UNIVERSITY OF LIVERPOOL GCP labs LCTU	**LCTU GCPLab** **Standard Operating Procedure** **PROCESSING OF SAMPLES FOR THE LPRG ACUTE PANCREATITIS BIOBANK**		
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Revision History

Version Number	Effective Date	Revision Date	Reason for Change	Change Control No:
1	07/12/2010	09/11/2011	Amended equipment section, statement ref blood samples & SOP numbers added	1750
2	17/05/2012	08/10/2012	SOP numbers added, additional clarification of equipment usage & changes to text	2371
3	03/12/2012	20/01/2014	LIMS procedure added. Amended retrospective section & SOP procedure	2475
4	03/03/2014	22/12/2015	Change in sample collection tubes	4338
5	20/05/2016	31/08/2016	New processing steps added	5641
6	31/10/2016	08/08/2018	Reference to PBRU amended to LPRG & author amended	8641

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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 Biobank and will process samples to store in the Acute Pancreatitis Biobank, UCD, 3rd Floor, and Royal Liverpool University Hospital.

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. 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, who will explain in detail, to the patient, the purpose of the biobank, the process of recruitment and various issues surrounding it. Please refer to SOP GCLPTSS116.

3. PURPOSE

The purpose of this SOP is to describe the procedure for the safe processing of samples in the LPRG laboratory room 3.302B UCD Building, Royal Liverpool University Hospital and storage in the Acute Pancreatitis Biobank freezer, located in room 3.373 (the GCP facility) UCD Building, Royal Liverpool University Hospital.

4. SCOPE

This SOP applies to all staff involved in processing samples for the LPRG Acute Pancreatitis Biobank when processing the samples in the LPRG Research Laboratories and when storing the samples in the GCP Facility.

5. PROCEDURE

5.1 RESPONSIBILITY

It is the responsibility of all staff on the delegation log to process samples for the Acute Pancreatitis Biobank to follow this SOP and enter all processing information into the Laboratory Information Management System (LIMS).

It is the responsibility of all staff delegated to store the processed sample tubes in the next available space in the freezer and to record the storage location in LIMS and on the checklist sheets.

5.2 PROTOCOL

5.2.1 Procedures to be followed to Minimise Risk to 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 should be performed in Class II cabinets, except where equipment is located outside the cabinets (ref: sections 5.2.5 and 5.2.6). The staff member processing the samples should wear a Howie style laboratory coat and gloves at all times.
- 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 Reception of the Blood Samples:

All blood tube samples collected in the Royal Liverpool University Hospital will be brought to the LPRG Lab Room 3.302A or 3.302B on the 3rd floor of the UCD building for processing, where the samples will be receipted 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 entered onto the Checklist sheet (see appendix 1)**. Upon completion of the processing of all samples the sheet is placed into the pending tray for the staff on the delegation log that has been delegated to transfer the appropriate information to LIMS. **All blood samples received from outside of the Royal Liverpool University Hospital must be processed immediately upon timed receipt into the LPRG laboratory, Room 3.302A.** All checklist sheets arriving with the samples must be first checked for whether consent has been obtained. Consent has been ethically approved for the Acute Pancreatitis Biobank to allow prospective, retrospective and a declaration of consultee consent (see GCLPTSS116).

If any consent is withdrawn at any time then again any samples stored must be destroyed. Sample Destruction forms (GCLPFAC005/F2 – GCLP Sample Destruction Form) can be obtained from the University of Liverpool SharePoint Online via a delegated member of staff. Once completed the form must be returned to the Quality Assurance Manager (QAM) and a copy kept in Office 3.347.

5.2.3 Studies Conducted Under the AP Biobank:

These studies (ref: SOPs GCLPTSS158 and GCLPTSS162) will only be conducted during the normal working week (not weekends) and only if the correct designated member of staff is available to process and analyse the samples. Kits P and Q

(GCLPTSS158) or S (GCLPTSS162) will be used to collect AP samples instead of Kits A, B or C.

Kits P and Q contain an additional blood tube, sodium citrate (blue top) 4.5 ml and this along with the EDTA tube (purple top) 4 ml is handed to the delegated staff member for processing samples for use on this study, in accordance with GCLPTSS158.
They will receipt these tubes in LIMS via the Flow Project LIMS workflow and enter all relevant processing information into LIMS.

S kit is identical to an A kit in its collection components; the 10ml is handed to the delegated staff member for processing samples in accordance with GCLPTSS158. They will receipt these tubes in LIMS via the SeaHorse sub study workflow and enter all relevant processing information into LIMS.
These studies will not be conducted on retrospective consent samples or on referral samples.

The rest of the kit is processed in accordance with the protocol described below from 5.2.4 onwards.

5.2.4 Receipt of Samples:

Upon receiving the patient sample into the laboratory the following procedure must be used to record the processing procedure into LIMS: 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 1). Select '**Trials and Studies**' from the drop down menu and select '**Panc Studies**'. Select '**PBRU**' and then select '**AP Biobank**'. The selection of '**AP Biobank**', results in the '**PBRU Workflow**' screen (Figure 2). 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 1: Live Screen

Figure 2: PBRU Workflow

Select the **Patient ID button** on the **Patient ID** screen will appear (Figure 3). Highlight the kit code used and select '**Set patient ID and confirm consent**' button.

This will bring up the Patient **ID Code** screen (Figure 4). 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 3). 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 5).

