Designation: F2383 – 11

Standard Guide for Assessment of Adventitious Agents in Tissue Engineered Medical Products (TEMPs)[1]

This standard is issued under the fixed designation F2383; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide is intended as a resource for individuals and organizations involved in the production, delivery, and regulation of tissue engineered medical products (TEMPs). The safety from contamination by potentially infectious adventitious agents is important in the development of all TEMPs as well as their components. This guide addresses how to assess safety risks associated with adventitious agents and their byproducts. These agents currently include bacteria, fungi, mycoplasma, viruses, endotoxins, transmissible spongiform encephalopathies (TSEs), and parasitic organisms. This guide does not address TEMPs with live animal cells, tissues or organs, or human cells, including stem cells, grown on any animal feeder cells. Also excluded is patient follow-up testing.

1.2 This guide does not apply to any medical products of human origin regulated by the U.S. Food and Drug Administration under 21 CFR Parts 16 and 1270 and 21 CFR Parts 207, 807 and 1271. This guide does apply to cellular therapies regulated under the PHS (Public Health Service) act.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1873 Guide for Detection of Nucleic Acid Sequences by the Polymerase Chain Reaction Technique

F2210 Guide for Processing Cells, Tissues, and Organs for Use in Tissue Engineered Medical Products

F2211 Classification for Tissue Engineered Medical Products (TEMPs)

F2312 Terminology Relating to Tissue Engineered Medical Products

F2386 Guide for Preservation of Tissue Engineered Medical Products (TEMPs)

2.2 *ANSI/AAMI Standard:*

ST72 Bacterial Endotoxin—Test Methodologies, Routine Monitoring and Alternatives to Batch Testing[3]

2.3 *Federal Regulations:*[4]

9 CFR Animals and Animal Products

21 CFR 210 Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs, General

21 CFR 211 Current Good Manufacturing Practice for Finished Pharmaceuticals

21 CFR 610.12 General Biological Products Standards—Sterility

21 CFR 610.13 (b) General Biological Products Standards—Purity Test for Pyrogenic Substances

21 CFR 820 Quality System Regulation

21 CFR 1270 Human Tissue Intended for Transplantation

21 CFR 1271 Human Cells, Tissues, and Cellular and Tissue-Based Products

2.4 *MDA Standard:*

Code of Practice for the Production of Human-Derived Therapeutic Products[5]

2.5 *U. S. Pharmacopeia Document:*

United States Pharmacopeia (USP), Edition XXIV (24)[6]

3. Terminology

3.1 *Definitions:*

[1] This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.45 on Adventitious Agents Safety.

Current edition approved March 1, 2011. Published March 2011. Originally approved in 2005. Last previous edition approved in 2005 as F2383 – 05. DOI: 10.1520/F2383-11.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

[4] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

[5] Available from Medicines and Healthcare Products Regulatory Agency (MHRA), Hannibal House, Elephant & Castle, London SE1 6TQ, U.K.

[6] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

3.1.1 *adventitious agents, n*—unintentionally introduced microbiological or other infectious contaminant. In the production of TEMPs, these agents may be unintentionally introduced into the manufacturing process or into the final product or both. (See Terminology F2312.)

3.1.1.1 *Discussion*—In this guide, adventitious agents also include microbiological or other infectious contaminants that may be endogenous to the starting cells or tissue.

3.1.2 *endotoxin, n*—pyrogenic high molar mass lipopolysaccharide (LPS) complex associated with the cell wall of gram-negative bacteria.

3.1.2.1 *Discussion*—Though endotoxins are pyrogens, not all pyrogens are endotoxins. Endotoxins are specifically detected through a Limulus Amebocyte Lysate (LAL) test.

4. Significance and Use

4.1 TEMPs may be composed of biological products (for example, human cells, organs, tissues, derivatives, and processed biologics), biomaterials (for example, substrates and scaffolds composed of polymers or collagen), and biomolecules (for example, recombinant proteins, alginates, and hyaluronates) (see Terminology F2312). Those TEMPs that contain human viable cells, organs, or tissues differ in terms of adventitious agent safety from other TEMPs because of the need to preserve viability of the organ, tissue, or cellular components. The need for preservation of viability limits processing options for the reduction or elimination of adventitious agents. Examples of TEMPs are listed in Classification F2211.

4.2 To ensure production and use of TEMPs with minimal risks associated with microorganisms and other adventitious agents, a multi-tiered approach is required. Donor testing, as well as testing of components and raw materials by sufficiently sensitive assays that are state of the art is usually necessary. Compliance with good manufacturing practices (GMPs) and good tissue practices (GTPs), where applicable, is required (21 CFR 210, 211, 820, 1270, and 1271). Although some of the components of the TEMPs may be processed to remove potential microbiological contaminants, viable tissues and cellular components are generally unable to withstand rigorous processing without losing functionality. For those TEMPs containing tissues or cells for which banking is not possible, even greater reliance on donor screening, component testing, and manufacturing controls is required. When more upfront testing is possible, there is generally greater confidence in the safety of the final product. Process validation can enhance confidence in the ability of the TEMPs' producer to minimize risks from adventitious agents.

