code

5.2.4.2 Receipt of all sample tubes into LIMS

1. For all sample tubes received into the lab, (e.g. PAXgene™, EDTA, Serum and Urine) select the appropriate receipt button and follow onscreen instructions to receipt the sample. For the urine sample, whether it has been received or not, you must still select the '**Receive Samples**' button in the urine section of the workflow and follow onscreen instructions.

If no urine has been received, you **MUST** select '**Delete Sample**' button (Figure 7) and follow instructions on each screen to indicate in LIMS that no sample was ever received.

2. For any sample tube not received, select '**Delete Sample**' button and follow the instructions on each screen that appears. Once all samples have been receipted return to the '**Sample Life Cycle**' screen (Figure 6) to begin the LIMS entry for the processing of samples.

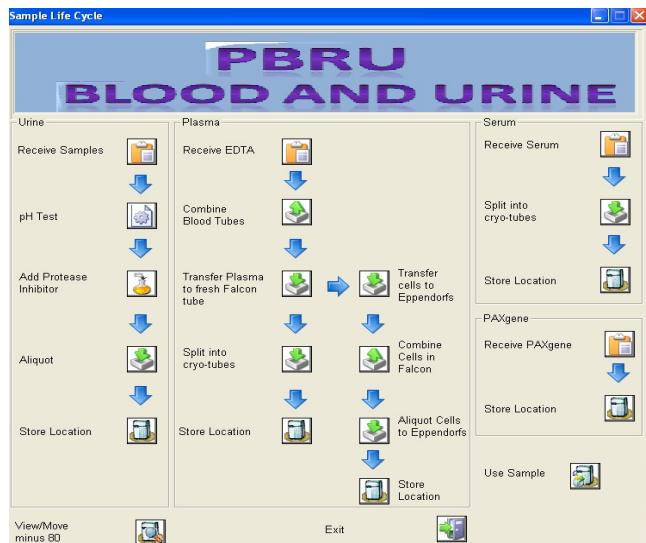


Figure 6: Sample Life Cycle

| Kit Code | Status |
|----------|--------------------|
| 1004 | Form Received RLUH |
| 1013 | Form Received RLUH |
| 1018 | Form Received RLUH |
| 1020 | Form Received RLUH |
| 1021 | Form Received RLUH |
| 1034 | Form Received RLUH |
| 1037 | Form Received RLUH |
| 1000 | Form Received RLUH |

| Equipment Code | Type | Patient ID |
|----------------|------|------------|
| | | |

Figure 7: Receive Samples

5.2.4.3 Processing of PAXgene Tube and storage in LIMS

1. Select '**Store Location**' icon in the PAXgene™ section of '**Sample Life Cycle**' (Figure 6) This will bring up the '**Store Location**' screen (Figure 8).
2. Highlight the appropriate PAXgene™ tube for the Patient ID and select the '**Store Location**' button. This will bring up the '**Store Location**' screen (Figure 9).

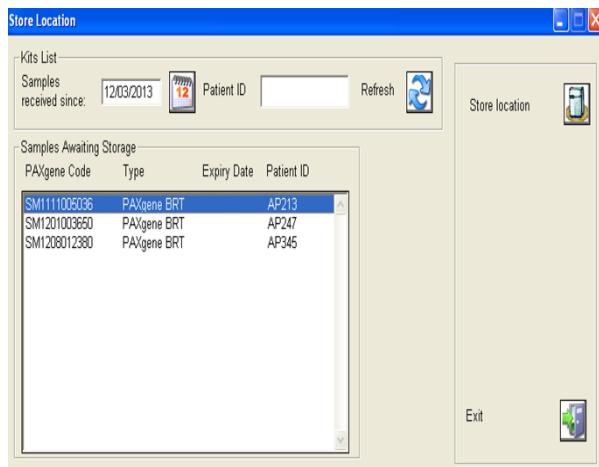


Figure 8: Store Location of PAXgene

3. Enter all information from the checklist sheet. Select 'Ok' to close the screen. Continue to select 'Exit' on following screens to exit the PAXgene™ section.

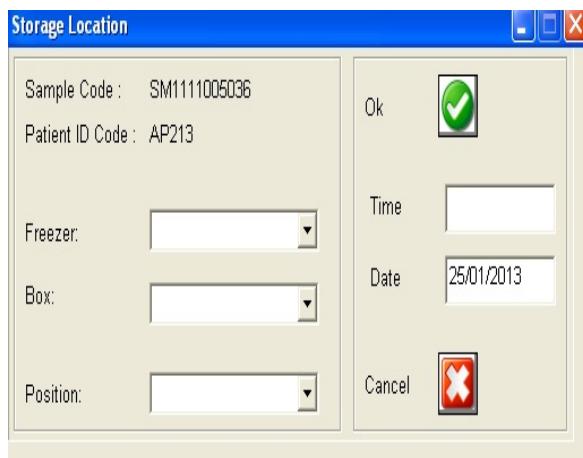


Figure 9: Storage Location

5.2.4.4 Processing of EDTA of Tubes for Plasma and Cell pellet

1. Select the button. This will bring up the 'Combine EDTA Tubes and confirm' screen (Figure 10). Highlight the appropriate patient ID code and moving to the right, highlight the LIMS tube number that now appears in the box. Click 'Aliquot'. This will bring up the screen 'Received Cryo-tubes' (Figure 11). Select 'Aliquot to Falcon' button to input the date of aliquoting the sample in LIMS.

