		<p align="center"><b>LCTU GCPLab</b></p> <p align="center"><b>Standard Operating Procedure</b></p> <p align="center"><b>PROCESSING OF SAMPLES FOR THE CHRONIC PANCREATITIS BIOBANK AND CONTROLS BIOBANK</b></p>	
<b>SOP Number:</b>	GCLPTSS040/3	<b>Issue Date:</b>	
<b>Effective Date:</b>		<b>Review Date:</b>	

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Revision History				
Version Number	Effective Date	Revision Date	Reason for Change	Change Control No:
1	26/09/2012	15/01/2016	Deletion of processing of Pancreatic juice from surgery and grammatical changes	3861
2	03/03/2016	18/06/2018	Anything pertaining to PBRU has been changed to LPRG & author name changed	8361

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## **1. WHO?**

This Standard Operating Procedure (SOP) applies to all designated Clinical Research Fellows, and to GCLP Technicians and Liverpool Pancreatitis Research Group (LPRG) staff trained to process samples for the Chronic Pancreatitis Biobank and the Controls Biobank.

## **2. BACKGROUND**

The Chronic and Control biobanks involves the collection and storage of human pancreas tissues for future, ethically approved, translational research. This involves Research Fellows, GCLP Laboratory staff and LPRG staff working as a team, to ensure that samples are collected according to Good Clinical Practice principles, legislation, only after full, informed consent is obtained and the rights and well-being of the patient is of paramount importance. The samples should be processed in a timely manner, the chain of custody maintained and the integrity of the sample assured. The samples should be stored to ensure that they can be accessed when needed for ethically approved future work.

## **3. PURPOSE**

This SOP describes the processing of samples donated by patients for storage in the Chronic Pancreatitis Biobank.

## **4. SCOPE**


This SOP applies to the designated Clinical Research Fellows, the GCLP Technicians and the LPRG staff and staff members delegated by the GCLP Operational Director to receive and process the sample. The sample may be processed either in the GCLP Facility or in the LPRG laboratory.

## **5. PROCEDURE**

### **5.1 RESPONSIBILITY**

- The appropriate technician should be informed of any planned sampling prior to the patients being approach, to ensure they are available for processing.
- It is the responsibility of the Clinical Research Fellows or Research Nurses to transfer the samples to the appropriate staff member processing the samples.
- If the sample(s) are ready for processing when the member of staff to process the sample is unavailable, then the sample can be left for up to 3 hours before the sample is processed from the time it was taken. If the designated member of staff is still not going to be available within the 3 hours, then it is the responsibility of the delegated (by the GCLP Operational Director) Research Fellow or Researcher to process the samples according to this SOP.
- It is the responsibility of the person processing the samples to follow the associated local SOPs depending on whether the samples are processed in the GCP Lab Facility or LPRG laboratory.

## 5.2 PROTOCOL

1. The person responsible for processing should be informed before samples are obtained to allow them to organise the time for processing, and once taken that individual should be sought to directly receive the samples.
2. The SARSTEDT Monovette Serum Z (9 ml) tube should have been immediately inverted by the research fellow 10 times after being taken. The processor must ensure that at least 30 minutes has elapsed since the time it was taken.
  - Check the sample sheet to ensure this has been done. If not the processor should invert the tube 10 times and leave the serum tube in an upright position for at least 30 minutes before processing.
3. All processes must be recorded on the LIMS (Laboratory Information Management System).
4. Login to LIMS (**SOP: Use of the LECMC GCLP LIMS**), select:
  - Select Matrix Plus icon
  - Select **'Access'**
  - Select Login, complete Login process
  - Select **'Live'**
  - Select **'Trials and Studies'**, from the drop-down menu, select **'Panc Studies'**, select **'Panc Rota'**
  - Select **'-80 degree'** icon , from the Panc Rota Workflow
    - The Sample Life Cycle screen for the Pancreas Rota Blood and Urine Workflow will appear (Figure 1).

