

[Skin Pathology Core] Protocols

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Receiving Study Samples

1. Make sure all study samples come with a Skin Pathology Request form that has been filled out by the investigator.
2. Assign a barcode label to each specimen bottle(s) and any accompanying clinical data.
3. Send clinical data to Core D.
4. Fill in this same number on the Request form under the column 'Skin Path Number (for SPL use only)'.
5. Scan each sample into the computer system using the barcode scanner.

Grossing Study Samples

The following protocol applies to formalin-fixed tissue only. For fresh tissue in Michele's Media, refer to the 'Frozen Sections' protocol.

1. Make sure the label number on each specimen bottle matches the number written on the Request form and the number in the computer.
2. Fill out bottom portion of the Request form.
3. In the bottle table, write the assigned SPL number under the 'SPL Number' column.
4. Print out cassettes with the assigned SPL numbers.
5. Match each bottle with its cassette, making sure to double check that the number on the bottle is the same as the number printed on the cassette.
6. Measure each specimen (length x width x height) and record the measurements in the 'Measurements' column of the Request form.
7. Ink the dermis of each specimen.
8. If a multi-sample block has been requested, ink each sample with a different color, and fill out the grossing sheet, indicating the color that corresponds to each sample.
9. Wrap the inked specimen(s) with Histo-wrap and place in the cassette.

10. With a pencil, write the number of pieces of tissue on the side of the cassette.
11. Attach the cassette lid and close.
12. Place each cassette in the metal cassette basket that is sitting in formalin to prevent the tissue from drying out.

Processing Study Samples

The following protocol applies to formalin-fixed tissue only. For fresh tissue in Michele's Media, refer to the 'Frozen Sections' protocol.

1. When you have grossed all of the samples, place metal lid onto cassette basket.
2. When you are ready to start the processor, take the cassette basket out of formalin.
3. Place in the Leica Processor, making sure the basket handle sits to the left.
4. Close the processor lid and turn the knob to lock it.
5. Follow the screen prompts to start the processor.
6. Let the processor run its cycle overnight.

Embedding Study Samples

The following protocol applies to formalin-fixed tissue only. For fresh tissue in Michele's Media, refer to the 'Frozen Sections' protocol.

1. When the processor has completed its run in the morning, take the basket out of the machine.
2. At the embedding station, remove the basket lid.
3. To prevent the paraffin from solidifying, keep the basket or cassettes in the warming tray of the embedding station.
4. Open the first cassette, and check to see that the number of pieces written on the side of the cassette corresponds to the number of pieces wrapped up in the Histo-wrap.

5. Remove the cassette lid from the base. The lid can be thrown out.
6. Take a metal mold from the warming tray big enough to fit the specimen(s).
7. Pour paraffin into the mold so that it fills most of the mold. Be sure to push away any air bubbles, if they form.
8. Use forceps to orient the tissue in the paraffin so that the long axis or cut side is parallel to the embedding surface, unless otherwise noted.
9. Slide the mold to the cold plate, making sure the tissue remains properly oriented. The cold plate will thicken the paraffin and help the tissue stay in place.
10. Place the cassette base on top of the mold. Pour some paraffin on top of the base, to make sure the base will properly adhere to the rest of the paraffin.
11. Move the mold to the cooling surface, so that the paraffin can solidify.
12. Once the paraffin has solidified enough, use a metal spatula/paint scraper to release the block from its mold.
13. Use the block cleaner to clean off the sides of the block.
14. If the block requested is a multi-sample block, be sure to fill out the diagram to record the placement of each sample in the block.
15. Proceed normally with attaching the cassette base, cooling the paraffin, and cleaning the block.

Cutting Blocks and Making Slides

The following protocol applies to paraffin-embedded tissue only. For cutting frozen tissue, refer to the 'Frozen Sections' protocol.

