Ultra-Rapid Microwave/Variable Pressure-Induced Histoprocessing: Description of a New Tissue Processor

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Abstract

We describe a new method of ultra-rapid histoprocessing that reduces the processing times for needle and endoscopic biopsies to 30 min and that of other surgical biopsy tissue blocks of up to 4 mm thick to 120 min. The MicroMED U R M Histoprocessor, which combines microwave irradiation with precise computer control of power, time, temperature, vacuum, and pressure, when used with a 1-step dehydrating/clearing reagent, consistently produces rapidly processed tissues with optimal cytomorphology and improved tissue sectioning properties. Staining properties are excellent with no deleterious effects on routine staining, histochemistry, or immunohistochemistry. This new processing technique represents a major change from conventional tissue processing and eliminates the use of hazardous reagents such as xylene. The ease of application and speed of this technique can significantly reduce turnaround times in diagnostic laboratories. (*The J Histotechnol* 21:219–224, 1998)

Key words: microwave irradiation, pressure, one-step dehydration and clearing, ultra-rapid histoprocessing, vacuum

Introduction

Over the past 10 yr microwave (MW) irradiation has been applied in histology for tissue fixation to accelerate techniques such as histochemical staining, immunohistochemistry staining, and antigen retrieval (1–22). Of particular importance has been its application to tissue processing where earlier investigators have utilized domestic or slightly modified ovens, attracting worldwide interest (12,23–30). The growing research in the applications of MWs for the acceleration of tissue processing has been fueled by the expectation that tissue processing times, which have changed very little over the past 50 yr, can be accelerated through advances in technology, providing significant improvements in specimen turnaround times and attendant cost benefits.

However, the adoption of MW irradiation for routine tissue processing has not been realized because of inconsistencies in results, which has been attributed to deficiencies in hardware design, lack of precise control of parameters such as temperature and power setting, and the uneven distribution of MWs in commercial ovens. Furthermore, published procedures for MW-stimulated tissue processing are either labor intensive, time-consuming or cumbersome when compared to conventional automated processing. Recent technological advances in hardware design and computer software have addressed these limiting factors, resulting in the development of a new ultra-rapid microwave tissue processor and a 1-step dehydration/clearing reagent. This study documents the evaluation of such a unit and the newly developed reagent and discusses their potential role in diagnostic pathology.

Materials and Methods

Reagents

The tissue processing method employs a recently developed 1-step dehydration/clearing reagent designated the "JFC solution," a proprietary name (Milestone, Sorisole, Italy), and comprises an organic solvent mixture of absolute ethanol, isopropyl alcohol and a long chain hydrocarbon (patent pending). Under the influence of MW irradiation the mixture becomes highly efficient in the simultaneous elimination of water and lipids from tissue, as a single step, without the necessity for further dehydration or clearing. All other reagents utilized are conventional in routine histology laboratories.

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Histoprocessor

Tissues were processed in the MicroMED URM (Ultra Rapid Microwave) Histoprocessor (developed by Milestone). The cavity of the unit incorporates a rotating glass dome processing chamber, fiber optic temperature sensor, and in-built MW absorbent magnetic stirrer (Figure 1). The glass processing chamber accommodates up to 60 tissue cassettes contained in a MW-transparent plastic rack, arranged as 2 concentric circles inside a glass dish (Figure 2). The tissues blocks are immersed in the JFC solution and subjected to MW irradiation, pressure, and vacuum, in sequence.

The unit is controlled through an interactive touch screen computer and dedicated software that ensures consistency and simplicity of operation. A menu of processing schedules can be activated by touch screen icons with preset parameters for microwave power, time, temperature, vacuum and pressure, all continuously monitored and regulated by the computer software (Figure 3). The completion of a selected procedure is indicated by an audible alarm. The system also provides protection of stored programs with access by means of a key card, ensuring only authorized access for modification or creation of customized processing schedules.

Tissues

Multiple tissue blocks were prepared from a wide range of formalin fixed surgical specimens, representing malignant and benign tissues, including breast, stomach, uterus, and spleen. Three size-formats were prepared, representing tissue blocks routinely encountered in surgical pathology laboratories:

1. Needle and endoscopic biopsies - measuring up to 20 mm in length and up to 2.0 mm in diameter
2. Intermediate size - measuring up to 15 × 10 × 2.5 mm
3. Large size - measuring up to 25 × 20 × 4 mm

Up to 60 tissue sections in universal plastic cassettes were placed in the MW-transparent plastic rack and rinsed in absolute ethanol to remove coating formalin and water. The rack was placed into the circular glass dish containing the JFC solution (20 ml/cassette) and positioned under the glass dome, with the appropriate selected processing schedule.

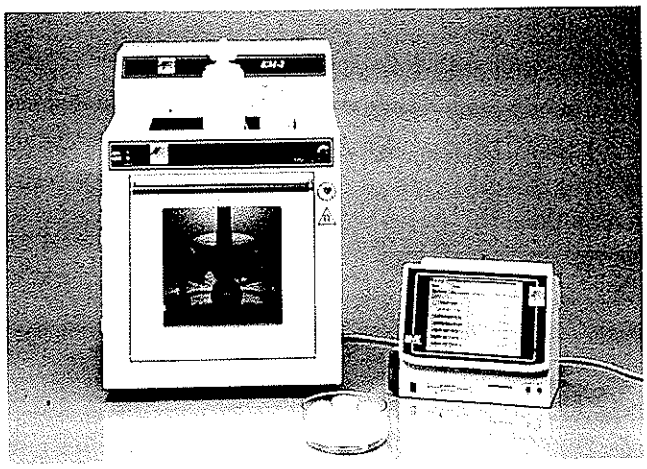

Figure 1. MicroMED U R M Histoprocessor comprising the microwave unit with pressure/vacuum resistant glass dome, touch screen computer, and glass cassette holder.

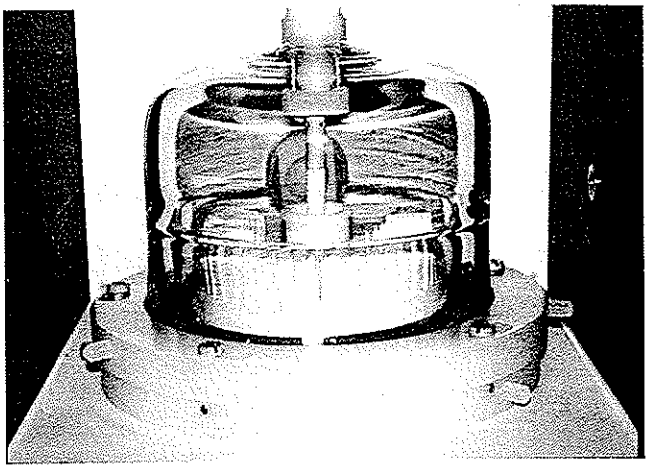

Figure 2. Sixty cassettes can be accomodated in the glass dish contained in a pressure/vacuum resistant glass dome with a central fiber optic temperature sensor. The glass dome sits on a carousel.

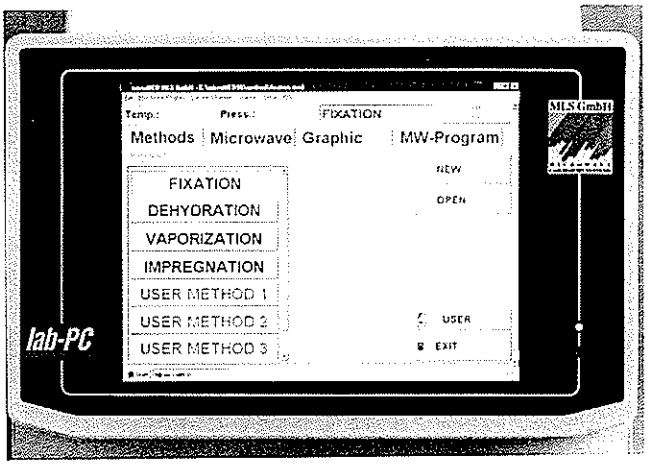

Figure 3. The touch screen computer in close up. The menu on the left side allows the selection of each of the 3 steps of dehydration, vaporization, and impregnation. In addition, there is provision for 3 customized protocols for each of these steps under "user method." There is also provision to set up programs for fixation in the unit. Fixation can be conducted under microwave stimulation either in normal saline or in a chemical such as formaldehyde. The menu on the right side allows the programming of new protocols for all of these steps.

Processing Schedules

Total processing times to paraffin for the specimens were: needle and endoscopic biopsies, 30 min; intermediate size, 90 min; and large size, 120 min. The processing schedule chosen was determined by the largest piece of tissue in the batch being processed. The entire process consisted of 3 main steps, with each controlled by its own preset program.

Step 1. Simultaneous 1-step dehydration and clearing in JFC solution: needle and endoscopic biopsies, 18 min; intermediate size, 65 min; and large size, 90 min. Temperature set at 70°C, MW power at 500 W, and pressure up to 2 Bar.

Step 2. Post dehydration vaporization: needle and endoscopic biopsies, 1.5 min; intermediate and large

size, 3 min. Temperature set at 60°C, MW power at 100 W, and vacuum at 0.1 Bar.

Step 3. One-step wax impregnation: needle and endoscopic biopsies, 9 min; intermediate size, 20 min; and large size, 25 min. Temperature set at 65°C, MW power at 400 W, and vacuum at 0.1 Bar.

On completion of Step 1 (dehydration/clearing), the rack was removed and briefly blotdried to remove excess reagent, placed into an empty glass dish, and repositioned under the glass dome before the commencement of Step 2 (post-dehydration vaporization). For Step 3, the cassette rack was removed, placed into a preheated glass jar containing histology grade molten wax at 65°C, and located under the glass dome before the commencement of the selected program. On completion, tissues were ready for embedding. Throughout Steps 1 and 3, the built-in magnetic stirrer ensured uniform heat distribution and acted as a source of heat, generated by a coating of MW-absorbent material (Weflon; MLS, Leutkirch, Germany) on the mixing bar.

Staining

Four-μm sections were stained with conventional hematoxylin and eosin stains and with routine histochemical stains such as Masson trichrome, Verhoffs elastic Van Gieson, and periodic acid Schiff. For immunohistochemistry, we used a peroxidase conjugated streptavidin-biotin system preceded by MW induced antigen retrieval, and antibodies to CD45, CD45RO, CD20, CEA, vimentin and cytokeratins were applied (21).

Results

Macroscopically, all paraffin blocks were uniformly and adequately dehydrated, with homogenous clearing and wax impregnation. Of particular note were the even and translucent features of fatty tissues, indicative of well processed tissue (Figure 4). The cutting properties of all tissue blocks were consistent, and all displayed a characteristic softness and smoothness during sectioning, best described as a "lubricated" effect, enabling ribbons of sections to be cut consistently, even for more difficult tissues such as myometrium. Microscopic examination revealed consistent and uniform preservation of tissue architecture and cytomorphology (Figures 5, 6, 7 and 8). Histochemical and immunohistochemical stains were consistent with expected patterns of antigenic staining intensity and distribution.

Discussion

Conventional tissue processing for wax impregnation relies on multiple changes of reagents (commonly graded alcohols and xylene) of varying concentrations depending on their diffusibility, followed by paraffin wax impregnation. The entire process is facilitated by vacuum and heat. An earlier study, employing a prototype MW tissue processor, the Lavis 1000 MicroMED (Milestone), was successful in producing well processed tissue from samples specially fixed in Kryofix (a mixture of ethanol, water and low molecular weight polyethylene glycol) or in 4% formalin for 48h (29,30). However, we have found that tissue blocks with a high lipid content failed to process uniformly and showed variable degrees of fat extraction (unpublished data). This limitation led us to develop the JFC solution, which allows a 1-step dehydration and clearing with consistent and optimal results. To accomodate and exploit the unique properties of this solution, we had to develop new hardware with the resultant, completely new MicroMED URM.

The miscibility of the 3 components of the JFC solution allows simultaneous 1-step dehydration and clearing, and represents a radical modification to conventional tissue processing. The action of this mixture can be explained by polarity of molecules. As a general rule, for optimal extraction to occur, the polarity of the solvent has to be similar to that of the "impurity" being removed; in histoprocessing, such impurities are mainly water and lipids. Tissue lipids consist primarily of 2 classes. Cellular glycerols are formed by the esterification of long chain fatty acids (C12–C18) with glycerin; the aliphatic portion of the fatty acids is non-polar, whereas glycerin is highly polar, together resulting in the formation of a moderately polar lipid. Phospolipids, the other major class of tissue lipids, are the main constituent of cell membranes, formed by the incorporation of phosphate groupings into lipid molecules: As a consequence, the entire molecule exhibits a high polarity. Isopropanol, a polar molecule, contributes to dehydration and also enhances the extraction of polar lipids. Ethanol, also a polar

