

PANCREAS DISSECTION AND ENZYME INJECTION

Safety routines

All work with human material carries the risk of transmitting infectious diseases. See [Skyddsföreskrift laboratoriearbete KITM](#) and [Hygienregler för Akademiska Sjukhuset AL9112](#).

Procedure

Preparation

The digest tubing system (kammarsystemet) needs to be assembled according to [Montering av digestionssystemet. Assembling the digestion system. AL5190](#) before the work described below begins.

1. Put one frozen ice pack into the "tarmdissektions" hood under the sterile drape.
2. Sterilely put a spike into a 1 L bottle of cold Ringer's Acetate and put back into the fridge.

Procedure

The person that does the dissection uses sterile gloves and sterile arm sleeves for the whole dissection process. This person should be assisted by another person to make sterile work possible. Removal of the duodenum from the pancreas and fine dissection of the pancreas are performed in different hoods.

1. Enter the start time of the dissection into the NICS-database. Transfer the pancreas and preservation solution into an empty kidney pan, keep cold, and remove excess fluid. Wash the pancreas three times with cold Ringer's Acetate solution (do not pour directly onto the pancreas, but pour solution beside the pancreas). The wash solution should be emptied into a specified waste container. Start the timer.
2. Dissect the duodenum from the pancreas in cold Ringer's acetate.
3. Pour off the Ringer's acetate.
4. Wash the pancreas two times with Jodopax (Iodine) 0,05% solution.
5. Pour off the Jodopax.
6. Wash the pancreas 3 times with cold Ringer's acetate.
7. Weigh the pancreas and note the weight in the NICS-database.
8. The bulldog clamp can be used to mark the location of the pancreatic duct.
9. Fill the plastic container with cold Ringer's acetate and place the pancreas into it. Attach the lid to the container.
10. Transfer the container with the pancreas to the digestion hood.
11. Change into new, sterile gloves and sterile sleeves.

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12. Transfer the pancreas to the metal kidney pan with cold Ringer's acetate.
13. Locate the main duct and take a hold of it with the fine pointed forceps.
14. Lift the pancreas with the forceps and carefully put in the metal cannula, use a different size metal cannula if the standard one does not fit. If there is resistance, the duct may be blocked by the pancreas weight. Lift and turn the pancreas to open the blockage until resistance is minimized.
15. When the cannula is in place, clamp it with the intestinal clamp to secure the duct and the cannula for enzyme injection. Clamping should be done so that a big enough piece of pancreas head is above the forceps to obtain proper biopsies. The intestinal forceps should only be locked to a point where the cannula is still movable with some resistance.
16. Ask the assistant to pour the enzyme solution into kidney pan with outlet.
17. Pour off the Ringer's acetate.
18. Draw about 10 ml cold enzyme solution into a 20 ml syringe and add methylene blue (about 20-50ul), mix and remove air from syringe.
19. Hold the intestinal forceps and let the pancreas hang in the air while slowly injecting about 10 ml enzyme solution into the pancreas via the cannula. Avoid injecting air.
20. Check for leakage in the pancreas. If there are leaks, use tissue glue or a clamp to seal and repair the damage. Note: immediately wipe clean any instruments that contain fresh tissue glue, it is difficult to remove after it has dried. Dry the pancreas around the leakage with sterile gauze and then apply the tissue glue.
21. When the leaks have been repaired, put the pancreas, with the head towards the pump, on the metal mesh above the kidney pan with an outlet.
22. Ask assistant to pour all "stamlösning" aka stock solution into the metal kidney bowl and draw 20 ml into the syringe to have available. Keep the solution in kidney bowl and syringe cold.
23. Continue with the dissection as described below, depending on which enzyme(s) is (are) to be used.
24. Try to follow these guidelines regarding pressure during enzyme perfusion, independent of manufacturer of the enzyme:

Pressure 60	5-8 minutes
Pressure 100	5-8 minutes
Pressure 150	5-8 minutes

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If Liberase from Roche is used:

25. Start the pump and make sure the fluid circulates in the tubing. Attach the tubing to the cannula when there is no air in the tubing. Let the Liberase solution infuse into the pancreas.
26. Cannulate and inject the enzyme solution in the secondary duct if there is no connection with the main duct.
27. Let the enzyme solution circulate (total maximum time about 20-25 minutes) during on-going dissection.
28. Avoid the injection of air to the pancreas and add more stock solution to the kidney pan if necessary.
29. Continue according to step 43 below.

If collagenase and neutral protease from Serva are to be used:

30. Start pumping the collagenase solution alone and make sure the fluid circulates in the tubing. Attach the tubing to the cannula when there is no air in the tubing. Let the collagenase solution infuse into the pancreas.
31. Cannulate and inject the enzyme solution in the secondary duct if there is no connection with the main duct.
32. When all collagenase is in the pancreas, stop the pump and pour the neutral protease solution (40 ml) into the kidney pan with outlet.
33. Start the pump and avoid the addition of air to the tubing.
34. Attach the tubing to the cannula and let the enzyme solution circulate (total maximum time about 20-25 minutes) during on-going dissection.
35. Avoid the injection of air to the pancreas and add more stock solution to the kidney pan if necessary.
36. Continue according to step 43 below.

