Maintaining Sample Integrity During Surface Decontamination of Cryopreserved Materials

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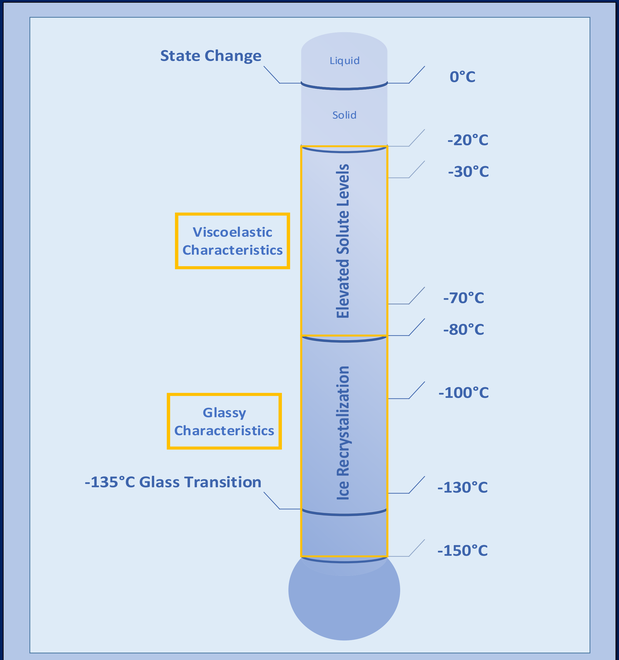
INTRODUCTION

Cryopreservation is a requirement for many biorepository samples including cells, tissues, and pathogens. Storage of biological materials in extremely low temperature has allowed scientists to preserve sample integrity for an extended period. Samples stored in cryopreservation devices are susceptible to cross-contamination between samples and contaminates found within the environment. Contamination of samples will result in economic losses and delays in productivity due to contamination of cultures, discarding samples, and transfer of contaminants amongst storage devices and facilities. Traditionally used disinfectants are ineffective at these extreme temperatures; therefore, samples are either only decontaminated prior to use or must be thawed, disinfected, and then returned to their cryopreservation device. Increases in sample temperatures compromise integrity as it passes the glass transition point and again as viscoelasticity increases (Phase Changes, Top Right). As a result, sample integrity is often jeopardized, or contaminants are left unchecked. There are three proposed solutions to allow decontamination while conserving sample integrity: utilizing a disinfectant that has a low freezing point, introducing deicers into an effective disinfectant, and sterile liquid nitrogen (SLN_2) washes.

Disinfectant (Active Agent and Concentration)	Freezing Point	Effective Disinfectant of the Following:			
		Viruses [1,4,5,6,9,10,11,12]	Bacteria [1,4,6,9,10,12,14,15,16,17,18,19]	Fungi [4,9,13,10,13]	Spore-Forming Bacteria [1,6,9,12,16,17,20]
Sodium hypochlorite (~5000 ppm)	−5°C	ASFV, ARI, FMDV, EBOV	Non-spore forming bacteria	Yes	Yes
Acetic acid (4-5%)	−3°C	ASFV, ARI, EBOV	*M. tuberculosis*	*C. albicans, Penicillium ssp.*	No
Potassium peroxymonosulfate, NaCl (1%)	−4°C	ASFV, ARI, FMDV, EBOV	*B. anthracis*	*Aspergillus sp., Penicillium ssp., Trichophyton ssp.*	Yes
Potassium peroxymonosulfate (1%), NaCl, propylene glycol (30-40%)	−25°C	ASFV, ARI, FMDV	*P. aeruginosa*	Yes	Yes
Potassium peroxymonosulfate (1%), NaCl, hydrogen peroxide (6.25%), MeOH (20%)	−20°C	ASFV, FMDV	Yes	TBT	Yes
Potassium peroxymonosulfate (1%), NaCl, hydrogen peroxide (6.25%), $CaCl_2$	−20°C	ASFV, FMDV	Yes	TBT	Yes
Hydrogen peroxide (23%), acetic acid (16%), peracetic acid (15%), sulfuric acid (1%)	−49°C	ARI, FMDV, EBOV	*B. anthracis*	TBT	Yes
Ethanol (70%)	−49°C	ARI, EBOV	*Y. enterocolitica*	Yes	No

Abbreviations: African Swine Fever Virus (ASFV), Acute Respiratory Infection viruses (ARI), Foot-and-Mouth Disease Virus (FMDV), Ebola Virus (EBOV), To Be Tested (TBT)



PHASE CHANGES

Passive rewarming in a decontamination bath maintained at −20°C will minimize the non-linear warming rate for the material's exposure time. Placement of a 1ml filled cryovial frozen at −150°C in a −20°C decontamination bath will minimize thawing of the material and warming through the glass transition point. Placement of a 1ml filled cryovial that is frozen at −70°C in a −20°C decontamination bath will minimize thawing of the material and lengthy exposure to elevated solute levels.

