Use of Isopropyl Alcohol in Histological Assays: Dehydration of Tissue, Enbessing into Paraffin, and Processing of Paraffin Sections

I. V. Viktorov and S. S. Proshin

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Isopropyl alcohol (isopropanol) can be used as a substitute for ethyl alcohol in tissue dehydration during embedding into paraffin and dehydration of stained sections. The use of isopropyl alcohol during paraffinization allows us to exclude treatment with intermediate solvents of paraffin (chloroform, xylene, and benzene), which reduces the degree of tissue compaction and simplifies and accelerates histological assay.

Key Words: *isopropyl alcohol; isopropanol; histological technique; tissue paraffinization*

Isopropyl alcohol (isopropanol) is an inexpensive and available substitute for ethyl alcohol that was brought into use for histological assays in the mid-20th century [7-11]. Manuals on microscopic studies recommend isopropanol for dehydration and paraffinization of tissues [3-5]. Isopropanol is a component of water-soluble dyes for lipid staining [3,4] and of Newcomer fluid that substitutes for Carnoy fluid for fixation of nucleic acids and preservation of chromosomes [3,4]. However, recommendations for the use of isopropanol in histological assays were not adequately described in Russian literature and were not widely used in laboratory practice. We believe that it is necessary to compensate for this deficiency.

Isopropyl alcohol (C_3H_8O) is characterized by extremely low acidity and can be mixed with water, ethyl alcohol, and organic solvents. Water content in anhydrous isopropanol (isopropanol-99) should not exceed 0.1% ("chemically pure") and 0.15% ("pure") [6]. Isopropanol is less toxic than xylene [7,8,10]. Hygroscopicity of isopropanol is lower than that of ethyl alcohol. Isopropanol is well preserved in the anhydrous state. Isopropanol poorly dissolves native paraffin, but it is easily mixed with molten paraffin. Isopropanol is recommended for dehydration and further paraffinization of tissues [3,5,7-11]. The use of isopropanol during dehydration and paraffinization of tissues simplifies and accelerates the process of histological processing. It excludes treatment with intermediate solvents of paraffin (chloroform, xylene, and benzene), which reduces tissue condensation, provides its greater plasticity in cutting [8,11], and allows obtaining thin serial sections (4-8 μ). Undoubtedly, the quality of sections depends on properties of paraffin used for tissue embedding. In our experiments we used paraplast (Paraplast Plus, Sigma, Cat. No. 3683).

Pure isopropanol is not used as a fixative, since it slowly diffuses into tissues. In histological and pathohistological assays of animal brain we use the method of vital transcardial perfusion [13] with a modified Tellesnitskii fixative [12], where ethyl alcohol is substituted for isopropanol (70% isopropanol, 20% formalin, and 10% glacial acetic acid). Autopsy specimens are fixed by embedding into this fixative. Fixation of the whole rat brain or tissue fragment takes 2.5-3.5 h. Since this fixative contains 70% isopropanol, tissue blocks are treated with 90% isopropanol (1 h), isopropanol-99 (1 h), isopropanol-99 (1.5 h), and isopropanol-99 (2 h). The volume of isopropanol should exceed the tissue volume by at least 50 times. Tissues can be maintained in the last portion of iso-

Laboratory of Experimental Neurocytology, Institute of Brain Research, Russian Academy of Medical Sciences, Moscow

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propanol overnight. After treatment with isopropyl alcohols, the tissue is kept in molten paraffin (56-60°C) for 4-5 h or overnight. Then the tissue is placed in freshly prepared paraffin for 30 min. This paraffin is used for final embedding and formation of paraffin blocks. The series of dehydrating compounds and first and second portions of paraffin can be used repeatedly.

After Doxtader's formalin fixation [8] the tissue should be thoroughly washed with flowing water to remove formalin, dehydrated, and embedded in paraffin. The tissue is treated with 60% isopropanol (1 h), isopropanol-99 (1 h), isopropanol-99 (2 h), isopropanol-99 (3 h), and paraffin (1 h). Then the tissue is kept in paraffin overnight and embedded in paraffin. During paraffinization low boiling point for isopropanol contributes to its rapid and complete removal from the tissue and paraffin [8].

Deparaffinization of sections with isopropanol includes 2 treatments with xylene and isopropanol-99, 90% isopropanol, 70% isopropanol, and distilled water.

After treatment with isopropanol deparaffinized sections of the brain and other organs were stained by the method of Nissl's with modifications using the buffer solution of cresyl violet [1], hematoxylin and eosin, and vanadium hematoxylin and acid fuchsin for acidophilic cells [13]. Myelin fibers of brain structures were stained with luxol fast blue (Kluver—Barrera stain) [2].

Stained preparation were dehydrated with 70% isopropanol and 2 portions of isopropanol-99 and xylene and mounted with Canadian balsam or synthetic resins (Entellan and Polymount) under a cover glass. By the quality of staining these preparations did not differ from those obtained after embedment in paraffin using ethyl alcohol and standard intermediate media (chloroform, xylene, and benzene).

Our experiments with isopropanol revealed advantages of this relatively inexpensive and available reagent that serves as a substitute for ethyl alcohol. Isopropyl alcohol is recommended for the use in histological assays.

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