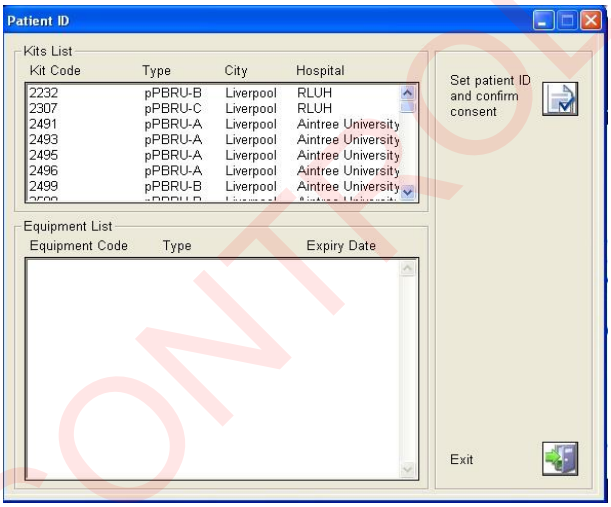


Figure 3: Patient ID screen

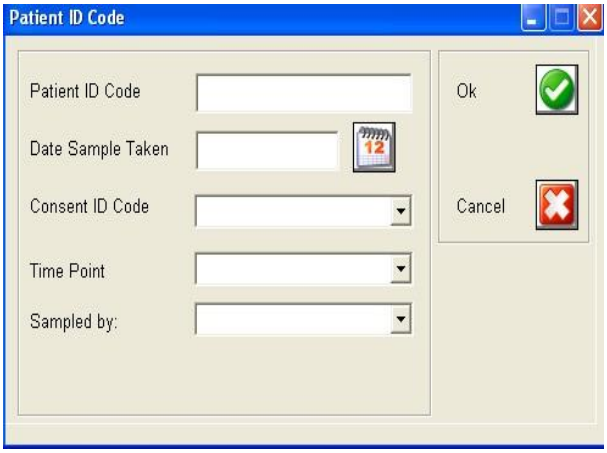


Figure 4: Patient ID code

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 6) and follow instructions on each screen to indicate in LIMS that no sample was ever received.

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 5) to being the LIMS entry for the processing of samples.

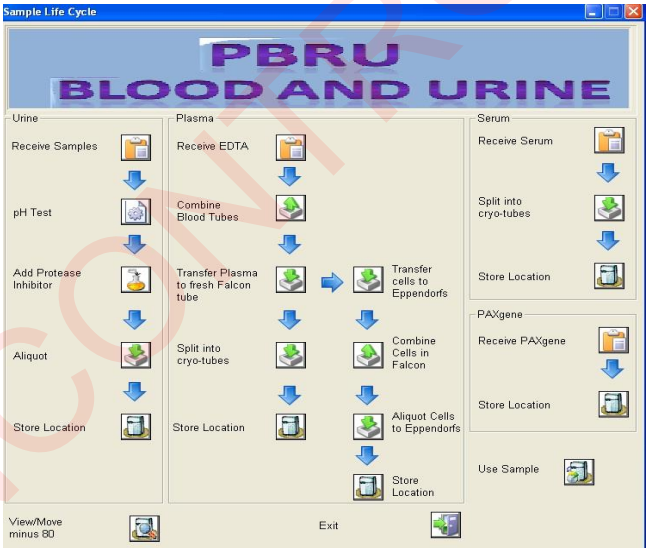


Figure 5: Sample Life Cycle

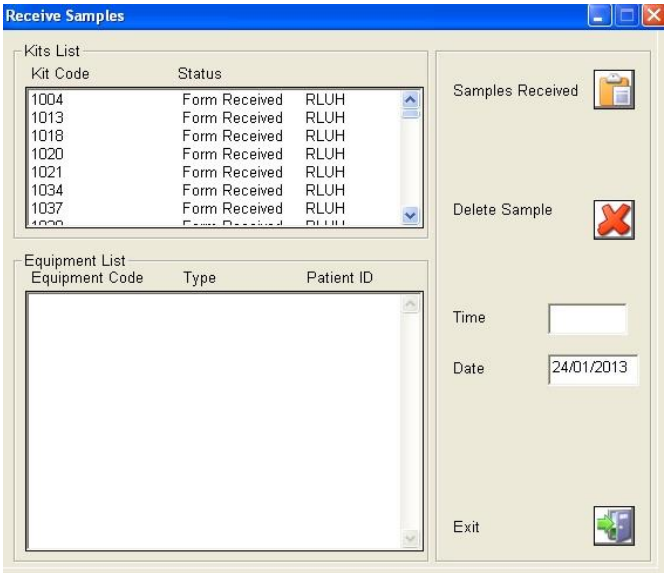


Figure 6: Receive Samples

5.2.5 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.

   Leave for 2 hours (from time of collection) in an upright position at room temperature. Store the PAXgene tube into the **Temporary Storage** box located in Freezer 9. LPRG staff should store the tube in the next available location in the freezer and complete the LIMS entry as follows:

   LIMS Entry of PAXgene™ Tube Storage:

   Select '**Store Location**' icon in the PAXgene™ section of '**Sample Life Cycle**' (Figure 5).

   This will bring up the '**Store Location**' screen (Figure 7).
   Highlight the appropriate PAXgene™ tube for the Patient ID and select the '**Store Location**' button. This will bring up the '**Store Location**' screen (Figure 8).

   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.

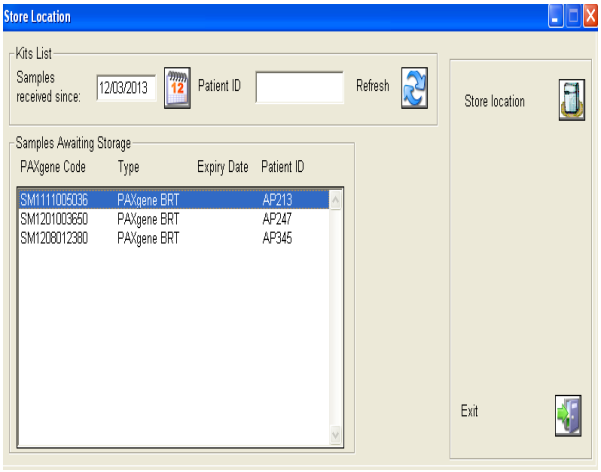


Figure 7: Store Location

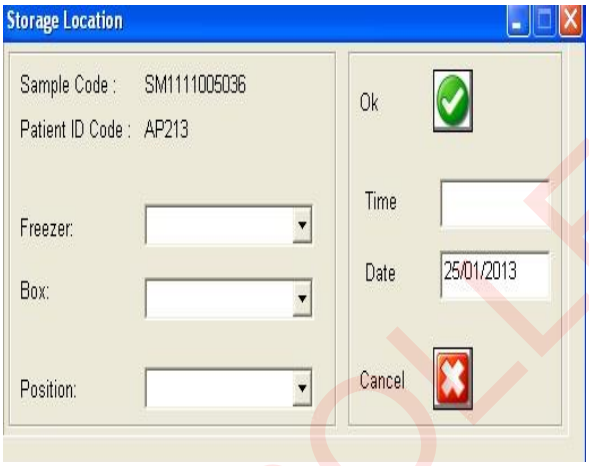


Figure 8: Storage Location

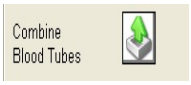
5.2.6 Extraction and Storage of Plasma and Cell Pellet (Processing of the EDTA Tube):

