



HISTOPATHOLOGY - FIRST COURSE

LAB -7- MICROTOMY FOR PARAFFIN AND FROZEN SECTIONS

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قسم تقنيات المختبرات الطبية

Third Stage

المرحلة الثالثة

محاضرة رقم 7

Lecture No. 6

الجانب العملي

Practical

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MICROTOMY

Microtomy :

Is the means by which tissue can be sectioned and attached to a surface for further microscopic examination.

Microtome:

- ✓ **Basic instrument used in Microtomy.**
- ✓ **Mechanical device for cutting thin uniform slices of tissue sections.**

Microtomy -paraffin wax

□ Factors involved in producing good paraffin-wax sections :

1. Temperature.
2. Knife angle.
3. Speed of cutting.
4. Slant.

◆ Temperature

1. Tissues are more easily sectioned at a lower temperature than that of the atmosphere.
2. Lowering temperature brings tissues of differing composition to a more uniform consistency, degree of hardness-ensures a uniform cutting process.
3. Blocks are cooled by keeping , face down on ice-tray (2-3min).

❖ Knife angle

1. Greater the rake angle(flatter the knife)more likely is a smooth plastic flow type cutting action.
2. Higher rake angles are more suitable to softer tissues.
3. Lower rake angles for harder tissues.

❖ Speed of cutting

1. Soft tissues are cut more easily at a slow speed.
2. Hard tissues are cut easily at a little fast rate.
3. If sections are cut at too fast speed, compression will become more marked.
4. If cut too slowly, difficult to maintain the rhythmic action required.

❖ **Slant**

- 1. Commonly used to refer to the relationship of the knife edge to the block when cutting nitrocellulose-embedded tissue on a sliding microtome.**
- 2. Advantages: larger area of the edge is employed.**
- 3. Resistance to cutting force is applied more gently.**

□ Paraffin section cutting:

Equipment required:

- 1. Microtome.**
- 2. Flotation(water bath)**
- 3. Slide drying oven or hot plate**
- 4. Fine pointed or curved forceps.**
- 5. Sable or camel haired brush.**
- 6. Scalpel.**
- 7. Slide rack.**
- 8. Clean slides.**
- 9. Teasing needle.**
- 10. Ice tray.**
- 11. Chemical resistant pencil or pen**

Cutting technique

1. Insert appropriate knife in the knife-holder of the microtome and screw it tightly in position.
2. Correctly set the adjustable knife angles.
3. Fix the block in the block holder of the microtome
4. Move the block holder forward or upward until the paraffin wax is almost touching the knife edge.
5. Ensure that the whole surface of the block will move parallel to the edge of the knife,
6. Bring the block face up until it nearly touches the knife edge.
7. Paraffin-wax embedded tissue , sections are normally cut at a thickness of 4-5 micrometer.
8. Thicker sections(10-20 micrometer) :demonstrate certain features of the central nervous system.

9. Thin sections(1-2 micrometer): for examining highly cellular tissue such as lymph nodes.

10. The amount of advance is operator determined most commonly in graduated 1 micrometer stages.

11. Paraffin wax embedded tissue: the properties of the wax causes each section to adhere by its edge to the previous forming a ribbon of sections Ribbons should be held gently with a fine moistened brush or with a pair of fine forceps.

12. Holding the ribbons with the finger is to be discouraged :

13. section and water bath may become contaminated with the operator's exfoliated squames.

14. Before being attached to the slides the creases must be removed and the sections flattened.

15. This is achieved by floating them on warm water.

Flotation(water bath)

- **Thermostatically controlled water baths for floating out tissue ribbons after sectioning. To remove the creases and flatten the sections.**
- **Temperature of water in the bath should be 10 degree celcius below the melting point of paraffin employed.**
- **Distilled water may be used to prevent water bubbles from being trapped under the sections.**
- **Alcohol or a small drop of detergent may be added to the water to reduce the surface tension to flatten out the sections with ease.**

- Sections which are curled will flatten on warm water, creases removed.
- To remove air bubble, thick sections of wax which curl into a roll during trimming are used.
- one roll in the end of a pair of forceps and bring the end of the wax roll up under the section to touch the air bubble.
- The bubble will adhere to the wax roll and come away with it when removed.

Mounting the section

Mounting the section on a slide:

- 1. A clean slide is half submerged in water and brought into contact with the edge of the section.**
- 2. Section approached from the edge of the forceps or dissecting needle.**
- 3. Section oriented on wet slide using the side, straight approach will push the section away.**
- 4. Section should be centrally positioned on the slide.**
- 5. Slide should be identified by inscribing the appropriate number on the slide with a diamond pencil.**

Any questions?

*Thank
You*