
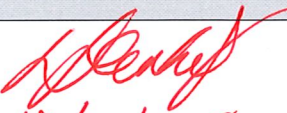
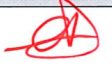
		<p align="center"><b>LCTU GCPLab</b></p> <p align="center"><b>Standard Operating Procedure</b></p> <p align="center"><b>PROCESSING OF SAMPLES FOR THE PANCREAS BANK</b></p>	
SOP Number:	GCLPTSS048/5	Issue Date:	11.12.18
Effective Date:	01.01.19	Review Date:	

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Revision History				
Version Number	Effective Date	Revision Date	Reason for Change	Change Control No:
1	25/10/2010	12/11/2012	Section 5.2.2 LIMS section simplified & processing of tissue added	2408a
2	19/02/2013	23/12/2014	The addition of 0.1M HCL to urine samples with high pH	3731
3	25/03/2015	02/05/2017	Minor grammatical & spelling changes. Removal of point 2 of section 5.2.1 & amended 'sterile' to 'clean' on section 5.2.5 point 3	6569
4	02/06/2017	20/11/2018	Addition of PAXgene Biopsy instructions	9006

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## **1. WHO?**

This Standard Operating Procedure (SOP) applies to all designated Clinical Research Fellows, and to GCP Technicians trained to process the samples from the 'Panc Rota' and store them in the Pancreas Bank.

## **2. BACKGROUND**

A major activity of the Department of Molecular and Clinical Cancer Medicine is the collection and storage of human pancreas tissue for future, ethically approved, translational research. This involves the Research Fellows and the GCP Laboratory staff working as a team, to ensure that samples are collected according to GCP 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 Pancreas Cancer Biobank.

## **4. SCOPE**

This SOP applies to the designated Clinical Research Fellow, and the GCP Technician delegated to receive and process the sample.

## **5. PROCEDURE**

### **5.1 RESPONSIBILITY**

- It is the responsibility of the Clinical Research Fellows to transfer samples to be processed to the GCP Freezer Room.
- It is the responsibility of the GCP Technician having received the sample before 5pm from the Research Fellow to process and store the samples according to this SOP.
- If the sample(s) are ready for processing either after 5pm or the GCP Technician is unavailable to process the sample(s) then it is the responsibility of the Research Fellow to process the samples according to this SOP.

### **5.2 PROTOCOL**

#### **5.2.1 Receipt of First Batch of Samples:**

1. The person responsible for processing will check the GCP Freezer Room at 09:30 for blood and urine samples.
2. The SARSTEDT Monovette Serum Z (7.5ml) 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.

3. All processes must be recorded on the LIMS (Laboratory Information Management System).

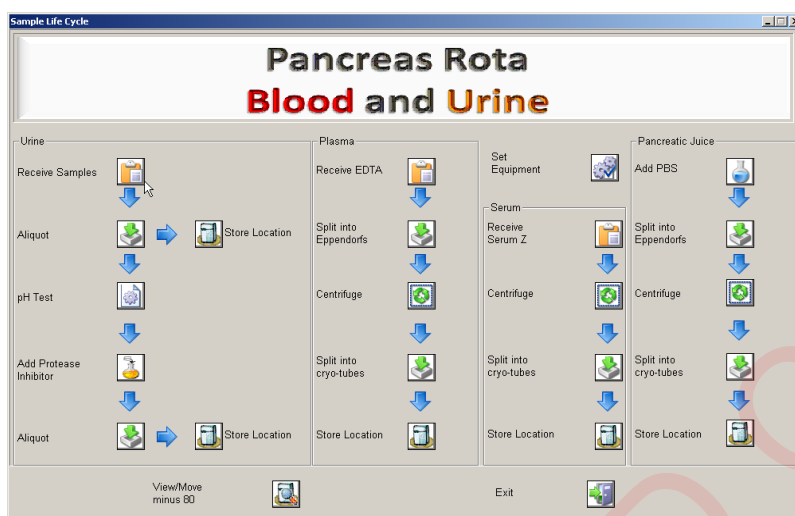


Figure 1: Pancreas Rota Blood and Urine Workflow

## 5.2.2 Processing of Serum:

1. Receive the serum tube on the LIMS and select the correct hood.
2. The SARSTEDT Monovette Serum Z 7.5ml tube should be centrifuged at 2000rcf for 10 minutes at ambient temperature in a centrifuge opposite an appropriate balance tube.
3. Centrifuge the sample on LIMS, ensure the correct centrifuge is selected (figure 2) and progress the sample (figure 3).

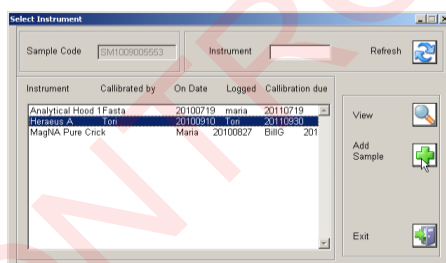


Figure 2: 'Select Instruments' screen

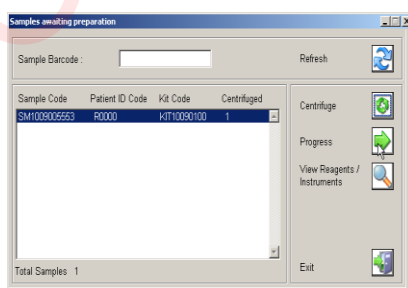


Figure 3: 'Samples awaiting preparation' screen

4. Once the centrifugation is complete the serum should be aliquoted into 4 x pre-labelled 1.8ml Nunc cryovials with white caps. Aliquot 1ml into each Nunc, if more than 4ml of serum available top up Nuncs. If less than 4ml is available dispose of empty Nuncs in accordance with local practice.

5. Leave the Nuncs at room temperature in a rack.
6. Return to the LIMS, select the 'Split into Cryo-tubes' icon and click on the appropriate cryovials/nuncs to aliquot.

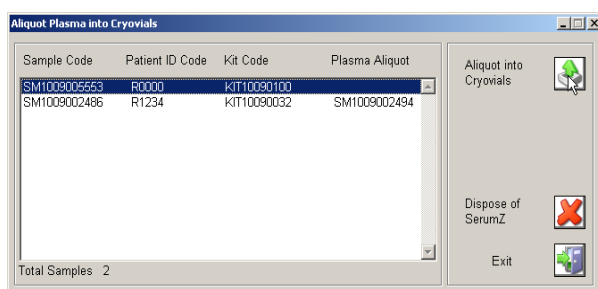


Figure 4: 'Aliquot serum into cryovials' screen

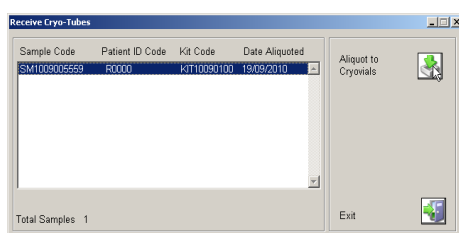


Figure 5: 'Receive Cryo-tubes' screen

7. Destroy the now empty Serum Z tube according to the SOP GCLPRPS004 Disposal of Hazardous Waste and confirm this on the LIMS.

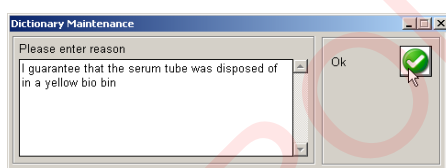


Figure 6: 'Dictionary Maintenance' screen

8. Store the white capped nuncs in the next available space at -80°C and record this on LIMS.

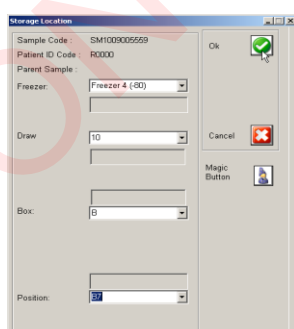


Figure 7: '-80°C Freezer Storage Location' screen

### 5.2.3 Processing of Plasma:

1. Invert the SARSTEDT Monovette EDTA K2-Gel 7.5ml tube 10 times to evenly mix the sample.
2. Aliquot the blood into 6 x 1.5ml Eppendorf tubes (~1ml per Eppendorf). The Eppendorf tubes should be spun at 16000rcf for 1 minute. Record this on LIMS, confirming the disposal of the EDTA tube.

3. The plasma should be carefully removed, without disturbing the cell pellet, and placed into 6 x pre-labelled 1.8ml nunc cryovials with red caps. Ensure the plasma from each Eppendorf is aliquoted into the correct nuncs on LIMS.
4. All Eppendorf tubes and nunc tubes should be stored in the next available space at -80°C and the locations recorded on LIMS.

## 5.2.4 Processing of Urine:

1. Transfer 10ml from the urine pot to falcon A (for subsequent pH testing and adjustment). Up to 50ml should be transferred into falcon T.
2. Record this on LIMS, note the amount of urine added to each falcon and then confirm disposal of the urine pot.

Figure 8: 'Combine Aliquots' screen

Figure 9: 'Aliquot to Falcon' screen

3. Calibrate the pH meter prior to use and note this on the correct form in the folder in the GCLP lab.
4. Test the pH of the urine in falcon A and adjust it to greater than pH 7 but no higher than pH 8 using 1M Tris-HCl buffer (pH8) located next to the pH meter.
5. If the pH of the urine is greater than pH 8, adjust the pH by the addition of 10µl drops of HCl (1M).
6. To create the 1M HCl, dilute the 10M HCL stock solution and store in a glass reagent bottle. (Avoid spillages and breathing in the fumes by using the HCL in the fume hood). Label the bottle with; contents, date and initial.
7. Agitate and measure pH between drops, stopping when pH falls below pH 8.



8. Record this on LIMS:

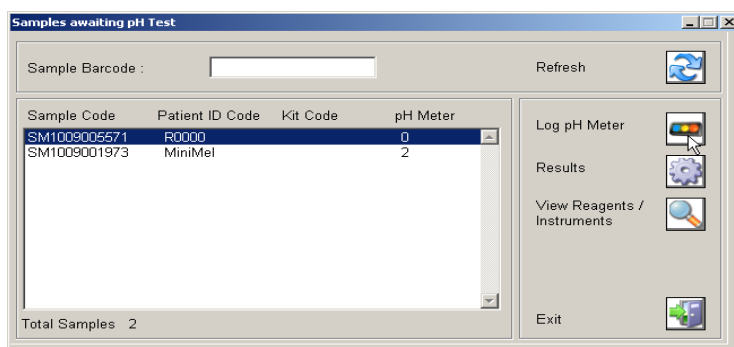


Figure 10: 'Samples awaiting pH Test' screen

Select the sample to be pH tested. Set the date of calibration and the instrument used.

Log results using the 'results' button.

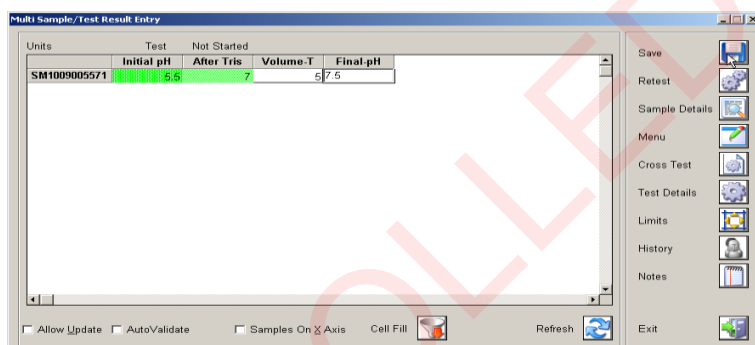


Figure 11: 'Multi Sample/Test Result Entry' screen

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', select 'Save', the sample should disappear from the screen.

9. Once the pH of the urine is balanced, a protease inhibitor tablet should be added and the tube agitated until fully dissolved.
10. Log this on LIMS recording the expiry date and batch number of the protease inhibitor tablets.

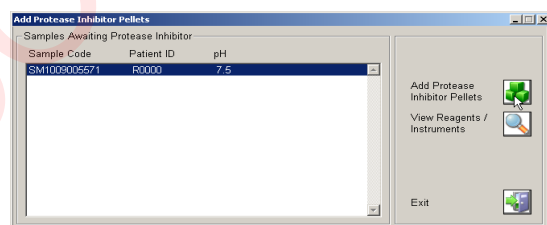


Figure 12: 'Add Protease Inhibitor Pellets' screen

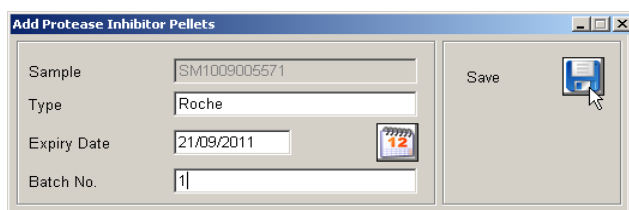


Figure 13: 'Add Protease Inhibitor Pellet' batch control screen

11. The pH balanced urine should then be aliquoted into 6 labelled 1.8ml nunc tubes with green inserts. Add 1.5ml of urine to each nunc. Log this on LIMS and confirm disposal of falcon tube.
12. Store the nunc tubes and falcon T in the next available spaces at -80°C and log these locations on LIMS using the two different store location buttons.

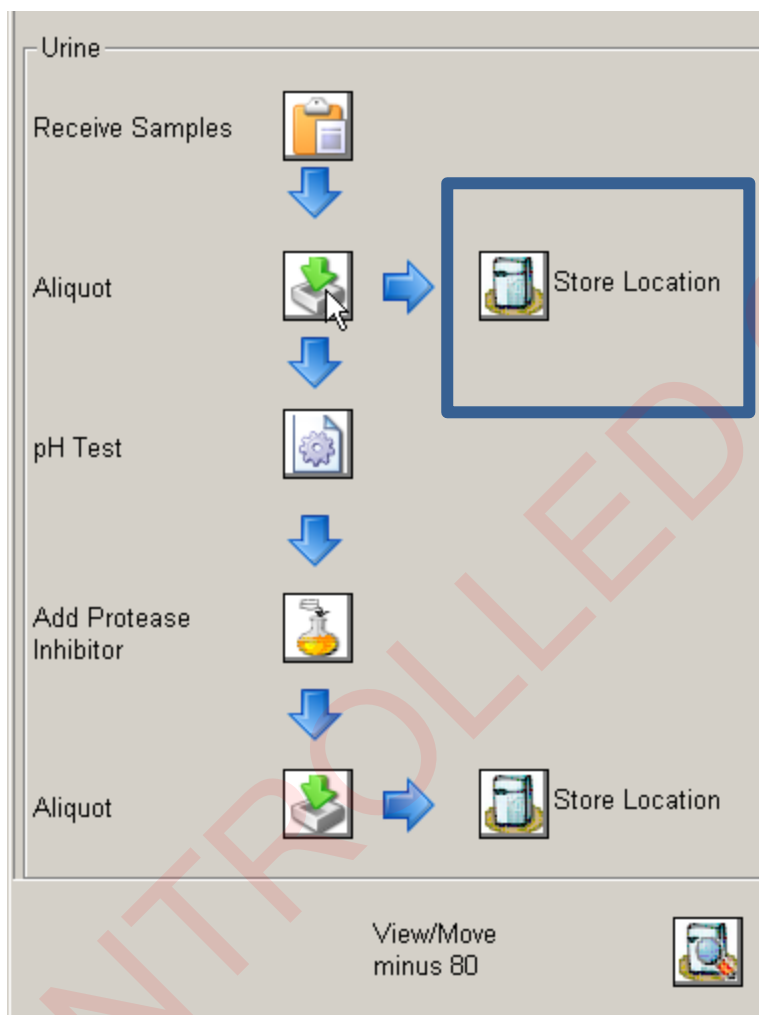


Figure 14: The correct 'Store Location' icon to store Falcon tube T

#### 5.2.5 Processing of Pancreatic Juice from Surgery:

1. If the juice is obtained during surgery it will be delivered directly to the GCP Freezer Room by the Research Fellow and the GCP Technician will be informed.
2. The designated sample processor must process the pancreatic juice sample as soon as possible after being informed that the sample has been taken and is ready for processing.
3. Clean scissors by wiping with 70% EtOH. Cut off the knot at the end of the plastic tube attached to the syringe.
4. If there is a volume of pancreatic juice less than 0.5ml in the syringe then gently aspirate 1ml of Dulbecco's sterile PBS into the syringe and dispense the whole volume of liquid into the 1.5ml pre-labelled Eppendorf.
  - If there is greater than 0.5ml of pancreatic juice then dispense directly into the pre-labelled Eppendorf, (if additional eppendorfs are needed refer to SOP 'Additional Samples for Pancreas Rota'.



- Record this on LIMS logging the PBS batch used and the estimated volume added to the eppendorf.
- Centrifuge eppendorf at 4000rcf for 5 minutes in a microcentrifuge.
- Record this on LIMS noting the centrifuge used and then 'progress' the sample.

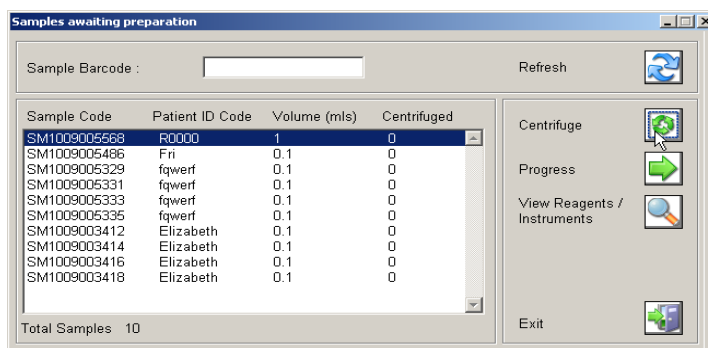


Figure 15: 'Samples awaiting preparation' screen for pancreas juice

- Once the eppendorf has been centrifuged, carefully remove the supernatant and place in a 1.8ml nunc. **DO NOT DISCARD THE EPPENDORF** – even if there is no visible pellet.
- Record this on LIMS ensuring the date aliquoted is correct.
- Store the eppendorf and nunc tubes in the next available space at -80°C and record this on LIMS.

#### 5.2.6 Receipt and Storage of Tissue:

- Carefully pour liquid nitrogen into the designated flask.
- Aliquot 1ml of isopentane into each of the pre-labelled nunc-Ts.
- Leave the liquid nitrogen flask and the nunc-Ts in the freezer room ready for the Research Fellow to take to surgery.
- When the Research Fellow brings the tissue back from surgery, remove nuncs from the liquid nitrogen and store in the next available space at -150°C.
- Log the locations on the LIMS indicating which sm number relates to which tissue type (blue capped: tumour, grey capped: true cuts, white capped: normal, yellow capped: duodenum, red capped: pancreas neck, green capped: CBD).
- If the GCP technician is not available the Research Fellow should take the nuncs out of liquid nitrogen and place them in a temporary storage box at -150°C. The GCP technician will store the tissue samples in their correct space and note this on the LIMS at the next available time.