- 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.
- If kit P or Q has been used for sample collection, hand the BD Vacutainer® K_2 EDTA Tube (purple top) 4ml to the BD LSR designated member of staff for processing. They will remove just 1ml for their purposes. Then continue with below with the BD Vacutainer® K_2 EDTA Tube (purple top) 10ml and the remaining contents of the 4ml tube.
- Prior to processing, remove red blood cell lysis buffer aliquot from the fridge and place in water bath set at 25ºC for 10 minutes.
- After receipt of the sample into LIMS (see 5.2.4) begin processing:

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 Heraeus Megafuge 16R refrigerated bench top centrifuge. Ensure that the centrifuge is properly balanced. Whilst the sample is undergoing

centrifugation, enter the processing information into LIMS, to confirm these steps have been taken.

LIMS Entry:

Select the 

button. This will bring up the 'Combine EDTA Tubes and confirm' screen (Figure 9).

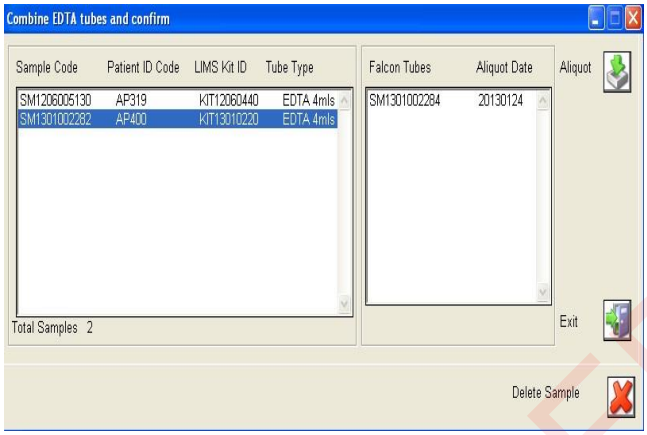


Figure 9: Combine EDTA tubes and confirm screen

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 10). Select '**Aliquot to Falcon**' button to input the date of aliquotting the sample in LIMS.

NOTE: *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 11). Select the '**Destroy EDTA Tube**' button. This will bring up a '**Dictionary Maintenance**' screen (Figure 12). On this screen a message will appear asking for confirmation of the EDTA being disposed in yellow sharps bins (Figure 12). Select '**Ok**' to confirm you have destroyed the EDTA tube. This will bring you back to the '**Destroy Samples**' screen (Figure 11). Click '**Ok**' to exit and return back to the '**Receive Cryo Tubes**' screen (Figure 10). This sequence should be followed for all EDTA tubes received.

NB 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 11).
2) Dictionary maintenance (Figure 12). Follow instructions for each screen ensuring the '**Exit**' icon is selected on each screen, to return back to the '**Sample Life Cycle**' screen (Figure 5).

Click 'Exit' to leave the section of the life cycle. **If entering 2 samples into LIMS at the same time, you MUST close this section in LIMS before re-opening this section to enter the details for the 2nd sample**.

