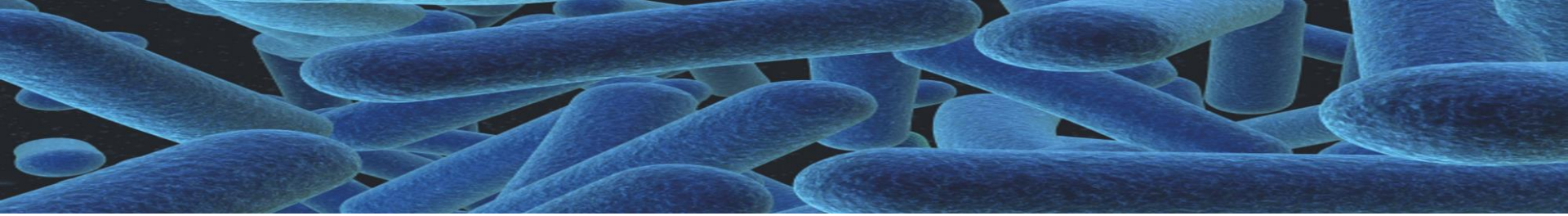
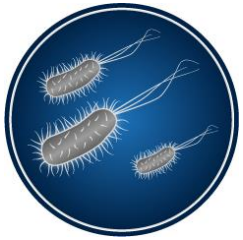


Regulatory and Compendial Guidance for Rapid Testing of Biologics and Advanced Therapy Medicinal Products

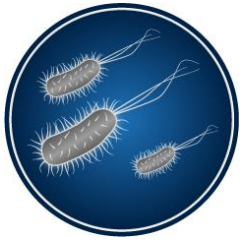
Michael J. Miller, Ph.D.

MICROBIOLOGY CONSULTANTS, LLC



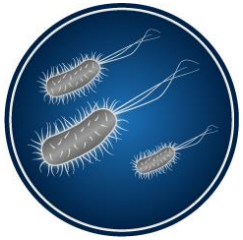
Biologics

- Used to diagnose, prevent, treat, and cure diseases and medical conditions
- Generally large, complex molecules
- May be produced using biotechnology in a microorganism, plant or animal cell
- Blood and blood components, human tissue and cells, vaccines, monoclonal antibodies, cytokines, growth factors, enzymes, ATMPs



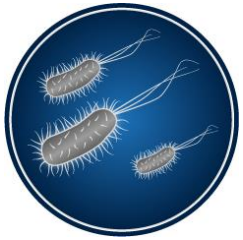
Advanced Therapy Medicinal Products (ATMP)

- Engineered cells or tissues
- Administered to patients with a view to regenerating, repairing or replacing a human tissue
- Bone, skin, corneas, ligaments, tendons, fascia, cartilage, dura mater, heart valves, veins and arteries, hematopoietic stem/progenitor cells derived from peripheral and cord blood



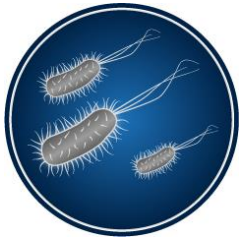
Advanced Therapy Medicinal Products (ATMP)

- Gene therapy is the transfer of genetic material (DNA or RNA) into the cells of a patient's body to treat the cause or symptoms of a specific disease
- Can be used to reduce levels of a disease-causing version of a protein, increase production of disease-fighting proteins, or produce new/modified proteins
- Genes can be introduced directly, packaged into artificially-created liposomes (sacs of fluid surrounded by a fatty membrane), or introduced in a viral vector



Advanced Therapy Medicinal Products (ATMP)

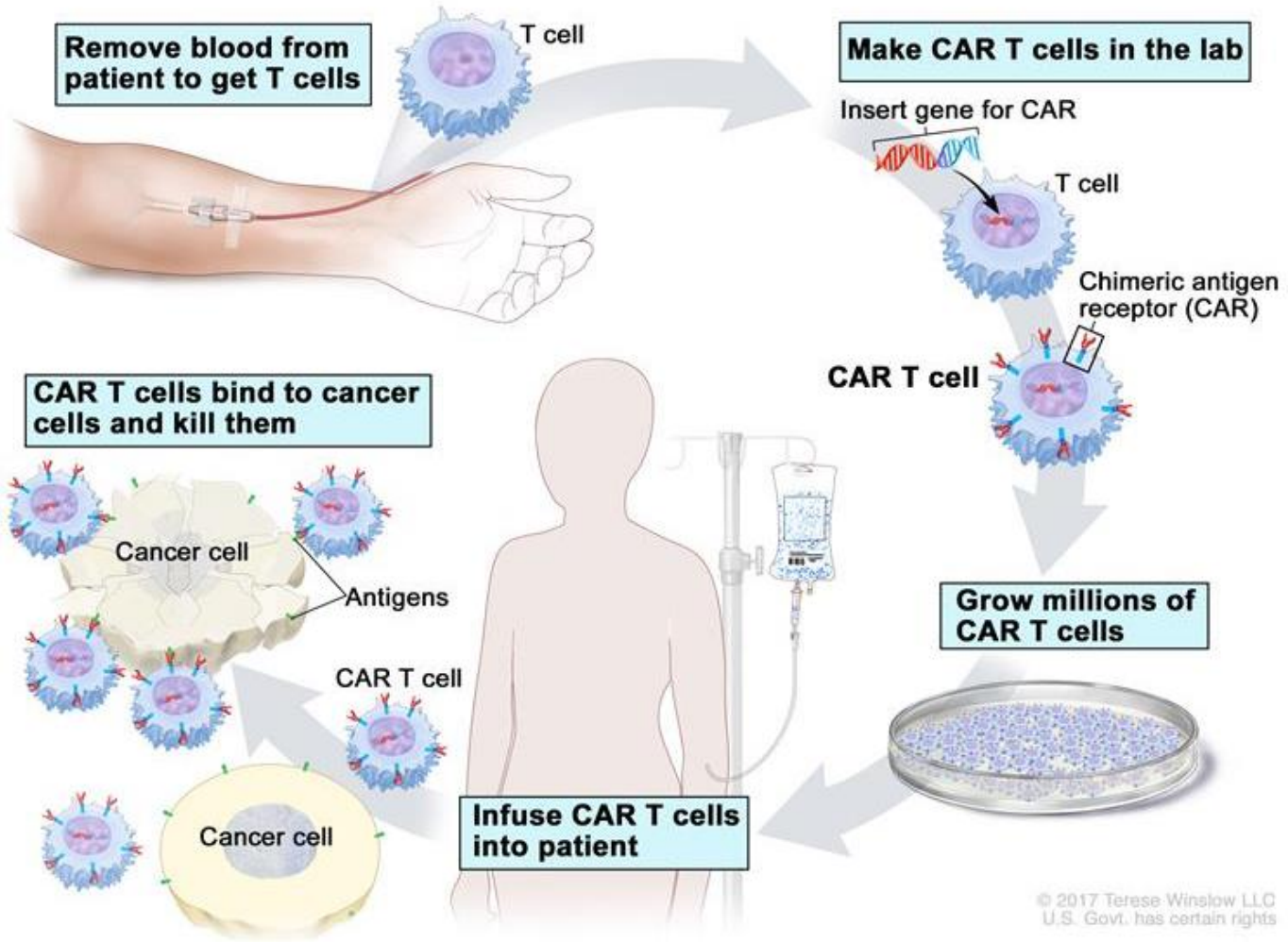
- Somatic cell therapy is the transfer of intact, live cells into a patient to help lessen or cure a disease
- The cells may originate from the patient (autologous), a human donor (allogeneic) or from another species, such as an animal (xenogeneic)
- Cells are manipulated or modified *ex vivo* for subsequent administration to patients; e.g., CAR-T therapy