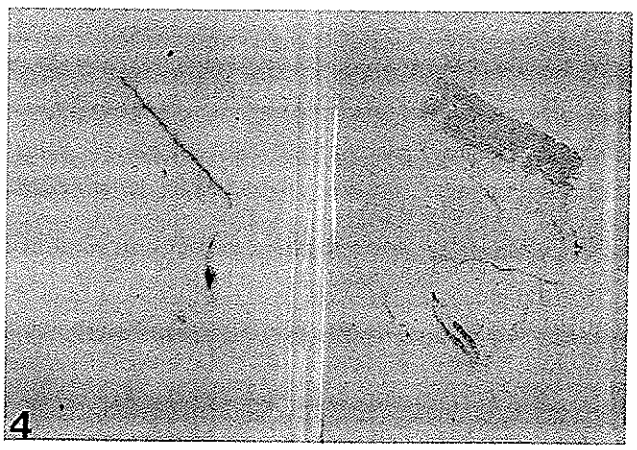

Figure 4. A paraffin embedded tissue block of a piece of skin and corresponding H & E stained section. Note the uniform processing of the fatty subcutaneous tissue, which sectioned with a soft "lubricated" consistency.

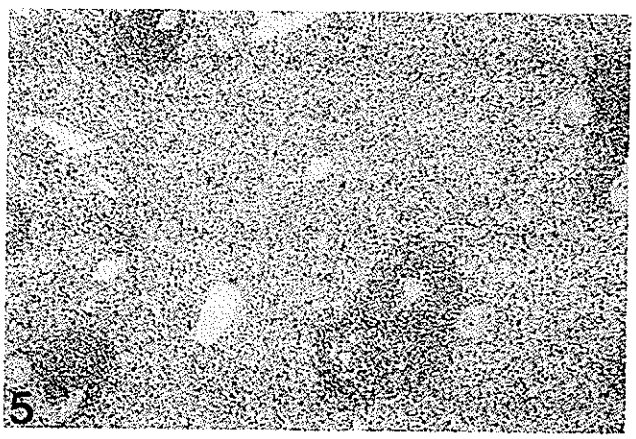

Figure 5. Section of spleen processed over 90 min to show the uniform and excellent cytomorphological preservation. H & E. Original magnification ×50.

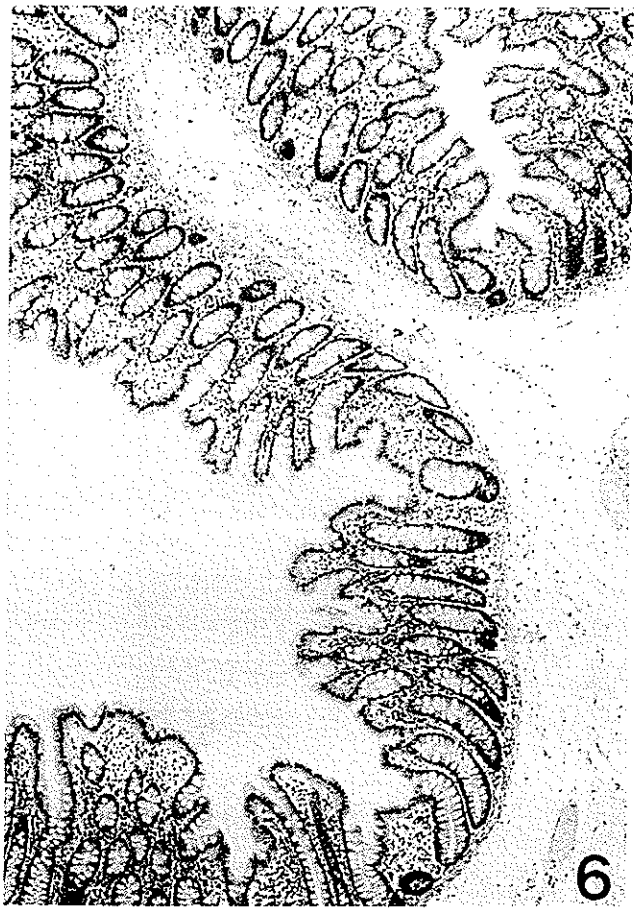

Figure 6. Section of caecum, processed over 30 min, showing excellent cytomorphological preservation. H & E. Original magnification ×50.

