

# Xylene-Free Tissue Processing: Good Results and Less Exposure for Staff and Environment

Pathology North Hesse, Kassel, Germany



## Pathology North Hesse Laboratory

Pathology North Hesse, in total, has a staff of 42 with an additional, five medical specialists and five assistant physicians. In the histology laboratory, reporting to laboratory heads Karin Flecke and Karoline Janas, there are twenty permanent medical technical assistants working full- or part-time, to process the incoming specimens quickly and reliably for diagnosis.

## Profile

Pathology North Hesse is a private institute for pathology, founded 2007 in Kassel, Germany with Prof. Dr. med. Josef Rüschoff as medical director. It is a consolidation and expansion of two independent pathology laboratories. Later, a branch laboratory in Eisenach, Thuringia, was added, in order to properly serve this region. The management of the Pathology North Hesse is in the hands of four associate partners – all medical specialists in pathology.

The service area of North Hesse Pathology includes hospitals and medical practices in Kassel and the surrounding region.

There are synergies with the discovery life science company, Targos, located in the same building, which specializes in the development of biomarkers and services in molecular pathology.

Technical equipment supporting the laboratory work includes seven tissue processors, three tissue embedding stations and four sectioning stations. Start of work is 6:00 A.M. – the first specimens are sectioned starting 6:30 A.M., continuing until noon. The team's goal is to process the tissue quickly, to enable a fast diagnosis and return of results. Under normal conditions, the specimens of the previous day are reported back around 1:00 P.M.

In the histology section, the workload varies between 300 to 400 specimen per day. In 2021, about 227,000 blocks were processed, resulting in about 358,000 slides.

Alongside the histology laboratory, there is a smaller cytology laboratory that handles about 30 samples a day, as well as a laboratory for IHC (Immunohistochemistry) samples. For immediate sectioning, two cryostats are available.



## Health Risks in Histology

In pathology, classification of tumors and definition of diseases comes from the analysis of tissue specimens. With the right diagnosis it is possible to develop a treatment for patients, and, in the best case, to initiate the healing of the patient. This is the aim of the pathologist, supported by the technical staff in the laboratory.

However, it should not be forgotten that the job of a medical technical assistant is one of the most unhealthy professions<sup>1</sup>. In processing tissue to be permanent and stable, hazardous fixation fluids and solvents are used. Therefore, the commitment to health of the patients can risk the health of laboratory personnel.

During the normal process, tissue is processed in four steps:

- First, the tissue is fixed, to stop degradation processes and to make the tissue stable. For this step, formalin/ formaldehyde is used in most of the pathology laboratories.
- After fixation the tissue is getting dehydrated – most often with the help of alcohol.
- The third step, degreasing and clearing is done with xylene.
- The last step, embedding into paraffin or other media, stabilizes the tissue and enables sectioning.

All those steps may include contact to formaldehyde and exposure to organic solvents and are, therefore, potentially hazardous for the user.

In the last few years, several methods have been developed to minimize the health risks in the laboratory. Technique can help here – e.g. a good ventilation system removes solvent vapors directly at their origin. Simple steps, like the usage of containers with lids preventing the fixative from evaporating at the work station, reduce the amount of solvent and formalin vapor in the atmosphere of the laboratory.

At the instrument level, there have been a number of technical enhancements improving safety: There are tissue processors on the market with built-in protection for users. The Excelsior™ ES, AS and Revos™ tissue processors from Epredia, for example, have downdraft ventilation included in the instrument. It

Since the health and life of patients depend on proper diagnoses, a solid quality management system is very important for Pathology North Hesse. Since 2013, the laboratory has been accredited by the DAkkS (Deutsche Akkreditierungsstelle GmbH; German accreditation body) following DIN EN ISO/IEC 17020 (conformity assessment). These accreditations require quality assurance and competency on highest levels. The Institute participates in internal as well as external audits and interlaboratory comparisons.

In relation to the work in the laboratory, this means – the laboratories work very precisely, cassettes are put into alphanumeric order before the start of the tissue processing, in the order of their laboratory entry, and different colored cassettes help to identify the different tissues types at one glance. The pre-processing arrangement does not add much time early in the work cycle – yet helps to save time in the end, since the workflow can proceed directly after tissue processing without any additional sorting steps. The arrangement of the cassettes also helps avoid mistakes.

automatically starts when the instrument is opened for loading or unloading of the samples. That way, the hazardous formalin and organic solvent vapors are removed in a manner that prevents the user contact. Those vapors are then routed through a variety of filters neutralizing the formalin and binding the xylene.



**Excelsior AS Tissue Processor from Epredia**

In the onboard storage area for solvent containers, hazardous vapors are removed and neutralized with filters as well. Additionally, the instruments use pre-filled containers which do not need to be decanted and can be placed directly into the instrument.