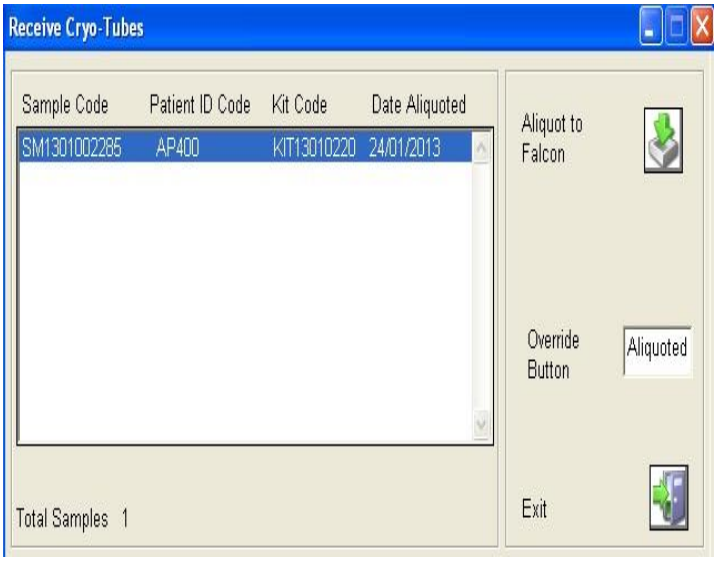


Figure 10: Receive Cryo Tubes

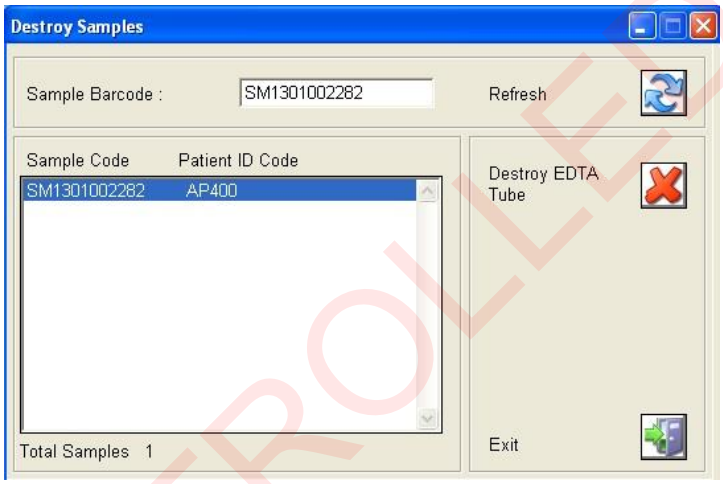


Figure 11: Destroy Samples

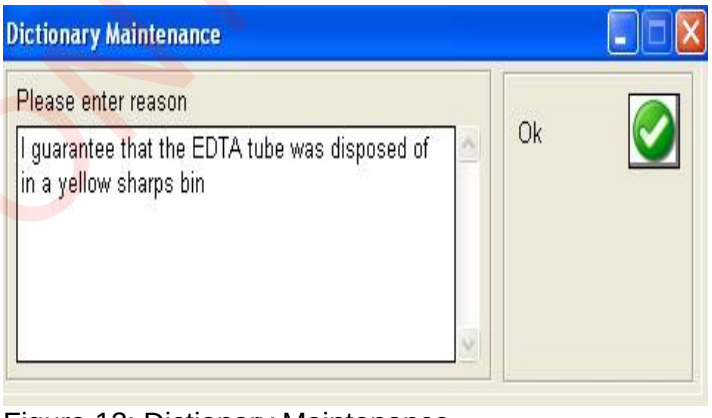


Figure 12: Dictionary Maintenance

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 13) 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 the tube back to the centrifuge and centrifuge for a further 10 minutes

in the Heraeus Megafuge 16R at 1500 x g at 24°C for 10 minutes. Ensure the centrifuge is balanced before proceeding.

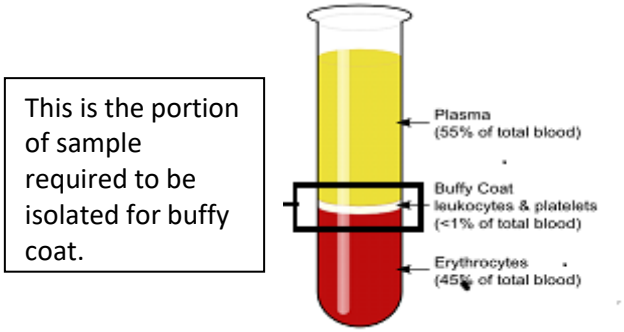


Figure 13: Blood separation after 30 minute centrifugation

4. Carefully remove the 'buffy coat' from the Falcon tube (Figure 13) 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.
5. 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 are a maximum of 5 tubes per kit.
6. 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).
7. Mix by inverting 3 times.
8. Allow the tubes to incubate on a rocker for 10 minutes at room temperature at 100 rpm.
9. Whilst the plasma is in the centrifuge and the buffy coat is incubating on the shaker in the red blood cell lysis buffer, enter into LIMS the appropriate information to confirm the processing steps taken above (4-9 inclusive).

LIMS Entry of Sample Processing:

Select 

button. The '**Centrifuge and to New Falcon**' screen (Figure 14) will appear. 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 10). Highlight the falcon tube used and follow the aliquotting 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 14).

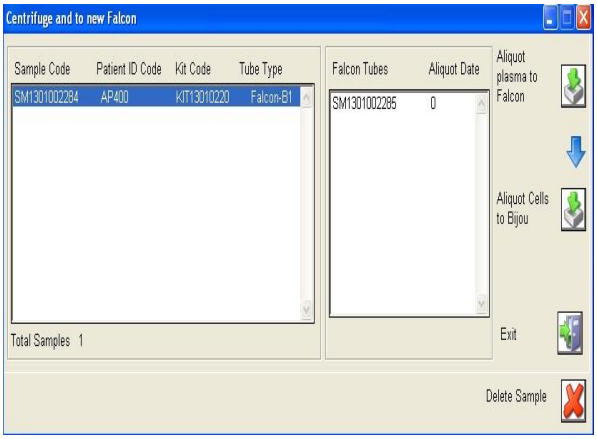


Figure 14: Centrifuge and to new falcon

For entering the information about the processing of the buffy coat, select the '**Aliquot Cells to Bijou**' icon. This will bring up the '**Transfer Cells to Bijou**' screen (Figure 15).

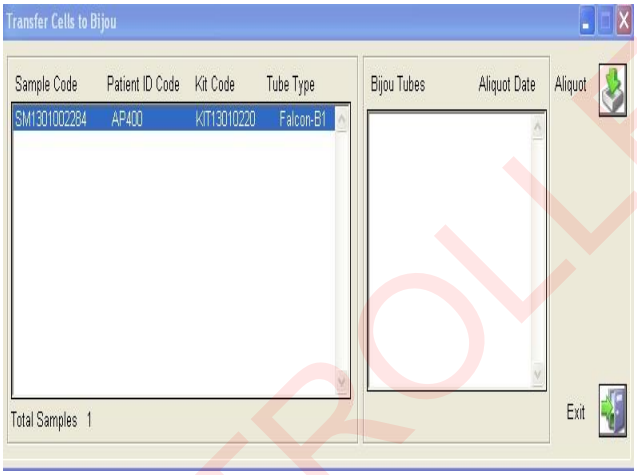


Figure 15: Transfer Cells to Bijou

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 16). Select '**Aliquot to Bijou**' button (Figure 16) for the appropriate Patient ID.

Upon exiting the screen the '**Destroy Samples'** screen (Figure 11) will appear. Follow the sequence of instructions as previously described, to register in LIMS that the bijou tube has been destroyed (Figure 11 and 12). Click '**Exit**' on all subsequent screens to return back to '**Sample Life Cycle**' (Figure 5).

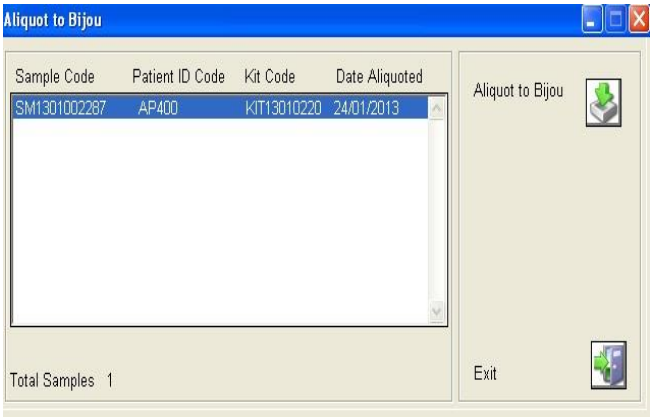


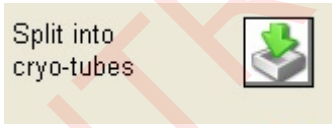
Figure 16: Aliquot to Bijou

Storage of Plasma:

10. Upon completion of centrifugation of the plasma, 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 Temporary Storage Box in Freezer 9. Complete the checklist sheet and LIMS entry for processing of the plasma samples.

    The delegated staff only should store tubes in the next available location in the storage boxes, in the LPRG Biobank -80ºC freezer located in UCD Building, Royal Liverpool University Hospital, and record the storage information on the checklist sheet and in LIMS as follows:

LIMS Entry for Plasma Processing:

Select 

in the '**PBRU Sample Life Cycle**' (Figure 5). This will bring up the screen '**Centrifuge and to a New Falcon**' screen (Figure 17) showing all the cryo-tubes available for use in the kit. Highlight the tube to which plasma was aliquoted and select '**Aliquot**'. This will bring up the '**Receive Cryo-Tubes**' screen (Figure 10). 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**. Once all tubes used have been aliquoted select '**Dispose of Falcon Tube**' button (Figure 17). This will take you through the screen sequence for confirming the falcon tube has been destroyed (Figure 11 and 12). Follow all on screen instructions.

LIMS Entry for Plasma Tube Storage:

To store the tubes in LIMS go to the Plasma workflow section of the '**Sample Life Cycle**' (Figure 5) and select the '**Store location**' icon, which will bring up the '**Store Cryo-tubes**' screen (Figure 18). Highlight each tube individually for the appropriate Patient ID processed and select '**Store Location**' button. Upon selecting '**Store Location**' button (Figure 18) this will bring up the '**Storage Location**' screen (Figure 19). Fill in the information in LIMS and record final storage location on the checklist sheet.

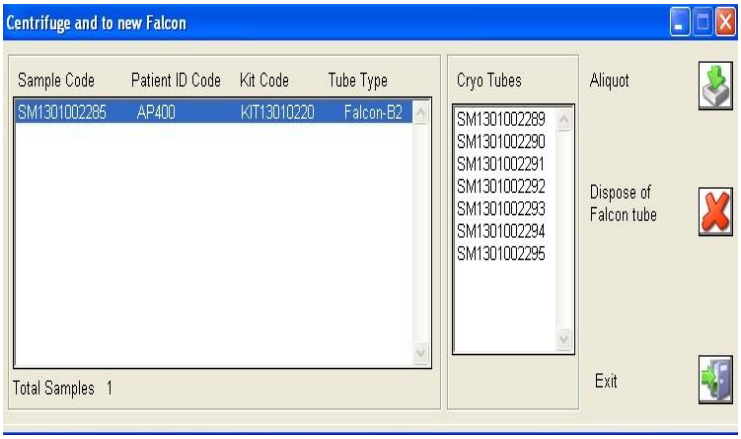


Figure 17: Centrifuge and to New Falcon

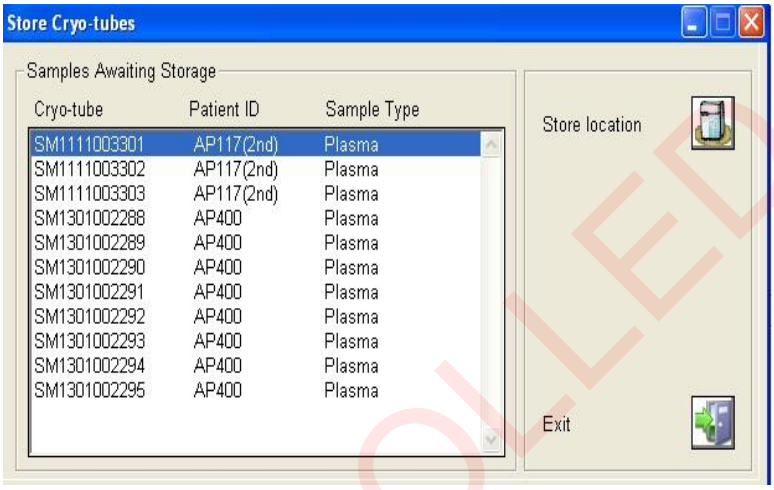


Figure 18: Store Cryo-tubes

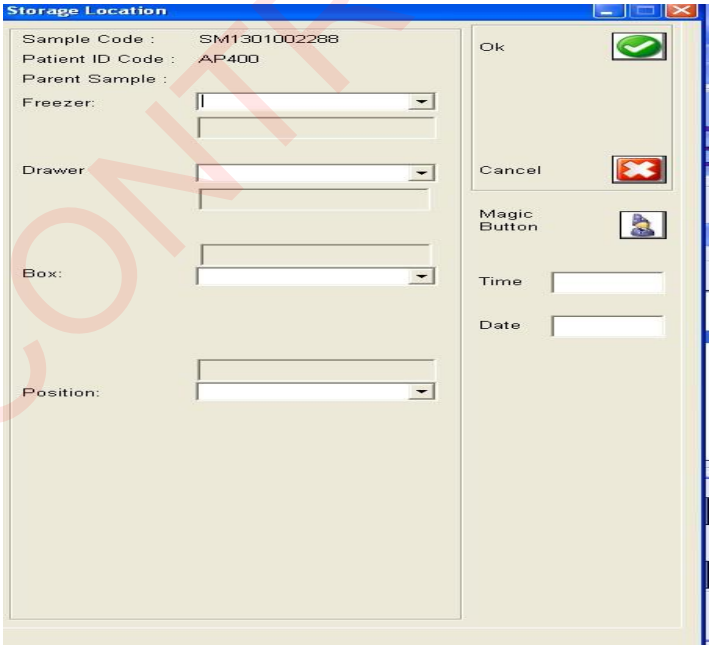


Figure 19: Storage Location

Do this for each sample tube that has been processed and stored in the LPRG Freezer.

Processing of Buffy Coat Containing Cell Pellet:

11. Once the buffy coat aliquots have been 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
12. 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.
13. 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.
14. Centrifuge the Eppendorfs at 600 x g for 3 minutes at room temperature in the micro-centrifuge.
15. 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.
16. Mix together all re-suspensions into one 15ml falcon tube, (labelled **Falcon-P**) using a 1ml pipette.
17. Double the amount of PBS (add an extra 1ml of PBS for every pellet added).
18. 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.**
19. Centrifuge the falcon tube at 600 x g at 24°C for 10 minutes, in the Heraeus Megafuge 16R Refrigerated Bench Top Centrifuge.
20. 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
21. Re-suspend the cell pellet with 1ml of sterile PBS, using a 1ml pipette and gently pipetting up and down.
22. 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 **Epp-P**) using a 1ml pipette. Centrifuge the Eppendorfs at 600 x g for 5 minutes at room temperature in the micro-centrifuge.
23. Using a 1ml pipette, carefully remove and properly dispose of the supernatant. Store tubes containing cell pellet in the **Temporary Storage** Box in the LPRG Biobank -80ºC freezer located in UCD Building. Record the processing steps of the buffy coat in LIMS as follows. LPRG staff should store the tubes in the final location in the freezer both on the checklist sheet and in LIMS.

LIMS Entry for Processing of PBMCs:

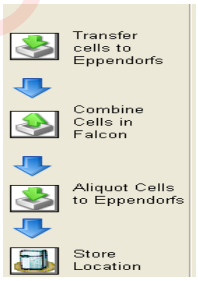


Figure 20: Cell Pellet Processing and Storage in Sample Life Cycle

Select '**Transfer Cells to Eppendorfs**' button (Figure 5 and 20). This will bring up the '**Transfer to Eppendorf**' screen (Figure 21). 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 22).

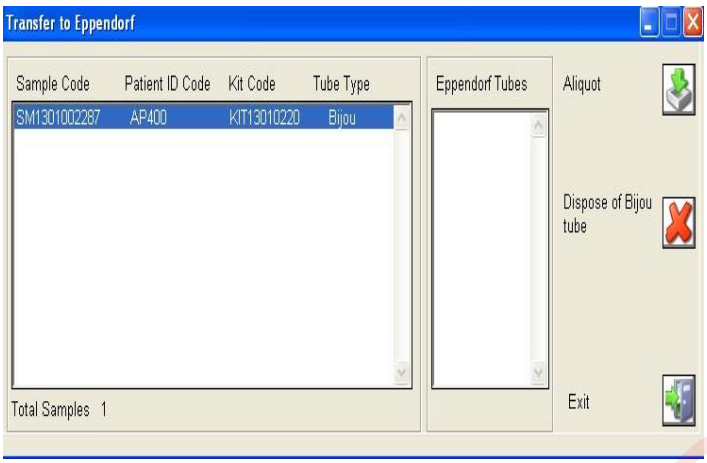


Figure 21: Transfer to Eppendorfs

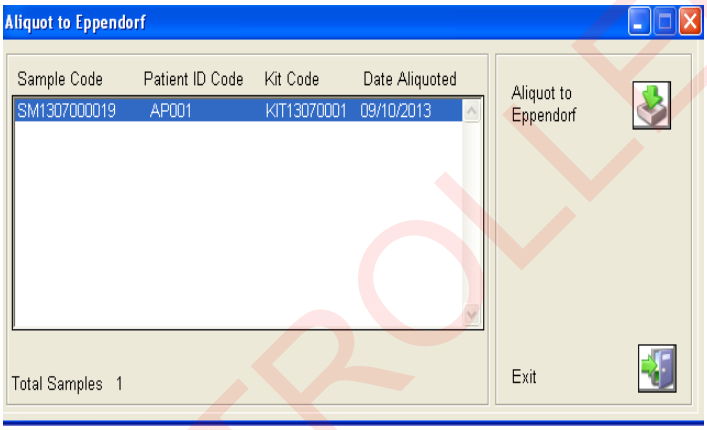


Figure 22: Aliquot to Eppendorf

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 21). Do this for all tubes aliquoted. Finally, select '**Dispose of Bijou Tube**' icon. Upon selection, this will take you through the screen sequence for disposing of tubes (Figure 11 and 12). Follow on screen instructions as previously described.

Select '**Combine Cells in Falcon**' button (Figure 5 and 21). This will bring up the '**Eppendorf to Falcon Tube**' screen (Figure 23). Highlight individually each tube that is aliquoted to the Falcon tube. 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 10). Follow on-screen instructions and sequence as previously described (Figure 11 and 12).

If there is more than 1 patient sample being processed in the workflow, complete 1 patient sample and click 'Exit' to exit the screen. Then re-open the screen (by selecting the 'Combine Cells in Falcon' button), and follow the exact steps as listed in the paragraph above to aliquot another patient sample to the Falcon tube.

Select '**Aliquot Cells to Eppendorfs**' icon in the PBMC processing and storage sample life cycle (Figures 5 and 20). This will bring up the '**Centrifuge and to a New Falcon**' screen (Figure 17) with 'Cryo-tubes' replaced by 'Eppendorf tubes' on the screen.

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 10). Follow the sequence for aliquotting 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 (Figure 11 and 12). Click '**Exit**' to leave this section.

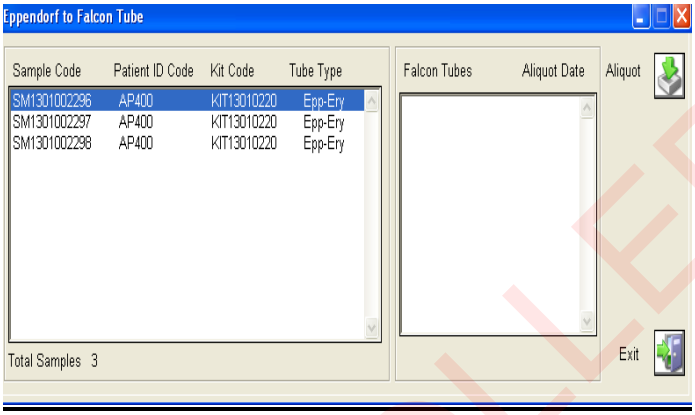


Figure 23: Eppendorf to Falcon Tube

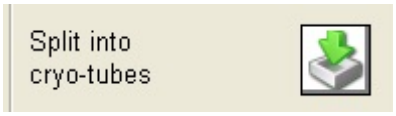
LIMS Entry for Storage of Samples:

Store samples by selecting the '**Store Location**' button and follow procedure as previously described for sample storage (Figure 19 and 20).

5.2.7 Extraction and Storage of Serum:

1. The clot activation should have been carried out at the time the blood was taken. Refer to the checklist 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 using the Heraeus Megafuge 16R Refrigerated Bench Top Centrifuge 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 **Temporary Storage** box and complete the checklist sheet.
5. LPRG staff should store the samples in the next available location in the LPRG -80°C freezer located in the UCD Building, Royal Liverpool University Hospital, and Room 3.373 and record the precise location of the tubes on the checklist sheet. Record the processing information into LIMS as follows:

LIMS Entry of Serum Processing:

Click on the 

button in the serum part of the workflow. This will bring up the '**Centrifuge and to a New Falcon'** screen (Figure 24). 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 10). Follow the sequence as described previously to aliquot samples.

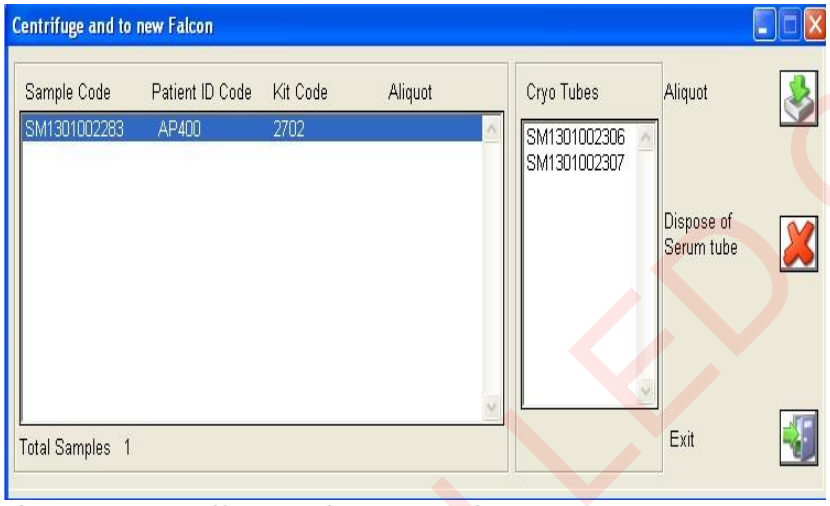


Figure 24: Centrifuge and to New Falcon

When complete select '**Dispose of Serum Tube**'. This will bring you through the screen sequence for disposing of a tube (Figures 10 and 11). Click '**Exit**' once serum has been destroyed to return back to the '**Sample Life Cycle**' screen (Figure 5).

LIMS Entry for Serum Storage:

Select '**Store Location**' and follow instructions as previously described for storing plasma and PBMC (Figure 18 and 9).

5.2.8 Processing of the Sodium Citrate Tube (blue top) 4.5ml:

1. If kit P or Q has been used for sample collection and you have a BD Vacutainer® Sodium Citrate Tube (blue top) 4.5ml to the BD LSR designated member of staff for processing.

5.2.9 Preparation and Storage of Urine:

If urine has been collected, the following steps must be taken.

It is the responsibility of LPRG staff to calibrate the pH meter. Check the calibration sheet next to the pH meter, as to the date of the calibration and record on the checklist sheet.

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 processing worksheet.**
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 **Temporary Storage** box in Freezer 9 for final storage by LPRG staff.

LIMS Entry for Sample Processing:

Select '**pH test**' icon in the Urine section of the '**Sample Life Cycle**' (Figure 5). This will bring up the '**Samples awaiting pH test**' (Figure 25). Select the appropriate Patient ID and select '**Results**' icon. This will bring up the '**Multi Sample/Test Result Entry**' (Figure 26).

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 25). Select to return to the '**Sample Life Cycle**' (Figure 5).

Select the '**Add Protease Inhibitor**' icon which will bring up the '**Add Protease Inhibitor Pellets**' screen (Figure 27). Select the '**Add Protease Inhibitor Pellets**' button, which will bring up another screen '**Add Protease Inhibitor Pellets**' screen (Figure 28). 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 5).

Select the '**Aliquot**' icon. This will bring up the '**Centrifuge and to a New Falcon Tube**' screen (Figure 17). Follow on-screen instructions for aliquotting samples as described previously (Figure 11, 12 and 17).

LIMS Entry for Storage of Urine:

Finally select the '**Store Location**' icon (Figure 18 and 19) and follow instructions as previously described for storing samples.

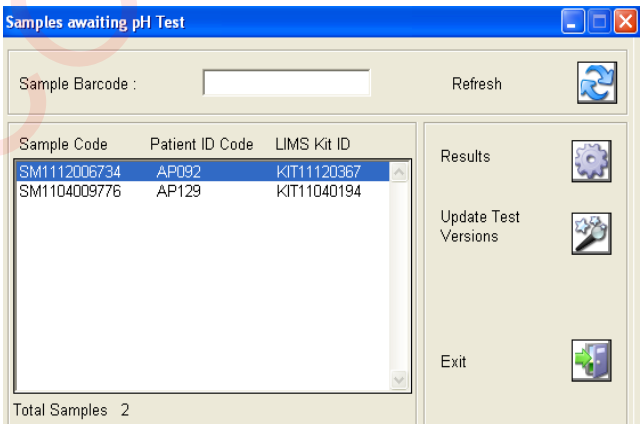


Figure 26: Samples awaiting pH test

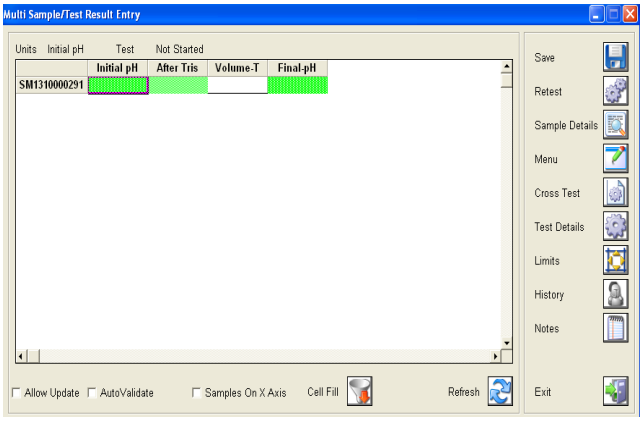


Figure 27: Multi Sample/Test

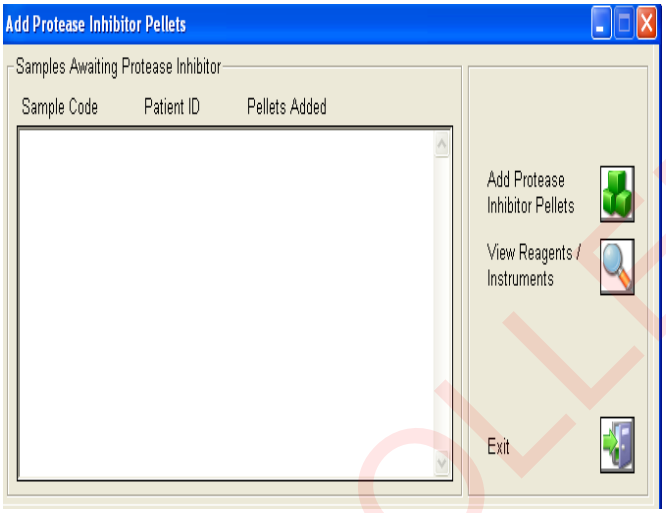


Figure 28: Add Protease Inhibitor

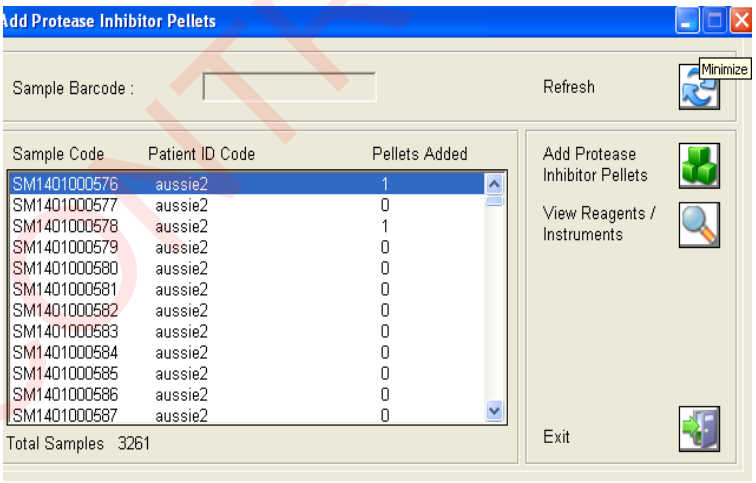


Figure 28: Add Protease Inhibitor Pellets

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 in accordance with the GCLPRPS024: Disposal of Hazardous Waste.

A Sample Destruction Form should be obtained from Document, completed and returned to the QAM with a copy being held in the Acute Pancreatitis Biobank records located in room 3.347 UCD building, Royal Liverpool Hospital.

7. ABBREVIATIONS

BD	Becton Dickinson
BRT	Blood RNA Tube
EDTA	Ethylenediaminetetraacetic acid
GCLP	Good Clinical Laboratory Practice
LPRG	Liverpool Pancreatitis Research Group

8. OTHER RELATED PROCEDURES AND DOCUMENTS

SOPs:

GCLPFAC005/F2	GCLP Sample Destruction Form
GCLPEQU037	Use of Pipettes
GCLPEQU038	Use of Centrifuges in PBRU
GCLPEQU040	Use of PBRU Biological Safety Cabinets
GCLPRPS024	Disposal of Hazardous Waste in the PBRU
GCLPTSS049/F1	Acute Pancreatitis Sample Processing Checklist
GCLPTSS116	Collection of samples for the LPRG acute pancreatitis biobank
GCLPTSS158	Processing of Samples for Leukocyte Phenotyping from PBRU Acute Pancreatitis Biobank
GCLPTSS161	Cell Counting in the PBRU
GCLPTSS162	Bioenergetic Profiling of Leukocytes in Peripheral Blood form PBRU

9. APPENDIX

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

ACUTE PANCREATITIS BIOBANK SAMPLE PROCESSING CHECKLIST

SAMPLE COLLECTION

	Date: **Date:**	**Time:** **Time:**

KIT CODE						**PATIENT CODE (AP NUMBER)**						
KIT TYPE	**A**		**B**		**C**		**P**		**Q**		**S**	

ORIGIN OF SAMPLE		**Sample Time Point:** (tick box, or complete week number)		
RLUH:		**24 hr**	**48 hr**	**Week No.**
Other:				

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

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 -80^{O}C PBRU Freezer 1	
Location of PAXGENE Tube (Freezer, Draw, Box, Co-ordinate)	
Record on PBRU Acute Pancreatitis Biobank section on LIMS (tick box)	

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 :	

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**			
Sodium Citrate (blue)	**4.5 ml**			
Urine collected				

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			%

ANSWER (A) X VOLUME (ml) = TOTAL number of cells

..... X	=	..... X 10^6 ANSWER (B)
Final concentration of cell pellet ANSWER B divide by 5	=	..... X 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 eppendorfs stored: **Number of cells/ eppendorf:** **Location of eppendorfs:** ➢ ➢ ➢ ➢ ➢ ➢ ➢ **Time eppendorfs 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 eppendorfs 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: