

<b>Acute Pancreatitis Sample Processing Checklist</b>
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## ACUTE PANCREATITIS RESEARCH BIOBANK SAMPLE PROCESSING CHECKLIST

<b>Person Collecting Sample:</b>  <b>Person Processing Sample (if different):</b>	<b>Date:</b>  <b>Date:</b>	<b>Time:</b>  <b>Time:</b>
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KIT CODE				PATIENT CODE (AP NUMBER)	
KIT TYPE	A		B		
	C				

ORIGIN OF SAMPLE		Sample Time Point: (tick appropriate box)			
RLUH:		Day 1	Day 4	Day 14	Interhospital Transfer
Other:					

  

	CONSENT (tick box)			Date
Patient Consent	No		Yes	
Patient Regaining Capacity to Consent	No		Yes	
Personal Consultee Consent	No		Yes	
Nominated Consultee	No		Yes	
Blood Collected	Amount	AP	Time	Invert x10 (tick box)
	t	Biobank	Taken	
EDTA Vacutainer (purple)	10 ml			
EDTA Vacutainer (purple)	6 ml			
EDTA Vacutainer (purple)	4 ml			
Serum Tube (golden)	3.5 ml			
PAXgene tube	2.5 ml			
Urine collected	na			

<b>SAMPLE PROCESSING AND STORAGE</b>
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(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°C Freezer and location	
Location of PAXGENE Tube (Freezer, Draw, Box, Co-ordinate)	
All information recorded on LIMS for the Acute Pancreatitis Research biobank	

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):	
Kit no:	
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 freezer and location:	
<b>Record Location of Plasma Tubes</b> ➤ ➤ ➤ ➤ ➤ ➤ ➤ ➤	
All information recorded on LIMS for the Acute pancreatitis research biobank	

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			
Resuspend 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			
<b>Count Cells: RECORD COUNTS IN THE TABLE BELOW</b>			
	FIRST COUNT	SECOND COUNT	AVERAGE
<b>TOTAL CELL COUNT ANSWER (A)</b>			X 10 <sup>6</sup> /ml
<b>Live Cells</b>			X 10 <sup>6</sup> /ml
<b>Dead Cells</b>			X 10 <sup>5</sup> /ml
<b>Viability</b>			%
<p>ANSWER (A) X VOLUME (ml) = TOTAL number of cells</p> <p>..... X ..... = ..... X 10<sup>6</sup> ANSWER (B)</p> <p><b>Final concentration of cell pellet</b></p> <p>ANSWER B divide by 5 = ..... X 10<sup>6</sup></p>			
Centrifuge falcon tube at 600 xg (ensure centrifuge is balanced)			
Remove supernatant and dispose of into 1% Virkon			
Resuspend 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 freezer and location (record the following information next to the bold type)	
<b>Number of eppendorfs stored:</b> <b>Number of cells/ eppendorf:</b> <b>Location of eppendorfs:</b> ➤ ➤ ➤ ➤ ➤ ➤ ➤	
<b>Time eppendorfs stored:</b>	
Location recorded on LIMS for the Acute Pancreatitis Research Biobank	
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)	
<b>Time centrifugation:</b>	
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:	
<b>Number of serum cryovials stored:</b> <b>Location of serum cryovials:</b> ➤ ➤ ➤ ➤ ➤ ➤ ➤	
<b>Time eppendorfs stored:</b>	
Record location on LIMS for the Acute Pancreatitis Research Biobank	