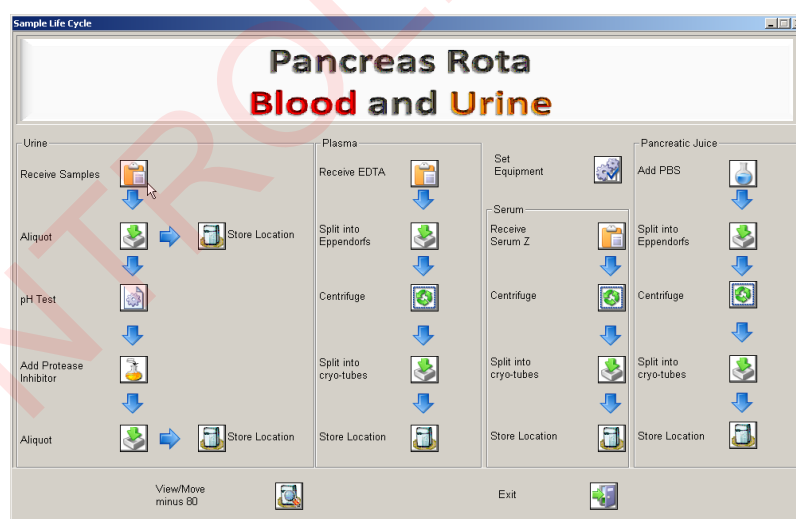

















Figure 1 – Pancreas Rota Blood and Urine Workflow

5. If a serum sample has been received, select the **'Receive Serum Z'** icon  in the Serum Workflow. Check the sample on the screen has the same ID as your tube and highlight it by clicking on it.
6. Select the **'Choose Hood'** icon  and select the class II cabinet in which the samples will be processed from the drop-down menu, and click on the **Ok'** icon . Press the **'Receive'** icon  which will cause the sample to disappear from the list. Click on the **'Exit'** icon  to leave the screen.

7. If an EDTA sample has been received, select the **'Receive EDTA'** icon  in the Plasma Workflow (Figure 1). Click on the sample having verified that the sample code on the label was the sample code on LIMS. Select the sample. Select the **'Choose Hood'** icon  and select the cabinet in which the samples will be processed, and click the **'Ok'** icon . Click the **'Receive'** icon  and close the window by clicking the **'Exit'** icon .
8. If a urine sample has been received, select the **'Receive Samples'** icon  on the urine work flow. Select the sample, checking the codes and click on the **'Samples Received'** icon . **'Exit'**  the screen.
9. Either log off LIMS or carry on with processing the sample or leave LIMS open if processing is carried out in the same laboratory.

## 5.2.1 Processing of Serum:

The 9ml SARSEDT Monovette Serum Z 9ml tube should be centrifuged at 2000rcf for 10 minutes at 20°C opposite an appropriate balance tube.

1. While the centrifuge is running, log on to LIMS and go to the Pancreas Rota work flow as before.
2. Select the **'Centrifuge'** icon  from the Serum Workflow, the **'Samples awaiting preparation'** screen will appear, (Figure 2) select **'Centrifuge'** . The **'Select Instrument'** screen will appear (Figure 3), select the centrifuge to be used and click on the **'Add Sample'** icon.

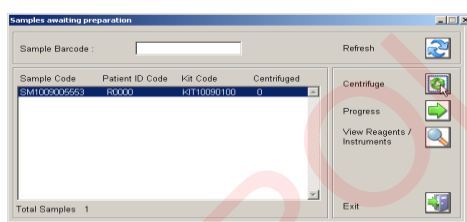


Figure 2 – 'Samples awaiting preparation' screen

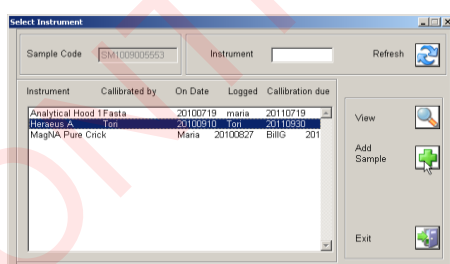




Figure 3 – 'Select Instruments' screen

3. The **'Add Reagent'** screen (Figure 4) will appear, Click on the **'Centrifuge'** icon the **'Samples Awaiting Preparation'** screen will appear (Figure 5). Select the **'Progress'** icon  then exit by selecting the **'Exit'** icon .

