MINI REVIEW

# Will adding Michel's solution to the laboratory reagents do any good for the neuropathologists?

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## ABSTRACT

The importance of intraoperative consultation through frozen sections can hardly be over-emphasized. The overall accuracy of frozen section diagnosis falls within the range of 92% to 97.98%; however, in brain tissues, the diagnostic accuracy is debatable, considering the artifacts due to ice crystal formation, crushing, and overstretching resulting in impaired histomorphology. On the other hand, reliance on immunohistochemistry on a frozen brain section is even more challenging because of reported interpretational problems. Hence, preserving the histomorphology of brain tissues and optimizing preanalytical variables is crucial for the accurate diagnosis of the patients.

**Keywords:** Brain, fixation, fixatives, frozen section, histomorphology, immunnohistochemistry, diagnosis.

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Appropriate fixation of tissues for histological examination is extremely important. The major objective of fixation in pathology is to maintain clear and consistent morphological features. In order to visualize the microanatomy of tissue, its stained sections must maintain the original microscopic relationships among cells, cellular components, in particular, the cytoplasm, nuclei, and extracellular material, with little disruption of the organization of the tissue along with maintenance of tissue's local chemical composition.[1,2]

Fixation of tissue can be accomplished by physical or chemical methods. Formaldehyde has been the "gold standard" fixative for decades. It is cheap, enables long-term storage of surgical material, preserves morphologic features well, allows special histologic stains, and in combination with antigen retrieval, allows for immunohistochemical analysis.[3] However, formaldehyde was classified as "carcinogenic to humans" by the International Agency for Research on Cancer and, therefore, represents a risk to anyone handling the solution. Furthermore, it is cross-linking masks antigens, which may hamper immunohistochemical analysis and cause fragmentation of nucleic acids that, in turn, impairs extraction of deoxyribonucleic acid (DNA) and ribonucleic acid. Other effects of using formaldehyde as a fixative include hardening, shrinkage, and color variation in the tissue sections.[4-6]

The frozen section for intraoperative consultation is widely used to assist in timely treatment decisions. Primary diagnosis, assessment of margins of excision, and nodal status assessment are the major indications for intraoperative consultations on several types of tissues.[7,8] Several studies have been conducted worldwide to assess the diagnostic accuracy of frozen sections in general, and the overall accuracy of frozen section diagnosis has been extensively studied. This accuracy rate falls within the range of 92% to 97.98%.[9,10] The diagnostic process can be greatly affected by the technical errors in frozen sections. Freezing artifacts due to the ice crystals introduced into the examined tissue, crushing artifacts, and overstretching artifacts seen in frozen preparations are common technical errors faced in the intraoperative consultations performed on specimens.[11]

Fresh frozen specimens are a better source of template DNA compared to archived formalin-fixed specimens.[8,12] Immunohistochemistry (IHC) on a frozen section material has the potential to reduce the number of late positive lymph nodes, especially concerning micro-metastasis and single-cell infiltration.[13,14] An interpretational problem with IHC on

frozen sections is impaired morphology combined with the presence of endogenous peroxidase, which might give false positive staining results, but snap freezing to reduce freezing artifacts and blocking endogenous peroxidase activity greatly reduces these artifacts.[15-17]

One of the other imperative considerations while performing IHC, especially on frozen sections, is the time consumption and cost of the procedure. Nowadays, intraoperative IHC examination methods have been developed. The use of IHC results in higher detection rates of metastases and fewer re-operations. A study conducted to evaluate the clinical and economic implications of intraoperative IHC showed an overall cost saving of approximately 40%.[18,19] The central nervous system (CNS) tumors pose diagnostic challenges because tumors of varying histogenesis show divergent differentiation and overlap in morphological features.[20] IHC is done, in addition to the routine histopathologic examination, to overcome the diagnostic difficulties since an accurate histologic diagnosis helps in predicting the clinical outcome of various CNS tumors.[21] The diagnosis of progressive glioblastoma is aided by the use of immunohistochemical stains. Specific genetic alterations that have been most thoroughly documented include isocitrate dehydrogenase 1 and 2 mutations, and promoter methylation status of methylguanine-DNA methyltransferase can also be determined by antigenic expression on tissues.[22]

Unfortunately, freezing of brain tumor tissue samples impairs histomorphology, and diagnostic tumor typing remains conclusive on the basis of tissues that are formalin-fixed and paraffin-embedded.[9,23] Thus, histomorphology and molecular analyses have to be performed from two different tissue samples, which could hamper a direct comparison of results.[24] Moreover, the maintenance of a frozen tissue bank is complex and costly. The collection of frozen brain tumor samples in the frame of multi-centric clinical trials with companion tissue-based translational research has proven problematic, in particular for logistic and economic reasons.[25] Alternative methods that do not impair nucleic acid quality and at the same time warrant high-quality histopathology and facilitate molecular analysis are needed. In recent years, several new fixatives and transport media have been proposed as an alternative to formalin fixation.[8,26,27] Among these alternatives, Michel's transport medium (MTM) seems to be a promising option. This medium, initially described by Michel et al.[28] has been simplified by Niedecken and Lange[29] and is currently used by most of the laboratories for the transport and detection of tissue-bound immune reactants by direct immunofluorescence microscopy and molecular analysis[30,31] and was subsequently used for transport of renal biopsy specimens for evaluation of immunoglobulin and complement deposition and even lymph nodes for detection of lymphocyte surface markers.[32] The MTM has not yet been extensively tested for brain biopsies but is recommended, in a single study, for use in a study for transporting small brain tissue sections which were 0.2 cm thick for long-term preservation in autopsy specimens.[33]

From a neurosurgical viewpoint, intraoperative consultation on neurosurgical specimens is a valuable guide for the best intra-or post-operative patient management. In addition, tumors such as astrocytomas and oligodendrogliomas can be intraoperatively diagnosed if artifacts and background staining in IHC can be knocked down.[34] Direct freezing of tissues leads to antigenic cryo-precipitation that can be a major issue in false positive/negative reporting of brain biopsies. Hence, the development of new fixatives and/or methods must be explored to minimize these artifacts and to maximize the retention of morphological details as well as antigenicity for reaching a conclusive diagnosis and differential diagnosis, which is critical for patients with CNS lesions, including tumors.

## Limitations of the Review

This review could not include data and efficacy statistics of MTM on different tissues as reported in the literature. A comprehensive systematic review should be carried out to generate reliable and authentic data regarding the utility of this fixative in routine labs.

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### List of Abbreviations

| | |
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| CNS | Central nervous system |
| DNA | Deoxyribonucleic acid |
| IHC | Immunohistochemistry |
| MTM | Michel's transport medium |

### Conflict of interests

None to declare.

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### Ethical approval

Not applicable.

### Authors' contribution

**ALL AUTHORS:** Conception, critical intellectual input, drafting of manuscript. Approval of the final version of the manuscript to be published.

**GR:** Conception and design of study. Acquisition of literature, critical intellectual input. He was co-supervisor of the M.Phil research project of the Principal author.

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