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Peripheral nerve biopsy technique

1. Biopsy procedure:

- * Local anesthesia with xylocaine or novocaine.
- * A whole cross section of the entire thickness of the nerve trunk is recommended instead of a fascicular biopsy of only some fascicles which gives more post-biopsy complications for the patient.
- * Remove first the proximal part and then the distal part.
- * Delicate excision and careful handling without crushing or stretching of the nerve specimen are essential to avoid artifacts. Use tweezers with smooth tips.
- * Total specimen length: 2 1/2 to 3 cm to be subdivided in:
 - one specimen of 1 1/2 cm for light microscopy (LM): classical histology
 - one specimen of 1 cm for classical electron microscopy (EM) or for immuno-EM and if enough tissue is available
 - one specimen of 1 cm for immuno-EM

2. Specimen preparation:

For classical LM: classical histology on *cryostat sections*: snap freezing of the specimen in liquid nitrogen -cooled isopentane.

For EM:

- * The specimen is immediately immersed for 2 hours:
 - in cooled (4°C) 4 % phosphate-buffered glutaraldehyde (4 % glutaraldehyde with 0.1 M phosphate buffer) **for conventional EM**

or in paraformaldehyde for **immuno-EM**

*After 2 hours of fixation in glutaraldehyde or paraformaldehyde, the specimen is further carefully subdivided in small blocks of 1 mm x 1 mm x 2 mm with a razor blade on a wax sheet under the dissecting microscope. The blocks are then replaced for 2 other hours in new glutaraldehyde or paraformaldehyde.

*After a total fixation period of 4 hours in glutaraldehyde or paraformaldehyde, the blocks are washed in phosphate-buffer added with sucrose. The blocks **may be stored** until the embedding for at least **3 weeks** in cooled (4° C) sucrose added-phosphate-buffer.

Embedding and resin polymerization see further.

3. Preparation of the fixatives

Millonig Buffer 0.1 M; pH 7.4 (buffer PO₄) to be kept at 4°C.

This buffer consists of:

- | | |
|---------------|--|
| 1. solution A | : 2.26 gr. NaH ₂ PO ₄ 1H ₂ O/ 100 ml H ₂ O bidistil. |
| or | 2.546 gr. NaH ₂ PO ₄ 2H ₂ O/ 100 ml H ₂ O bidistil. |
| 2. solution B | : 2.52 gr. NaOH/ 100 ml H ₂ O bidistil. |
| buffer | : 83 ml solution A
+ 17 ml solution B |

100 ml Millonig buffer at pH 7.4

This buffer must be adjusted to pH 7.4 with one of the 2 solutions (A or B)

Glutaraldehyde = gluta (GA) **fixative** to be kept at 4°C.

Single dose ampoule of 2 ml gluta 70% to dissolve in
----- 4%
33 ml Millonig buffer 0.1 M

Control of pH; if necessary adjust with one of the solutions of the Millonig buffer.
Gluta may be stored at 4 ° C during 2 weeks if pH is regularly controlled.

Paraformaldehyde (PF) fixative to be kept at 4°C.

Preparation of 4 gr. paraformaldehyde for 100 ml Millonig buffer on a hot plate and using a magnetic stirrer. If the solution is not limpid :

addition of some drops of NaOH 0.1N + 0.7 ml glutaraldehyde at 70%
The solution must be adapted to pH 7.4

Araldite embedding

Araldite CY 212	10 ml	1L= 1,14 Kg	11,4 g
Araldite-härtner HY 964	10 ml	1L= 0.98 kg	9,8 g
Araldite-accelerator DY 964	0,5 ml		0,5 ml

4. Embedding and resin polymerization procedure

4. 1. Conventional EM:

*The tissue blocks are post-fixed in 2 % osmium tetroxide at pH 7.4 for 2 hours, dehydrated (15 minutes in alcohol 50°; 15 minutes in alcohol 70°; 15 minutes in alcohol 90°; 2 x 15 minutes in absolute alcohol) infiltrated and embedded in araldite.

*Blocks are transferred during 60 minutes in a mixture Araldite (araldite MCY 212; araldite härtner; araldite accelerator) / propylen oxid (1/1) in glass tubes.

* Then blocks are transferred for 16 to 24 hours in araldite without propylen oxid.

* Finally blocks are transferred in flat embeddingmolds filled with araldite. The specimens are aligned properly for transverse and longitudinal sectioning. Polymerisation: 3 days at 60° C in a standard oven.

4. 2 Immuno-EM: according low temperature embedding method

Embedding in unicryl

- wash 2 x 5 min in Millonig buffer
- rinse 2 x 5 min in aqua dest.

Dehydration and infiltration

- 30 min 30% ethanol at 4° C
- 30 min 50% ethanol at 4° C
- 30 min 70 % ethanol at - 20° C
- 30 min 90 % ethanol at - 20° C
- 30 min 100 % ethanol at - 20° C
- 30 min 100 % ethanol at - 20° C
- 30 min 70/ 30 ethanol/ unicryl at - 20° C
- 30 min 50/ 50 ethanol/ unicryl at - 20° C
- 30 min 30/ 70 ethanol/ unicryl at - 20° C
- 1 h 100% unicryl at - 20° C
- overnight 100% unicryl at - 20° C

Add 0.1 % BEE (benzoïn ethyl ether) to unicryl

- after overnighting in unicryl, one droplet of unicryl is put in the beem-capsule
- the tissue is placed in the capsule which is filled up with unicryl
- polymerisation: 2 - 3 days by UVirradiation at - 20° C