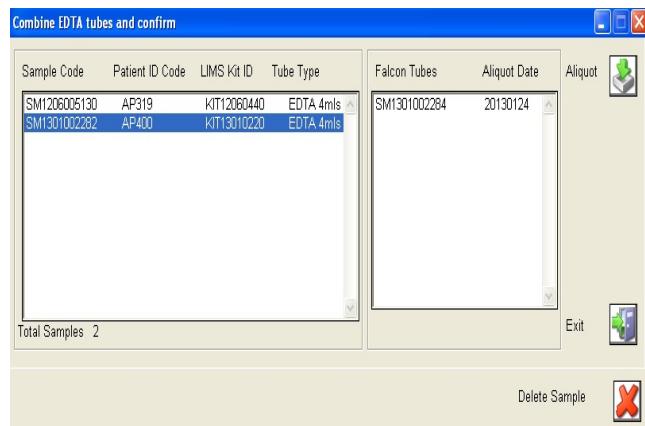


Figure 10: Combine EDTA tubes and confirm screen

NOTE 1: Any aliquot date entered into LIMS will always be the date that you are entering the information into LIMS.

After the aliquot date has been entered the '**Destroy Samples**' screen will appear (Figure 12). Select the '**Destroy EDTA Tube**' button. This will bring up a **Dictionary Maintenance** screen (Figure 13).

2. On this screen a message will appear asking for confirmation of the EDTA being disposed in yellow sharps bins (Figure 13). Select '**Ok**' to confirm you have destroyed the EDTA tube. This will bring you back to the '**Destroy Samples**' screen (Figure 12). 4. Click '**Ok**' to exit and return back to the '**Receive Cryo Tubes**' screen (Figure 11). This sequence should be followed for all EDTA tubes received.

Note 2: LIMS disposing or destroying tubes. When destroying or disposing tubes at any stage in recording the processing in LIMS in the Plasma and Serum sections, the screen sequences will be always as follows:

- 1) Destroy samples (Figure 12).
- 2) Dictionary maintenance (Figure 13). Follow instructions for each screen ensuring the '**Exit**' icon is selected on each screen, to return back to the '**Sample Life Cycle**' screen (Figure 6).

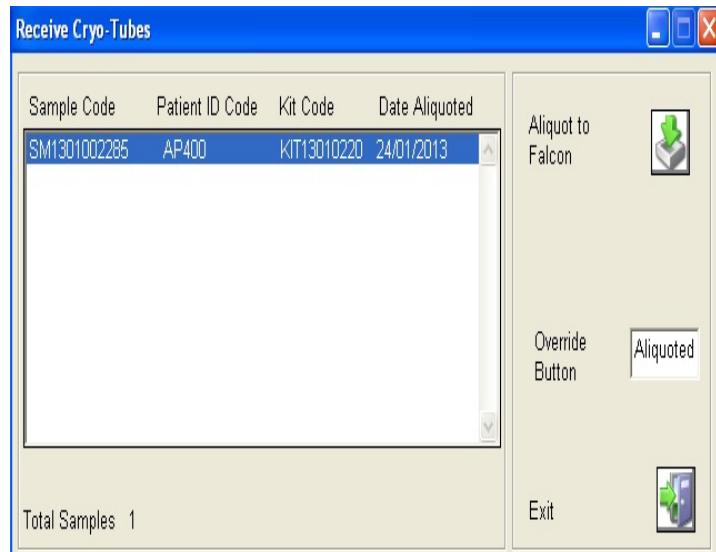


Figure 11: Receive Cryo Tubes

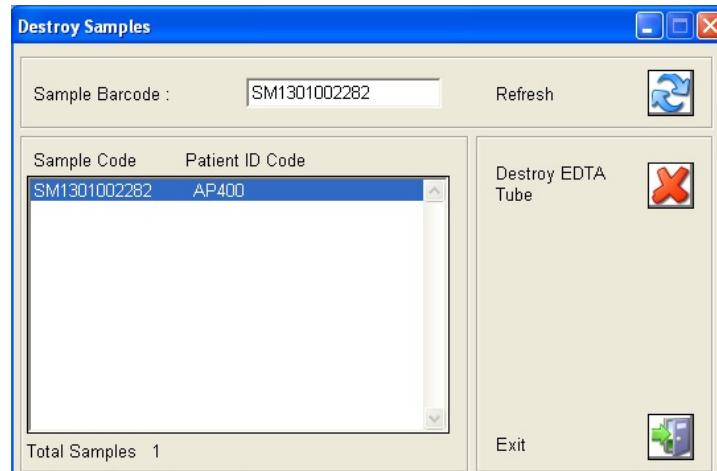


Figure 12: Destroy Samples

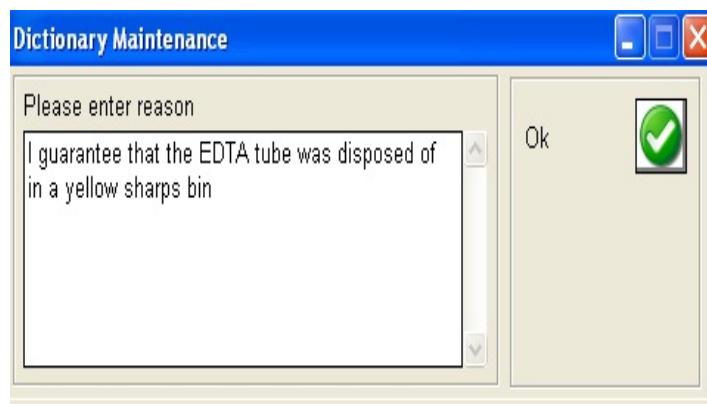
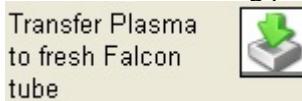


