Designation: F3515 – 21

Standard Guide for Characterization and Testing of Porcine Fibrinogen as a Starting Material for Use in Biomedical and Tissue-Engineered Medical Product Applications[1]

This standard is issued under the fixed designation F3515; 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 covers the evaluation of porcine fibrinogen suitable for use in biomedical or pharmaceutical applications including, but not limited to, tissue-engineered medical products (TEMPs).

1.2 This guide addresses key parameters relevant for functionality, characterization, and purity of porcine fibrinogen.

1.3 As with any material, some characteristics of porcine fibrinogen may be altered by processing techniques, such as electrospinning (**1**)[2] and sterilization, required for the production of a specific formulation or device. Therefore, properties of fabricated forms of this protein should be evaluated using test methods that are appropriate to ensure safety and efficacy and are not addressed in this guide.

1.4 The primary focus of this document is fibrinogen derived from porcine blood, which is similar to human fibrinogen. The biggest advantage that pigs have over other species (such as cattle, sheep, goats, elk, and deer) is that they are less likely to transmit transmissible spongiform encephalitis (TSE) (ISO 22442-1 Annex D; WHO Guidelines, 2003; WHO Guidelines, 2006; WHO Guidelines, 2010). The document may also discuss fibrinogen from other sources when useful information is available. Fibrin is also discussed in some sections.

1.5 *Units*—The values stated in SI units are to be regarded as the standard. No other units of measurement are included in this standard.

1.6 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1298 Guide for Determination of Purity, Impurities, and Contaminants in Biological Drug Products (Withdrawn 2014)[4]

F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Insertion into Bone

F1983 Practice for Assessment of Selected Tissue Effects of Absorbable Biomaterials for Implant Applications

F2212 Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs)

F3163 Guide for Classification of Cellular and/or Tissue-Based Products (CTPs) for Skin Wounds

2.2 *ISO Standards:*[5]

ISO 10993-1 Biological evaluation of medical devices—Part 1: Evaluation and testing with a risk management process

ISO 11137-1:2006 Sterilization of health care products—Radiation—Part 1: Requirements for validation and routine control of a sterilization process for medical devices

ISO 11737-1:2006 Sterilization of health care products—Microbiological methods—Part 1: Determination of a population of microorganisms on products

ISO 11737-2:1998 Sterilization of health care products—

[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 F04.42 on Biomaterials and Biomolecules for TEMPs.

Current edition approved Aug. 1, 2021. Published August 2021. DOI: 10.1520/F3515-21.

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

[3] 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.

[4] The last approved version of this historical standard is referenced on www.astm.org.

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

Microbiological methods—Part 2: Tests of sterility performed in the definition, validation and maintenance of a sterilization process

ISO 13485 Medical devices—Quality management systems—Requirements for regulatory purposes

ISO 14160:2020 Sterilization of health care products—Liquid chemical sterilizing agents for single-use medical devices utilizing animal tissues and their derivatives—Requirements for characterization, development, validation and routine control of a sterilization process for medical devices

ISO 14644-1 Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness by particle concentration

ISO 14644-2 Cleanrooms and associated controlled environments—Part 2: Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration

ISO 14644-3 Cleanrooms and associated controlled environments—Part 3: Test methods

ISO 14644-4 Cleanrooms and associated controlled environments—Part 4: Design, construction and start-up

ISO 14644-5 Cleanrooms and associated controlled environments—Part 5: Operations

ISO 14644-7 Cleanrooms and associated controlled environments—Part 7: Separative devices (clean air hoods, gloveboxes, isolators and mini-environments)

ISO 14644-8 Cleanrooms and associated controlled environments—Part 8: Classification of air cleanliness by chemical concentration (ACC)

ISO 14644-9 Cleanrooms and associated controlled environments—Part 9: Cleaning of surfaces to achieve defined levels of cleanliness in terms of particle and chemical classifications

ISO 14644-10 Cleanrooms and associated controlled environments—Part 10: Classification of surface cleanliness by chemical concentration

ISO 14644-13 Cleanrooms and associated controlled environments—Part 13: Cleaning of surfaces to achieve defined levels of cleanliness in terms of particle and chemical classifications

ISO 14644-14 Cleanrooms and associated controlled environments—Part 14: Assessment of suitability for use of equipment by airborne particle concentration

ISO 14644-15 Cleanrooms and associated controlled environments—Part 15: Assessment of suitability for use of equipment and materials by airborne chemical concentration

ISO 14698-1 Cleanrooms and associated controlled environments—Biocontamination control—Part 1: General principles and methods

