Terminal Sterilization: One Eye Bank's Experience

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Microorganisms are frequently referred to as "bugs". Terminal sterilization is a method of inactivating any microorganisms on a given object. Corneal tissue is not considered sterile and, by definition, harbors microorganisms. While infections related to corneal transplants are rare, they do happen. For some types of corneal transplants, cellular viability is not required. Examples of this are tectonic grafts and glaucoma shunt covers. For these non-viable grafts, eye banks may wish to consider a sterilization protocol in order to reduce the already low likelihood of graft related infections. Additionally, sterilization of corneal tissue makes shelf-stability easier to achieve as bioburden growth will no longer compromise the tissue once sterilized. Making a sterility claim is not to be taken lightly. Please use this information in careful consultation of experts in the field of tissue sterilization.

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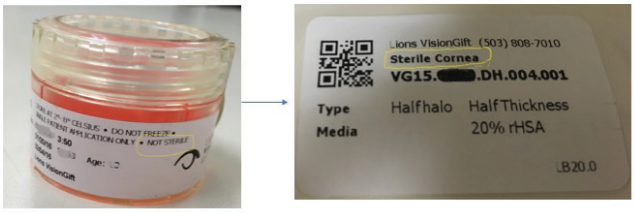
Background

- Eye tissue historically labelled as "not sterile". EBAA Medical Standards actually required label to state that all tissue must have this warning (J1.000).
- Standards did not accommodate the concept of sterile tissue and had to be changed.



In June 0f 2014, Eye Bank Association of America medical standards were changed to accommodate grafts that are sterile.

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Choose a Method

- We used the VD_{max} method for establishing the dose.
- Method 1 and Method 2 are also options which are not discussed in this presentation.

For this talk, we use VDmax as our chosen method for documenting that sterility can be assured. VDmax is an acronym for "verification dose maximum". There is more than one method to make a sterility claim. Each firm must describe for itself which approach makes the best sense for their product.

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What does this have to do with validation?

- Eye banks historically don't have much experience with sterilization.
- Expertise IS available. You don't have to do this alone and we certainly didn't figure this out on our own.
  - Consultants are very beneficial.
  - Testing laboratories with experience in these types of validations are essential.

Making a sterility claim requires careful consideration of a number of factors. While the FDA does require validation of procedures, they do NOT prescribe specific methods to make a sterility claim. There are a number of excellent references to help guide the experienced and inexperienced alike. For those embarking on this journey for the first time, partnering with a reputable testing facility is essential. Additionally, it may be helpful to work with a consultant or consulting company to help walk through these steps for the first time.

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Overall Sterilization Plan (VDmax)

- Step 1: Define Product Family
- Step 2: Bioburden recovery efficiency and B/F testing of non-irradiated grafts
- Step 3: Establish bioburden
- Step 4: Establish dose and verification dose
- Step 5: Dose maps for verification and standard doses
- Step 6: Irradiate samples at verification dose
- Step 7: B/F testing of irradiated samples
- Step 8: Sterility testing of samples irradiated verification dose
- Step 9: If no growth in 9/10 samples, accept results
- Step 10: Ongoing monitoring of bioburden and periodic dose audits with tests of sterility

This slide breaks the process of validating the sterility claim into multiple logical steps. It may be possible to break the steps into slightly different phases according to your desired methodology.

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Step 1: Define Product Family

- If needed, justify why mixture of tissue types is acceptable.
- If bioburden among tissue types varies, use the bioburden of the most "challenging" tissue type.

Our validation addresses both cornea and sclera. To determine a dose of irradiation, the tissue bioburden must be ascertained. Different tissue types may have different standard bioburden (e.g. aseptically recovered bone may have lower bioburden than skin which has a natural expected microbiological flora). It may be acceptable to mix tissue types, but there should be a justification documented in the validation for utilizing two (or more) tissue types.

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Step 2: Bioburden recovery efficiency and B/F testing of non-irradiated grafts

- Needs to be performed on both irradiated and non-irradiated product.
- If recovery of bioburden is not 100%, this will establish a correction factor for interpreting later bioburden results.
- Bacteriostasis/Fungistasis (B/F) testing establishes that the test articles themselves do no inhibit growth of organisms of interest. Ensure B/F tests are for all types of organisms of interest (e.g. Fungi, aerobes and anaerobes).

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Step 3: Establish bioburden

- Tests of tissue samples processed but not irradiated are used for establishing bioburden.
- Samples must come from multiple processing batches.
- Sample item portions (SIP) of <1 must be justified and documented for calculation of total bioburden.