Figure 13: Dictionary Maintenance

1. To continue entering processing data, select on Life cycle screen (Figure.6)


The 'Centrifuge and to New Falcon' screen (Figure 15) will appear.
2. Select and highlight the appropriate Patient ID code if more than one tube is in the list. Select the Falcon tube in the sub screen to the right and select '**Aliquot Plasma to Falcon**'. This will bring up a '**Receive Cryo-tubes**' screen (Figure 11).
3. Highlight the falcon tube used and follow the aliquoting sequencing as described previously for the '**Combine Blood Tube**' icon. Select '**Exit**' to leave the screen and return to '**Centrifuge and to New Falcon**' screen (Figure 15).

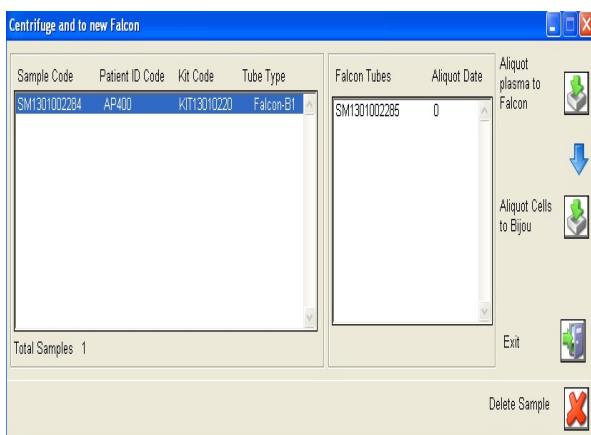


Figure 15: Centrifuge and to new falcon

4. For entering the information about the processing of the buffy coat and cell pellet, select the '**Aliquot Cells to Bijou**' icon. This will bring up the '**Transfer Cells to Bijou**' screen (Figure 16).

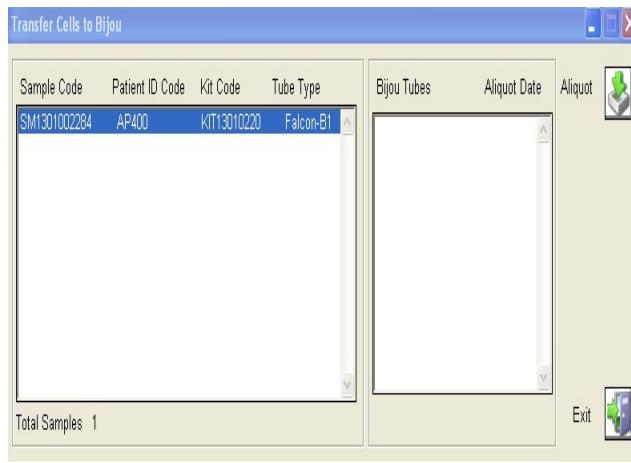


Figure 16: Transfer Cells to Bijou

5. Select the falcon tube used and the bijou tube LIMS code will appear in the right box on the screen. Select the '**Aliquot**' button. This will bring up the '**Aliquot to Bijou**' screen (Figure 17).
6. Select '**Aliquot to Bijou**' button (Figure 17) for the appropriate Patient ID.
7. Upon exiting the screen the '**Destroy Samples**' screen (Figure 12) will appear. Follow the sequence of instructions as previously described, to register in LIMS that the bijou tube has been destroyed (Figure 12 and 13). Click '**Exit**' on all subsequent screens to return back to '**Sample Life Cycle**' (Figure 6).