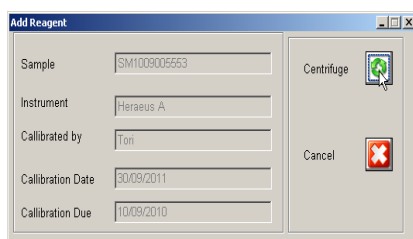


Figure 4 – 'Add reagent' screen

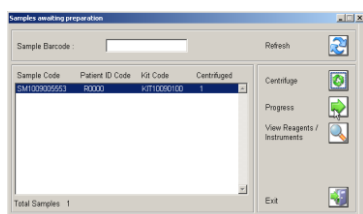




Figure 5 – 'Samples awaiting preparation' screen

4. Once the centrifugation is complete the serum should be aliquoted into a maximum of 4 x 1.8ml NUNC cryovials with white caps **note the sample number of the cryovials used (e.g. in the example below SM1009005559)**.
5. Leave the cryovials at room temperature in a rack.
6. Return to LIMS, Select the '**Split into Cryo-tubes**' icon . Select the '**Aliquot into Cryovials**'  icon in the '**Aliquot serum into cryovials**' screen (Figure 6).

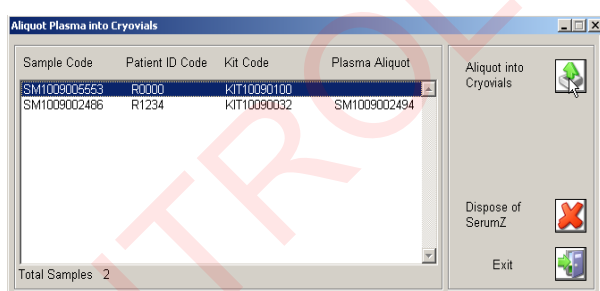




Figure 6 – 'Aliquot serum into cryovials' screen

7. The '**Storage location screen**' will appear, select the numbered cryovial and select the '**Aliquot**' icon . The '**Receive Cryo Tubes**' screen will appear (Figure 7), highlight the sample by clicking on it and choose on the '**Aliquot to Cryovials**' icon, and the current day's date will appear in the '**Date Aliquoted**' section. Click on '**Exit**'  to close the screen.

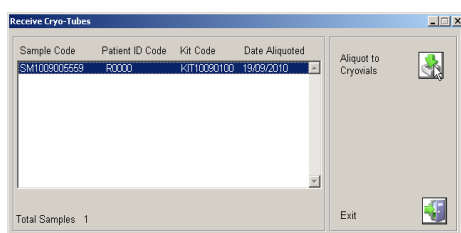


Figure 7 – 'Receive Cryo-Tubes' screen

8. Destroy the now empty SerumZ tube according to the **SOP: Disposal of Hazardous Waste**.
9. Select the **'Split Cryo-tubes'** icon from the main Serum Workflow, and select the dispose of **'SerumZ'** icon ✖, bringing up the **'Destroy Samples'** screen. Select the correct sample and click the **'Destroy Samples'** icon ✖. The **'Dictionary Maintenance'** screen (Figure 8) will appear with a text statement guaranteeing the destruction of the tube. Click on the **'Ok'** icon ✔.

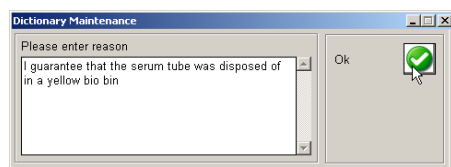


Figure 8 - 'Dictionary Maintenance' screen

10. Select the **'View/Move minus 80'** icon 🧊 from the bottom of the Workflow.
11. Check the next available space from the list (Figure 9).

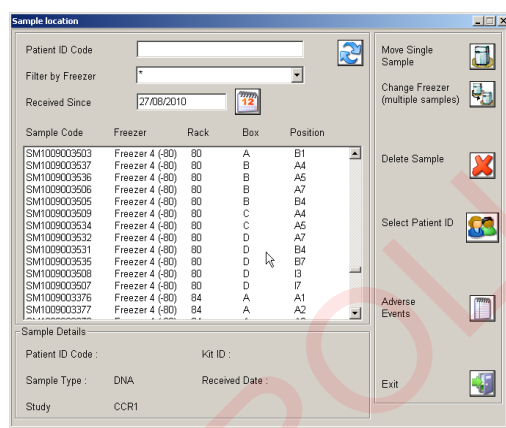


Figure 9 - Freezer List

12. Select the **'Store Location'** icon 🧊 in the main workflow (Figure 1). On the **'Storage location'** screen (Figure 10) complete the drop-down menu to log the locations of the stored samples on LIMS. Select the **'Ok'** icon ✔. Note locations stored for each sample. When all samples stored, are logged into the LIMS system, click on the **'Exit'** icon.
13. Store the samples in the -80°C freezer in the GCLP Facility (room 3.373) in the locations specified on LIMS.

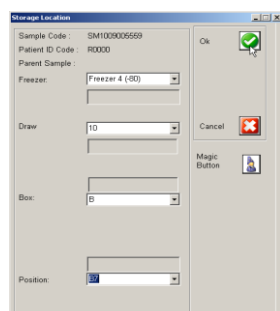




Figure 10 – 'Storage location' screen

## 5.2.2 Processing of Plasma:

1. The 9 ml of blood in a SARSTEDT Monovette EDTA K2 9 ml tube should be inverted 10 times to evenly mix the sample and then be aliquoted out into 6 x 1.5ml eppendorf tubes (~1ml per eppendorf). The Eppendorf tubes should be spun at 16000rcf in the centrifuge for 1 minute.
2. The plasma should be carefully removed from the pellets into a maximum of 6 x 1.8ml NUNC cryovials with red caps.
3. Click on the **'Split into Eppendorfs'** icon  (see Figure 1), the **'Aliquot into Eppendorf'** screen will appear (Figure 11), select the sample and select the **'Aliquot into Eppendorfs'** icon .

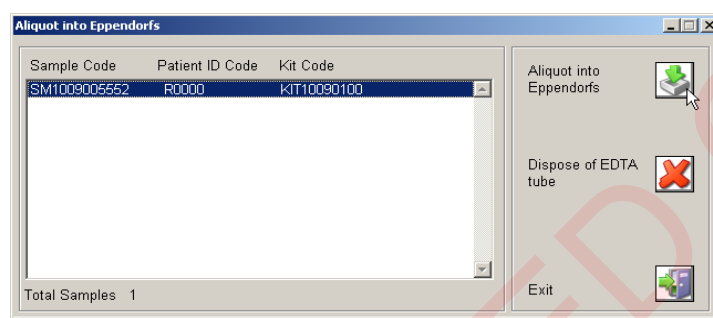


Figure 11- **'Aliquot to Eppendorfs'** screen

4. Each eppendorf code will appear, verify that the codes are correlate, select the **'Aliquot into Eppendorfs'** icon  and today's date will appear in the screen (Figure 12). Click on the **'Exit'** icon .

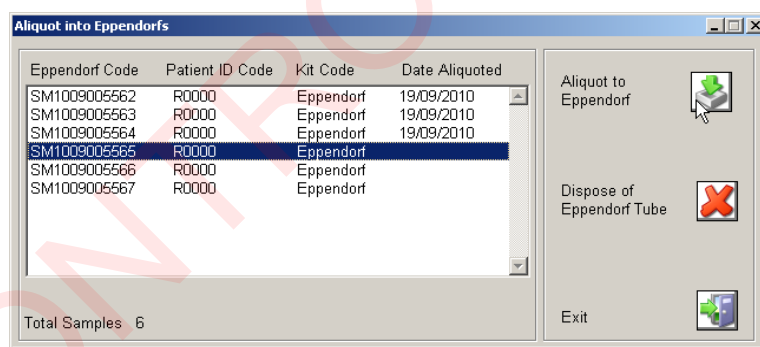






Figure 12 – Selection of each sample aliquot and the date aliquoted

5. Select the **'Centrifuge'** icon  from the Plasma Workflow, making the **'Samples Awaiting Preparation'** screen appear. Highlight the sample to be centrifuged by clicking on it and select the centrifuge icon  (Figure 13). This will cause **'Add Reagent'** screen (Figure 4) to appear to match the centrifuge to the sample. Select the **'Centrifuge'** icon. A number 1 will appear on the **'Samples Awaiting Preparation'** screen indicating the sample has been centrifuged. For each sample centrifuged and logged, click on the **'Progress'** icon  and then **'Exit'** .



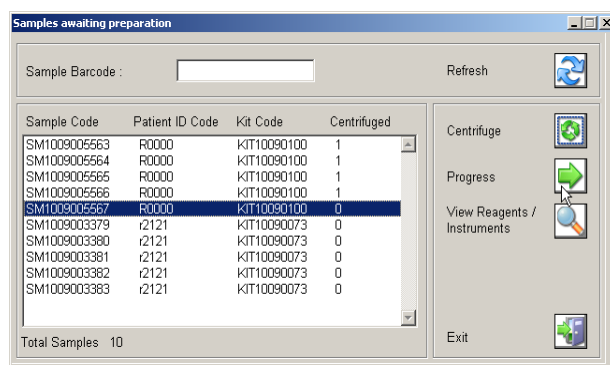




Figure 13 - 'Samples Awaiting Preparation' screen

6. The supernatant from each eppendorf should be aliquoted into a corresponding red topped NUNC, and this should be logged on LIMS by selecting the '**Split into Cryo-tubes**' icon  from the Plasma Workflow on the main screen (Figure 1). The '**Aliquot Plasma into Cryovials**' screen (Figure 14) will appear, click on the sample to be aliquoted and select the '**Aliquot into Cryovials**' icon .

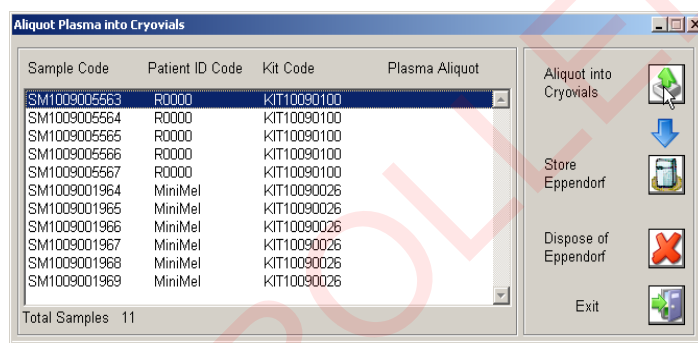




Figure 14 – 'Aliquot Plasma into Cryovials' screen

7. The '**Storage Location**' screen (Figure 15) will appear showing the cryovial codes, select the cryovial and click on the '**Aliquot**' icon .
8. The '**Receive Cryovials**' screen will appear, (Figure 16) double click on the '**Aliquot to Cryovials**' icon  and today's date will appear showing the date that the sample was aliquoted. Repeat this for all the samples.

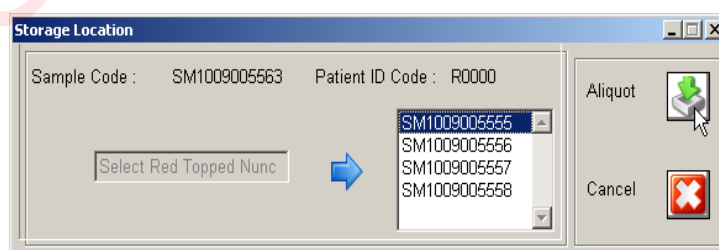


Figure 15 - 'Storage location' screen

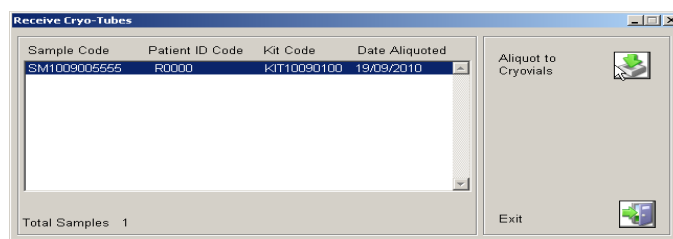






Figure 16 'Receive Cryo Tubes' screen

9. Select the '**View/Move minus 80**' icon  from the main Workflow and note down the next free space in the freezer.
10. Return to the '**Aliquot Plasma into Cryovials**' screen (Figure 14). Click on the '**Store Eppendorf**' icon . Select the freezer storage locations for all the eppendorfs from the drop-down menu (Figure 10).
11. Select the '**Store Location**' icon , the '**Store Cryo-tubes**' screen will appear (Figure 17), select the cryovial to be stored then click on the '**Store Locations**' icon . Log the positions of all the samples in the drop-down menu (Figure 10).

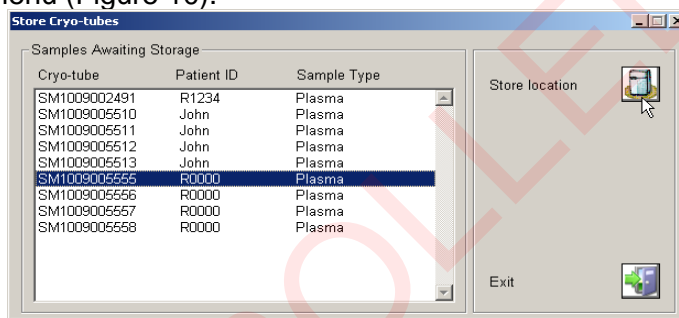





Figure 17 'Store Cryo-tubes' screen

12. All eppendorf tubes and NUNC tubes should be stored at -80°C in the locations stored in LIMS.
13. Dispose of the empty EDTA tube and return to the '**Aliquot of Eppendorfs**' screen from the Plasma Workflow, select the '**Dispose of EDTA tube**' icon  and follow the prompts to record the disposal of the EDTA tube (similar to Figure 8) according to the **SOPs: GCLPRPS024**.

### 5.2.3 Processing of Urine:

1. Transfer 10ml from the urine pot to falcon A (for subsequent pH testing and adjustment see below). Up to 50ml should be transferred into a pre-labelled falcon tube, T.
2. Go to the Sample Lifecycle screen (Figure 1). Select the first '**Store Location**' icon  (shown in the blue square Figure 18). The '**Store Location**' screen will appear (Figure 19). Click on the sample to be stored and then select the '**Store Location**' icon .

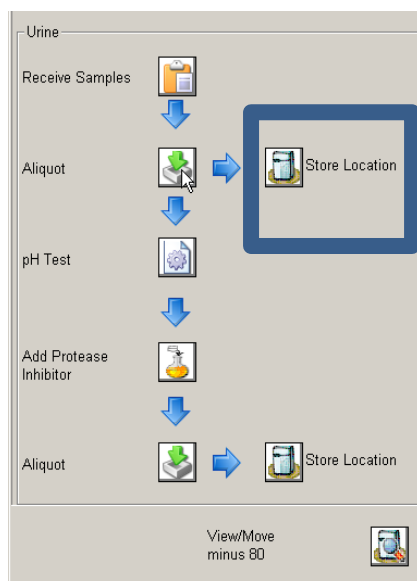


Figure 18 - Showing the correct 'Store Location' icon to select to store Falcon tube T

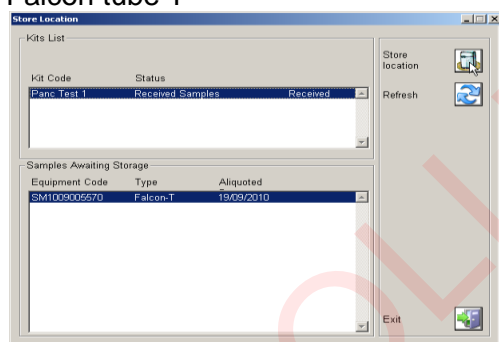




Figure 19 – 'Store Location' screen for Falcon tube T

3. The 'Storage Location' screen will appear select the freezer, box (UT number) and position to log the falcon storage then click on the 'Ok' icon and then exit by selecting the 'Exit' icon.
4. Store the Falcon tube T at -80°C in the designated box and location as set on LIMS.
5. Select the 'Aliquot' icon from the Lifecycle Workflow  the 'Combine Aliquots' screen will appear (Figure 20). Select the urine sample then click on the 'Add 10 mls of urine to Falcon-A (for aliquoting)' icon .


Sample Code	Sample Type	Patient ID	Sample Taken
SM1009001971	U-Pot	MiniMel	
SM1009002564	U-Pot	Clowis	
SM1009002701	U-Pot	MacsAreShite	
SM1009005569	U-Pot	R0000	19/09/2010

Sample Details

Patient ID Code : R0000      Parent Sample :

Sample Type : U-Pot      BatchCode : KIT10090100







Figure 20 'Combine Aliquots' screen

- On the 'Aliquot into Falcon' screen (Figure 21) add the volume taken (10mls), select 'Aliquot to Falcon' icon , and the date aliquoted will appear. Select 'Exit' to return to 'Combine Aliquots' screen.

Eppendorf Code	Patient ID Code	Kit Code	Date Aliquoted
SM1009005571	R0000	KIT10090100	

Volume taken: 10 mls

Figure 21 – 'Aliquot to Falcon' screen

- Select the  icon 'Add rest of urine to Falcon-T (for storage)'. A similar screen to Figure 21 will appear, complete the volume taken box (usually 50ml) and select the 'Aliquot to Falcon' icon , the date aliquoted will appear. Click on the 'Exit' icon to leave the Workflow.
- Select the 'Aliquot' icon on the 'Sample Lifecycle' screen , then select the sample and click on the 'Dispose of Urine Pot' icon , the 'Destroy Samples' screen will appear (Figure 22). Click on the 'Destroy Samples' icon , the 'Dictionary Maintenance' screen will appear (similar to Figure 8). Click on the 'Ok' icon  to confirm that the urine pot has been destroyed according to the **SOP: Disposal of Hazardous Waste**. Click on the 'Exit' icon on the 'Destroy Samples' screen to close the window.

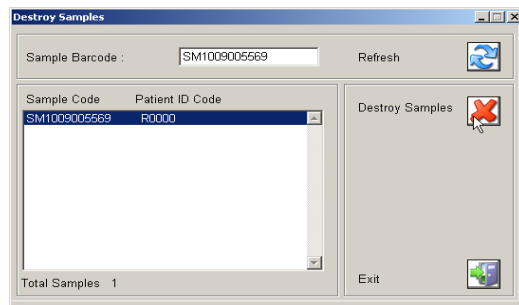



Figure 22 - 'Destroy Samples' screen

9. The pH should be balanced to greater than 7, but no higher than pH 8 using 1M Tris-HCl buffer (pH8) located next to the pH meter.
10. Select the '**pH Test**' icon on the Sample Lifecycle screen (Figure 1) . The '**Samples Awaiting pH Test**' screen should appear (Figure 23).

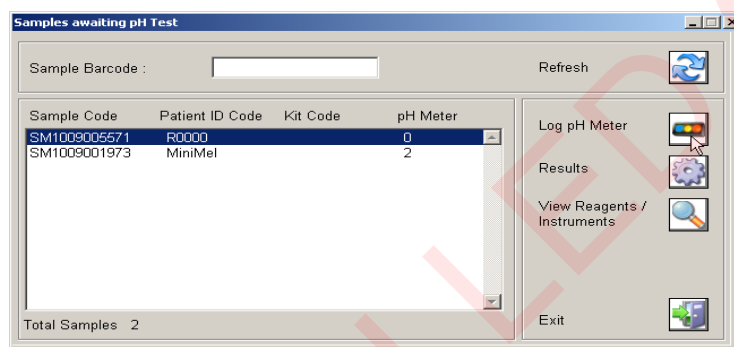




Figure 23 – 'Samples awaiting pH Test' screen

11. Select the sample to be pH tested. Select the '**Log pH meter**' icon  and set the date of calibration and the instrument used from the drop-down menu. Select the '**Results**' icon , the '**Multi Sample/Test Result Entry**' screen will appear (Figure 24).

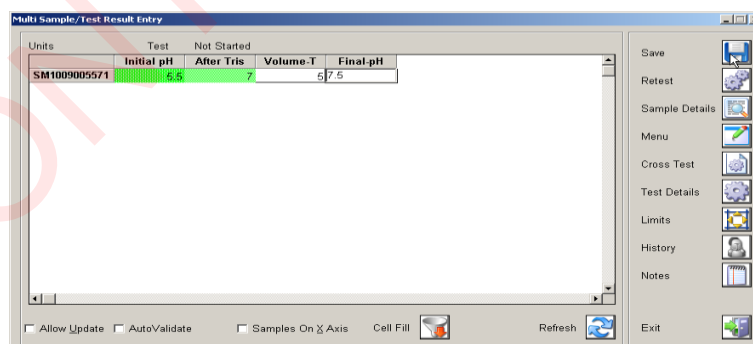







Figure 24 - 'Multi Sample/ Test Result Entry' screen

12. When all boxes are completed, click on the '**Volume T**' box the '**Final pH**' box should then be filled with green. Click on the '**Cross Test**' icon , click on the '**Final pH**' number, select '**Save**' icon , the sample should disappear from the screen. Click the '**Exit**' icon to leave the Workflow.

13. Once the pH of the urine is balanced an EDTA-free protease inhibitor tablet should be added and the tube agitated until fully dissolved.
14. The **'Add Protease Inhibitor'** icon should be selected from the Sample Lifecycle Workflow , the **'Add Protease Inhibitor Pellet'** Screen (Figure 25), will appear. Select sample and select the Protease Inhibitor Pellets icon . The **'Add Protease Inhibitor Pellets'** batch control screen will appear (Figure 26). Complete the table and click on the **'Save'** icon .

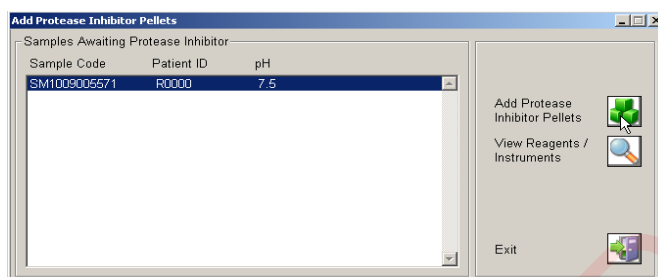


Figure 25 - **'Add Protease Inhibitor Pellets'** screen

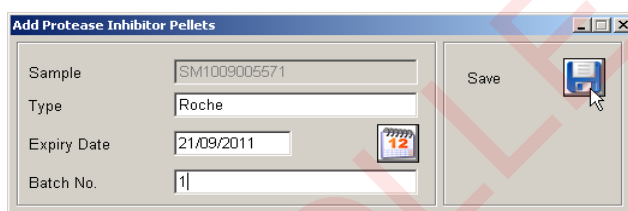





Figure 26 – **'Add Protease Inhibitor Pellet'** batch control screen

15. The pH balanced urine then should be aliquoted into 6 labelled 1.8ml NUNC tubes (so that there is an equal volume of urine per tube) and stored at -80°C.
16. The **'Aliquot'** icon should be selected from the main Urine Workflow , the **'Aliquot into Cryovials'** screen will pop up (Figure 27). Select the sample then select the **'Aliquot into Cryovials'** icon . The list of cryovials to store the urine will appear (Figure 28) and the sample kit code. Select a cryovial and click on the **'Aliquot to Cryovials'** icon , the date aliquoted will appear.

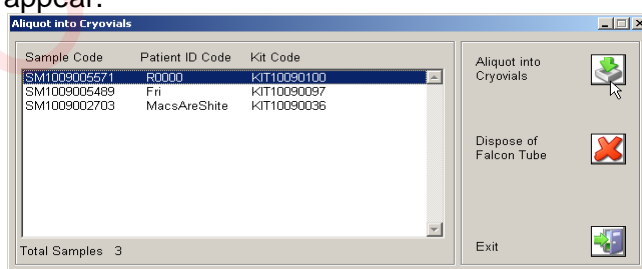


Figure 27 - **'Aliquot into Cryovials'** screen

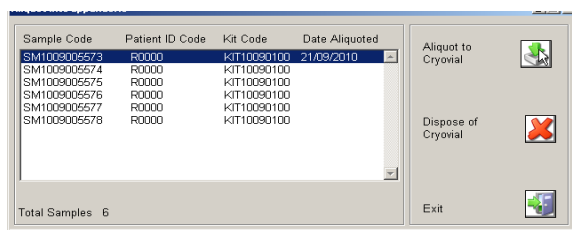







Figure 28 - 'Aliquot into selected Cryovials' screen

17. Return to the '**Aliquot to Cryovials** screen'. Confirm the destruction of Falcon tube A, (Figure 21) by clicking on the '**Disposal of Falcon Tube**' icon . Select the '**Destroy Samples**' icon  on the '**Destroy Samples**' screen. Then click on the '**Ok**' icon  on the '**Dictionary Maintenance**' screen (similar to Figure 8).
18. Select the '**Store Location**' icon at the end of the Urine Workflow . The '**Store Cryo-tubes**' screen will appear. Select a cryovial to store and click on the Store location icon . The -80°C freezer '**Storage Location**' screen will appear, log the location of the sample in the freezer (and all subsequent urine samples) by selecting the freezer, drawer, box and position (Figure 10).

#### 5.2.4 Completing the Pancreas Biobank Database:

Please refer to the **SOP: Data Entry for Chronic Pancreatitis Database** for the completion of the relevant database to record the information on the samples taken.

## 6. ABBREVIATIONS

<b>LPRG</b>	Liverpool Pancreatitis research group
<b>GCP</b>	Good Clinical Practice
<b>GCLP</b>	Good Clinical Laboratory Practice
<b>EDTA</b>	Ethylenediaminetetraacetic acid
<b>LIMS</b>	Laboratory Information Management System

## 7. OTHER RELATED PROCEDURES AND DOCUMENTS

### SOPs:

GCLPEQU010	Use of the Heraeus 3SR+ Refrigerated Bench Top Centrifuge
GCLPEQU038	Use of Centrifuges in the PBRU
GCLPRPS011	Use of the LIMS to Record Kit Construction, QC, Storage and Dispatch
GCLPRPS024	Disposal of Hazardous Waste in the PBRU
GCLPTSS047	Sample Collection, Reception and Tissue Storage for the Pancreas Bank