This is Annex I of SUBI's Part to the Final Report of the initial STORE Project (EURATOM Grant Agreement No. 232628)

Prepared by

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Note:

Relevant for the preparation of the paraffin blocks are steps 1-5 (B. Grosche, 04 Aug 2016)

Technology of preparation of histological slides of dead animals' tissues and organs

Preparation of histological slides was performed using methods accepted in hospital prosectoriums and in morbid anatomy departments of research institutions of USSR and Russia. Experts in this field used the following papers as guidelines: "Microscopic Technique" by Romeis B., translated from German. M., 1953, and "A course of patholgico-histological technique" by Merkulov G.A., publishing house "Medicine", 1969.

1. Fixation

Fixation is the first stage of processing pieces cut from different organs and tissues for microscopic investigation. It is aimed at tissue structure fixation in the state it was at the moment of putting the pieces into the preservative solution, as well as at preserving the pieces from destruction.

10% formalin solution pH 6 was used for pieces fixation, for this 1 portion of manufactured formalin was diluted by 9 portions of tape water. The volume of fixative solution was 20 – 40 times greater than pieces for fixation volume. Each piece for fixation wasn't thicker than 0.5 – 1 cm. Fixation time was 24 – 48 hours.

2. Washing

After fixation the pieces were washed in the cold running water during the period of 24 – 48 hours.

3. Desiccation

The next step of the material processing was its desiccation in ethyl alcohol of ascending concentration, aimed at tissue preparation for impregnation with celloidin and paraffin:

1) 70° alcohol – 24 hours
2) 80° alcohol – 24 hours
3) 96° alcohol I – 24 hours
4) 96° alcohol II – 24 hours
5) 100° alcohol I – 3 hours
6) 100° alcohol II – 3 hours
7) 100° alcohol + ether (1:1) – 30 minutes

After desiccation the pieces were put into castor oil with 2% celloidin solution. For is preparation 2 gr of celloidin were dissolved in 100 ml mixture of 100° ethyl alcohol with ethyl ether in 1:1 ratio. The pieces were kept in this solution no less than for 10 days.

4. Embedding

The embedding was performed successively using homogenized paraffin (steps 5÷8):

1) 100° alcohol + ether (1:1) – 30 minutes
2) Chloroform I – 30 minutes
3) Chloroform II – 3 hours
4) Chloroform III – 3 hours
5) Paraffin + chloroform (1:1) during night in thermostat at 37° C
6) Paraffin I – 2 hours in thermostat at 56° C
7) Paraffin II – 2 hours at 56° C
8) Paraffin III – 2 hours at 56° C

The pieces from III portion of paraffin were put into crystallizing dish with cold running water for cooling, and then placed into paper bags and labeled.

5. Gluing blocks. Cutting.

The pieces were glued to wooden blocks with paraffin, and then 5-7 micrometer thick cuts were made with sliding microtome and put onto microscope slide.

6. Preparation of cuts for microscopy

1) Ehrlich's hematoxylin preparation: 2 gr of hematoxylin was dissolved in 100 ml of 96° alcohol, then 100 ml of distilled water, 100 ml of pure glycerin, 3 gr of potassium alumen ($KA1(SO_4)_2 \cdot 12H_2O$) and 10 ml of glacial acetic acid were added. The solution was left in the light for 14 days, it was regularly shaken.
2) 0.1% eosin solution preparation: 0.1 gr of eosin was dissolved in 100 ml of distilled water.

3) Deparaffination of cuts
    - Toluene I – 5 minutes
    - Toluene II – 5 minutes
    - Toluene III – 5 minutes
4) Cuts delipidization
    - 100° alcohol + ether (1:1) – 1 minute
    - 100° alcohol – 1 minute
    - 96° alcohol – 1 minute
    - 70° alcohol – 1 minute
5) Hematoxylin-eosin staining of cuts
    - hematoxylin – 15 – 20 minutes
    - distilled water – rinsing
    - running water – washing till the cuts were blue
    - differentiation
    - 1% muriatic alcohol 5 – 30 seconds (1 ml HCl for 100 ml of 70° alcohol)
    - Distilled water – rinsing
    - Running water + 5 drops of ammonia – 10 minutes
    - Distilled water – rinsing
    - Distilled water – 5 minutes
    - 0.1% aqueous solution of eosin – 1 -2 minutes
    - 96° alcohol I – to wash eosin off – rinsing
    - 96° alcohol II – rinsing
    - 100° alcohol – rinsing; blot with blotting paper
6) Cuts clearing
    - Carbol-xylol – 5 – 7 minutes
    - Toluene I – 10 - 15 minutes
    - Toluene II – 10 - 15 minutes
    - Toluene III – 10 - 15 minutes

7. Putting the cuts into balsam