THE FUNDAMENTALS OF ...

Tissue Processors

Robert M. Dondelinger

Before a pathologist can perform a microscopic examination of tissue removed from a body, such as biopsy tissue for diagnostic purposes, the tissue must be prepared. This preparation must simultaneously leave the tissue unaffected and preserve the tissue for storage and later review. The process of preparation involves fixing (stabilizing or setting), dehydrating, clearing, and infiltrating tissue specimens (using some potent and hazardous chemicals) so they can be imbedded in either paraffin for thin slicing by a microtome for visual or in plastic for electron microscopy.

Tissue is often first fixed with one or more formalin baths. Each bath can last from one to four hours. Next, the tissue is dehydrated using multiple solutions of between 70% and 100% pure ethanol. Dehydration is usually followed by two clearings of toluene or chloroform. These clearings remove all traces of the alcohol and some solutions even make the tissue transparent for visual examination. Lastly, the sample is transferred to a paraffin bath for up to four hours, allowing the paraffin to infuse into the sample tissue.

Once the tissue has processed for the required time, which varies by tissue sample and specific technique, one of two actions are taken. If the sample is to be visually examined, it is centered in a plastic or stainless steel blocking mold to which more paraffin is added. After the paraffin has cooled, the specimen is removed from the mold, mounted to a microtome, and thinly sliced for optical microscopic observation. If the sample is to be examined with an electron microscope, the processing steps are substantially similar, but different chemicals are used, and the processed specimen is mounted in a resin or plastic material instead of paraffin. Epoxies and acrylics are commonly used for mounting specimens for electron microscopy, depending on the application.

Current Technology

Without automation, tissue processing would be a labor-intensive, error-prone, dangerous task performed by low-paid laboratory assistants. The modern tissue processor automates this task, reducing both human error and the lab technician's exposure to the hazardous chemicals. Automated tissue processing uses the same chemicals and procedures as manual processing, but prepares tissue for microscopic examination in a much safer manner. Product designs fall into one of two basic classifications of tissue processors, either stationary-chamber or moving-basket. In either case, the basic purpose of a tissue processor remains the same—to automate the process of fixing a tissue specimen for examination. Cycle time for tissue processors varies between three and 72 hours (with 12 hours being typical) and is determined by the rate at which each reagent penetrates the sample, which is a function of both the type of tissue and the reagents employed. During a typical processing cycle, immersion time is usually around one hour,

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although some reagents with high penetration rates can reduce the time to only 15 minutes per step. Gentle agitation aids penetration as does drawing a partial vacuum on the specimen chamber. The vacuum also helps to remove dangerous vapors from the area, both an Occupational Safety and Health Administration (OSHA) and fire prevention consideration.

As the name implies, stationary-chamber processors employ a single chamber or container to process the specimen. Each specimen is placed in a perforated cassette and then placed in the processing chamber. During the processing cycle, the chamber is alternatively filled, drained, and purged of the particular processing agent, be it a fixative, a dehydrating or cleaning agent, or an embedding fluid. This occurs through a centrally controlled system of tubing, valves, and pumps connected to individual holding containers for each solution. An additional clean-and-flush step after each processing reagent removes the residue of the previous reagent and readies the chamber for the next reagent.

Variations of this basic design reduce the number of individual tubing lines needed. These designs employ a common tube and one or more solenoid operated and/or multiport valves to switch between reagent drain (evacuation), clean-and-flush solutions, and the incoming reagent. A recent variant of the stationary-chamber design employs microwave technology at specific points in the processing cycle. By placing the specimen container within the cavity of a microwave oven, chemical action and penetration is increased, which results in an overall reduction of the processing time. Some designs employ a vacuum system to increase the fill rate and pressure to speed the removal of reagents. This same vacuum system also removes fumes from the chamber and immediate area around the machine and either evacuates them to a safe location (such as outdoors) or traps them in a charcoal filter.

Stationary-chamber processors typically utilize microprocessor technology to control the processing cycle. This requires the laboratory technician to individually program each step of the operation—which solution, what chamber temperature, immersion time, agitation or no agitation, drain time, and purging. One advantage to microprocessor control of processing is that it allows for automatic self-checking and validation of the process and the triggering of audible and visual alarms as well as automatic shutdown of the device in the event of a catastrophic malfunction. Many microprocessor-controlled tissue processors allow the operator to store frequently used processing parameters into recallable programs for quick selection, maintain their memory with an uninterruptable power supply (UPS) or backup battery in the event of a power failure, and provide a report of the various operating parameters during a processor run.

The moving-basket processor design employs one container (normally glass, ceramic, stainless steel, or plastic) per reagent, placed in processing sequence in either a rectangular or circular pattern. The first processing chemical (for example, formalin) is placed in the first container in the rotation order; the second chemical (another formalin solution, for example) is placed in the second container, etc., until all processing chemicals are in their respective containers. The laboratory technician places the tissue to be processed in a perforated cassette just as in the stationary-chamber processor, but on moving-basket processors, the cassette is designed either to be hung from an overhead mechanism or placed in one or more perforated baskets attached to the overhead mechanism.

