



Play It Safe: Cryostat Disinfection Techniques

By Jan Minshew, HT, HTL (ASCP), *Leica Microsystems, Marketing Manager, Bannockburn, USA*

Properly frozen tissue specimens can be sectioned in a cryostat because, like formalin-fixed paraffin embedded specimens, cellular structures are well supported and can withstand the cutting force without distortion. The trick, of course, is freezing at an extremely rapid rate to prevent the formation of damaging ice crystals and sectioning at the appropriate temperature so the tissue is neither brittle (temperature too low) nor soft (temperature too high).

Frozen sectioning techniques are most often performed on fresh specimens to obtain rapid diagnosis for intraoperative consultation, although frozen sectioning is used for other procedures as well. Since fresh specimens are not exposed to chemical fixatives, processing solutions or heat (like formalin-fixed paraffin embedded specimens), many enzymes, diffusible compounds, and antigens are well preserved within the specimen. This makes the technique ideal for enzyme histochemistry, immunohistochemistry, and the demonstration of various neurological elements and lipids.

For the histotechnician, there are special concerns when working with fresh, frozen tissue. Pathogens also remain well preserved within a frozen specimen and are quite capable of infecting the cryostat operator, service personnel, and other individuals in the working area.

Cryostat disinfection techniques are extremely important. The CAP Laboratory Accreditation Program Inspection Checklist requires a documented procedure for routine decontamination of a cryostat at defined intervals, and also requires evidence of decontamination records.

The following disinfection guidelines can be used to maintain compliance with CAP inspection criteria and improve laboratory safety: **Wear Personal Protective Equipment (PPE)**
Personal Protective Equipment, such as gowns, puncture and penetration resistant gloves, and eye protection must be worn when performing cryostat disinfection procedures.

Cryostat Preparation

Remove used blades/knives from their holder. Although not a requirement, steel mesh gloves should be worn when changing knife blades. Dispose of blades according to the regulations of your institution or disinfect knives before reusing by soaking in disinfecting solution. Remove ALL debris and utensils (pencils, forceps, brushes, gauze, etc.) from the chamber. Debris must be removed because organic material (blood and proteins) may contain high concentrations of microorganisms and could inactivate the chemical disinfectant or prevent access to contaminated surfaces. Debris should be treated as a biohazard and disposed of according to the policies and procedures of your institution. Utensils must be disinfected before reusing. 70% ethyl or reagent alcohol can be used to clean the cryostat and partially disinfect the cryostat. The germicidal activity of ethyl alcohol is most effective in the 70% range because it can penetrate tubercle bacteria and has an advantage over isopropyl alcohol by its ability to kill hydrophilic viruses.

Chemical Disinfection

To disinfect a cryostat using a chemical disinfectant, the instrument **MUST** be at room temperature before the process is started. Do not create an airborne mist by spraying disinfectant (or anything else) in an open cryostat chamber. Instead, pour disinfectants onto surfaces or absorbent disposable towels and allow them to remain in contact with contaminated surfaces for the length of time specified in the instructions of the individual agents.

Properly dispose of paper towels.

Use a tuberculocidal disinfectant that is non-corrosive. The EPA posts a list of Antimicrobial Chemical/Registration Number Indexes (<http://www.epa.gov/oppad001/chemregindex.htm>) and updates it regularly. From this link you can find agents effective against bloodborne pathogens such as mycobacterium tuberculosis, human HIV-1 virus, and

Hepatitis B or Hepatitis C virus. Critical: NONE of the listed solutions have been tested at low temperatures and can ONLY be used at room temperature.

Following Chemical Disinfection

After the disinfection procedure is complete, the cryostat must be thoroughly dried and lubricated before being put back into service at cold temperatures. Only use lubricants that are recommended by the cryostat manufacturer and only in the recommended amounts. For optimum sectioning, allow at least two and a half hours for the metal microtome parts to reach the cold temperature setting.

Built-in Disinfection

There are several cryostats manufactured with built-in disinfection systems, including Leica's CM1850 UV and the CM1900 UV. Prior to production of these instruments, an independent laboratory, Ecoscope, Laboratory for Microbiology and Ecotoxicology in Amtzell, Germany, performed tests in various positions in the chambers to validate the efficiency of the UVC surface disinfection. As a result of those tests, the Amtzell Laboratory established a certificate attesting to the UVC's efficiency and the recommended irradiation times to inactivate all kinds of bacteria, spores, fungi, and viruses, including the Avian Influenza A (H5N1). Although regularly scheduled chemical disinfection is still suggested, UVC disinfection is an excellent means of rapidly reducing the exposure to dangerous pathogens without warming or defrosting the cryostat. Please feel free to contact your Leica representative or authorized dealer for information regarding the cryostats or the certificates.



Leica CM1900 UV cryostat: the efficient germicidal effect of UVC rays for surface disinfection in the Leica CM1900 UV cryostat has been proven by an independent laboratory.