It is essential that bioburden is assessed from tissue samples that have been subjected to the whole process right up to sterilization without having actually been subjected to radiation. This will capture the bioburden that is inherent to the tissue itself as well as any bioburden that has been introduced by the process the tissue has been subjected to. Testing methodology must be selected carefully in order to ensure a proper growth medium for all organisms of interest.

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Step 4: Establish dose and verification dose

- Bioburden calculations and Sterility Assurance Level (usually 10^{-6}) will establish irradiation dose or substantiate a predetermined dose.
- A verification dose must be established. $1/10^{th}$ the dose of a standard sterilization dose is a common rule of thumb, but the actual number is based off of a reference table.

ISO ANSI/AAMI/ISO 11137:2:2006 Sterilization of Health Care Products—Radiation—Part 2: Establishing the Sterilization Dose is used to establish an irradiation dose in kiloGrays based on the bioburden present and the desired sterility assurance level. Sterility assurance levels are determined by the firm but are commonly set at 10-6. This means that we can expect about one graft in a million to contain a viable colony forming unit. A verification dose is also established based on bioburden.

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Step 5: Dose maps for verification and standard doses

- Collaboration with sterilization facility will establish standard sterilization and verification run protocols.
- A dose map will be performed to establish that the dose is met throughout the entire product load.
- A vendor audit for compliance with these protocols is a good idea.

A dose map is used to demonstrate that your product is irradiated with the minimum required dose to assure sterility throughout the entire load. Standardization of protocols are essential to assure that all runs are irradiated in the same reproducible manner.

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Steps 6: Irradiate samples at verification dose

- A verification dose is quite low relative to sterilization dose (usually ~1/10th).
- It requires its own dose map and protocol for the sterilization vendor.
- Care in selection of a vendor that can hit these really tight doses is important.

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Step 7: B/F testing of irradiated samples

- You have to do this. It's true.

A repeat of the bacterostasis and fungistasis testing after irradiation must be completed to ensure that radiation does not inhibit growth in samples where viable bioburden is known to be present.

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Step 8: Sterility testing of irradiated samples

- 14 day testing for growth.
- Negative tests of sterility required for 9/10 samples.
- Recall that the verification dose is ~1/10th the sterilization dose or 0.1kGy (whichever is greater). The verification dose gives a 10^{-1} SAL.

SAL is the sterility assurance level.

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Step 9: Interpret Results

- Based on established criteria, accept results as evidence of a validated procedure.
- Requires certificate of irradiation documenting the intended dose was met and not exceeded by 10% for verification dose.

SAL is the sterility assurance level.

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You now have a validated sterility claim

- You aren't done, yet.
- You still need to do a performance qualification/process validation.
- You will never be "done", as you will have to monitor to ensure that your process remains in control.

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Step 10: Ongoing monitoring

- Product release is based on certificate of irradiation at established sterilization dose.
- Ongoing bioburden and sterility monitoring is performed on a routine basis.
- Changes in the procedure may require re-validation or addendums to the established validation (e.g. changing sterilization facility).

This validation is intended to allow for "parametric" release of sterilized products. In other words, once documented evidence of sterilization parameters has been provided by the sterilization facility, no additional sterility testing is required to release products. However, ongoing bioburden and sterility testing of samples irradiated at the verification dose is still performed on a scheduled basis.

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Challenges we faced

- Determining the bioburden sampling approach.
- Determining an appropriate bioburden sample. Is it reasonable to utilize a whole cornea for every bioburden sample?
- Ensuring that we had a reproducible set up for the sterilization batches.
- Determining the product families and tracking bioburden accordingly.

Validation plans often pose unique challenges. Careful collaboration between the quality, operations, and laboratory teams along with informed guidance from Medical and Scientific Directors will help address these issues in order to ensure a safe, high quality and compliant end product.

Slide 18:

References

- ANSI/AAMI/ISO 11137-1:2006, Sterilization of health care products — Radiation — Part 1: Requirements for development, validation, and routine control of a sterilization process for medical devices
- ANSI/AAMI/ISO 11137-2:2006, Sterilization of health care products — Radiation — Part 2: Establishing the sterilization dose
- ANSI/AAMI/ISO 11137-3:2006, Sterilization of health care products — Radiation — Part 3: Guidance on dosimetric aspects
- AAMI TIR 37:2007 Sterilization of health care products – Radiation – Guidance on sterilization of human tissue-based products
- AAMI TIR 33:2005 Sterilization of health care products – Radiation – Substantiation of a selected sterilization dose – VD_{max}

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Thank you

Dead Bug

My contact info: chris@visiongift.org

We have shown a method for validating a sterility claim. By following this method, it is safe to say with a high level of assurance that any "bugs" that were present on the donor material are nonviable.