A rectangular moving-basket tissue processor showing the individual containers within the hood.

If the containers are placed in a rectangular pattern, several motor and gearbox assemblies are used to raise and lower the specimen and to move it in two dimensions from one processing chemical position to another. The mechanism moving the specimen from one position to another in rectangular pattern processors is similar to an overhead-track crane in a factory that moves materiel in X, Y, and Z coordinates, with the Z corresponding to raising and lowering the basket. The X and Y coordinates, of course, correspond to the location of individual processing chemical containers. Because a rectangular pattern processor employs a single basket, moving from one processing position to another, it can only process one cassette or basket at a time, but it is more flexible since individual containers may be skipped as required by the processing protocol. Typically, the entire area above the chemical containers is covered by a shroud to vent fumes and reduce the risk of fire. Some models are even fitted with a built-in fire-suppression system within the shroud.

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When the containers are configured in a circular pattern, typically two motor and gearbox assemblies are connected to a center shaft upon which is connected a series of spoke-like arms, each holding a basket. Often, this shaft-and-arm assembly is covered by a shroud to help contain fumes. One motor and gearbox assembly causes the central shaft to go up and down, while the other rotates the arms either clockwise or counterclockwise, depending on the design. Because the containers are arranged in a circular array, several baskets can be individually processed as long as processing times are the same. Like the rectangular pattern processor, the laboratory technician places the tissue to be processed in a perforated cassette and/or-basket, but the overhead mechanism looks and operates differently.

The processing begins when one motor and gearbox lowers the basket into the first processing solution, and may employ agitation by providing minor up and down motion during processing. At the end of the processing time for the first solution, the same mechanism raises the basket well above the container. There, it pauses for a programmed drain time, then the other motor and gearbox rotates the arms to position the basket above the second solution container, and the first mechanism lowers the basket into the second solution container. Meanwhile, another specimen, which may be placed in the second basket, is lowered into the first processing solution. Again, gentle up and down agitation may be provided by this mechanism. Once the processing time has elapsed, the entire sequence is repeated and continues until the processing cycles through all the chemicals, reagents, and paraffin bath is complete.

Moving-basket processors generally rely on one of two methods for controlling the processing cycle. Legacy and some modern units employ a traditional notched circular disk that is mechanically rotated by a clock motor. The laboratory technician "programs" the processor by cutting notches corresponding to points in the cycle where the processing cycle changes (triggering the removal of the tissue specimen from one container, draining, and moving it to the next container) out of the disk, with the width of the notch corresponding to the duration of each process. As the disk rotates, a sensor—mechanical in legacy units and optical in some modern units—detects the notches and initiates the cycle advancement. The second method of control is microprocessor technology as in the stationary-chamber processor. Like all control methods used for tissue processors, the laboratory technician must individually program each advance of the cycle, but unlike the stationary-chamber processor, certain solutions cannot be skipped or used out of sequence from their circular setup pattern. Microprocessor control of moving-basket processors feature the same technological advantages of the stationary-chamber tissue processor, such as automatic self-checking and validation of the process, the triggering of audible and visual alarms, and automatic shutdown of the device in the event of a catastrophic malfunction.

How to Manage the Device

Low-end tissue processor prices begin around $11,000 and go up to the $150,000 range. As such, they represent a substantial investment to the healthcare facility. Therefore, maintenance should be scheduled for the item (by unique identification or serial number) and a detailed maintenance history should be maintained. Microprocessor-controlled, high-end tissue processors may benefit from a maintenance contract with the original equipment manufacturer if it includes software upgrades at no additional charge. Since these upgrades are cumulative, they must either be purchased when they are released or included in a service contract. A cost-benefit study should be performed as part of the in-house versus contract decision-making process.

Regulations

In the United States, both regulatory and nonregulatory agencies are involved where tissue processors are used. Each has its own concerns and requirements; fortunately, they tend to be complementary rather than conflicting. These agencies both ensure the safety of the laboratory technicians and others in the area and that of the patients whose outcomes are dependent upon the proper processing of tissue prior to examination and evaluation:

- OSHA is concerned about hazardous chemicals and worker exposure to fumes. Chemicals such as formalin and various alcohols are used in fixation and are considered hazardous chemicals, but are necessary to provide a readable slide to the pathologist. In fact, most of the chemicals used in modern laboratories present both a health and safety hazard to users. To protect workers, OSHA imposes limits on the amount of

time an employee may be exposed to these chemicals at work. The use of shrouds and the removal of hazardous vapors improve operator safety.