1. After the blocks have been cleaned, bring them over to a microtome.
2. Determine how many slides of each block you will need to make, and label them properly. Use the blue charged slides.
3. To make sectioning easier, and to fit more sections per slide, use a single razor blade to box the paraffin block. To do this, press the razor blade down at 45° angle into each side of the paraffin block. Make sure to leave space between the

- tissue and the edge of the paraffin. If the tissue samples fill up most of the area of the block, boxing is not necessary.
4. Take each block and orient in the microtome, with the label edge of the cassette on the right.
 5. Carefully insert a microtome blade into the microtome and lock it in place.
 6. Use the arrows on the control pad to move the block closer or further away from the blade.
 7. Once the block is just about to touch the blade, rotate the microtome's rotary wheel to bring the block closer.
 8. Change the thickness setting to 30 μ m and rotate the rotary wheel a few more times to reach the start of the block.
 9. Rotate the wheel a few more times to shave the block down until you reach the tissue. Look at the block to determine if you need to shave the block more. You do not want to shave too far down, but do want a complete tissue section, with a complete epidermis.
 10. When you are ready to take sections, change the thickness to 4 μ m. Press both the 'Start' and 'Stop' buttons at the same time to start automatic rotation of the handle.
 11. Use your forceps to grab one edge of the ribbon that forms as each section of paraffin attaches to the previous section. Gently hold onto the ribbon until it is the desired length, and you have as many tissue sections as you would like.
 12. Press the 'Stop' button.
 13. Use your other pair of forceps to grab the end of the ribbon.
 14. Carefully bring the ribbon over to the water bath that has been preheated to around 40°C.
 15. Roll the ribbon onto the water, placing one end down first and letting the rest of the ribbon follow. This method helps to minimize air bubbles in the paraffin.
 16. Use the curved forceps to separate the sections.
 17. Take a slide and gently place in the water under the floating tissue section. Use the forceps to help you align the tissue section with the slide.

18. Once the slide and tissue section are aligned, lift the slide up so that the tissue section sticks to the glass.
19. Place slide in a slide rack so that it can dry before any further steps.
20. Continue the process until you have all the slides needed.
21. If unstained slides were requested, let the slides dry in the slide rack for a couple of days before packaging them.

IHC Staining

1. Put slides into the oven for 30 minutes at 70°C.
2. Slides that need a low pH antigen retrieval solution should be put in a separate slide rack and placed in the Hacker linear stainer to deparaffinize. The slides that need the high pH solution will be deparaffinized in the antigen retrieval solution itself.
3. Preheat both the high and low pH solutions in the PT Link.
4. Place the high pH slides onto the high pH solution. Place the low pH slides into the low pH solution.
5. Close the PT Link and lock it.
6. On the screen, press the 'Run' buttons for both tanks.
7. After about 40 minutes, the heating and cooling cycle will have finished, and the PT Link will unlock.
8. Take the slides out of the PT Link and place in cold wash buffer for a few minutes so that the slides can cool down further.
9. Program the run into the computer, using the Dako Autostainer Program. For detailed instructions on how to use the Program, see the *IMPOX protocol* in the SPL Protocol binder.
10. Prepare reagents needed for experiment.
11. Load slides and reagents into the Dako Autostainer.

12. Start the program run, making note of when the 'Batch Time' is. You will need to come back to the machine at this time with the chromagen. If using the permanent red chromagen, be sure to make up the solution only around 5 minutes before the batch time.
13. The computer will pause for the batch. Add the chromagen solutions, and press 'OK' so the machine will continue the staining process.
14. When the run is done, take out the slides, and run them through alcohol and citrus clearing solvent.
15. Let slides dry for a few minutes.
16. Hand coverslip with mounting media.

H&E Staining

The following protocol applies to H&E staining for paraffin-embedded blocks only. For H&E staining of frozen sections, refer to the 'Frozen Sections' protocol.