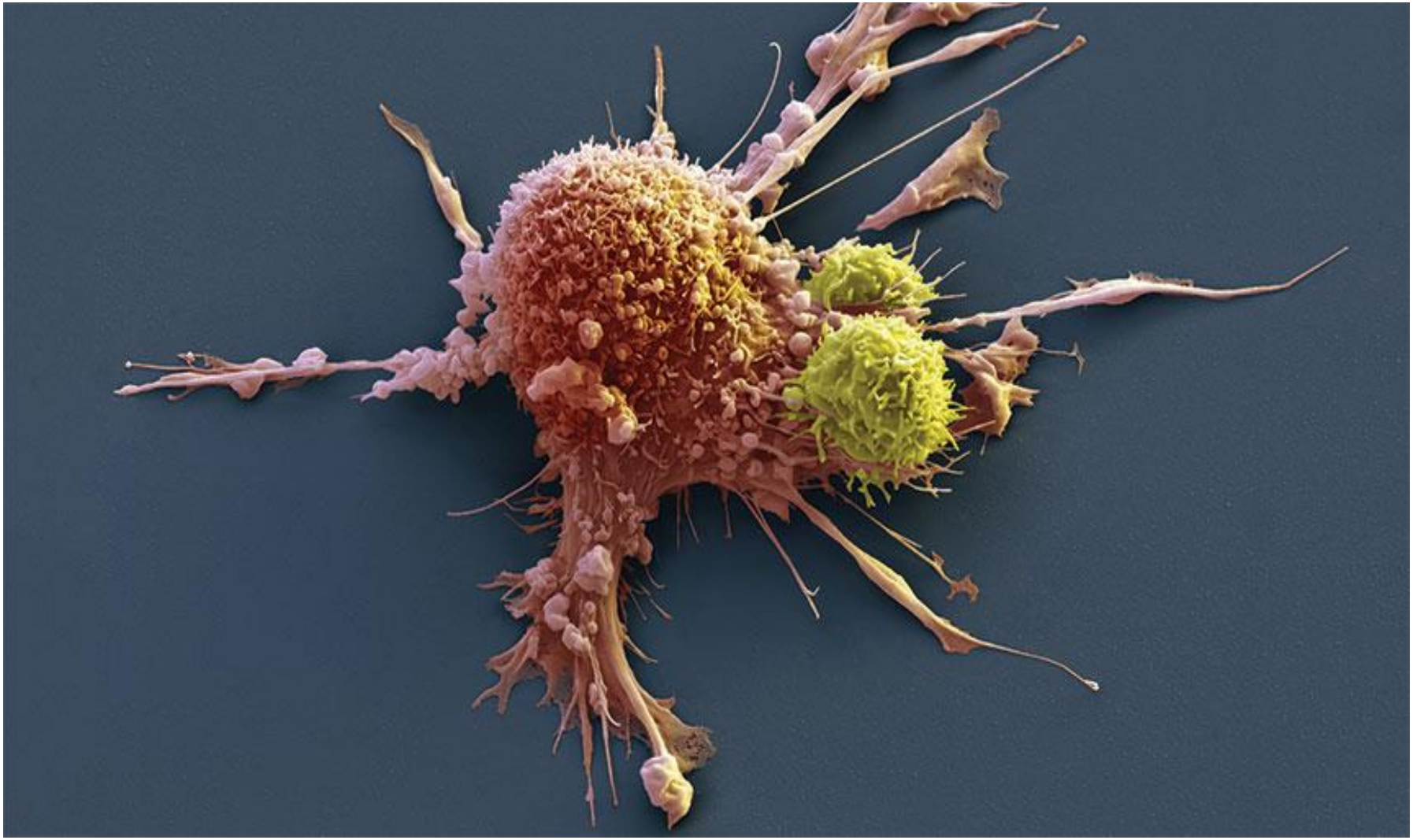
Chimeric Antigen Receptor (CAR) T cell therapy

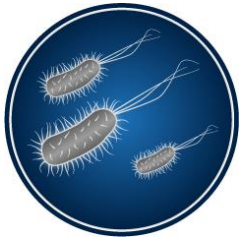
- Modifying the patient's own immune cells (T cells) to express a receptor on their surface that recognizes tumor antigens on the surface of cancer cells
- Once the receptor binds to a tumor antigen, the T-cell is stimulated to attack the cancer cells
- Currently, only two FDA-approved CAR T therapies
  - Kymriah (Novartis) to treat B cell acute lymphoblastic leukemia (2018)
  - Yescarta (Kite Pharma/Gilead) to treat relapsed or refractory large B cell lymphoma (2017)