- The Clinical Laboratory Improvement Amendments (CLIA) of 1988 identify three levels of complexity for all laboratory procedures: waived, moderate complexity, and high complexity. A procedure level is based upon a number of factors including laboratory technician training and experience, as well as the quality control (QC) necessary to ensure accurate results. Since a high degree of training and experience is required to prepare the tissue sample—the processing includes quite a number of steps, the chemicals must be precisely prepared, and a high level of operator intervention is required—tissue processing is classified as a "high complexity" procedure.
- The National Fire Protection Association (NFPA) requires an automatic fire extinguishing system in the area where a tissue processor is used. Additionally, they prohibit the storage of combustible materials within five feet of open tissue processors. Although the NFPA is not a regulatory agency per se, many states and municipalities have incorporated NFPA standards in their fire code. The authority having jurisdiction over the facility using a tissue processor can be queried to determine if compliance with NFPA is mandatory or advisory. Here again, the use of shrouding and vapor removal improves safety by reducing the risk of fire.
- The College of American Pathologists (CAP) accreditation assessment includes an evaluation of how well the laboratory prepares tissue specimens for examination. They also operate an educational program to improve laboratory technicians' skills in the preparation of histologic slides. Furthermore, laboratories are encouraged to submit sample slides for critique by a peer committee to facilitate program improvement. The committee—consisting of histotechnologists, histotechnicians, and pathologists—evaluates the slides for fixation, tissue processing and embedding, microtomy, staining, and coverslipping and offers suggestions for improvement.

Risk Management Issues

The art of processing tissue for examination, either manually or using an automated process, involves a plethora of risks, all of which must be managed to ensure both safety and positive outcomes. First, there are the processing chemicals themselves. For the most part, they are highly flammable, carcinogenic, or both. The risk of both laboratory technician exposure and fire is reduced by the use of personal protective equipment worn by the workers handling the chemicals, adequate ventilation of fumes from the area, and fire suppression systems in the processing area.

The second risk to be managed is the processing procedure itself. In a multistep process such as preparing tissue for examination, a missed step, a mistimed processing step resulting in either under or overexposure to a chemical, or the use of exhausted chemicals can compromise the quality of the resulting slide. This can, and has, caused a misdiagnosis of the patient's condition.

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Yet another risk requiring management is the disposal of processing waste. The chemicals (alcohols, toluene, xylene, formalin, etc.) used in tissue processors represent a unique disposal problem. By the nature of the basic chemicals, they are hazardous wastes (meeting hazmat criteria) that are now also contaminated by human tissue, qualifying them as medical waste as well. In the United States, this is considered mixed waste and is regulated by the Environmental Protection Agency (EPA) and the state in which the laboratory (the generator of the waste) is located. Proper disposal procedures, to include manifesting, must be followed to both ensure they are safely disposed of and to keep the generator from paying a heavy fine for improper disposal.

Troubleshooting

Today's modern tissue processors are remarkably reliable and trouble free. The most

Origin and Evolution

Unlike many medical devices which are key in determining the diagnosis of emergent states—such as computed tomography (CT) scans for head trauma after a traffic accident—or assisting physicians in the heroic treatment of patients (e.g., a defibrillator), tissue processors typically perform their important duties in back areas of the laboratory, away from the glitz and glamour of the emergency room or intensive care unit. After approximately 45 years of evolution, today's tissue processors are remarkably similar in physical appearance and general operation to the original models developed in the mid-1960s. The basic principles of the moving-basket processors remain unchanged. While they have undergone minor incremental improvements—such as the replacement of microswitches with optical sensors and motor-driven mechanical timers with computer controls—they are still remarkably similar to the original models. Tissue processors seem to be a classic example of a well thought out, timeless, basic design.

dangerous problem with stationary chamber processors is leaks caused by deteriorating tubing. Although the tubing used in these devices is resistant to the chemicals, it is not completely impervious and will eventually break down and leak. Mechanically and electronically, stationary chamber processors are quite reliable since pinch-valves are often used throughout the device.

Of the moving-basket processors, those employing the notched circular disk for programming are quite reliable and easy to repair when they do fail. Probably the most important aspect of troubleshooting these is for the biomedical equipment technician to be mindful of the correct sequence of events as it relates to how the device actually operates. The sequence of events is the most revealing aspect of determining both the malfunction (not just the symptoms) and the root cause of the malfunction.

The reliability of microprocessor-controlled moving basket processors is on a par with the notched circular disk programmed units, but remediation of failures is totally different. Problems with the former tend to be more software related (corrupted software) and less component-related; problems in the later-designed units are electro-mechanical (damaged or malfunctioning microswitches, mechanical failure of the motors, etc.) in nature.

Training and Equipment

Generally, a medical electronic background coupled with a good mechanical aptitude is necessary to service tissue processors since they are true electromechanical devices. For some models, knowledge of computer repair would be a plus since the heart of many high-end processors is a central processing unit (CPU) perceiving things such as positioning motors, heaters, and air pumps (for agitation) as peripheral devices. Well-equipped biomedical maintenance facilities should already have all test equipment and service aids required to maintain tissue processors.

Future Development

Many tissue processors feature some form of microprocessor control. This trend is expected to increase since there are a number of present and future advantages to microprocessor control of the processing cycle. Additionally, improvements in fractional horsepower-motor technology will provide motors that produce the same or more torque at lower currents. This, coupled with microprocessor technology, will allow future units to operate during a power outage, not just "remember" in what processing stage they were in when the power was lost. As new and safer processing chemicals are available, cycle parameters of future microprocessor-based tissue processors will be adaptable to these new chemistries. Lastly, future tissue processors will fully document, archive, and recall all parameters of past processing cycles as required for litigation purposes. ■

Resources

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