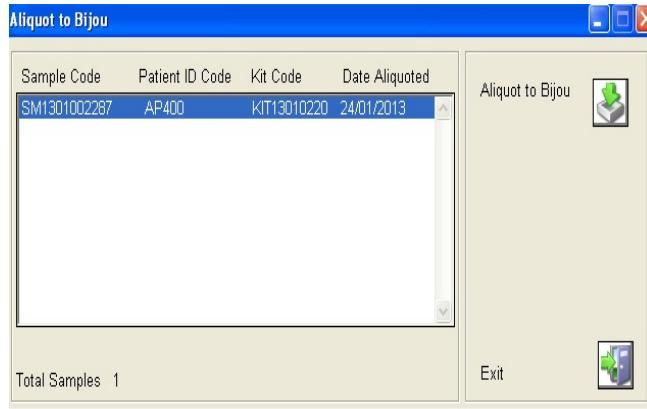


Figure 17: Aliquot to Bijou

8. For entering plasma processing data Select  in the '**PBRU Sample Life Cycle**' (Figure 6). This will bring up the screen '**Centrifuge and to a New Falcon**' screen (Figure 18) showing all the cryo-tubes available for use in the kit.
9. Highlight the tube to which plasma was aliquoted and select '**Aliquot**'. This will bring up the '**Receive Cryo-Tubes**' screen (Figure 11). Proceed as described previously to aliquot samples in LIMS for the '**Combined Blood Tubes**' icon, repeating this for all tubes in which plasma was aliquoted **only**.
10. Once all tubes used have been aliquoted select '**Dispose of Falcon Tube**' button (Figure 18). This will take you through the screen sequence for confirming the falcon tube has been destroyed (Figure 12 and 13). Follow all on screen instructions.
11. To store the tubes in LIMS go to the Plasma workflow section of the '**Sample Life Cycle**' (Figure 6) and select the '**Store location**' icon, which will bring up the '**Store Cryo-tubes**' screen (Figure 19).
12. Highlight each tube individually for the appropriate Patient ID processed and select '**Store Location**' button. Upon selecting '**Store Location**' button (Figure 19) this will bring up the '**Storage Location**' screen (Figure 20).
13. Fill in the information as entered on the checklist sheet.

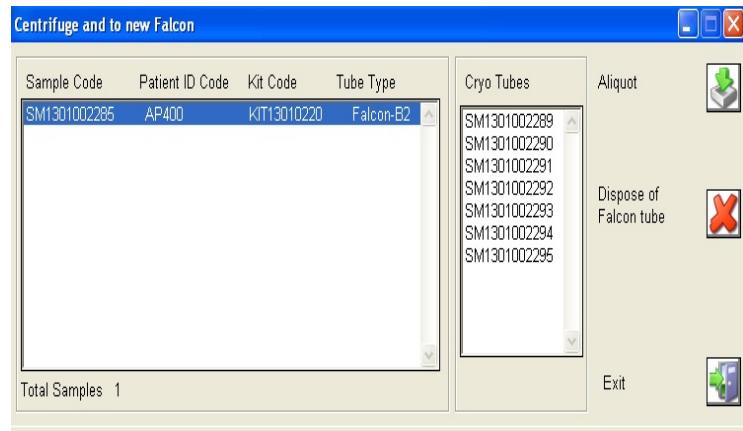


Figure 18: Centrifuge and to New Falcon

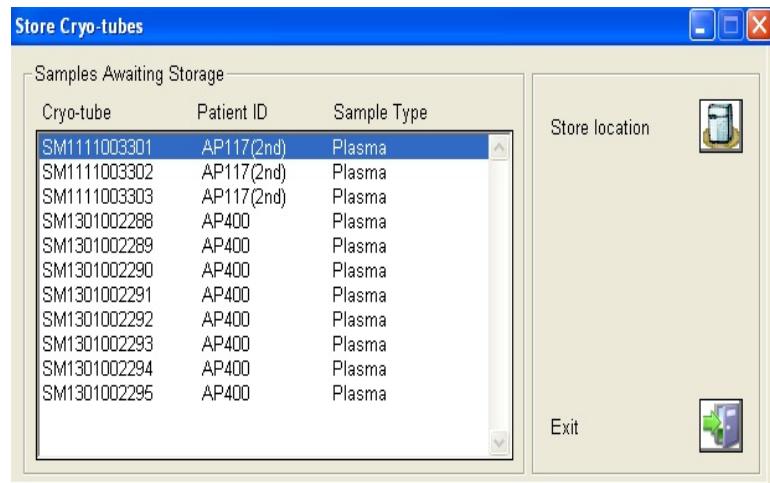


Figure 19: Store Cryo-tubes

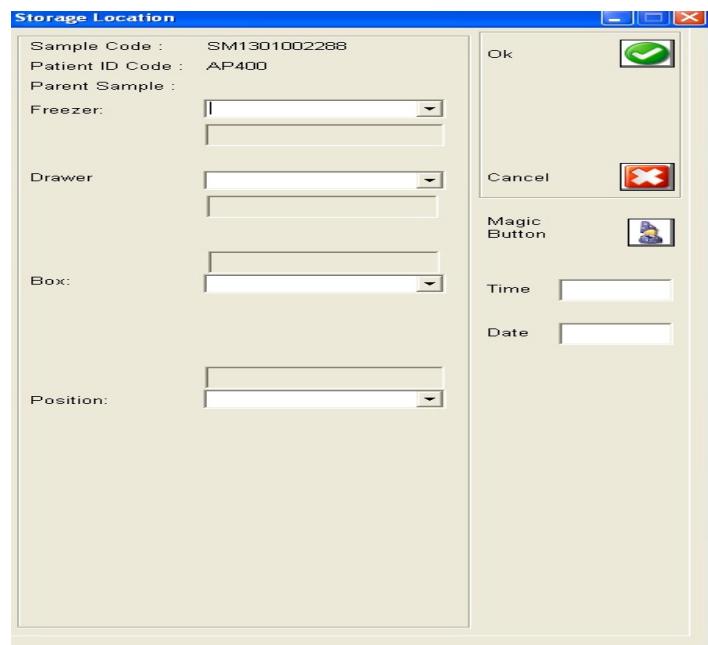


Figure 20: Storage Location

Do this for each sample tube that has been processed and stored in GCP facility freezer room (WHD).