#### 5.2.7 Receipt and Shipment of Paxgene Tissue:

- Leave the PAXgene tube with the liquid nitrogen flask in the freezer room ready for the Research Fellow to take to surgery.
- When the Research Fellow brings the tissue back from surgery the tissue should remain in the PAXgene Tissue FIX for 8-24 hours.
- After this time the PAXgene Tissue FIX should be discarded, according to local guidelines, and replaced with PAXgene Tissue Stabilizer.
- This can be kept at ambient temperature for up to 7 days, if it is kept on site any longer than 7 days the sample should be stored at -150 oC.

5. This will be sent on to Sanger institute by the GCP Technician responsible via first class post no later than the following day.
6. The Paxgene sheet and LIMS should be filled out by the GCP Technician sending the sample with the required information.
7. On receipt at the Sanger institute the sheet should be filled out and faxed/e-mailed back to the GCP Technician to confirm sample receipt.

#### **5.2.8 Completing the Pancreas Bank Database:**

1. Open the 'Names' database and enter the password.
2. Check using the Surname, Forename and D.O.B. that the patient is not already registered.
3. Using the 'Patient data sheet for pancreatic research' form complete the 'Names' database with the relevant details.
4. Close the 'Names' database.
5. Open the 'Anonymous' database and enter the password.
6. Open the 'Query Pancreas Rota'.
7. Complete the row, entering all available data in the fields detailed below. Complete as much as possible.
  - Paired (to be filled in when both tissue and blood is received, if only a single type of sample e.g. blood is taken then state the type of sample)
  - Author
  - Time/Date sample taken
  - Time/Date sample processed
  - Hospital
  - Patient gender
  - DOB
  - Year of Birth (YOB)
  - Date registered on database (current days date)
  - Year registered
  - Age
  - Last contact with patient
  - Year of last contact
  - Event time
  - Diabetic (Y/N)
  - Year diabetes diagnosed
  - Units insulin
  - Comments on insulin
  - Malabsorption (Y/N)
  - Comments
  - Year diagnosed
  - Date
  - Supplements used
  - Dose creon
  - Smoke (drop-down menu)
  - Qualitative smoking (drop-down menu)
  - No. per day
  - Age started
  - Age stopped
  - Alcohol (drop-down)
  - Qualitative drinking units/week
  - Age started

- Age stopped
  - Pancreatitis
  - Operative history
  - Date principle operation
  - Year
  - Date tumour test
  - Date tumour marker test
  - Year tumour marker test
  - CA199
  - CEA
  - Other markers
  - Platelet count
  - WBC
  - Bilirubin
  - Date bilirubin
  - Lap US Report
  - Pre-op ERCP
  - Trandominal Ultrasound report
  - Date of CT
  - Year of CT
  - Report of CT
  - Urine for Tanya (fill in location)
  - Locations of samples
8. The EUROPAC Research Fellow will at three month intervals follow up patient with survival.

## 6. ABBREVIATIONS

<b>DMCCM</b>	Department of Molecular and Clinical Cancer Medicine
<b>DOB</b>	Date of Birth
<b>LIMS</b>	Laboratory Information Management System
<b>PBS</b>	Phosphate Buffered Saline
<b>WBC</b>	White Blood Cells
<b>YOB</b>	Year of Birth

## 7. OTHER RELATED PROCEDURES AND DOCUMENTS

### SOPs:

GCLPEQU019	Use of pH Meter
GCLPRPS004	Disposal of Hazardous Waste
GCLPRPS011	Use of the LIMS to Record Kit Construction, QC, Storage and Dispatch
GCLPTSS047	Sample Collection and Storage for the Pancreas Cancer Bank