CAR T Cell Therapy



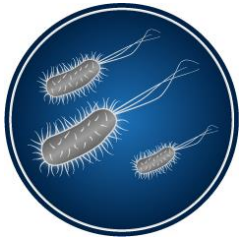
CAR T Cell Therapy





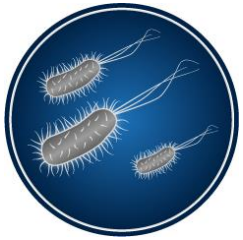
Gene and Cell Therapy Challenges

- Short product shelf-life
- Must be administered before the end of sterility testing
- Low production volumes
  - Cannot meet compendial requirements for sample size
  - Less product = Lower success rate for a positive clinical outcome
- Recent regulatory policies have taken these challenges into consideration



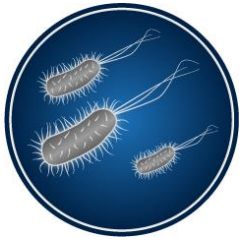
FDA Sterility Testing for Biologics

- On June 4, 2012, FDA amended the sterility test requirements for biologics in its Final Rule, *Amendments to Sterility Test Requirements for Biological Products*
- 21 CFR 610.12
- We will discuss the most relevant sections



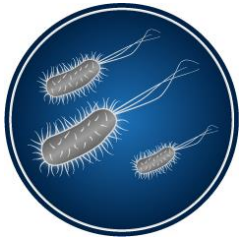
FDA Sterility Testing for Biologics

- “Advances in technology in recent years have allowed the development of new sterility test methods that yield accurate and reliable test results in less time and with less operator intervention than the currently prescribed methods”
- “Examples of novel methods include the Adenosine Triphosphate bioluminescence, chemiluminescence, and carbon dioxide head space measurement”
- “Manufacturers may benefit from using such sterility test methods with rapid and advanced detection capabilities”



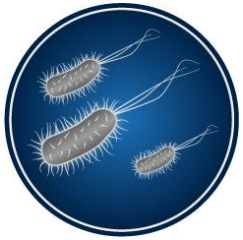
FDA Sterility Testing for Biologics

- **§ 610.12 (a) The test**
- Manufacturers must perform sterility testing of each lot of each biological product's final container material or other material, as appropriate and as approved in the biologics license application (BLA) or supplement for that product
- "We recognize that due to the nature of some biological products, testing the final container material may not always be feasible or appropriate"
- e.g., intermediates, API, bulk drug substance



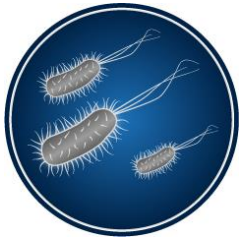
FDA Sterility Testing for Biologics

- If sterility testing needs to be performed on material other than final product, include the following information in a BLA or BLA supplement:
  - A description of the details of the test method
  - The procedure for testing the alternate material instead of the final container material
  - The scientific rationale for selecting the specific test material instead of the final container material
    - e.g., will testing the final growth medium in a cell-based preparation be indicative of contamination in the final product?



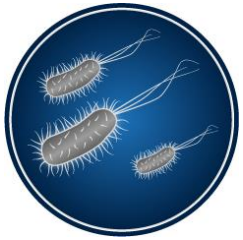
FDA Sterility Testing for Biologics

- A manufacturer who wants to use an alternate sterility test method other than the one approved in its BLA must submit a prior-approval supplement to an approved application
- Submitting the change to FDA in an annual report is not sufficient because FDA views the change as a potential significant impact to the sterility assurance level of a product



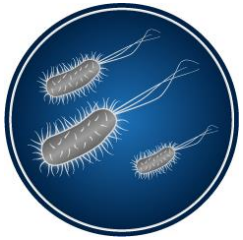
FDA Sterility Testing for Biologics

- **§ 610.12 (b) Test requirements**
- (1) The sterility test must be appropriate to the material being tested such that the material does not interfere with or otherwise hinder the test
  - False positives, background noise
  - False negatives
- Method suitability should be performed on all new products, and repeated whenever there are changes in the test method or production method that could potentially inhibit or enhance the detection of microorganisms



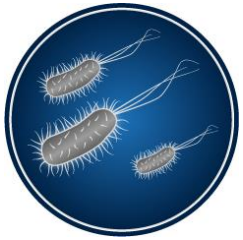
FDA Sterility Testing for Biologics

- (2) The sterility test must be validated to demonstrate that the test is capable of reliably and consistently detecting the presence of viable contaminating microorganisms
- New methods (e.g., non-culture-based methods) or those that deviate from the official compendial method will require validation
  - LOD, specificity, ruggedness, robustness



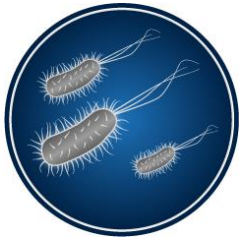
FDA Sterility Testing for Biologics

- FDA's position on culture-based methods
- Validation protocols should include aerobic and anaerobic organisms that grow at differing rates
- Challenge organisms should be added directly to the product prior to membrane filtration or direct inoculation
- If this is not possible due to inhibition by the product, then the organisms should be added to the final portion of sterile diluent used to rinse the filter, or directly to the media containing the product



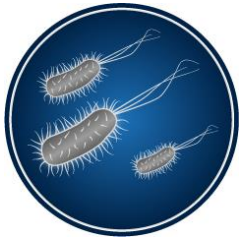
FDA Sterility Testing for Biologics

- FDA's position on non-culture-based methods
- The feasibility of identifying microorganisms from a contaminated sample should be evaluated during validation
- If a method does not have the capability to identify organisms to the species level, the protocol should require that an additional method for species identification be utilized for investigation of detected contaminants