If collagenase and thermolysin from VitaCyte are to be used:

37. Start pumping the combined collagenase and thermolysine solutions and make sure the fluid circulates in the tubing. Attach the tubing to the cannula when there is no air in the tubing. Let the enzyme solution infuse into the pancreas.
38. Cannulate and inject the enzyme solution in the secondary duct if there is no connection with the main duct.
39. Start the pump and avoid the addition of air to the tubing.
40. Attach the tubing to the cannula and let the enzyme solution circulate (total maximum time about 20-25 minutes).
41. Avoid the injection of air to the pancreas and add more stock solution to the kidney pan if necessary.
42. Continue with step 43.

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Common procedure independent of enzyme manufacturer.

43. Start dissecting fat and membranes using the forceps and scissors. Work by carefully separating tissue from the pancreas, cutting away loose tissue. Be careful not to damage the pancreas. The most important thing is to remove fat and membranes. If there is not enough time during the dissection, leave blood vessels and bile duct.
44. If the tail part of the pancreas is not distended properly, continue the dissection as usual for about 20 minutes. Then stop the pump, divide the pancreas in 2 parts at the middle and put the catheter into the tail part. Perfuse the tail for an additional 5 minutes or until properly perfused.
45. Stop the pump, remove the cannula and all clamps and **cut away the intestinal forceps.**
46. Carefully dissect away **all** tissue glue if necessary.
47. The assistant now opens the chamber inside the hood.
48. Cut the pancreas into 5-6 pieces with a scalpel and transfer them to the chamber. Then tilt the pan back so that the outlet comes above the fluid surface and pump the solution in the tubing into the kidney pan. Pour the solution in the kidney pan with outlet into the chamber.
49. Put 6-9 metal/silicon nitride marbles into the chamber and the three metal mesh screens in the following order, large, medium and fine mesh at the top. Screw the top of the chamber to the bottom. Take it out of the hood and attach the chamber to the shaking apparatus. Start the clock. Note the time in the NICS database and then continue according to [Digerering av pankreas. Pancreas digestion. AL5182.](#)
50. Take 2 pancreas biopsies (about 10x10x5 mm) from the portion of the pancreas taken when removing the intestinal forceps. Put these on the sterile cloth (to remove fluid). Put one sample in formalin tube and the other sample into a cryotube (for further information, see [Fixering och parafininbäddning av pankreasbiopsi. Fixation and parafine imbedding of pancreatic biopsy. AL5187](#) and [Frysning av pankreasbiopsi av pankreasbiopsi för kvalitetskontroll. Pancreas biopsy for quality control. AL5176](#)
51. Remove excess solution and weigh the trimmed fat and other tissue dissected away in the previous steps and enter the weight into the NICS database.
52. If research consent is given, continue with biopsy collection according to the template in the lab. If no research consent is given, collect the trimmed tissue from both the hoods, place it in a plastic bag, seal the bag and put it in the riskavfall (trash bin for hazardous material).

Protocol and archiving

Protocols must be stored for a minimum of 10 years.

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Equipment

Apparatus

Electronic quick balance	746092
LAF Holten 2010	MTA 074204, 075957
Perfusion pump Watson Marlow 323	MTA 073376
Timer	

Material

Arm Sleeve	746241
BD Venflon Pro 18 GA (green), as needed	746270
Cooling plate (white)	
Gauze pad, sterile, as needed	746188
Metal catheter	746343
Fine dissection kit (from Sterilcentralen)	1 each
Gross Intestine kit (from Sterilcentralen)	1 each
Injection Cannula 1.2 x 50 mm pink, 1 each	746006
Slang Tygon R-3603 2.4x1.6mm	
Sterile Gloves	746183
Syringe, 1 ml, 1 each	746082
Syringe, 20 ml, 1 each	746154
Suture, as needed	746231
Towel, Clinical Drape (sterile towel), 3 each	746184
Vinyl Gloves	746157
Biopsy tube, 1 each	746096
Cyrotube, 2 ml	746030

Reagents/Supplements

Jodopax (Iodine) 0,05%	758222
Methylene Chloride	758232
Ringer's Acetate	767583
Tissue Glue	758241
Formalin	758034
Liberase	758229
Serva collagenase	758325
Serva neutral protease	758326
Vitacyte collagenase	758361
Vitacyte thermolysin	758362

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"Good to know"

- It is acceptable if all enzyme solution is not infused into the pancreas.

References

[Montering av digestionssystemet. Assembling the digestion system. AL5190](#)

[Digerering av pankreas. Pancreas digestion. AL5182](#)

[Fixering och parafininbäddning av pankreasbiopsi. Fixation and parafine imbedding of pancreatic biopsy. AL5187](#)

[Frysning av pankreasbiopsi av pankreasbiopsi för kvalitetskontroll. Pancreas biopsy for quality control. AL5176](#)

[Skyddsföreskrift laboratoriearbete KITM](#)

[Hygienregler för Akademiska Sjukhuset AL9112.](#)

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