LIMS Entry for Processing of PBMCs:

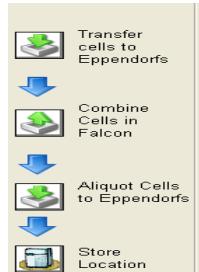


Figure 21: Cell Pellet Processing and Storage in Sample Life Cycle

14. Select '**Transfer Cells to Eppendorfs**' button (Figures 6 and 21). This will bring up the '**Transfer to Eppendorf**' screen (Figure 22).
15. Highlight the appropriate AP code and the Eppendorf tubes for that kit will appear in the box on the right in the screen. Select individually all tubes that are used and click the '**Aliquot**' button to aliquot the sample to the Eppendorf. This will bring up the screen '**Aliquot to Eppendorf**' (Figure 23).

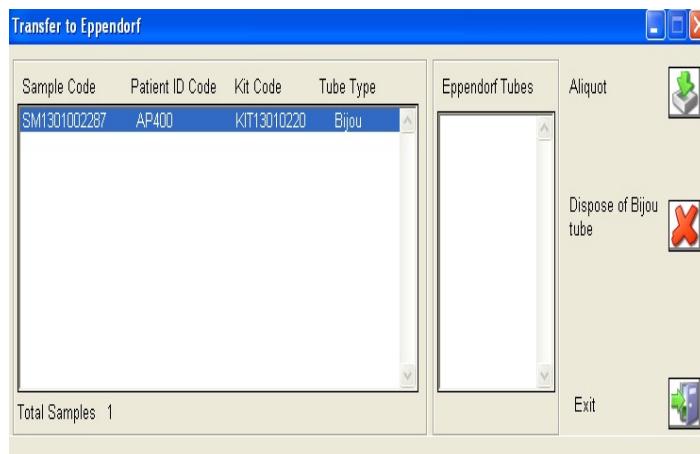


Figure 22: Transfer to Eppendorfs

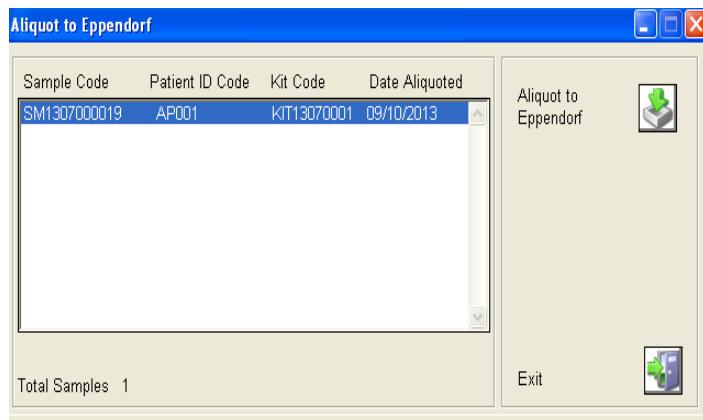


Figure 23: Aliquot to Eppendorf

16. Select the '**Aliquot to Eppendorf**' (Figure 22) icon which will enter the date of aliquoting. Click '**Exit**' to return to the '**Transfer to Eppendorf**' screen (Figure 22). Do this for all tubes aliquoted.
17. Finally, select '**Dispose of Bijou Tube**' icon. Upon selection, this will take you through the screen sequence for disposing of tubes (Figures 12 and 13). Follow on screen instructions as previously described.
18. Select '**Combine Cells in Falcon**' button (Figures 6 and 21). This will bring up the '**Eppendorf to Falcon Tube**' screen (Figure 24). Highlight individually each tube that is aliquoted to the Falcon tube.
19. Select the '**Aliquot**' button to aliquot the sample. Ensure that only tubes containing the same AP number are highlighted and aliquoted to the Falcon tube. This will bring up the '**Receive Cryo-tubes**' screen (Figure 11). Follow on-screen instructions and sequence as previously described (Figures 12 and 13).
20. Select '**Aliquot Cells to Eppendorfs**' icon in the PBMC processing and storage sample life cycle (Figures 6 and 21). This will bring up the '**Centrifuge and to a New Falcon**' screen (Figure 18) with 'Cryo-tubes' replaced by 'Eppendorf tubes' on the screen.
21. Select the appropriate Patient ID code. This will bring up the Eppendorfs which are available for final storage of the PBMCs in the right box. Highlight the patient ID code and individually highlight each tube used and then select '**Aliquot**'. This will bring up the '**Receive Cryo-tubes**' screen (Figure 11).
22. Follow the sequence for aliquoting samples, as previously described. Once all tubes that have been used have been aliquoted select '**Dispose of Falcon Tube**' button and dispose of the Falcon tube as previously described (Figures 12 and 13). Click '**Exit**' to leave this section.

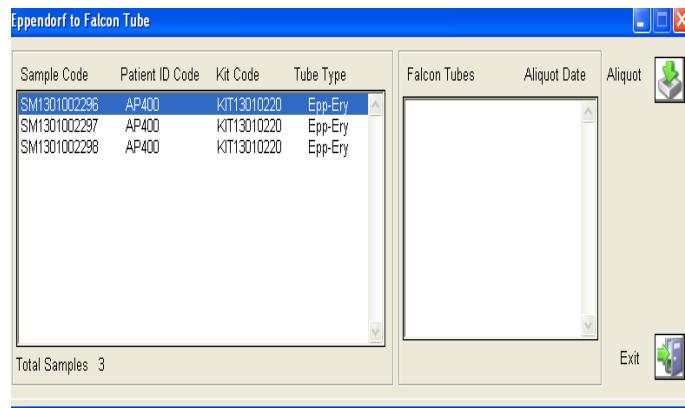


Figure 24: Eppendorf to Falcon Tube

23. Samples are stored by selecting the '**Store Location**' button and follow procedure as previously described for sample storage (Figures 19 and 20).

