II. Application

Roti®-Histol can be used like xylene for
- paraffin embedding
- clearing prior to coverslipping with hydrophobic mounting media
- dewaxing

Treating with Roti®-Histol prevents tissue samples from hardening and brittleness.

After coverslipping Roti®-Histol evaporates more slowly than xylene, therefore, the samples take more time to dry thoroughly. Roti®-Histol produces excellent results with Roti®-Histokitt mounting medium (Art. No. 6638). But it is also possible to use other xylene-based mounting media.

Roti®-Histol is appropriate to immunohistochemical assays (e.g. to work with antisera and monoclonal antibodies).

Roti®-Histol is particularly suitable for all histological hand work which usually exposes the user to solvent vapours in high concentrations.

When coming in contact with air, Roti®-Histol has a tendency to autoxidation. Thus it should not be used regularly in automates, it might cause deposits in tubes. For application in automates we recommend the xylene alternative Roticlear® (Art.-No. A538).

III. Storing

Roti®-Histol should be stored in tightly closed glass bottles. We do not recommend long-term storing in plastic bottles, for plastics are non-resistant to Roti®-Histol.

⚠️ Danger H304-H226-H410-H315-H317 P210-P273-P280-P301+P310-P311-P331-P333-P313

Roti®-Histol | 6640.1 | 1 l
| 6640.4 | 2.5 l
| 6640.5 | 5 l
| 6640.2 | 10 l
| 6640.3 | 22 kg

IV. Experiences with Roti®-Histol:

Roti®-Histol in lieu of xylene for paraffin embedding and embedding of undecalcified cortical bones

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Up to now Xylene (1) has always been used as an intermediate when dewaxing organic tissue cuttings and when embedding undecalcified cortical bone sections or whole cortical bones.

Xylene, however, causes severe irritation to the respiratory tracts, even when used under a closed hood, and requires disposal as a hazardous waste. It was therefore necessary to find a xylene substitute. Roti®-Histol (2) and Agi Agbon (3) are now available on the market. A methodical study was carried out to establish and confirm whether dewaxing tissue cuttings with Roti®-Histol is in fact as positive an experience as claimed and if Roti®-Histol can also substitute xylene as a dehydrating agent for undecalcified block-stained cortical sections and for whole cortical bones.

Material

Roti®-Histol is a terpene with a strong mandarin fragrance. From a chemical point of view it is a limonene in which pure methacrylate are soluble. It therefore fulfils the physical requirements of a xylene substitute (2). Roti®-Histol is said to be non-toxic, it does, however, have an irritating effect in high concentrations and is thus marked accordingly on the container. As it is primarily produced from lime and is biodegradable, it can be added quite safely to the sewage if an activated sludge process treatment is available (2)—please notice footnote*. We always dispose of Roti®-Histol as hazardous waste. Roti®-Histol is produced in Germany by Carl Roth GmbH & Co and is sold as a xylene substitute.

Methods
**Paraffin histology:**
Dewaxing and dehydrating process of supernatant solutions:

Before haemalun staining with Meyer’s acid haematoxylin can be carried out, those cuttings embedded in paraffin must first be dewaxed in xylene. In the past the tissue cuttings on the slides were placed in cuvettes filled with xylene (xylene I and xylene II) prior to a descending alcohol run for 2-3 minutes. Upon completion of haemalun staining we removed the supernatant dye by dipping the slides with the stained cuttings in two cuvettes with xylene (xylene I and II) for 2-3 minutes.

Parallel to this test we placed cuttings from the same histological preparation in Roti®-Histol (Roti®-Histol I and Roti®-Histol II) instead of xylene (xylene I and xylene II) to dewax them in addition to doing this after haemalun staining.

**Results**
After microscopic analysis of these preparations we concluded that there was no difference in dewaxing and in staining with Meyer’s acid haematoxylin when using either xylene or Roti®-Histol. We can therefore confirm that Roti®-Histol can be used as a substitute for xylene without restriction in paraffin histology as claimed in Reference (2).

**Cleaning embedding moulds**
Up to now we have always used xylene to clean all plastic or metal frames used in paraffin histology. Since lime, which is the basic chemical material of Roti®-Histol, dissolves paraffin we tried to dissolve the paraffin on the embedding frames by inserting them in Roti®-Histol. It was a complete success. However, in those cases where Roti®-Histol was not replaced often enough the dissolved paraffin suddenly coagulated.

**Methyl methacrylate – embedding method of undecalcified cortical tissue:**
For the histology of undecalcified cortical cuttings of bones, cortical sections or whole cortical bones (1) must first be stained in an ascending fuchsine alcohol series. In order to remove the supernatant water and dye completely from the cortical bones after staining we placed the cortical sections, in a series of tests, in xylene (xylene I, xylene II, xylene III) for 3 days at 24 hours a day. As a parallel test, we also inserted the cortical sections for dehydration and removal of supernatant dye in Roti®-Histol instead of xylene.

**Results**
The bone structure of undecalcified cortical cross-section preparations was no different with regard to staining irrespective of the dehydrating agent used – xylene or Roti®-Histol. Such is also the case for the polymerisation process of methyl methacrylate. In both series of tests it was equally possible to cut the cortical cross-section preparations at a thickness of 500 μ.

**Summary**
Roti®-Histol (1) and Adi Agbon (2, 1) are available on the market as a xylene substitute. Its mandarin fragrance makes Roti®-Histol pleasant for the respiratory tracts. Extensive contact with the skin should, however, be avoided as this can lead to an allergic reaction (2). In addition to the positive experience made with Roti®-Histol in this study with regard to staining and the embedding of paraffin cuttings and undecalcified cortical bones, it is also important to emphasize its easy disposal*. This is particularly crucial when a large volume is required during bone histology. We have been using Roti®-Histol both for dewaxing organic tissue cuttings as well as during the dehydrating process of undecalcified cortical sections or even whole cortical bones since the completion of the above-mentioned tests.

If Roti®-Histol is to be used as a xylene substitute for cleaning embedding moulds which are severely soiled with paraffin, it should be replaced frequently and promptly before the dissolved paraffin precipitates due to supersaturation. The pleasant handling and processing of Roti®-Histol, however, makes the extra effort worthwhile.

* Important supplement to the field report of Professor Hutzschenreuter:

In 2007 Roti®-Histol was classified as “Dangerous for the environment”, for in a long term it may have a harmful effect on water. Therefore, it is to be disposed as hazardous waste.

Carl Roth GmbH

**References**