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Skin biopsy technique

1. Procedure: punch biopsy

* Under local anesthesia with xylocaine or novocaine a small [1 x 2 x 5 mm] piece of skin is sampled from the shoulder area (or axilla, exclusively in polyglucosan body disease and Lafora disease) and submitted to standard electron microscopic procedures. The only special precaution is to take a **“full thickness” biopsy** of epidermis, dermis and hypodermis.

2. Specimen preparation:

* 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** and/ 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. Special precaution is need to preserve during sectioning such an **orientation** of the fragments that a **full thickness section** of epidermis, dermis and hypodermis could simultaneously be examined in the smaller blocks. 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 additioned 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:

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|---------------|--|
| 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. Flat embedding molds are used to provide for **an adequate orientation** of the various parts of the skin including simultaneously epidermis, dermis and hypodermis. This special precaution allows a full thickness section of the biopsy. Polymerisation: 3 days at 60° C in a standard oven.

4. 2 Immuno-EM: low temperature embedding

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 overnighing 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 UV irradiation at **- 20° C**