5.2.4.5 Processing of serum tubes and storage

1. Click on the  button in the serum part of the Sample life cycle workflow (Figure 6) This will bring up the '**Centrifuge and to a New Falcon**' screen (Figure 25).
2. Highlight the sample code of the serum tube used and select the cryotube that was aliquoted and click the '**Aliquot**' button. This brings up '**Receive Cryo-tubes**' (Figure 11). Follow the sequence as described previously to aliquot samples.

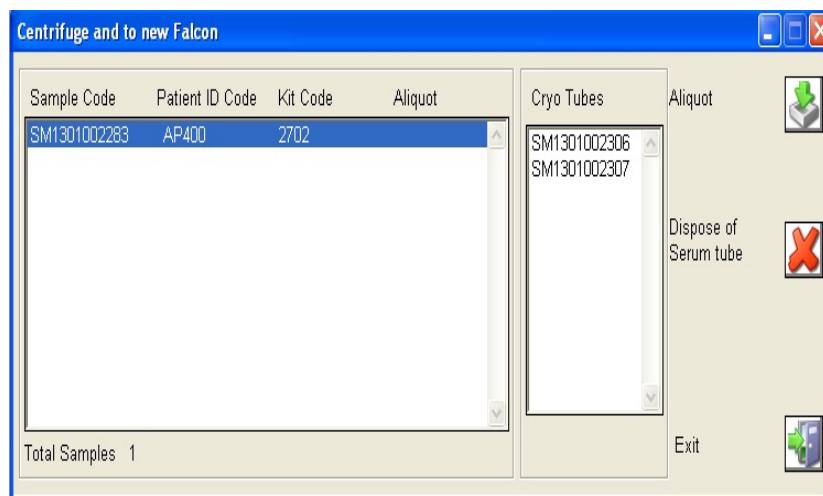


Figure 24: Centrifuge and to New Falcon

3. When complete select '**Dispose of Serum Tube**'. This will bring you through the screen sequence for disposing of a tube (Figures 11 and 12). Click '**Exit**' once serum has been destroyed to return back to the '**Sample Life Cycle**' screen (Figure 6).
4. Select '**Store Location**' and follow instructions on the screen and as previously described for storing plasma and PBMC (Figures 19 and 20).

5.2.4.6 Processing and storing of urine samples

1. Select '**pH test**' icon in the Urine section of the '**Sample Life Cycle**' (Figure 6). This will bring up the '**Samples awaiting pH test**' (Figure 25).
2. Select the appropriate Patient ID and select '**Results**' icon. This will bring up the '**Multi Sample/Test Result Entry**' (Figure 27). Enter all information as recorded on the checklist sheet and select '**Save**'. Select '**Exit**' which will return you to the '**Samples Awaiting pH test**' screen (Figure 26). Select to return to the '**Sample Life Cycle**' (Figure 6).
3. Select the '**Add Protease Inhibitor**' icon which will bring up the '**Add Protease Inhibitor Pellets**' screen (Figure 28).
4. Select the '**Add Protease Inhibitor Pellets**' button, which will bring up another screen '**Add Protease Inhibitor Pellets**' screen (Figure 29). Select the '**Add Protease Inhibitor Pellets**' icon and record all information as asked and then select '**Save**'. Select '**Exit**' on all subsequent screens until you return to the '**Sample Life Cycle**' (Figure 6).
5. Select the '**Aliquot**' icon on the Sample life cycle screen. This will bring up the '**Centrifuge and to a New Falcon Tube**' screen (Figure 18). Follow on-screen instructions for aliquoting samples as described previously (Figures 12, 13 and 18).
6. Finally select the '**Store Location**' icon on the life cycle screen (Figure 6) and follow instructions as previously described for storing samples.