Utilizing Disinfectant With A Low Freezing Point

PROS

- Biological material will remain in freezing temperature, conserving integrity.

CONS

- Require additional research in order to confirm effectiveness at extreme temperatures.
- Volatile solvents can compromise labels or containers.

Low Freezing Point: A possible approach to decontamination of frozen material is to utilize disinfectants that have a naturally occurring low freezing points in solution such as acetic acid and alcohols (Table). For example, a 60% solution of acetic acid has a freezing point of −26.5°C. A 60% ethanol solution has a freezing point of −37°C and a 100% ethanol solution has a freezing point of −115°C. Peracetic acid has a natural freezing point of 0°C and has been a target of low temperature disinfection. VigorOx®, a commercially available disinfectant (composed of hydrogen peroxide, acetic acid, peracetic acid, and sulfuric acid), has a freezing point of −49°C and is established as an effective disinfectant in wastewater treatments[8]. As a result, acetic acid, ethanol, peracetic acid, and compounds with similar properties require more research in order to determine their effectiveness as decontaminating solutions at extreme temperatures.

Introducing Deicers Into Disinfectant

- Proven to be effective at room temperature
- Material integrity conserved by remaining frozen

- Reduced effectiveness as deicer concentration increases
- More research is required

Deicers: The introduction of deicers to lower the freezing point of disinfectants that has been established as effective disinfectants may be used to preserve sample integrity (Table). As a result of low-temperature outbreaks such as, Foot-and-Mouth Disease Virus (FMDV) outbreaks in Republic of Korea and Avian Influenza Virus (AIV) outbreaks in Canada, researchers began to investigate low temperature disinfectants. Several studies have confirmed that numerous disinfectants that are known to be effective lose their properties as deicers are added. For example, sodium hypochlorite is an effective disinfectant for AIV at room temperature. However, when adding organic material to lower the freezing point the effectiveness of the disinfectant is also decreased[2]. Sodium Carbonate reacts in the same manner and is not effective against viruses when mixed with deicers such as ethylene glycol, sodium chloride, calcium chloride, and typical washer fluid[3]. Other disinfectant compounds have been shown to retain their properties as deicers are added and have the potential of disinfecting at extremely low temperatures. For example, citric acid has been shown to effectively eliminate FMDV at 37°C, 4°C, and −20°C when mixed with deicers like sodium chloride and ethyl alcohol[3].

Performing Sterile LN_2 Washes

- Material integrity conserved by remaining frozen

- Requires access to sterile LN_2
- May result in container failure due to sample submersion

Sterile LN_2 Washes: A novel approach proposed for the decontamination of frozen materials is to rinse the samples in sterile LN_2 (SLN_2). Preliminary research suggests that SLN_2 washes can effectively decontaminate samples from bacteria and fungi. In a study conducted in 2012, vitrification carriers were dipped into LN_2 contaminated with bacteria or fungi. The carriers were then assigned into two groups: no wash (control) and 3-time wash in SLN_2. While the control group had a contamination rate of 76% for bacteria and 100% for fungi, all carriers washed in SLN_2 were found to be sterile[7]. Although an effective disinfectant, this method requires dipping samples into SLN_2 three consecutive times for 30 seconds each. This method may compromise sample integrity due to container failures.

Alternatives Considered: The possibility of utilizing vaporized hydrogen peroxide or chlorine dioxide has been considered. However, vaporized disinfectants are likely ineffective due to the limited contact time before the disinfectant condenses under ultra-low temperatures. If vaporized hydrogen peroxide were used it will be present in the freezer and may be an effective decontaminate when samples are thawed. This approach creates a hazard that must be taken into consideration. Hydrogen peroxide can undergo explosive thermal decomposition and creates an unnecessary risk to the equipment and personnel. In addition, many freezers are not designed to hold an excessive amount of hydrogen peroxide or chlorine dioxide, which can be corrosive to metals, and may lead to mechanical failures.

By investigating disinfectants and decontamination techniques that will allow for decontamination at ultra-low temperature researchers will be able to better conserve sample integrity. Decontamination without repeated thawing and freezing will preserve sample protein structures, nucleic acids, and cell membranes.

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