Beyond instrument features and methods that help users avoid exposure to hazardous substances, there are ways to avoid hazardous substances completely and use harmless

substitutes. For user safety, xylene should be banned from use in laboratories wherever possible. For example, in most of the tissue processing procedures, xylene can be substituted by the less-harmful isopropanol.

## Xylene-free working procedures

At Pathology North Hesse, the dehydration of tissues has moved to a xylene-free procedure wherever possible to protect the laboratory staff. In 2004, the first Excelsior ES was purchased. From that point forward, most tissue samples were processed without xylene usage. Meanwhile, in the histology laboratory, there are six Excelsior and one Revos tissue processor, of which five are operated without xylene. Xylene is needed for efficient clearing only when processing of very fatty tissue like breast and intestine.

In the Eisenach Branch Laboratory, two of the three tissue processors are running permanently xylene-free – the team has found the xylene-free procedure is not only safer in handling, but also results in higher-quality tissue.

“Among others, gynaecological, urological and dermatological tissue samples, treated with alcohol only, are easier to section. If you dehydrate with xylene, the specimen gets harder and therefore more difficult to cut”, says Ms. Karoline Janas, one of the two laboratory heads at Pathology North Hesse.

From time to time, Ms. Janas gets calls from colleagues, who have issues with the quality of their embedded tissues. “Very often, this is just an issue with the dehydration step”, according to Ms. Janas – and this can easily be fixed by using a xylene-free process for tissue processing.

“...tissue samples, treated with alcohol only, are easier to section. If you dehydrate with xylene, the specimen gets harder and, therefore, more difficult to cut”

Karoline Jonas, Deputy Head of Entry Laboratory



## Protocol for xylene-free embedding and classic embedding with xylene

Xylene-Free Dehydration		Classic Dehydration with Xylene	
Solvent	Time (hrs)	Solvent	Time (hrs)
70% Isopropanol	00:30	70% Isopropanol	00:30
80% Isopropanol	00:45	80% Isopropanol	00:45
90% Isopropanol	00:45	90% Isopropanol	00:45
96% Isopropanol	00:45	96% Isopropanol	01:00
100% Isopropanol	00:45	100% Isopropanol	01:00
100% Isopropanol	01:00	100% Isopropanol	01:30
100% Isopropanol	01:00	Xylene	00:45
100% Isopropanol	01:30	Xylene	01:00
100% Isopropanol	01:30	Xylene	01:15
Paraffin	01:00	Paraffin	00:45
Paraffin	01:15	Paraffin	01:00
Paraffin	01:15	Paraffin	01:00
<b>Total time</b>	<b>12:00</b>	<b>Total time</b>	<b>11:15</b>

*The dehydration of the tissue with both protocols runs overnight.*

*The fixation time is not included in this table – it varies with the type of the tissue.*

Protocols used in the laboratory for xylene-free and classic dehydration are very similar. Both protocols take about the same amount of time to dehydrate the tissue. Since both protocols are optimized for an overnight run, time is not a major factor.

In addition to the potentially slower time for tissue processing, higher safety for the lab personnel, and the better sectioning quality – there are two more reasons for the change to dehydrating tissue just with isopropanol. If the tissues allow: Running only with isopropanol saves reagents – per reagent rotation, just one bottle of isopropanol is used, instead of two bottles – isopropanol and xylene. This saves time for changing of one bottle (and, depending on the source of the solvents, time for decanting). It also saves money, since the costs for xylene and its disposal can be omitted.

Many institutes with xylene-free dehydration replace xylene with ethyl alcohol. Ethyl alcohol can also be replaced by isopropanol at each step of the dehydration process.

## Conclusion

At Pathology North Hesse in Kassel, the consumption of xylene is reduced to the minimum. In many cases, during tissue processing, xylene can be replaced with isopropanol as the only alcohol for dehydration. Isopropanol is the only alcohol where the user can go without the intermedium (xylene).

The resulting tissue blocks often have better usability than those processed with xylene, due to being less hard and rigid. Those tissue preparations can be used the same way as classically dehydrated tissues. They are also usable for the IHC without any restriction. “There have never been issues.”, says laboratory head Ms. Flecke.

Only when used for very fatty tissue have the results without usage of xylene been less satisfying than with classical processing methods.

“We have used this method for fourteen years”, according to Ms. Janas, “and we only see benefits. Tissue quality and safety at work are always our number one priority.”

### Pathology North Hesse

info@patho-nordhessen.de

<sup>1</sup> <https://www.businessinsider.de/most-unhealthy-jobs-in-america-2017-4?op=1>  
<https://finance.yahoo.com/news/the-15-jobs-that-are-most-damaging-to-your-health-155706120.html?guccounter=1>

Find out more at **epredia.com**