ISO 14698-2 Cleanrooms and associated controlled environments—Biocontamination control—Part 2: Evaluation and interpretation of biocontamination data

ISO 14971 Medical devices—Application of risk management to medical devices

ISO 22442-1 Medical devices utilizing animal tissues and their derivatives—Part 1: Application of risk management

ISO 22442-2 Medical devices utilizing animal tissues and their derivatives—Part 2: Controls on sourcing, collection and handling

ISO 22442-3 Medical devices utilizing animal tissues and their derivatives—Part 3: Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents

2.3 *Other Documents:*

AAMI TIR 19:1998 Guidance for ANSI/AAMI/ISO 10993-7:1995, Biological evaluation of medical devices—Part 7: Ethylene oxide sterilization residuals[6]

21 CFR 211 Current Good Manufacturing Practice for Finished Pharmaceuticals[7]

21 CFR 820 Quality System Regulation[7]

European Commission, OJ L 2011/C 73/01 Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3)[8]

European Pharmacopoeia 9.0, 2017 Fibrin Sealant Kit[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.2.3 Potentiometric Determination of pH[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.2.40 Near-Infrared Spectroscopy[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.4.8 Heavy Metals[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.5.9 Determination of Nitrogen by Sulphuric Acid Digestion[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.5.12 Water: Semi-micro Determination[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.5.32 Loss on Drying[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.6.1 Sterility[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.6.12 Total Viable Aerobic Count[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.6.13 Test for Specified Microorganisms[9]

European Pharmacopoeia 9.0, 2017, Chapter 2.6.14 Bacterial Endotoxins[9]

European Pharmacopoeia 10.0, Supplement 10.3, 2020, Chapter 2.6.32 Test for Bacterial Endotoxins Using Recombinant Factor C[9]

European Pharmacopoeia 9.0, 2017, Chapter 5.1.6 Alternative Methods for Control of Microbiological Quality[9]

FDA Guidance Document, 1991 Shelf Life of Medical Devices[10]

FDA Guidance Document, 2008 Guidance for Industry: Container and Closure System Integrity Testing in Lieu of

[6] Available from Association for the Advancement of Medical Instrumentation (AAMI), 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633, http://www.aami.org.

[7] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, http://www.access.gpo.gov.

[8] Available from the European Medicines Agency (EMA), Domenico Scarlattilaan 6 1083 HS Amsterdam, The Netherlands, https://www.ema.europa.eu.

[9] Available from the EDQM Council of Europe, 7 allée Kastner, CS 30026, F-67081 Strasbourg, France, https://pharmeuropa.edqm.eu.

[10] Available from U.S. Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993, http://www.fda.gov.

Sterility Testing as a Component of the Stability Protocol for Sterile Products[10]
FDA Guidance Document, 2012 Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers[10]
ICH Q1A(R2), 2003 Stability Testing of New Drug Substances and Products[8]
ICH Q7, 2000 Good Manufacturing Practice for Active Pharmaceutical Ingredients[8]
USP <61> Microbial Limit Tests[11]
USP <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms[11]
USP <71> Sterility Tests[11]
USP <85> Bacterial Endotoxins Test[11]
USP <231> Heavy Metals[11]
USP <731> Loss on Drying[11]
USP <791> pH[11]
USP <1211> Sterilization and Sterility Assurance of Compendial Articles[11]
World Health Organization, 2003 WHO Guidelines on Transmissible Spongiform Encephalopathies in Relation to Biological and Pharmaceutical Products[12]
World Health Organization, 2006 WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies[12]
World Health Organization, 2010 WHO Tables on Tissue Infectivity Distribution in Transmissible Spongiform 188 Encephalopathies[12]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *electrospinning, n*—a fiber manufacturing process to produce ultrafine fibres (fiber diameter range of nanometers to micrometers) by charging and ejecting a polymer melt or polymer solution through a spinneret under a high-voltage electric field and to solidify or coagulate it to form a filament (**2**).

3.1.2 *factor XIII, n*—a factor that polymerizes fibrin monomers so that they become stable and insoluble in urea, thus enabling fibrin to form a firm blood clot (also known as fibrin-stabilizing factor (FSF)).

3.1.3 *fibrin, n*—the insoluble protein formed from fibrinogen by the proteolytic action of thrombin during normal clotting of blood; it forms the essential portion of the blood clot.

3.1.4 *fibrinogen, n*—a plasma protein with an approximate molecular mass of 340 000 g/mol, composed of three subunits (α, β, γ) encoded by separate genes, which is converted to fibrin through the action of thrombin and is a key protein in the blood clotting cascade.

3.1.5 *soft tissue, n*—the tissues that connect, support, or surround other structures and organs of the body, not being hard tissue such as bone. Soft tissue includes tendons, ligaments, fascia, skin, fibrous tissues, fat, and synovial membranes (which are connective tissue), and muscles, nerves, and blood vessels (which are not connective tissue).

3.1.6 *tissue-inducing biomaterials, n*—lifeless biomaterials capable of inducing tissue regeneration in vivo without addition of growth factors or living cells (**3**).

3.1.7 *tissue regeneration, n*—a healing process in which lost or damaged tissue is placed by migration, differentiation, proliferation, and patterning of cells that deposit extracellular matrix with normal architecture, function, and topological appearance. **F3163**

4. Summary of Guide

4.1 Fibrinogen, a soluble and complex plasma glycoprotein in vertebrate blood, is a hexamer, containing two sets of three different chains (α, β, and γ), having a total molecular mass of 340 000 g/mol. The use of fibrinogen derived from porcine blood in surgery started in approximately 2000 when fibrin gel formed by mixing fibrinogen and thrombin could effectively stop bleeding during surgery. More recently, fibrinogen, as a raw material, has been blended with a degradable polymer and prepared into a micro- to nano-scale three-dimensional network structure by electrospinning (**4, 5**) for use in the regeneration of soft tissue as a tissue-inducing biomaterial (**4, 6**). The aim of this guide is to identify key parameters relevant for the functionality and characterization of fibrinogen during development of new products containing porcine fibrinogen for biomedical applications.

5. Significance and Use

5.1 The purpose of this guide is to provide guidance on characterization of the properties of porcine fibrinogen as a starting material for surgical implants and as a matrix for tissue-engineered medical products (TEMPs). This guide contains a set of physical and chemical parameters directly related to the function of porcine fibrinogen. This guide can be used to help select and characterize appropriate fibrinogen starting materials for specific purposes. Not all tests or parameters are suitable for all uses of fibrinogen.

5.2 Fibrinogen described in this guide may be used in various types of medical products including, but not limited to, implants, tissue-engineered medical products (TEMPs), and cell, drug, or DNA delivery vectors. The recommendations in this guide shall not be construed to guarantee the successful clinical application of any tissue-engineered medical product.

5.3 In determining whether fibrinogen meets the requirements for use in a TEMP, the relevant regulatory authorities or other appropriate guidelines relating to the production, regulation, and approval of TEMP products shall be taken into account (Guide E1298, Practice F981, Practice F1983).

6. Physical and Chemical Characterization Methods

6.1 *Characteristics of Fibrinogen:*

6.1.1 *Solubility (European Pharmacopoeia 9.0, Fibrin Sealant Kit)*—Fibrinogen should be dissolved at an appropriate temperature (often 37 °C) in an appropriate medium (such as an aqueous buffer solution) for an appropriate amount of time

[11] Available from U.S. Pharmacopeial Convention (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.
[12] Available from World Health Organization (WHO), Avenue Appia 20 1211 Geneva, Switzerland, https://www.who.int.

(often 20 min), and the clarity of the solution should be reported by visual assessment. Typically, soluble fibrinogen solutions are nearly colorless, clear, or slightly turbid.

6.1.2 *pH*—The pH of the solution following fibrinogen dissolution should be recorded and will often be between 6.5 to 8.0 (EP 9.0 Chapter 2.2.3; USP <791>).

6.1.3 *Water Content*—Water content of lyophilized fibrinogen preparations must be determined by a suitable method, such as semi-micro determination (EP 9.0 Chapter 2.5.12), loss on drying (EP 9.0 Chapter 2.5.32; USP <731>), or near-infrared spectrophotometry (EP 9.0 Chapter 2.2.40). Notice that the water content will change during storage, depending upon the storage condition.

6.1.4 *Microbial Limits*—The fibrinogen as a starting material should comply with compendial specification for microbial quality as assessed by an appropriate test (USP <61> and <62>; EP 9.0 Chapters 2.6.12 and 2.6.13).

6.1.5 *Immunological Precipitation Reactions*—The identity of fibrinogen should be confirmed with an anti-fibrinogen antibody using an immunological precipitation reaction (**7**). Only anti-pig serum or plasma should be used, not anti-horse, anti-cow, or anti-sheep serum or plasma.

6.1.6 *Clottable Protein Amount*—The amount of clottable protein in a fibrinogen preparation should be determined (EP 9.0, Fibrin Sealant Kit). Typically, the amount of clottable protein is between 70 % and 130 % of the value given on the product label. A common method is to mix 0.2 mL of the reconstituted preparation with 2 mL of a suitable buffer solution (pH 6.6 to 7.4) containing thrombin (approximately 3 IU⁄mL) and calcium (0.05 mol/L). Maintain at 37 °C for 20 min, separate the precipitate by centrifugation (5000 g for 20 min), wash thoroughly with a sodium chloride solution (9 g⁄L), and determine protein by the nitrogen method following sulphuric acid digestion (EP 9.0 Chapter 2.5.9). Calculate the protein content by multiplying the result by 6.0. If this method cannot be applied for a particular preparation, use another validated method for determination of fibrinogen.

6.1.7 *Factor XIII*—If the label indicates that coagulation Factor XIII activity is greater than 10 units/mL, the activity is estimated to be no less than 80 % and no more than 120 % of the activity indicated on the label (EP 9.0, Fibrin Sealant Kit). A common approach for measuring Factor XIII activity is to make at least three suitable dilutions of thawed or reconstituted fibrinogen and of porcine normal plasma (reference preparation) using as diluent coagulation Factor XIII-deficient plasma or another suitable diluent. Add to each dilution suitable amounts of the following reagents:

6.1.7.1 Activator reagent, containing bovine or porcine thrombin, a suitable buffer, calcium chloride, and a suitable inhibitor such as Gly-Pro-Arg-Pro-Ala-NH_2, which inhibits clotting of the sample but does not prevent coagulation Factor XIII activation by thrombin;

6.1.7.2 Detection reagent, containing a suitable Factor XIII-specific peptide substrate, such as Leu-Gly-Pro-Gly-Glu-Ser-Lys-Val-Ile-Gly-NH_2, and glycine ethyl ester as a second substrate in a suitable buffer solution; and

6.1.7.3 Nicotinamide adenine dinucleotide hydrogen (NADH) reagent, containing glutamate dehydrogenase, α-ketoglutarate, and NADH in a suitable buffer solution.

6.1.8 After mixing, the absorbance changes (Δ A/min) are measured at a wavelength of 340 nm, after the linear phase of the reaction is reached. One unit of Factor XIII is equal to the activity of 1 mL of porcine normal plasma. Calculate the activity of the test preparation by appropriate statistical methods. Typical acceptance criteria for a test are when the 95 % confidence limits (P = 0.95) are between 80 % and 125 % of the indicated activity.

6.2 *Physical and Chemical Characterization of Fibrinogen:*

6.2.1 *Appearance*—Freeze-dried fibrinogen is a hygroscopic, white or pale yellow powder or a friable solid. Frozen fibrinogen is a colorless or pale yellow, opaque solid. Fibrinogen in aqueous solution is colorless or pale yellow. For the freeze-dried or frozen fibrinogen, reconstitute or thaw as stated on the label immediately before carrying out measurements, except for the solubility test or water content test.

6.2.2 *Short-Term Stability Test*—No gel formation appears at room temperature for 120 min following thawing or reconstitution.

6.3 *Impurity Profile:*

6.3.1 *Endotoxin*—Endotoxin contamination is difficult to prevent because it is ubiquitous in nature, stable, and small enough to pass through sterilizing filters. There are several tests to determine the presence of endotoxin in the samples. The test for bacterial endotoxins is used to detect or quantify endotoxins of gram-negative bacterial origin using amoebocyte lysate from horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). There are three techniques for this test: (*1*) the gel-clot technique, which is based on gel formation; (*2*) the turbidimetric technique, based on the development of turbidity after cleavage of an endogenous substrate; and (*3*) the chromogenic technique, based on the development of color after cleavage of a synthetic peptide-chromogen complex. In the event of doubt or dispute, the final decision is made based upon the gel-clot method unless otherwise indicated in the monograph. The test is carried out in a manner that avoids endotoxin contamination (EP 9.0 Chapter 2.6.14; USP <85>). A test for bacterial endotoxins using recombinant factor C (rFC) can be used in the same way as LAL-based methods, after demonstration of its fitness for use for the specific substance or product. The use of rFC for BET testing does not need to be validated, thus facilitating the implementation of rFC-based methods by users (EP 10.0 Supplement 10.3, Chapter 2.6.32).

6.3.2 *Heavy Metal Content*—The presence of heavy metal impurities should be assessed and their levels should not exceed the limits given in the product specification (EP 9.0 Chapter 2.4.8; USP <231>). Substances that typically respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum. A control solution to calibrate results should be assessed. The control solutions may be standard lead solutions of known concentrations and they may be analyzed and visually inspected alongside the test samples. Additional heavy metal contaminants may be introduced during handling and processing. If necessary, the user