1. After the slides have been cut and put into a slide rack, place them in the oven for 30 minutes at 70°C
2. Put a white plastic bar onto the slide rack.
3. Place the slide rack in the first basin of xylene for 5 minutes.
4. Move the slide rack to the Hacker linear stainer. The stainer will move the slide rack through the various reagents.
5. When the stainer's alarm goes off, the slides will be sitting in the very last basin of alcohol in the stainer.
6. Move the slide rack into the basin of xylene and let it sit for 3 minutes.
7. Load the slide rack into the automatic coverslipper and start the machine.
8. When the machine beeps, take the coverslipped slides out and lay them out on a tray so that the glue can dry.

Special Stains

1. Place slides into slide rack, and put the rack into the oven for at least 30 minutes at 70°C.
2. Refer to the '*Specials Stains*' binder for specific protocols for individual stains.
3. Use mounting media to coverslip completed slides.

Frozen Sections

1. Fresh tissue should arrive in Michele's Media and be put into the refrigerator after being labeled and scanned into the computer.
2. Measure the tissue (length x width x height).
3. Wash with PBS several times. If tissue is especially bloody, wash until very little blood is left (this will help with cutting).
4. Identify the epidermis. Orient the tissue so that the epidermis is either to the left or right. Place tissue on the steel panel of the cryostat and cover with Optimal Cutting Temperature media. The media will freeze into a white compound in about 5 to 10 minutes.
5. While the tissue is freezing, label a cassette and a thin strip of paper with the tissue's number.
6. When the block has frozen, mark the orientation of the tissue on the side of the block with a red marker. The tissue should be oriented so that the epidermis is facing either the left or the right. When the tissue is cut, you want to go through all the layers at the same time, i.e. left side: epidermis--dermis--fat: right side). Then use a razor blade to gently scrape the block off the steel.
7. Put OCT media on a cold chuck and place the frozen block on the chuck. Use enough media to make sure the block is attached well. Allow the media to freeze for 2 to 3 minutes.
8. If the tissue is exposed, put a small amount of OCT media on top of the block and place a slide on top of the new media (this is to create a buffer zone so when you shave the block you don't waste tissue).

9. Spray the slide with freeze spray to freeze the media underneath. Remove the slide by prying it off with a razor blade. Keep this slide in the cryostat for later use.
10. Place the chuck in the chuck holder so that the red mark is facing up and tighten to screw well. Use the lever on the side of the chuck holder to align the block parallel to the blade holder. Tighten the lever well.
11. Put a low-profile blade in the blade holder and tighten well.
12. Set the section thickness to 14 μ m.
13. Use the arrow buttons and rotate the rotary wheel of the cryostat until you begin to get sections.
14. Shave the block down until you reach the tissue. Try to get full circle sections.
15. Use a brush to clean off excess frost from the stage.
16. Label blue charged slides.
17. Rotate the wheel in a steady forward motion and use a small brush to guide the section onto the stage.
18. Once you have a section, press the slide onto the section. The section will stick to the glass slide.
19. If the tissue is separating from the media in the sections, use freeze spray for 1 second on the section. Wipe the excess off with a brush and turn the wheel once. You don't want to take the section right after using the freeze spray because they will be too thick and will have freezing artifacts. After cutting a throw-away section, the next few sections should not separate as much.
20. Check under the microscope to make sure the epidermis, dermis, and any underlying tissue are present.
21. Continue cutting until you have all the slides you need.
22. When you have finished cutting, remove the blade. Discard.
23. Remove the chuck from the holder and remove the block from the chuck.
24. Label the block with the thin strip of paper, and place it in its cassette. Store the cassette into the appropriate box in the -80°C freezer.

25. If an H&E stain has been requested, put the slide(s) in a plastic slide rack with a white bar. Put the slide rack into the first water basin in the stainer (marked 'Start H&E'). Let the rack go all the way to the end of the machine.
26. Coverslip with the automatic coverslipper.
27. If an IHC stain has been requested, fix the tissue in cold acetone at 4° for 10 minutes.
28. Proceed with established or experimental IHC protocol.