Guidelines for the Blood Transfusion Services

21.5: General guidelines for tissue processing

http://aws-lon-jpac.targetservers.uk/red-book/chapter-21/21-5-general-guidelines-for-tissue-processing

21.5: General guidelines for tissue processing

Processing must not change the physical properties of the tissue so as to make it unacceptable for clinical use. Processing steps must be validated to demonstrate that the final product does not have any clinically significant residual toxicity.

21.5.1: Aseptic processing facilities

Facilities for aseptic processing must comply with the *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2015*[5], EC Guidelines to Good Manufacturing Practice[6] and the Human Tissue (Quality and Safety for Human Application) Regulations 2007 (as amended)[7]. They must provide separate work areas with defined physical and microbiological parameters. Facilities must have:

- floors, walls and ceilings of non-porous smooth surfaces that are easily sanitised
- temperature control
- air filtered through high-efficiency particulate air (HEPA) filters with appropriate pressure differential between zones, which must be documented
- a documented system for monitoring temperature, air supply conditions, particle numbers and bacterial colony-forming units (environmental monitoring)
- a documented system for cleaning and disinfecting rooms and equipment
- a documented system for gowning and laundry
- adequate space for staff and storage of sterile garments
- access limited to authorised personnel
- documented system for general staff hygiene practices.

21.5.2: Tissue not destined for terminal microbial processing

Critical work areas are those where tissue is manipulated openly either following a disinfection or sterilisation step or in those cases where tissue has been procured aseptically and will not be further disinfected or sterilised. Critical work areas on which sterile containers, aseptically procured tissue or disinfected tissue are exposed to the environment, must have an air quality of Grade A and should have a Grade B background. (For further information see *Rules and Guidance for Pharmaceutical Manufacturers and Distributors 2015*[5] and the EC Guidelines to Good Manufacturing Practice.[6]) Any lowering of this standard in the background environment must be documented and it must be demonstrated that the

chosen environment achieves the quality and safety required, at least taking into account the intended purpose, mode of application and immune status of the recipient.

Wherever possible, representative samples of tissue should be removed and tested for bacterial and fungal contamination using protocols authorised by the designated medical officer or designated microbiologist. Swabs or other validated non-destructive sampling methods should be used where it is impossible to remove tissue without damaging the graft.

Procedures must ensure that no cross-contamination between batches of tissue from different donors can occur. Key process parameters and acceptance limits must be identified and validated. A full record of each process applied to each tissue or batch must be retained.

21.5.3: Tissue destined for terminal microbial processing

Work areas in which tissue materials and containers are prepared should have an environment with air quality of at least Grade C in the vicinity of exposed tissue.

Terminal antimicrobial processing must follow the filling of the final container. The procurement, processing and filling environment must be of sufficient quality to minimise the microbial contamination of the tissue to ensure that the subsequent antimicrobial processing is effective.

The tissue in its final container must be subjected to a validated procedure utilising an agent such as gamma irradiation.

21.5.3.1: Terminal sterilisation

Sterilisation is a statistical phenomenon, expressed as the probability of microorganisms surviving the procedure. The sterility assurance level (SAL) is the probability of a microorganism on one item within a batch or within a defined population. The accepted level for considering medical devices to be ‘sterile’ is a SAL of 10^{-6} (i.e. less than one item per million items will have a surviving microorganism on it). For medical devices, the microorganisms under consideration are contaminants (i.e. bacteria and fungi and their spores). Unless specifically stated, viruses are not routinely considered.

Because of the large numbers involved, demonstrating SAL of 10^{-6} must use procedures that extrapolate from smaller batches. For sterilisation procedures that show a log10/linear decrease in microbial viability, extrapolation can be achieved using the D-value (decimal reduction value) concept.

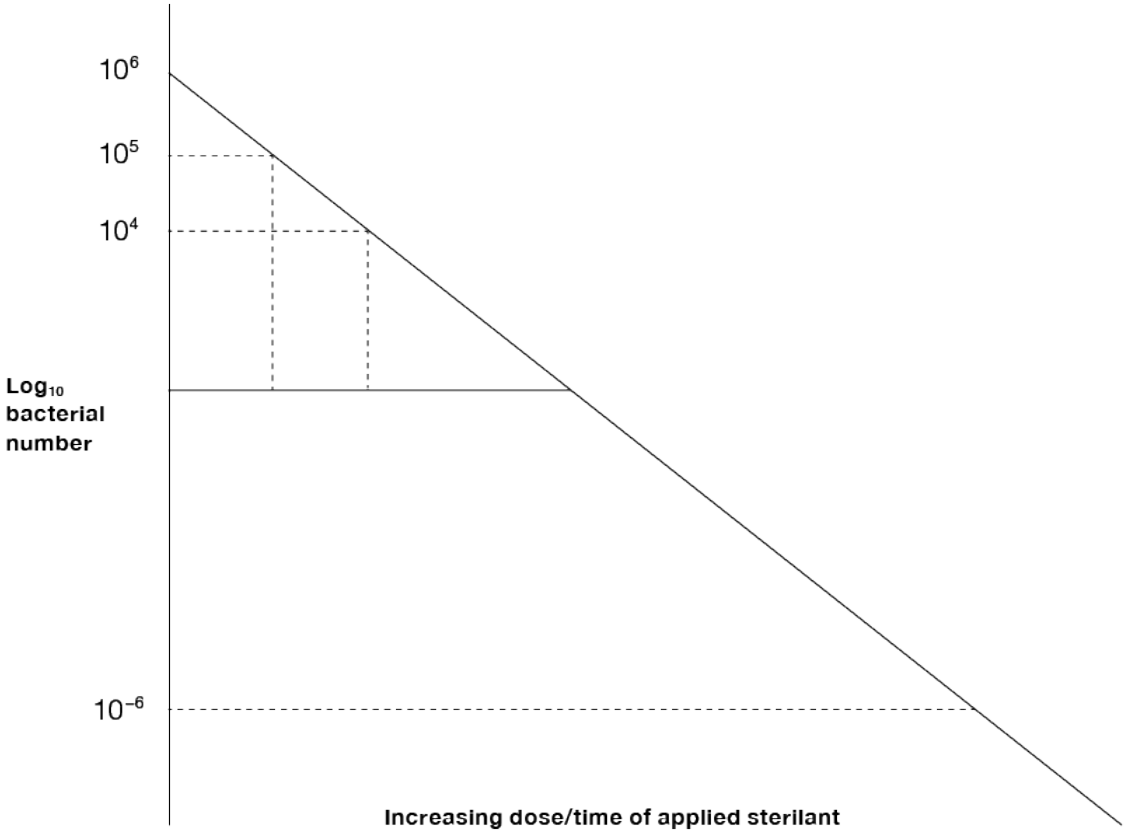

Figure 21.1 An example of increasing inactivation of bacteria related to increasing the dose of the sterilant

In the example shown in Figure 21.1, each log reduction requires an additional unit of the sterilant to be applied, hence D-value = 1.0. Therefore moving from an initial bioburden of 106 bacteria to a SAL of 10^{-6} would require 12 × D-value of the sterilant.

In practice, the processing that is applied to tissue grafts prior to application of the terminal sterilisation step often reduces the bioburden to close to zero. Therefore application of a sterilisation procedure sufficient to provide a 6-log reduction of bacteria is often satisfactory to achieve a SAL of 10^{-6}.

Very often, validation studies will be carried out using the microorganism that is known to be most resistant to the sterilisation procedure (often bacterial spores). This is therefore a 'worst-case' validation. Achieving a SAL of 10^{-6} for this microorganism will guarantee a significant overkill for more sensitive microbes.

21.5.3.2: Validation of terminal sterilisation

Whenever a novel terminal sterilisation step is introduced the following validations need to be addressed:

- That the sterilisation technique achieves a SAL of 10^{-6} for the most resistant microorganisms.
- That the sterilisation technique can be applied to the tissue graft in its final packaging without subsequent exposure, and that the integrity of the packaging is not adversely affected by the process.

- That the sterilisation technique does not adversely affect the essential properties of the graft and does not leave toxic residuals.
- That the sterilisation technique inactivates all categories of microorganisms commonly found on tissue grafts including vegetative Gram positive and Gram negative bacteria, vegetative fungi, and bacterial and fungal spores. This must be demonstrated either by literature review or validation.

21.5.4: Gamma irradiation

Gamma irradiation must be performed in a controlled manner to ensure that all tissue receives at least the minimum specified dose of radiation. This requires the use of standard packaging materials and irradiator load configuration and is usually validated using calibrated dosimeters placed throughout the load. The dose should never be less than 15 kGy, unless pre-irradiation processing has been validated to consistently yield a low microbial bioburden such that there is the required assurance, in accordance with medical device standards, that the dose will result in the tissue being sterile.

Tissue must be irradiated in its final packaging, which must bear a suitable indicator to demonstrate that it has been irradiated. This must be checked before release of the tissue.

If a dose in excess of 25 kGy is required, then consideration must be given to the possible detrimental effect on the biological and physical properties of the tissue.

Many viruses are resistant to irradiation and therefore any claim of viral inactivation must be supported by validation data obtained using appropriate marker viruses.

21.5.5: Pooling

Pooling of tissues from different donors is not recommended and should only be considered if this is the only way in which clinical efficacy can be achieved.

21.5.6: Preservation methods

Where specific attributes of a tissue are claimed, the process should be validated to show these attributes are preserved.

21.5.6.1: Freezing

For the purposes of this guidance this term applies to tissues that are frozen and stored under conditions that are unlikely to be compatible with preservation of cells. Frozen tissue must be stored below –20°C and the length of storage permitted depends on the temperature the tissues are stored at (see Tables 21.1 and 21.2).

21.5.6.2: Cryopreservation

For the purposes of this guidance this term applies to tissues that are treated with a cryoprotectant and/or cooled at a controlled rate in order to preserve cells. Cryopreserved tissue must be stored below –135°C. For storage at higher temperatures, validation must be performed to demonstrate that the required properties of the graft are maintained for the stated expiry.

21.5.6.3: Freeze-drying

Where tissues are freeze-dried, a sample of each type of tissue from each freeze-drying run must be analysed for residual water which must be less than 5% (weight/weight) of the dry weight of the graft or residual water activity of between 0 and 0.5 Aw.

21.5.6.4: Glycerolisation

Where tissues are preserved by high concentrations of glycerol the procedure should be validated to demonstrate achievement of the specified glycerol concentration within the tissue or an acceptable range within the tissue.

21.5.7: Solutions

Rinse solutions, antibiotic mixtures, nutrient media and cryopreservation solutions must be stored at a specified temperature and with a storage period consistent with functional requirements. They must be discarded if not used within 24 hours of opening. Any solutions coming into direct contact with tissues during retrieval or processing must be sterile and fully identified in the associated records.