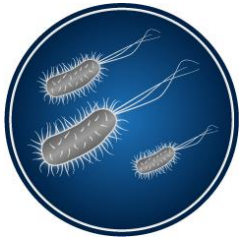
FDA Sterility Testing for Biologics

- Use challenge organisms recovered from the manufacturing environment and from product
  - A mix of ATCC and local facility isolates
- There is no mention of using “stressed” organisms
- But these may be appropriate to confirm if longer incubation times are required for validating culture-based methods



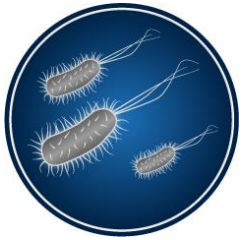
FDA Sterility Testing for Biologics

- “We believe methods validation is a well recognized activity and can be performed without comparison to a ‘referee’ test method”
- “There is no single ‘referee’ test method that would work for all products and that some novel methods cannot be easily compared to culture-based methods such as USP Chapter 71 because these testing methods do not measure microbial growth”



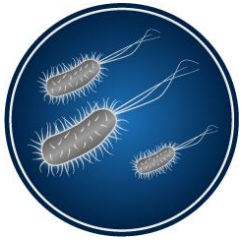
FDA Sterility Testing for Biologics

- “Therefore, we believe that it is neither necessary nor appropriate to add a reference to a standard or ‘‘baseline’’ in this final rule”
- **However, in another section . . .**
- “FDA may decide, as appropriate, to encourage the use of the compendial method as a benchmark or starting point for validation of novel methods and certain other methods”



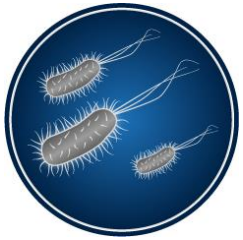
FDA Sterility Testing for Biologics

- **But wait; there's more . . .**
- "While FDA may decide, as appropriate, to encourage the use of the compendial method as a benchmark or starting point for validation of some novel or other methods, we also may decide not to encourage such use for some (for example, non-culture-based) methods that cannot easily be compared to culture-based methods such as the USP compendial method"
- This is a good reason to discuss your validation plan with FDA



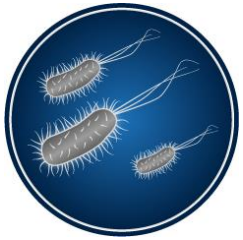
FDA Sterility Testing for Biologics

- (3) The sterility test and test components must be verified to demonstrate that the test method can consistently detect the presence of viable contaminating microorganisms
- Verification occurs over the lifetime of the method
- May be necessary on a periodic basis or each time a sample is tested, depending upon the method
  - Controls to demonstrate organisms will be detected
  - Growth promotion of media over its shelf life
  - For non-culture-based methods, controls must demonstrate the ability to detect viable microorganisms



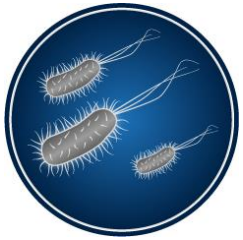
FDA Sterility Testing for Biologics

- **§ 610.12 (c) Written procedures**
- Manufacturers must establish, implement, and follow written procedures for sterility testing that describe, at a minimum, the following:
- (1) The sterility test method to be used;
- (i) If culture-based, include, at a minimum:
  - (A) Composition of the culture media;
  - (B) Growth-promotion test requirements; and
  - (C) Incubation conditions (time and temperature)



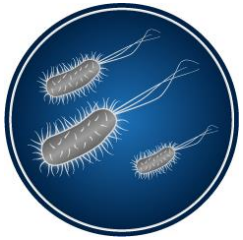
FDA Sterility Testing for Biologics

- (ii) If non-culture-based, include, at a minimum:
  - (A) Composition of test components;
  - (B) Test parameters, including acceptance criteria; and
  - (C) Controls used to verify the method's ability to detect the presence of viable contaminating microorganisms



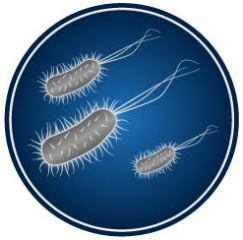
FDA Sterility Testing for Biologics

- (2) The method of sampling, including the number, volume, and size of articles to be tested
- (3) Written specifications for the acceptance or rejection of each lot
- (4) A statement of any other function critical to the particular sterility test method to ensure consistent and accurate results



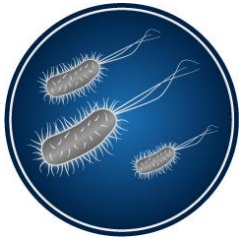
FDA Sterility Testing for Biologics

- **§ 610.12 (d) The sample**
- The sample must be appropriate to the material being tested, considering, at a minimum:
- (1) The size and volume of the final product lot
  - Note: ATMPs may not meet USP 71 Tables 2-3
- (2) The duration of manufacturing of the drug
  - FDA expects samples be taken from different points of manufacture (i.e., at least from the beginning, middle and end). This may be an issue for cell therapy products.



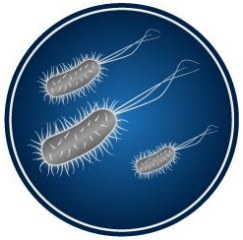
FDA Sterility Testing for Biologics

- (3) The final container configuration and size
  - May need to establish an appropriate testing plan for very low production volumes
- (4) The quantity or concentration of inhibitors, neutralizers, and preservatives, if present
  - May need to test the sample in a manner that will eliminate interference with the test



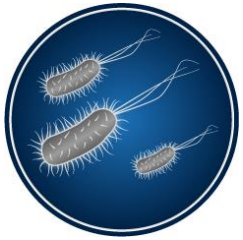
FDA Sterility Testing for Biologics

- (5) For a culture-based method, the volume of test material that results in a dilution that is not bacteriostatic or fungistatic
- (6) For a non-culture-based method, the volume of test material that results in a dilution that does not inhibit or otherwise hinder the detection of viable contaminating microorganisms