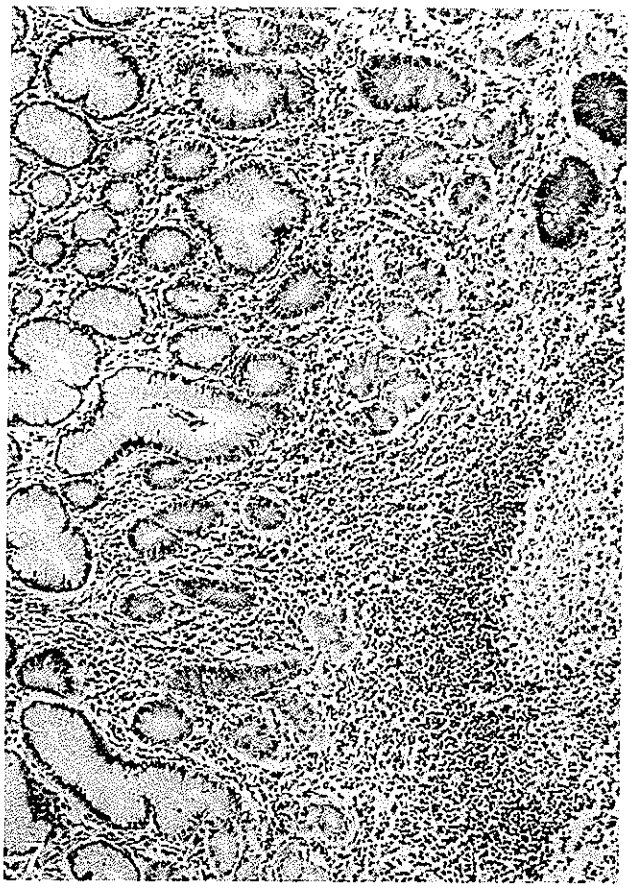

Figure 7. Tissue block taken from a gastrectomy specimen, processed over 90 min, demonstrating chronic gastritis with lymphoid follicles. H & E. Original magnification ×125.

molecule, primarily serves to dehydrate, with the action of both alcohols becoming highly efficient under MW stimulation. The third component of the JFC solution, an organic solvent, is a chemically inert, non-polar, and relatively non-toxic molecule. Although it has a relatively poor affinity for lipids under normal conditions, its delipidating activity is greatly enhanced by MW irradiation in the presence of ethanol and isopropanol.

The MicroMED URM Histoprocessor provides the combination of MW and alternating pressure and vacuum essential to exploit the properties of the JFC solution allowing optimal 1-step dehydration-clearing to be achieved at 70°C. The interaction of ethanol with the polar molecules of water within the tissue results in accelerated dehydration, as opposed to the diffusion that occurs with the conventional methods of tissue processing. The delipidating activity of the long chain hydrocarbon on non-polar fatty chains, combined with the action of isopropanol on the polar portions of the lipid molecule, results in highly efficient extraction of all tissue lipids. The action of the JFC solution at its optimal temperature of activity not only results in the simultaneous accelerated extraction of lipids and water molecules but also produces some condensation of the alcoholic components. The procedure is performed under pressure at 2 Bar; the positive pressure raising the boiling point to avoid reagent vaporization.

The second step of post dehydration drying prepares the tissues for wax impregnation. This is achieved through the removal of residual JFC solution present in the tissue by vaporization. The tissue blocks contained in cassettes are placed into an empty glass container and subjected to MW irradiation and vacuum at 0.1 Bar. The latter lowers the boiling and vaporization point temperatures of the JFC reagent and produces tissue dehydration and the evaporation of the JFC reagent. The desiccating action results in minimal macroscopic shrinkage of the tissue blocks, however, there is no disruption of their structural integrity.

Wax impregnation is performed as a single step under MW influence and at vacuum of 0.1 Bar. This vaporizes any residual traces of the JFC solution and assists with impregnation, the latter process restoring the tissue blocks to their original volume and shape, with no deleterious effects on their cytomorphology.