4.3 Throughout this guide, the reader is referred to other documents that may provide specific information that can be applied in the manufacture and testing of TEMPs. Although many of these documents were not written with TEMPs in mind, parts are often applicable. Most of the potentially applicable position papers and guidance documents from many regions of the world can be accessed via the internet. New documents are continually produced. The MDCA (U.K. Medical Devices Agency, now part of MHRA, Medicines and Healthcare Products Regulatory Agency) Code of Practice for the Production of Human-Derived Therapeutic Products provides information on quality control, microbiological safety of donations, production, and processing practices. Two Rijksinstituut voor Volksgezondheid en Milieu (RIVM) reports provide valuable information. One of these reports addresses preclinical safety assessment of TEMPs, and the other provides an approach to risk management for TEMPs (**1**, **2**).[7]

4.4 References may be made to draft guidances and rules. These should not be read as requirements.

5. Sources of Risk

5.1 *Donor*—In some cases, donors with potential previous exposure to certain infectious agents must be excluded. Guidance on donor selection is available from the American Association of Tissue Banks (AATB) and the U.K. Department of Health (**3**, **4**). The U.S. Food and Drug Administration (FDA) has produced many documents that provide useful information on donor testing (**5-11**). There may be specific requirements for different regions of the world. For TEMPs with autologous cells, donor testing is also recommended because of the potential for expansion of adventitious agents during manufacturing (**12**), and the potential for cross-contamination of other products manufactured concurrently in the same facility.

5.2 *Nonviable Animal Material*—The sponsor (product owner) has the responsibility to substantially reduce risks from adventitious agents, including TSEs, in nonviable animal materials. Mitigation of such risks can include scrutiny over donor sourcing or proven, rigorous processing, or both.

5.3 *Cell Banks*—Many TEMPs include cells that can be banked. Master Cell Banks (MCB) and Working Cell Banks (WCB) can be prepared and extensively tested for the presence of adventitious agents. Although TEMPs are not included in the scope of International Conference on Harmonization (ICH) guidelines, the ICH guideline on cell substrate characterization does provide useful information on the production, testing, and storage of cell banks (**13**). Further information may be found in an FDA publication on cell line characterization and the ICH guideline on viral safety (**14**, **15**).

5.4 *Raw Materials:*

5.4.1 The raw materials used in the manufacture of the cellular components of TEMPs are controlled by a number of requirements that describe the microbiological safety testing of components of cell culture media and reagents used in the manipulation of the cells during culture. Some of these materials are manufactured synthetically, for example, amino acid supplements of culture medium. Much more frequently, however, materials are of animal origin such as bovine serum (essential for many mammalian culture systems) or, in the case of anchorage-dependent cell lines, trypsin.

5.4.2 Raw materials of human and animal origin are of particular concern to the manufacturer and licensing authorities, owing to their potential contamination with extraneous agents from the source animal. Most manufacturers of

[7] The boldface numbers in parentheses refer to the list of references at the end of this standard.

biotechnology-derived products do not themselves produce raw materials, but depend on external suppliers. A certificate of analysis indicating all tests performed, with results, and including data on the adventitious agent testing should also be obtained. A critical examination of the microbiological safety testing carried out by the supplier is recommended. As this may not be appropriate to satisfy the regulatory agencies, manufacturers may have to repeat and extend the adventitious agent testing performed by the supplier to ensure that raw materials meet the performance and safety requirements for the production process. Alternatively, it may be appropriate for the TEMPs manufacturer to demonstrate that their processing methods reduce the risk to an acceptable level. Tissue culture components are also discussed in Guide F2210.

5.4.3 Other raw materials should be tested to demonstrate they are free of adventitious agents. In some cases, the testing performed by the raw material supplier is sufficient. If a raw material is available only as research grade, then the sponsor should test that material for adventitious agents.

5.5 *Transport*—After packaging, the external surfaces of the containers may need to be decontaminated or cleaned or both. Container integrity and shipping validation are also important elements for ensuring TEMPs with a minimized defined risk from adventitious agent contamination are delivered to the handlers and users of the final products.

5.6 *Processing*—Adventitious agents can be introduced into the TEMPs during processing. The agents can be derived from contaminated environments, personnel, raw materials, and processing materials, including water, and cross-contamination from previously or concurrently processed products. Processing is addressed in detail later.

5.7 *Storage*—Adventitious agents can be introduced during the storage of all materials. Storage of the starting tissues or cells, raw materials, and final product requires a standardized procedure. The outer surface of the container may need to be decontaminated again prior to use.

6. Processing and Process Validation

6.1 Compliance with GMPs or GTPs, or both, when in effect, is essential for the production of TEMPs that have a minimized defined risk of transmitting adventitious agents. One of the major considerations is aseptic processing. Aseptic processing must be validated and then periodically revalidated to prevent contamination by microbial contamination. Refs **(16-18)** should be evaluated and applied to TEMPs, where feasible. Relevant elements are raw materials testing; suitable control of the processing environment and routine environmental monitoring; operator training; implemented documentation systems; use of suitable, validated analytical test methods; process design; equipment qualification and process validation; and container-closure system validation. Guide F2210, GMPs, and GTPs address many of these issues. Process design, equipment qualification, and process validation are discussed in the following sections.

6.2 *Process Design:*

6.2.1 Each step in the process should be evaluated for potential exposure to environmental contamination, including introduction of contamination by personnel. Processing materials, such as water and buffers, should be free from adventitious agents. Water quality should be evaluated. Depending on the intended use, either water for injection (WFI) or sterile purified water may be appropriate. To ensure further confidence in the process, where feasible, inactivation steps that are suitable for adventitious agents should be incorporated into the process, and decontamination and cleaning protocols established for any contact surfaces.

6.2.2 When multiple processing steps are used, storage conditions that protect the product intermediates from adventitious agents must be incorporated into the process scheme. When multiple products are being processed in the same environment, even more stringent environmental controls may need to be implemented. The use of disposable equipment may be advantageous for ensuring microbiological safety of the TEMPs, but that equipment must be suitably disposed of so that no other facilities or individuals are put at risk **(19)**.

6.3 *Equipment Design and Qualification*—Where disposable equipment is not appropriate, equipment design should be considered to prevent contamination by adventitious agents. Dead legs, crevices, and threaded fittings are likely sites to harbor adventitious agents. Contact surfaces must be accessible and compatible with decontamination and cleaning agents. Equipment is qualified by performing a design qualification, an installation qualification, and an operational qualification. These qualification operations are defined in an ICH document on good manufacturing practices for active pharmaceutical ingredients **(20)**.

6.4 *Use of Suitable, Validated Analytical Test Methods*—Processes cannot be validated without the use of validated assays. During development, those assays that provide the most relevant information should be established and validated. In some cases, the data from traditionally used assays for adventitious agents will not be available in time to release TEMPs containing viable cells for patient use. Other, more rapid assays may have to be validated against the traditional assays. Data from in-process testing are particularly useful for the manufacture of products containing viable cells since final product testing results may not all be available prior to product use. Appropriate in-process testing is strongly recommended for such products. For further guidance, see Reference **(21)**.

6.5 *Process Validation Issues Relevant to Adventitious Agent and Contamination Control:*

6.5.1 In the case of cellular- and tissue-based TEMPs, it may be necessary to perform many more runs than the traditional three to five consecutive runs to ensure the process controls and outputs are sufficient to minimize the risks from adventitious agents and their byproducts. For TEMPs, the use of medium or a product reference standard may be the most logical approach to maintaining a validated state. Medium or the standard can be periodically run through the process to provide documented evidence that the process provides a product meeting specifications that are related to adventitious agent control.

6.5.2 Information on process validation can be found in several documents. FDA has published guides on general

principles of process validation and on validation of human tissue products (**22**, **23**). The ICH document on active pharmaceutical ingredients provides a good framework and defines qualification and validation activities. Two ICH guidelines provide information on validation of analytical methods (**24**, **25**).

6.5.3 Periodic revalidation of the process with the in-house reference standard or media will also provide some confidence in the capability of the process to provide TEMPs with minimized risk from adventitious agents. Whenever there are new reagents, new processes, process changes, new personnel, or other situations, such as an out-of-specification result, process validation should be repeated. If a reference standard is used, it should be properly stored and its stability validated.

6.5.4 The manufacturing process used will depend on the properties of the components and the requirements for the final TEMPs. There are a wide range of processes because of the variability in the sources and properties of different TEMPs and their components. However, in many cases, unit operations will consist of expansion of cells, removal of excess culture fluid, purification of scaffold, assembly of final product, packaging, and shipping. Each unit operation must be validated and appropriate validated in-process assays used, where feasible, to minimize risks from adventitious agents. Validated storage times and conditions must be used between each process step. The capability of antimicrobial preservatives to inhibit microbial growth should be validated along with the preservation process. If cryopreservation is used, it is important to protect the product from microbial contamination in the liquid nitrogen vessel. When feasible, products should be tested after thawing (**4**). The American Association of Tissue Banks (AATB) also provides guidance on storage (**26**).

6.5.5 Potential risks from cross-contamination by adventitious agents can be minimized by segregation of different products by time or space or both. Personnel and equipment should be dedicated to one product at a time. Closeout inventories and cleaning validation between products are important elements in the prevention of cross-contamination by potential adventitious agents.

6.5.6 Process validation should be performed for any inactivation or removal steps that minimize risks from adventitious agents and their byproducts. Spiking studies are often performed on a model system to demonstrate the effectiveness of inactivation or removal steps or both. Inactivation or removal of potential adventitious agents in viable tissue or cellular components of the TEMPs may not be possible.

6.5.7 Equipment cleaning and decontamination validation can, in some cases, be accomplished with a combination of the small-scale coupon studies (see 6.2.2) and in-process monitoring during the conformance batches. Data generated during validation should provide evidence that decontamination agents do not impair functionality of equipment. Routine monitoring should be continued after validation is complete.

6.6 *Container-Closure System Validation*—Validation of the container-closure system must also be performed. Details can be found in Refs (**27**, **28**). In most cases, TEMPs cannot be terminally sterilized.

6.7 *Preservation*—Preservation of TEMPs is addressed in Guide F2386.

7. Final Product

7.1 For the design of TEMPs with the lowest possible risks for disease transmission, it is a prerequisite that in addition to assessing the safety of the individual components and the processing procedures, the final product is also tested.

7.2 In many cases, traditional test methods will not be sufficiently rapid, and newer technologies will be used to release the product. In addition to adventitious agent testing, test methods may include assays for byproducts of adventitious agents, for example, endotoxin. Stability testing is also addressed by FDA and ICH documents, and part of a stability profile includes a demonstration that it remains uncontaminated by adventitious agents during its storage period (**29**, **30**).

7.3 In-process sterility testing at critical points during manufacturing of viable cellular products is useful when tests on the final product will not be available prior to use of the product in patients. For example, this might be done routinely during extended culture periods and after critical points in manufacturing, such as when cells have undergone activation or other modification. The results of this in-process testing should meet acceptance criteria as part of required final product specifications (**31**). When in process testing is used for product release, testing on the final product must also be performed, and a system put in place to report occurrences of sterility failure detected after product release.

7.4 Representative retention samples, where appropriate, should be maintained under appropriate conditions so that a thorough investigation can be performed in the event that an adverse patient reaction is observed. The size of a batch may be small for many TEMPs, particularly those containing autologous cells, but the TEMPs sponsor should ensure that adequate product is archived. Archival time should be established based on current regulatory expectations and functional lifetime or beyond, when feasible.

8. Adventitious Agents, Byproducts, and Detection Methods

8.1 In this section, an overview of potential adventitious agents and testing methods is presented (see Table 1). Fungi, bacteria, mycoplasma, viruses, TSEs, and parasites are included. Byproducts of some of these agents, for example, endotoxin or other pyrogenic material from bacteria, are also considered. The TEMPs' manufacturer should determine which, if any, of these agents should be tested for and where in the process that testing should occur. A risk assessment and discussions with relevant regulatory agencies should enable the manufacturer to make appropriate choices. Although the sections below provide examples of some newer test methods, it is important to realize that there is rapid progress in this field.

8.2 Knowing exactly which tests to perform can require significant expertise. Some tests are better suited to viable materials while others are more suitable for nonviable components of TEMPs. Since scientific progress is rapid in this field,

TABLE 1 Summary Table

Risk	Potential Risk Source(s)	Test Method[A]
Bacteria and fungi	Human donor, animal materials, cell banks, raw materials, human contact during transport, processing and storage	Sterility, bioburden: Compendial assays: USP, JP, EP, 21CFR 610.12. Rapid methods (for example, colorimetric, bioluminescence).
Mycoplasma	Human donor, animal materials, cell banks, raw materials, human contact during transport, processing and storage	Compendial assays: USP, JP, EP. U.S. FDA Points to Consider in the Characterization of Cell Lines. PCR
Pyrogens	Human donor, animal materials, cell banks, raw materials, human contact during transport, processing and storage	Rabbit pyrogen test LAL test for endotoxins RIVM assay MM6-CA8 assay
Viruses	Human donor, animal materials, cell banks, raw materials, human contact during transport, processing and storage	Infectivity and PCR assays for specific viruses. *In vitro* and *in vivo* infectivity assays for general virus screening. 9 CFR assays for bovine and porcine viruses.
Transmissible spongiform encephalopathies	Human donor, animal materials	No sufficiently sensitive screening methods available at this time. Clearance studies are performed with scrapie agent (for example, hamster 293k strain) or Western Blot or both.
Parasites	Human donor, animal materials, processing	Microscopic examination PCR detection methods Antibody detection methods

[A] A review of donor sources' relevant medical records for applicable risk assessment should be included where relevant.

the test methods here are listed for information only. Traditional test methods are not always suited for TEMPs or their components. Traditional test methods include those for bacteria and fungi, mycoplasma, endotoxin and other pyrogenic materials, and endogenous and adventitious viruses. Sponsors of a TEMP should discuss their suggested testing plan with the appropriate regulatory agency.

8.3 *Sterility (Bacteria and Fungi):*

8.3.1 A vast number of bacterial and fungal species are known to potentially infect cells and raw materials of human origin as well as those derived from animal sources. These would be too numerous to list in this guide, however, these agents should be shown to be absent from any biotherapeutic materials. When manual steps are used in production, there is a greater risk of microbial contamination. Culturing of cells, especially those that are not banked and thoroughly tested, increases the chances of bacterial proliferation. If bacteria are detected in a general screening assay, speciation is required.

8.3.2 Currently accepted compendial test methods for detection of the presence of bacteria and fungi are not designed for TEMPs and may be inappropriate. They are performed according to the European Pharmacopoeia (EP), Japanese Pharmacopoeia (JP), U.S. Pharmacopoeia (USP), or 21 CFR 610.12. These test methods test for microbial limits or microbial sterility. For TEMPs, preliminary sterility test results (for example, 48 h) may have to be used to release products with viable cells. In this case, the test is continued for the specified time (for example, 14 days) and the data reported to ensure patient follow-up if necessary. Alternative rapid test methods are being established to release products with short shelf life. Growth-based test methods include ATP bioluminescence and colorimetric detection of carbon dioxide production. Some references are provided here, but the reader is urged to evaluate new methods continually as they are developed (**32-34**). The reader should also be aware that different tests may be appropriate for different components of the TEMPs, and should discuss their use with the appropriate regulatory agency.

8.3.3 As described in the USP-NF <1046> 1st supplement, automated sterility testing methods that rely on colorimetric detection or continuous monitoring may be accepted if they are validated. This should, however, be discussed with the appropriate regulatory agency. A PDA Technical Report (**35**) describes approaches for evaluation, validation, and implementation of new microbiological testing methods. This approach has been applied for at least one such test method that detects viable microbial cells and does not require an extended incubation period. It has been applied to both process water and detection of microbial contaminants in animal cell culture processes (**36**).

8.4 *Mycoplasma:*

8.4.1 As a subgroup of the bacteria, mycoplasma are considered specifically because of their ability to infect human cells. Detection methods for mycoplasma are significantly different from other bacteria.

8.4.2 Mycoplasma detection methods are described in the European and Japanese Pharmacopoeias and in the U.S. FDA Points to Consider in the Characterization of Cell lines (**14**). Both agar and broth procedures are used as well as the indicator cell culture procedure. The required time for performance of the tests may exceed the shelf life of some TEMPs. There is considerable work in the field on alternative methods. PCR (polymerase chain reaction) is just one of those methods that have been applied to myoplasma testing. In an ATCC (American Type Culture Collection) newsletter, PCR was compared to the traditional Hoechst staining and direct culture methods (both broth and agar). As discussed in the article, there are four species of mycoplasma that constitute 85 % of the mycoplasma that infect cells in culture. The PCR methods are capable of picking up these species, and it would appear that applying this method to TEMPs may enhance patient safety (**37**). Consideration should be given to the possibility that materials in the TEMPs may interfere with the assay and may result in false positive or false negative results. Steps should be taken to validate the appropriateness of the test for each product. However, further improvements and validation studies are needed to reach the assurance of currently accepted methods (agar and broth procedures and the indicator cell culture procedure).

8.5 *Pyrogen:*

8.5.1 The rabbit pyrogen test is a procedure to investigate if any material capable of increasing body temperature is present in the test materials (see 21 CFR 610.13(b)). The most likely pyrogenic substance will be gram-negative bacterial endotoxin (see 8.6), but the possibility that other materials present in the TEMPs may be pyrogenic should be considered. Therefore, a test should be performed on all new TEMPs to rule out nonendotoxin pyrogenic substances. Once this is done, the manufacturer can usually replace that test with an LAL (Limulus Amebocyte Lysate) method. In some cases, the rabbit pyrogen test may be required. Another test method for detection of pyrogenic materials is described by RIVM (Rijksinstituut voor Volksgezondheid en Milieu) **(38)**. The MM6-CA8 assay is a good supplement for evaluating pyrogenicity in humans (see 8.6.2).

8.6 *Endotoxin:*

8.6.1 Natural biomaterials are potentially contaminated with a significant amount of endotoxin, and once contaminated, it is quite difficult to remove the endotoxin during the manufacturing process. Endotoxin should generally be assessed in the final product. The method of sample preparation is a key point for accurate endotoxin detection. In the case of collagen products, the proper sample preparation method requires enzymatic digestion. In the case of other biomaterials, sample homogenization is often a powerful test method to recover the contaminating endotoxin. Consideration should be given to the possibility that materials in the TEMPs may interfere with the assay. Steps should be taken to validate the appropriateness of the test for each product.

8.6.2 The bacterial endotoxin test (BET) detects endotoxin based on a clotting reaction elicited in lysates of amoebocytes from the horseshoe crab. This test has been adopted as an official test and is now used widely as a simple and highly sensitive method for detection of endotoxin. Three basic techniques are used for this test. The gel clot technique is based on gel formation. The turbidity technique measures turbidity formation after cleavage of an endogenous substrate. The chromogenic technique is based on the development of color after cleavage of a synthetic peptide-chromogen complex. When using the kinetic colorimetric assay, the relative standard deviation (RSD) can be estimated by an equation **(39)**.

8.6.3 Further guidance on endotoxin testing is available from ANSI/AAMI ST72 and an FDA document on the validation of the BET test **(40)**. In the latter document, the endotoxin limit for medical devices is 0.5 endotoxin units (EU)/mL, except for devices in contact with cerebral spinal fluid for which the limit is 0.06 EU/mL. The current endotoxin administration limit for biological products is 5.0 EU/kg/h by the parenteral route or or 0.2 EU/kg/dose for intrathecally administered products. USP Rev 27 contains further information on the BET test <85>, rabbit pyrogen test <151>, and <161> allows for 20 EU/device to account for dilutions.

8.7 It has been reported that the results from the BET test do not exactly parallel *in vivo* pyrogenic activity. Environmental endotoxin has been shown to be less pyrogenic than purified endotoxin when the endotoxins are administered into rabbits at the same EU dose level. To assess the pyrogenicity in humans, the IL-6 inducibility of the extracts in human monocytic MM6-CA8 cells was examined, since the responses of cells to pyrogens have been demonstrated to be highly relevant to human pyrogenic responses. Data from the experiments showed that the pyrogenicity and IL-6-inducibility of the extracts approximately agreed with the endotoxin concentrations in the extracts determined by the BET test. Another assay described in the RIVM report is the human whole blood pyrogen assay. It is based on the induction of IL-1b production. This assay is claimed to be more accurate for solid materials to which pyrogens may be firmly attached. If either the MM6-CA8 or human whole blood pyrogen assays are used, they should be validated for the particular product by the sponsor.

8.8 *Viruses*—Viruses are perhaps the agents that give rise to the greatest concern in microbiological screening. The list below is extensive and may go beyond those requested for a particular product by specific regulatory agencies. Current scientific knowledge may also dictate that other viruses be assayed. A risk assessment and discussions with regulatory reviewers will enable those that are relevant to be tested.

8.9 *Human Viruses:*

8.9.1 The following is a list of viruses that are generally recognized as having the highest potential threats. Human immunodeficiency virus (HIV) 1 and 2, human T cell leukemia virus I and II, hepatitis A virus, hepatitis B virus, hepatitis C virus, cytomegalovirus, Epstein-Barr virus, human herpes virus Types 6, 7 and 8, and human parvovirus B-19. Viral safety testing should be performed for these and other viruses depending on the tissue type of the source cells or raw materials.

8.9.2 Cells or other materials harvested from the respiratory tract should also be screened for human picornaviruses, parainfluenza viruses, respiratory syncytial viruses, orthomyxoviruses (influenza), coronaviruses, human spumaviruses, reoviruses, and adeno viruses.

8.9.3 Cells or other materials harvested from the gastrointestinal tract should also be screened for human picornaviruses (in particular, human enteroviruses), human adenoviruses, and caliciviruses.

8.9.4 Cells or other materials harvested from the skin or dermal layers should also be screened for human herpes simplex virus Types 1 and 2, varicella zoster virus, human papilloma viruses, and poxviruses.

8.9.5 Cells or other materials harvested from the circulatory system, including whole blood, should also be screened for human circovirus (TTV) and human and animal arboviruses including West Nile Fever virus. Other viruses should be considered for detection depending on the geographical location, and advice should also be taken from local disease surveillance organizations.

8.9.5.1 Cells or other materials harvested from neurological tissues should be screened for HSV 1 and 2, and human polyoma viruses (JC and BK).

8.9.6 Human viruses should be shown to be excluded from any biologically sourced materials. In some cases, infectivity assays are not available and other assays are used. For example, in some cases, the polymerase chain reaction (PCR) assay can provide some useful information. The use of PCR

without infectivity assays, however, assumes that one knows what sequences are being sought. (See Guide E1873.)

8.10 *Bovine Viruses:*

8.10.1 There are a number of viruses that are recognized as being a risk in the contamination of bovine materials. Included in these are bovine viral diarrhoea virus (BVDV), bovine parvovirus (BPV), bovine adenovirus (BAV), blue tongue virus (BTV), rabies virus, bovine respiratory syncytial virus (BRSV), and reovirus. Other viruses, which are more recently recognized as having the potential to contaminate bovine materials include bovine polyoma virus, bovine herpes virus Type IV, Borna disease virus, and bovine circovirus. Animal arboviruses, including West Nile Fever virus, and other viruses should be considered for detection depending on the geographical location of the cows providing the material. Advice should also be taken from local disease surveillance organizations.

8.10.2 The U.S. Code of Federal Regulations (Animals and Animal Products 9 CFR part 113.53) specifies the required testing for bovine viruses. In 9 CFR section 113.53, it states that monolayers of the vero (African green monkey kidney) cell line and either primary cells or a cell line of the same species of origin as the ingredient shall be used in the test. The inoculated cultures are tested for the presence of viral contaminants by characteristic cytopathic effect (CPE) and by specific confirmatory assays. Bovine viruses other than those detected by the 9 CFR assay can be assayed for using PCR reactions, as described in 8.9.6.

8.11 *Porcine Viruses:*

8.11.1 A number of viruses have been characterized in pigs and many of these are capable of infection and causing disease in humans. Many of the identified viruses, however, are of little concern as the main material sourced from pigs is trypsin (frequently used to disassociate mammalian cells) and this is subject to a stringent manufacturing process which is capable of virus removal. The main virus concern is porcine parvovirus, which is known to be present at high levels in slaughter pigs and is resistant to both chemical and mechanical removal steps. An additional concern is porcine circovirus.

8.11.2 9 CFR specifies the required testing for porcine viruses. The purpose of the regulation is to detect the presence of a number of porcine viruses in many different materials, but is particularly designed to detect porcine parvovirus (PPV) in trypsin. The primary porcine kidney (PPK) cell line described in 9 CFR is permissive for a comprehensive range of porcine viruses that includes parvovirus, enteroviruses, pseudorabies, transmissible gastroenteritis (TGE), reoviruses, and adenoviruses. Porcine viruses other than those detected by the 9 CFR assay can be assayed for using PCR reactions, as described in 8.9.6.

8.12 *Transmissible Spongiform Encephalopathies (TSEs):*

8.12.1 There are a number of neurodegenerative diseases that fall under the broad umbrella of human TSEs. The incidence of TSE disease in the human population has fallen under close scrutiny in recent years, with the description of the new disease variant Creutzfeld-Jakob disease (vCJD). TSEs are caused by an infectious agent that is manifested as the accumulation of an abnormal isoform of the constitutive cellular protein Pr. Human TSE disease exists as three main types: sporadic, genetic, and transmitted (iatrogenic). For example, the incidence of CJD in the normal human population, that is, the sporadic rate, is 1 in 10^6 per year. The disease most frequently affects the older section (median age 68 years) of the population. The genetic or familial risks of CJD or other TSE disease have been well documented and have been associated with codon mutations or other chromosomal characteristics. The affected patient groups are well characterized. Transmitted TSE disease is best described by the examples of transmission by dura mater and pituitary growth hormone and of vCJD. Transmission of vCJD is linked to the consumption of bovine materials with bovine spongifom encephalopathy (BSE). Unlike CJD, vCJD has generally affected humans under the age of 55 years. Cells and other materials from human sources can be contaminated with abnormal PrP. Until recognized methods are developed for screening cell lines and other biologicals, reliance is placed on properly screening human donor sources for potential histories of risk (for example, travel, receipt of high risk products) **(41)**.

8.12.2 BSE, although relatively recently described, has dramatically affected the way in which the safety of bovine materials is viewed. BSE is essentially the bovine equivalent of CJD with similar etiology and pathology. There are clearly risks associated with the use of bovine materials in that they may be contaminated with the BSE agent. Currently available detection methods are not sufficiently sensitive to pick up low levels.

8.13 *Parasites:*

8.13.1 Generally, parasites are not considered to be a major safety issue. However, in the case of TEMPs that incorporate live cells, frequently cultured only for a short period of time, there may be the possibility that some parasitic organisms survive and contaminate the product. The potential list of possible parasitic organisms that can contaminate donated cellular material may be extensive. Emphasis should be placed on the correct risk assessment and screening of the donor of the materials. There are many different parasites taking many forms. Properly administered screening questionnaires for blood, organ, and tissue donation can reduce the risk of parasitic infections in the donor source. There should also be procedures in place to ensure the processing does not introduce parasitic contamination. For example, source water must be free of parasites.

8.13.2 Detection of parasites can be done by a variety of means including microscopic examination. However, some parasitic stages can be intracellular and present in very low number. Increasingly, molecular techniques based on PCR technology are used for detection of parasites, and antibody detection is useful for indicating exposure to a specific parasite, but these methods do not necessarily indicate an active infection.

8.13.3 Useful information sites include several from the U.S. Centers for Disease Control (CDC) **(42-45)**.

8.13.4 As described at the CDC Division of Parasitic Diseases site, “Microscopic examination is still considered the “gold standard” for the diagnosis of parasitic diseases.” If an

unequivocal identification of the parasite cannot be made, the specimen can be analyzed using molecular techniques such as polymerase chain reaction (PCR). PCR-amplified fragments can be analyzed by using restriction fragment length polymorphisms (RFLP) or DNA sequencing if further characterization is needed.

8.13.5 Diagnosis of parasitic infections is definitively made by identification of parasites in host tissue or excreta. Detection of antibodies can be very useful as an indicator that an individual has been infected with a specific parasite. A positive result in a person with no exposure to the parasite before recent travel in a disease-endemic area may be interpreted as indicating recent infection. However, detection of specific antibodies in a person native to an area in which the parasite is endemic may reflect only a past infection unrelated to current clinical status. In general, detection of antibodies to parasitic diseases indicates only infection at some indeterminate time and not necessarily acute or current infection. Levels of antibodies to parasites slowly decline after the patient is cured of the infection but generally last for at least six months to many years, depending on the infecting parasite, and thus are not generally useful, real-time indicators of successful cure.

9. Keywords

9.1 adventitious agents; microorganisms; TEMPs; testing

REFERENCES

(1) RIVM Report 640080 002/2001, "Pre-Clinical Safety Assessment of Tissue-Engineered Medical Products (TEMPs). An Investigation on Assays and Guidelines for Biocompatibility Testing," available at www.rivm.nl.

(2) RIVM Report 605 148 009/2001, "Tissue Engineered Medical Products (TEMPs): A Prelude to Risk Management," available at www.rivm.nl.

(3) *Standards for Tissue Banking*, 10th edition, American Association of Tissue Banks, April 2002.

(4) Advisory Committee on the Microbiological Safety of Blood and Tissues for Transplantation, "Guidance on the Microbiological Safety of Human Organs, Tissues and Cells Used in Transplantation," U.K. Department of Health, August 2000, http://www.doh.gov.uk/msbt/msbt.pdf.

(5) "Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)" Final Rule published in Federal Register Vol 69: No. 101, 29786-29833, May 25, 2004. http://www.fda.gov/cber/rules/suitdonor.pdf.

(6) Draft Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps), May 20, 2004. http://www.fda.gov/cber/gdlns/tissdonor.htm.

(7) "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeld-Jakob Disease (CJD) and New Variant Creutzfeld-Jakob Disease (nvCJD) by Blood and Blood Products," January 2002, www.fda.gov/cber/infosheets/cjdchange.htm and http://www.fda.gov/cber/gdlns/cjdvcjd.pdf.

(8) "Guidance for Human Somatic Cell Therapy and Gene Therapy," CBER, FDA, March 1998, www.fda.gov/cber/gdlns/somgene.pdf.

(9) "A Proposed Approach to the Regulation of Cellular and Tissue-based Products," Feb. 28, 1997, (62 FR 9721), www.fda.gov/cber/gdlns/CELLTISSUE.pdf.

(10) "Tissue Action Plan: FDA Approach to the Regulation of Cellular and Tissue-Based Products," http://www.fda.gov/cber/tissue/tissue.htm.

(11) "Draft Guidance for Industry: Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)," 6/14/2002, http://www.fda.gov/cber/gdlns/cjdvcjd0602.pdf.

(12) Benton, K., "FDA Perspectives on Characterization and Comparability of Cellular Therapy Products," *BioProcessing Journal*, 2(1), 2003, pp. 44-47.

(13) ICH, Q5D, "Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products," 1997, http://www.ich.org/.

(14) "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)," (58 FR 42974), 5/17/1993, http://www.fda.gov/cber/gdlns/ptccell.pdf.

(15) ICH Document Q5A, "Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin," 1997, http://www.ich.org/.

(16) PDA Technical Report No. 24, "Current Practices in the Validation of Aseptic Processing," *J. Pharm Sci & Technol*., Supplement Vol. 51, 1997.

(17) PDA Technical Report No. 22, "Process Simulation Testing for Aseptically Filled Products," *J. Pharm Sci & Technol*., Supplement Vol. 50, No. S1, 1996, www.pda.org.

(18) "Guidance for Industry Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice," Draft Guidance, FDA, September 2004, http://www.fda.gov/cber/gdlns/steraseptic.pdf.

(19) *Biosafety in Microbiological and Biomedical Laboratories*, CDC, NIH, 4th edition, 1999.

(20) ICH Document Q7A, ″Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients,″ 2002, http://www.ich.org/.

(21) FDA/CBER Draft Guidance for Reviewers "Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy Investigational New Drug Applications (INDs) (2003)" http://www.fda.gov/cber/gdlns/cmcsomcell.pdf.

(22) Guidelines on General Principles of Process Validation. FDA. 1987 (http://www.fda.gov/cber/guidelines.htm#m4).

(23) U.S. FDA, CBER, "Guidance for Industry: Validation of Procedures for Processing of Human Tissues Intended for Transplantation," March 2002, www.fda.gov/cber/gdlns/tissval.pdf.

(24) ICH Q2A, "Text on Validation of Analytical Procedures," 1994, http://www.ich.org/.

(25) ICH Q2B, "Validation of Analytical Procedures: Methodology," 1996, http://www.ich.org/.

(26) AATB Standards for Tissue Banking, http://www.aatb.org/.

(27) "Guidance for Industry: Container Closure Systems for Packaging Human Drugs and Biologics; Chemistry, Manufacturing, and Controls Documentation," July 7, 1999, http://www.fda.gov/cber/gdlns/cntanr.pdf.

(28) "Pharmaceutical Inspection Convention (PIC) Recommendation on Sterility Testing," 2002, www.picscheme.org/docs/pdf/pi%20012-1%20recommendation%20on%20sterility%20testing.pdf.

(29) "Guidance for Industry: Stability Testing of Drug Substances and Drug Products," Draft Guidance, FDA, CDER, CBER, June 1998, www.fda.gov/cber/gdlns/stabdft.pdf.

(30) ICH Q5C, "Stability Testing of Biotechnological/Biological Products," 1995, http://www.ich.org/.

(31) ICH Q6B, "Guidance on Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products," Federal Register, Vol. 63, No. 110, June 9, 1998, www.fda.gov/cber/gdlns/ichbiotest.pdf; and http://www.fda.gov/cber/gdlns/ichbiotest.txt.

(32) Onadipe, A. and Ulvedal, K., "A Method for Rapid Detection of Microbial Contaminants in Animal Cell Culture Processes," *J. Pharm Sci & Technol*., 55(6), 2001, pp. 337-345.

(33) Mehta, H. B. and Jeong, H., "Strategies for Rapid Microbial Detection in the Pharma/Biotech Industry," *American Pharmaceutical Review*, 6(1), 2003, pp. 73-77.

(34) Olson, W. P., Ed., *Rapid Analytical Microbiology*, PDA/Davis Horwood Publishing Ltd., 2002.

(35) PDA Technical Report No. 33, "Evaluation, Validation, and Implementation of New Microbiological Testing Methods," *J. Parent. Sci Tech*., Vol. 54, No. 3, 2000.

(36) Costanzo, S. P., Borazjani, R. N., and McCormick, P. J., " Validation of the Scan RDI for Routine Microbiological Analysis of Process Water," *J. Parent. Sci Tech*., Vol. 56, No. 4, 2002, pp. 206-219.

(37) Ikonomi, P. and Polayes, D., "Detecting Mycoplasma in Cell Cultures Using the ATCC Mycoplasma Detection Kit," *ATCC Connection*, 22(1), 1, 5, 2002.

(38) RIVM Report 640080 002/2001, "Pre-Clinical Safety Assessment of Tissue-Engineered Medical Products (TEMPs). An Investigation on Assays and Guidelines for Biocompatibility Testing," p. 16.

(39) Haishimia, Y., et al., "Estimation of Uncertainty in Kinetic-Colorimetric Assay of Bacterial Endotoxins," *J.Pharm.Biomed. Anal*., 32, 2003, pp. 495-503.

(40) "Guideline on the Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices," U.S. FDA, 1987.

(41) Pruisner, S.B., "Detecting Mad Cow Disease," Scientific American, July 2004, pp.86-93.

(42) http://www.cdc.gov/ncidod/dpd/professional/default.htm.

(43) http://www.dpd.cdc.gov/dpdx/.

(44) http://www.dpd.cdc.gov/dpdx/HTML/DiagnosticProcedures.htm.

(45) http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s7c.htm.