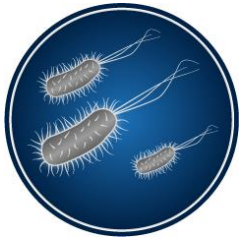
FDA Sterility Testing for Biologics

- **§ 610.12 (e) Verification**
- (1) For culture-based test methods, demonstrate that media will consistently detect the presence of microorganisms, including tests for each lot of culture media to verify its growth promoting properties over its shelf-life
- (2) For non-culture-based test methods, appropriate controls must be used to demonstrate the ability of the test method to continue to consistently detect the presence of microorganisms



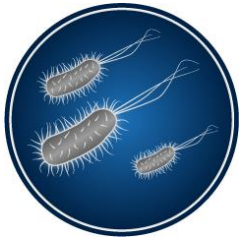
FDA Draft Guidance on Gene Therapy

- July 2018, *Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications*
- "Analytical procedures different than those outlined in the USP, FDA guidance, or Code of Federal Regulations (CFR) may be acceptable under IND if sponsors provide adequate information on test specificity, sensitivity, and robustness."



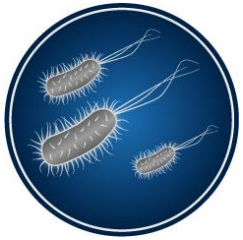
FDA Draft Guidance on Gene Therapy

- Examples of alternative methods, which may be needed for live cells, include:
  - Rapid sterility tests
  - Rapid mycoplasma tests (including PCR-based tests)
  - Rapid endotoxin tests
- "FDA recommends that sponsors demonstrate equal or greater assurance of the test methodology, compared to a compendial method, prior to licensure."



FDA Draft Guidance on Gene Therapy

- “For ex vivo genetically modified cells administered immediately after manufacturing, in-process sterility testing on sample taken 48 to 72 hours prior to final harvest is recommended for product release.”
- Also recommend testing the final product:
  - A Gram stain or other rapid detection test
  - A sterility test, compliant with 21 CFR 610.12

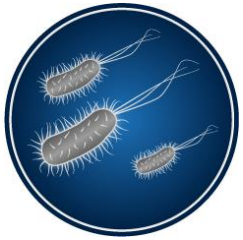


FDA Draft Guidance on Gene Therapy

- “Under this approach, the release criteria for sterility would be based on a negative result of the Gram stain and a no-growth result from the 48 to 72 hour in-process sterility test.”
- The final product sterility test should incubate for the full duration (“14 days”), even after the product has been administered to the patient
  - **NOTE:** 21 CFR 610.12 allows for rapid sterility tests, after validation

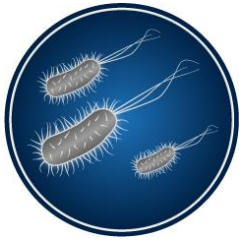
EU Guidelines for ATMP

- EudraLex. The Rules Governing Medicinal Products in the European Union. Volume 4. GMP. "*Guidelines on Good Manufacturing Practice Specific to Advanced Therapy Medicinal Products*"
- Effective May 2018
- Applies to the manufacturing of ATMPs that have been granted a marketing authorisation and of ATMPs used in a clinical trial setting



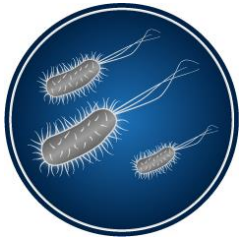
EU Guidelines for ATMP

- “Application of the sterility test to the finished product (Ph. Eur. 2.6.1) may not always be possible due to the scarcity of materials available, or it may not be possible to wait for the final result before the product is released due to short shelf-life or medical need.”
- “In these cases, the strategy regarding sterility assurance has to be adapted.”
  - Use alternative methods for preliminary results, combined with sterility testing of media or intermediates at relevant time points



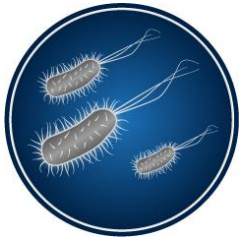
EU Guidelines for ATMP

- When testing intermediates (instead of the finished product) or in-process controls (instead of batch release testing), you must demonstrate the test results are relevant to the critical quality attributes (e.g., sterility) of the finished product
- Also referenced real time testing of materials/products that have a short shelf life



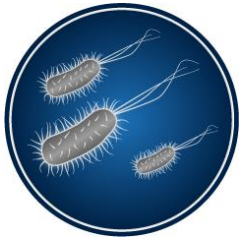
EU Guidelines for ATMP

- The use of validated alternative rapid microbiological methods according to Ph. Eur. 2.6.27 may also be considered, as long as method suitability for the product has been demonstrated
- “If the results of the sterility test of the product are not available at release, mitigation measures should be implemented, including informing the treating physician.”



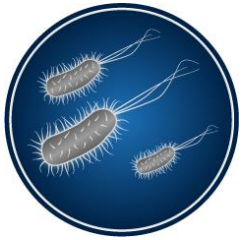
EU Guidelines for ATMP

- “Where aseptic operations are performed, monitoring should be frequent using methods such as settle plates, volumetric air and surface sampling (e.g. swabs and contact plates).”
- “Rapid microbial monitoring methods should be considered and may be adopted after validation of the premises.”
  - e.g., real-time intrinsic fluorescence air monitoring systems



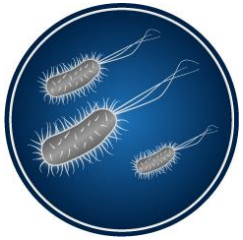
Ph. Eur. 2.6.27

- *Microbiological Examination of Cell-Based Preparations* (July 2017)
- Takes into account the characteristics and limitations of these preparations
  - Shelf-life, which may not always allow for completion of conventional microbiological tests before administration to the patient
  - Amounts available for testing
  - Testing time to result v. patient administration needs
  - Sampling or technical related issues, such as initial turbidity or antimicrobial activity



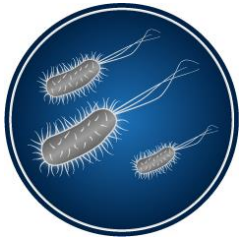
Ph. Eur. 2.6.27

- “Microbial contaminants may be found either inside or on the surface of cells or other components of the cell-based preparation and may not be detected if only supernatants, such as culture or transport media, are analysed. The sample tested must be representative of all of the components of the cell-based preparation, unless otherwise justified.”
- “The test sample must be representative of all of the components of the cell-based preparation and be taken from the final preparation.”



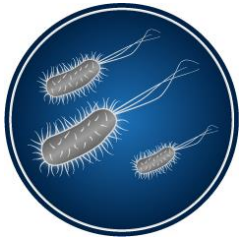
Ph. Eur. 2.6.27

- However, “where this is not possible, surrogate testing may be performed, for example on the liquids last in contact with the cells being processed.”
- This is similar to the EU Guidelines for ATMPs



Ph. Eur. 2.6.27

- Small sample sizes available for testing (due to single donor or manufacturing-related capacities) must still be sufficient to ensure suitable sensitivity and specificity of the test method
- If it is necessary to store samples, the impact of the storage on potential contaminants must be evaluated

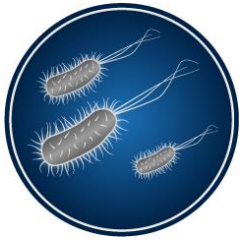


Ph. Eur. 2.6.27

- When the total volume of the batch is between 1 mL and 1 L in a single container:

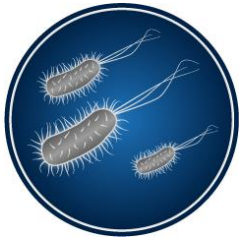
Total Product Volume (mL)	Minimum Test Sample Volume (Divided Between Aerobic and Anaerobic Bottles)
$10 \leq V \leq 1000$	1% of total product volume
$1 \leq V < 10$	100 µL
$V < 1$	Not applicable

- **NOTE:** be mindful of contamination risks when taking the test sample from the final container, if the container will be dispensed for use



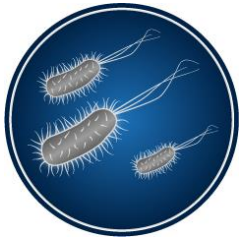
Ph. Eur. 2.6.27

- “For other volumes or multiple containers, alternative approaches should be used and have to be justified.”
  - **NOTE:** consideration should be given for the number of containers, fill volumes and what can be sacrificed for release testing vs. patient needs
- “For preparation volumes less than 1 mL, where final sampling is not possible, surrogate testing, in-process testing or other appropriate testing should be used and has to be justified.”



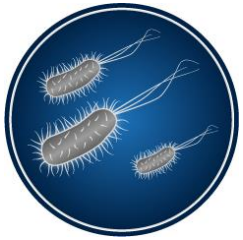
Ph. Eur. 2.6.27

- Can use automated growth-based methods
- Can use alternative methods
  - A combination of pre-culturing and detection by alternative methods (see 5.1.6)
  - Direct detection by alternative methods (see 5.1.6)
  - Methods based on the sterility test (see 2.6.1)



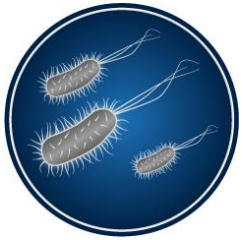
Ph. Eur. 2.6.27

- For an automated growth-based method, at least two types of media should be used to detect fungi and aerobic and anaerobic bacteria
- < 100 CFU of each of the strains listed in 2.6.27 is used for growth promotion; incubate for a maximum of 7 days for at the appropriate temperature for testing
- Method suitability is performed with product, using less than 100 CFU. Three replicates. Use the list of organisms in 2.6.27 (other organisms may be applicable if previously found or are associated with the cell preparation).



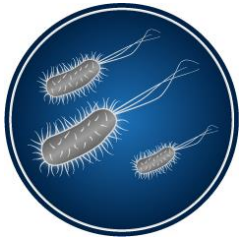
Ph. Eur. 2.6.27

- A variety of options for incubation temperatures are provided; these include two temperatures or a single, elevated temperature to obtain a relevant readout
- Test samples are incubated for not less than 7 days; may need to extend to 14 days
- If inoculated bottles are stored >12 hours prior to being placed in an automated culturing system, subculture of each incubated bottle is performed to check for false negatives (i.e., a fast-growing organism that replicates during storage but is not detected in the system)



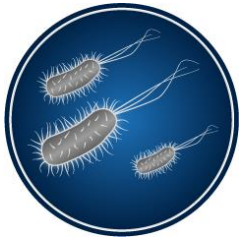
Ph. Eur. 2.6.27

- Alternative Methods: Combination of pre-culturing and detection
- Samples are incubated in aerobic and anaerobic liquid or solid media for a short period of time (e.g. 12-24 h depending on the sensitivity of the alternative approach)
- An alternative method suitable for rapid detection of micro-organisms is then performed (nucleic acid amplification, flow cytometry, bioluminescence)



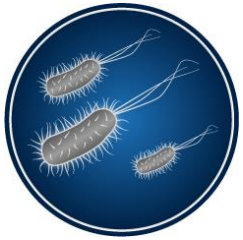
Ph. Eur. 2.6.27

- Alternative Methods: Direct detection
- Where a cell-based preparation has a very short shelf-life (e.g. a few hours), non-growth-based, direct detection methods may be performed (nucleic acid amplification, flow cytometry, bioluminescence)
- May result in lower sensitivity, in comparison to growth-based methods, because both viable and non-viable microorganisms may be detected
  - **NOTE:** this is resolved during method suitability testing
  - In reality, the same or better sensitivity (LOD or LOQ) can be achieved



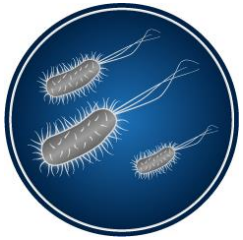
Ph. Eur. 2.6.27

- Alternative Methods: Validation
- Follow the recommendations provided in chapter 5.1.6 and in 2.6.27 under Method Suitability for automated growth-based methods
- **NOTE:** 5.1.6 provides guidance on inoculum levels
  - LOD: the lowest number of organisms that can be detected under the stated analytical conditions
  - LOQ: the lowest number of CFUs which can be enumerated with suitable Precision and Accuracy
  - Equivalence: use low levels (e.g., less than 5 CFU)



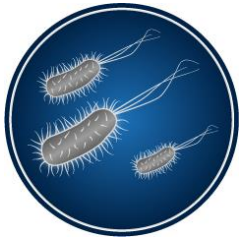
Ph. Eur. 2.6.27

- Provides guidance on releasing cell-based preparations prior to the end of a sterility test
- When justified for a cell-based preparation with a short shelf life, you may be able to release product based on a “Negative-to-date” result, which is an intermediate reading of the sterility test
- Results from additional microbiological in-process control testing may also be needed



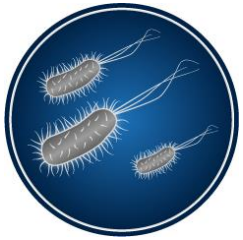
USP <1071>

- USP <1071>, *Rapid Sterility Testing of Short-Life Products: A Risk-Based Approach*. Sep-Oct 2018 Pharmacopeial Forum; comments until Nov 30, 2018.
- Provides guidance when the compendial sterility test is unsuitable for product release
  - Compounded sterile preparations (CSPs)
  - Positron emission tomographic (PET) products
  - Gene and cell therapies



USP <1071>

- “Current growth-based sterility tests with an incubation period of at least 14 days are not suitable for short-life products or for products prepared for immediate use.”
- “Rapid sterility tests (RSTs) should be risk-based.”
- Stakeholders should select their preferred technology considering time to result, specificity, limit of detection (LOD), sample size, and product attributes

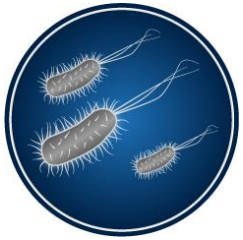


USP <1071>

- The chapter refers to the 1% sampling plan in Ph. Eur. 2.6.27 and provides the following examples:

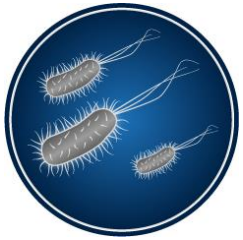
No. of Containers	Container Volume	Total Batch Size	1% of Batch	Container Used for Sample
1	15 mL	15 mL	0.15 mL	1
3	25 mL	75 mL	0.75 mL	1

- **NOTE:** use caution if sampling from a container that will be dispensed; other sampling strategies may be required



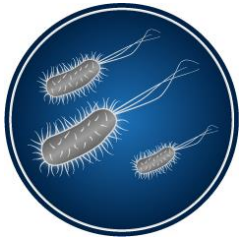
USP <1071>

- Examples of relevant technologies:
  - Adenosine triphosphate (ATP) bioluminescence
  - Flow cytometry
  - Solid phase cytometry
  - Isothermal microcalorimetry
  - Nucleic acid amplification (universal primers/probes)
  - Respiration (e.g., detection of CO_2 during microbial growth)



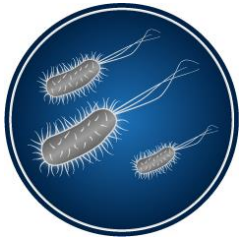
USP <1071>

- “RSTs or other rapid microbiological methods may be used as in-process controls prior to the final product release sterility test to provide early detection of gross contamination or probability that a product may fail sterility”
- Perform method suitability, in the presence of product using < 100 cfu



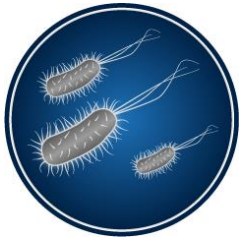
USP <1071>

- “Growth-based sterility tests can be shown to have at least a theoretical LOD of 1–3 cfu based on a Poisson distribution. Setting an LOD of a single viable cell with all technologies is an unrealistic barrier of entry for any sterility test, especially when the signal is not the colony-forming unit that is amplified by cultural enrichment.”
  - Agree, as long as the method does not have a purported detection sensitivity level of a single cell
  - Disagree, as some non-growth based RSTs have proven single cell detection capability (solid phase cytometry)



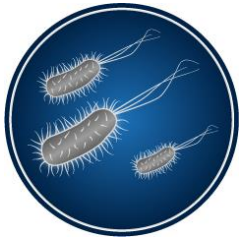
USP <1071>

- The chapter also provides support for not validating a RST down to a single cell, based on an infectious dose:
  - “there is little or no evidence that 1 cfu is an infectious dose for injectable products . . . ”
  - “infectious dose may be 10^2 to 10^3 viable microorganisms, depending on the virulence of the microorganism”
- I recommend considering the technology capability and regulatory expectations when validating LOD



Case Studies

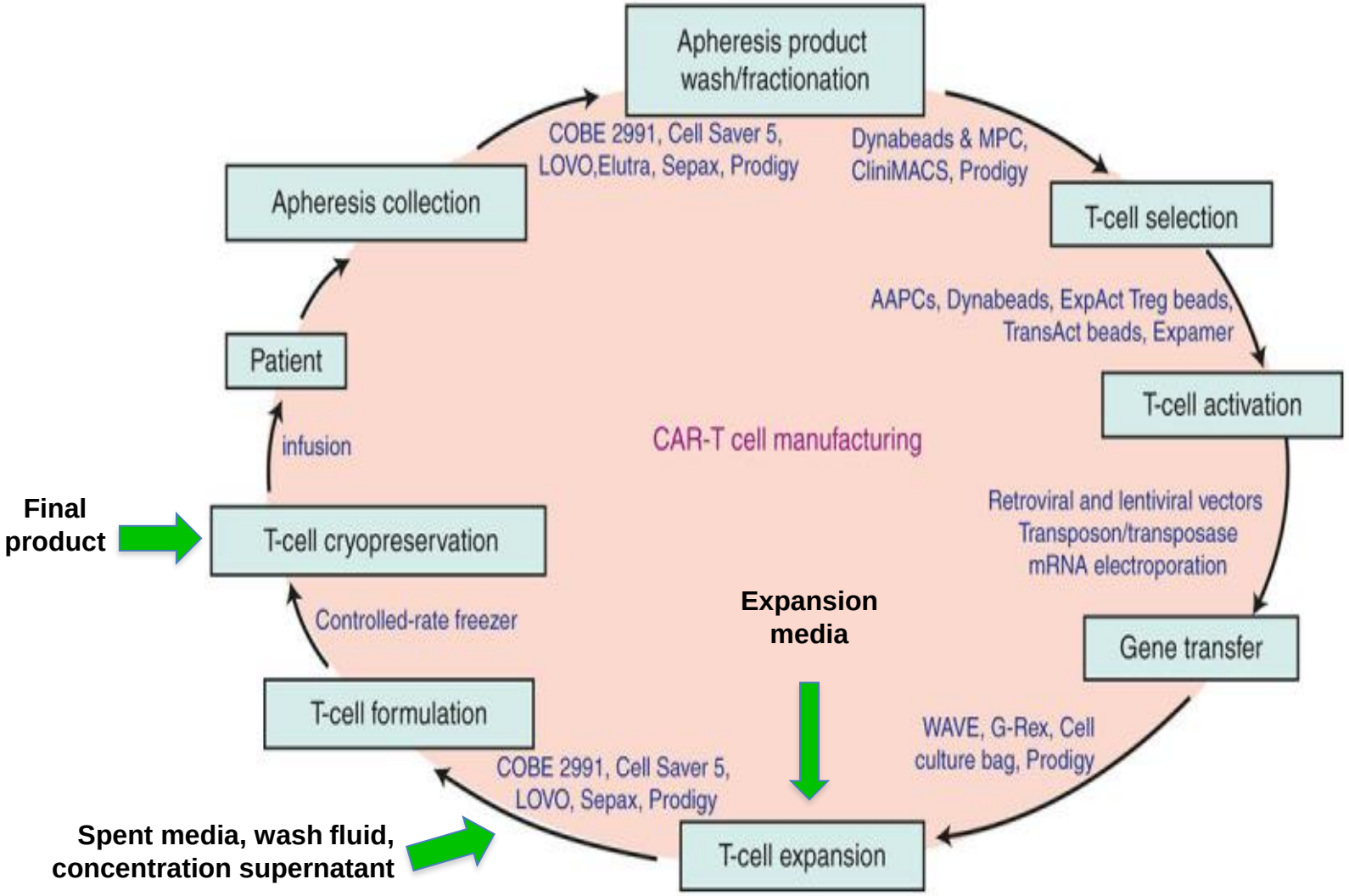
- Based on my consulting with several cell therapy companies
- Selected RMM technologies that matched the URS (e.g., time to result, limit of detection and product compatibility)
- Identification of the test sample and sample size, conducted feasibility testing
- Confirmation of sampling and testing strategies with FDA
- Validated the method, performed product suitability studies, demonstrated equivalence with the compendial test

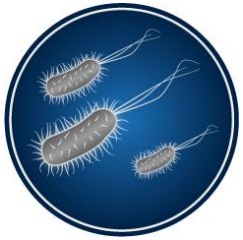


Case Studies – Test Sample

- Considered upstream surrogate or in-process samples
  - In some cases, there was no limitation on available sample
  - Had to demonstrate microorganisms detected in these samples would also be present in the finished product
- Most companies chose to test finished product, even if upstream samples were analysed
- Considered testing residual product from the bulk vessel prior to filling; may be appropriate if there is low risk of contamination from downstream processes

Illustration from Wang X, Rivière I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. *Molecular Therapy Oncolytics*. 2016. 3:16015.



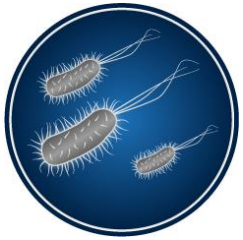


Case Studies – Sample Size

- Initially referred to compendial sterility test requirements

Product	Minimum Number of Articles to be Tested	Minimum Quantity (For Each Medium)	Testing and Patient Risks
4 IV bags 60 mL fill 240 mL batch	**4 containers**	**20 mL from each bag 160 mL total[1]**	**Must sample every bag (potential contamination). Loss of 67% of the batch.**
15 vials 4 mL fill 60 mL batch	**4 containers**	**2 mL from 4 vials 16 mL total[2]**	**Must sacrifice 4 vials. Loss of 27% of the batch.**

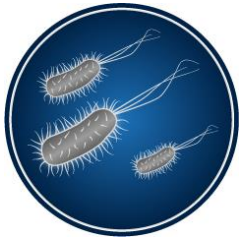
[1] 20 mL × 2 media × 4 bags.
[2] 2 mL × 2 media × 4 vials.



Case Studies – Sample Size

Product	Minimum Sample Size Assume 1% of Batch	Testing and Patient Risks	Comment
4 IV bags 60 mL fill 240 mL batch	2.4 mL from one bag	Must sample one bag (potential contamination). Minimal batch loss (1%).	Could test greater volume if one bag is sacrificed (depends on required dose; loss of 25% of batch).
15 vials 4 mL fill 60 mL batch	0.6 mL from 1 vial	Sacrifice 1 vial. Minimal batch loss (7%).	Could test greater volume from the vial.

- **NOTE:** total batch size may be variable (depends on initial concentration of patient cells and expansion efficiency)



Case Studies

- Sample size may also impact the test method
  - Solid phase cytometry: 1 test sample
  - ATP bioluminescence (with filtration and enrichment): sample is divided between 3 incubation parameters
  - Respiration / CO_2 detection: sample is divided between 2 media / 2 incubation temps
- Regulators may be concerned that the smaller the sample size, the lower the sensitivity of the sterility test; always discuss your strategy