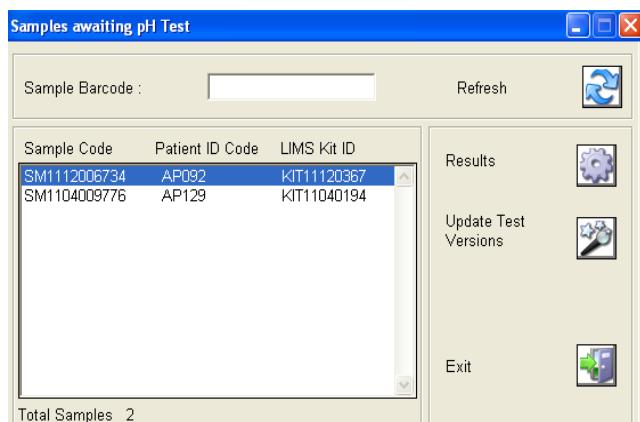


Figure 27: Samples awaiting pH test

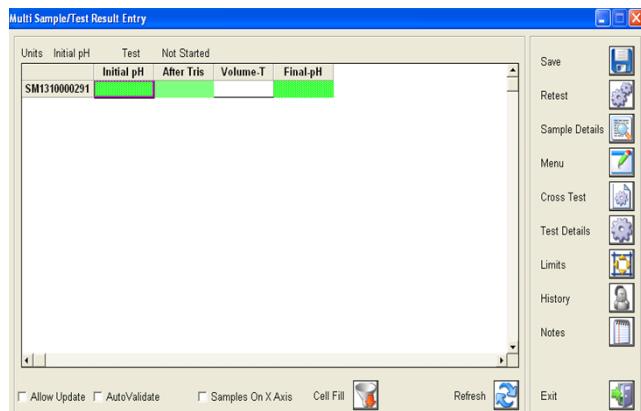


Figure 28: Multi Sample/Test

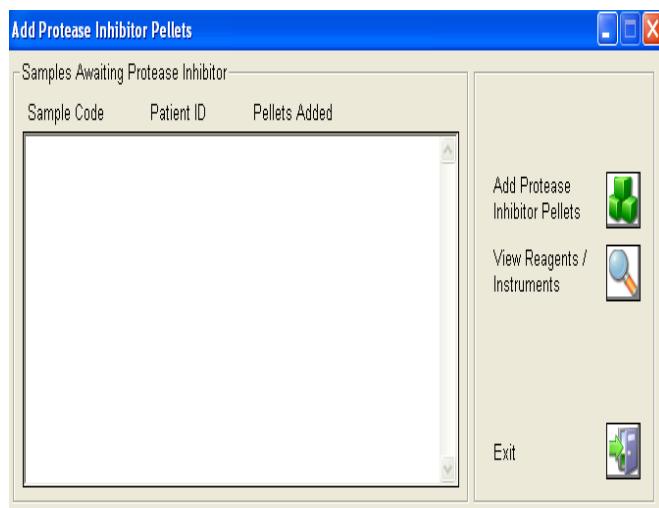


Figure 29: Add Protease Inhibitor

6. WITHDRAWAL OF INFORMED CONSENT

If informed consent is withdrawn at any time then all samples from this patient including any stored data must be destroyed and reported in the Q Pulse document management system in accordance with GCLPPP017 and in accordance with the GCLPRPS004: Disposal of Hazardous Waste. LIMS must be updated at the same time with this information.

7. ABBREVIATIONS

| | |
|-------------|---------------------------------------|
| EDTA | Ethylenediaminetetraacetic acid |
| GCP | Good Clinical Practice |
| LPRG | Liverpool Pancreatitis Research Group |
| WHD | William Henry Duncan Building |

8. OTHER RELATED PROCEDURES AND DOCUMENTS

SOPs:

| | |
|---------------|---------------------------------------------------------------|
| GCLPEQU037 | Use of Pipettes in the LPRG |
| GCLPEQU038 | Use of Centrifuges in LPRG |
| GCLPRPS004 | Disposal of Hazardous Waste in the LPRG |
| GCLPTSS049/F1 | Acute Pancreatitis Sample Processing Checklist |
| GCLPTSS116 | Collection of samples for the LPRG acute pancreatitis biobank |
| GCLPTSS161 | Cell Counting in the LPRG |
| GCLPPPD017 | Recording and Reporting of Quality Incidents |

9.Appendix

9.1 Appendix 1: Example of Acute Pancreatitis Biobank Sample Processing Checklist:

ACUTE PANCREATITIS BIOBANK SAMPLE PROCESSING CHECKLIST

SAMPLE COLLECTION

| | | |
|--|-------|-------|
| | Date: | Time: |
| | Date: | Time: |

| KIT CODE | | | | | | PATIENT CODE (AP NUMBER) | | | |
|----------|---|--|---|--|---|--------------------------|--|--|--|
| KIT TYPE | A | | B | | C | | | | |
| | | | | | | | | | |

| ORIGIN OF SAMPLE | Sample Time Point: (tick box, or complete week number) | | | |
|------------------|-----------------------------------------------------------|-------|-------|--------|
| RLUH: | | Day 1 | Day 4 | Day 14 |
| Other: | | | | |

| | CONSENT (tick box) | | | Date |
|-----------------------|--------------------|--|-----|------|
| Patient Consent | No | | Yes | |
| Retrospective Consent | No | | Yes | |
| Consultee Consent | No | | Yes | |

| Blood Collected | Amount | Tick box | Time Taken | Invert x10 (tick box) |
|--------------------------|--------|----------|------------|--------------------------|
| EDTA Vacutainer (purple) | 10 ml | | | |
| EDTA Vacutainer (purple) | 4 ml | | | |
| Serum Tube (golden) | 3.5 ml | | | |
| PAXgene tube | 2.5 ml | | | |
| Urine collected | | | | |