Processing time for needle core biopsies can be further reduced to as low as 15 min for samples 1 mm in diameter.

The histoprocessor has been designed to ensure uniform MW irradiation, accurate temperature recording through a fiber optic temperature sensor. Uniform temperature distribution is achieved through a unique magnetic stirrer built-in within the electromagnetic (MW) field. The development of specifically dedicated software and preset programs to monitor and control the essential parameters of processing, ie, power, time, temperature, vacuum and pressure, has eliminated guesswork, creating a consistently reliable, and

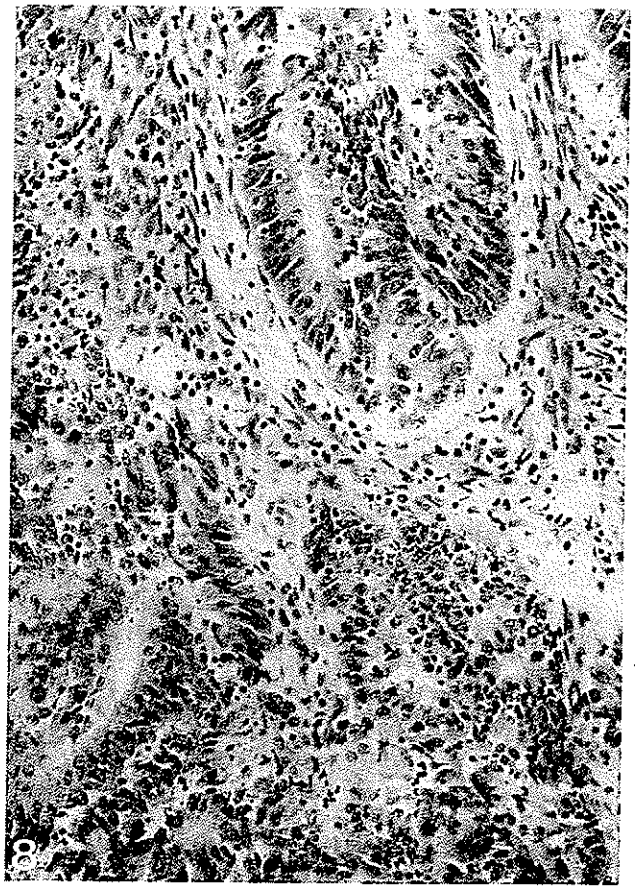

Figure 8. Section of colonic carcinoma processed in 90 min. Despite the large areas of necrosis and inflammation, the tissue held together during processing and cytomorphologic preservation was satisfactory. H & E. Original magnification ×125.

easy to use processor. The system is almost fully automated and merely requires the manual transference of the rack of tissue cassettes after each of the 3 steps in the procedure and activation of the relevant screen icon. Once the unit is programmed, the operator can go on to other tasks knowing that the completion of each step will be indicated by an audible alarm. An additional feature of the software is the graphic display of all parameters during the processing and their documentation as a permanent record for compliance with Quality Assurance Programs.

There is provision for a fixation cycle in the unit. Accelerated fixation of the tissue blocks can be conducted under the stimulation of MWs with duration and temperature controlled by the computer. Fixation can be conducted according to any of the previously published protocols in normal saline or in a chemical fixative such as formaldehyde or alcohol (2–9). However, only formalin fixed tissues were included in the study.

The histoprocessor is associated with a reduction in reagent usage and costs. The single, 1-step wax impregnation process without contamination allows reuse of the wax, requiring only the occasional "top-up" to replace wax impregnated into the tissues blocks. The savings from significantly reduced wax consumption can be enormous over the long term. This contrasts with the conventional method where a change of at least 1 wax container per processing run is required to eliminate xylene carry-over. Xylene, the conventional clearing agent, contributes substantially to environmental pollution at a global level, and its toxicity is well known. Xylene can be as expensive to dispose of as it is to purchase, so that its elimination from tissue processing represents important progress and significant cost savings.

Conclusions

The MicroMED URM Histoprocessor was developed from experience with MW applications in the fields of histoprocessing and analytical chemistry. The hardware is reliable and user-friendly, allowing consistency in the application of MW irradiation with pressure and vacuum. Together with the highly efficient JFC solution this new histoprocessor allows a rapid single step clearing/dehydration for greatly accelerated histoprocessing with its attendant benefits.

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