SAMPLE PROCESSING AND STORAGE

(TICK THE BOX WHEN EACH STAGE COMPLETED OR COMPLETE WITH APPROPRIATE INFORMATION)

| PAXGENE TUBE | |
|-------------------------------------------------------------------------|--------------------------|
| Confirm 2 hour incubation at room temperature Y/N | |
| TIME STORED in -80OC PBRU Freezer 1 | |
| Location of PAXGENE Tube (Freezer, Draw, Box, Co-ordinate) | |
| Record on PBRU Acute Pancreatitis Biobank section on LIMS (tick box) | <input type="checkbox"/> |

| | |
|--------------------------------------------------------------------------|--|
| PLASMA (tick box when completed or record information) | |
| Time process begun: | |
| Pour blood from the two EDTA tubes (purple tops) into a 15ml falcon tube | |
| Volume of blood (ml): | |
| Centrifuge 600 xg for 30 minutes (ensure centrifuge is balanced) | |
| Dispose of blood tube | |
| Remove plasma layer into a fresh 15ml falcon tube | |
| Dispose of falcon tube | |
| Centrifuge 1500 xg for 10 minutes (ensure centrifuge is balanced) | |
| Aliquot plasma into cryovials (red top) (max=8) | |
| Number of plasma samples stored at -80°C in PBRU Freezer 1 : | |
| Record Location of Plasma Tubes | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| Record location on the PBRU Acute Pancreatitis Biobank LIMS | |

| CELL PELLET | |
|---------------------------------------------------------------------------------------------------------|--|
| Remove buffy coat into a 7ml Bijoux tube mix by inversion x3 | |
| Dispose of falcon tube | |
| Transfer 500µl aliquots into Eppendorf tubes (max = 5) | |
| Add 1ml red blood cell lysis buffer mix by inversion x3 | |
| Place on roller for 10 minutes at room temperature | |
| Centrifuge 600 xg for 5 minutes room temperature (ensure centrifuge is balanced) | |
| Remove supernatant into 1% Virkon waste pot | |
| Add 1ml red blood cell lysis buffer mix by flicking tube (tap sharply) | |
| Centrifuge 600 xg for 3 minutes room temperature (ensure centrifuge is balanced) | |
| Remove supernatant into 1% Virkon waste pot | |
| Re-suspend each cell pellet in 1ml PBS | |
| Mix all cell suspensions into a 15ml falcon, add an extra 1ml PBS for every ml of cell suspension added | |

Count Cells: RECORD COUNTS IN THE TABLE BELOW

| | FIRST COUNT | SECOND COUNT | AVERAGE |
|--------------------------------------------|------------------------|-------------------------|-----------------------|
| TOTAL CELL COUNT ANSWER (A) | | | X 10 ⁶ /ml |
| Live Cells | | | X 10 ⁶ /ml |
| Dead Cells | | | X 10 ⁵ /ml |
| Viability | | | % |

$$\text{ANSWER (A)} \times \text{VOLUME (ml)} = \text{TOTAL number of cells}$$

$$\dots \times \dots = \dots \times 10^6 \text{ ANSWER (B)}$$

Final concentration of cell pellet

$$\text{ANSWER B divide by 5} = \dots \times 10^6$$

| | |
|------------------------------------------------------------------|--|
| Centrifuge falcon tube at 600 xg (ensure centrifuge is balanced) | |
| Remove supernatant and dispose of into 1% Virkon | |
| Re-suspend cell pellet in 1ml PBS | |
| Add 4ml PBS to cell pellet | |
| Aliquot 1ml into 5x fresh Eppendorf tubes | |

| | |
|-------------------------------------------------------------------------------------------|--|
| Centrifuge 600 xg for 5 minutes at room temperature (ensure centrifuge is balanced) | |
| Remove supernatant and dispose of into 1% Virkon | |
| Store at -80°C in PBRU Freezer 1 (record the following information next to the bold type) | |
| Number of Eppendorf's stored: | |
| Number of cells/ Eppendorf: | |
| Location of Eppendorf's: | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| Time Eppendorf's stored: | |
| Record location on the PBRU Acute Pancreatitis Biobank LIMS | |
| Dispose of any remaining tubes | |

| SERUM | |
|------------------------------------------------------------------------------------------|--|
| Check clot activation carried out (recorded as time sample inverted 10 times above) | |
| Centrifuge 1500 xg 10 minutes room temperature (ensure centrifuge is balanced) | |
| Time centrifugation: | |
| Add 1ml serum into cryovials (white top) (max = 2) | |
| Store at -80°C in PBRU Freezer 1 Record the following information next to the bold type: | |
| Number of serum cryovials stored: | |
| Location of serum cryovials: | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| Time Eppendorf's stored: | |
| Record location on the PBRU Acute Pancreatitis Biobank LIMS | |

| URINE | |
|---------------------|--|
| pH meter calibrated | |

| | |
|-----------------------------------------------------------------------|--|
| Time started: | |
| Transfer 10ml urine to 50ml falcon tube | |
| pH measured (adjust to pH7 if necessary with Tris pH7 in nurses' box) | |
| Initial pH Reading: | |
| pH Reading after 1ml Tris Buffer (pH7) added: | |
| Total Volume of Tris Buffer (pH7) added: | |
| Final pH Reading: | |
| Add protease inhibitor cocktail tablet for each 10ml urine | |
| Add 1ml urine to each cryovials (green top)(max = 5) | |
| Store at -80°C in PBRU Freezer 1 LECMC GCLP Freezer Room (3.373) | |
| Record location on the PBRU Acute Pancreatitis Biobank LIMS | |
| Number of urine cryovials stored: | |
| Location of urine cryovials: | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| ➤ | |
| Time urine cryovials stored: | |

Comments: