(mildly reactive). Repeat the procedure if the suitability of the system is not confirmed. For dose-response evaluations, repeat the procedure, using quantitative dilutions of the sample extract.

Table 2. Reactivity Grades for Elution Test

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

⟨88⟩ BIOLOGICAL REACTIVITY TESTS, IN VIVO

The following tests are designed to determine the biological response of animals to elastomerics, plastics, and other polymeric material with direct or indirect patient contact, or by the injection of specific extracts prepared from the material under test. It is essential to make available the specific surface area for extraction. When the surface area of the specimen cannot be determined, use 0.1 g of elastomer or 0.2 g of plastic or other material for every mL of extraction fluid. Also, it is essential to exercise care in the preparation of the materials to be injected or instilled to prevent contamination with microorganisms and other foreign matter. Three tests are described. The *Systemic Injection Test* and the *Intracutaneous Test* are used for elastomeric materials, especially to elastomeric closures for which the appropriate *Biological Reactivity Tests, In Vitro* ⟨87⟩ have indicated significant biological reactivity. These two tests are used for plastics and other polymers in addition to a third test, the *Implantation Test,* to test the suitability of these materials intended for use in fabricating containers and accessories thereto, for use in parenteral preparations, and for use in medical devices, implants, and other systems.

These three tests are applied to materials or medical devices, if there is a need for classification of plastics and other polymers based on in vivo biological reactivity testing.

For the purpose of this chapter, these definitions apply: the *Sample* is the specimen under test or an extract prepared from such a specimen. A *Blank* consists of the same quantity of the same extracting medium that is used for the extraction of the specimen under test, treated in the same manner as the extracting medium containing the specimen under test. A *Negative Control*[1] is a specimen that gives no reaction under the conditions of the test.

Change to read:

CLASSIFICATION OF PLASTICS

Six Plastic Classes are defined (see *Table 1*). This classification is based on responses to a series of in vivo tests for which extracts, materials, and routes of administration are specified. These tests are directly related to the intended end-use of the plastic articles. The choice of extractants is representative of the vehicles in preparations with which the plastics are likely to be in contact. The *Table 1* classification facilitates communication among suppliers, users, and manufacturers of plastics by summarizing the tests to be performed for containers for injections and medical devices if a need for classification exists.

With the exception of the *Implantation Test*, the procedures are based on the use of extracts that, depending on the heat resistance of the material, are prepared at one of three standard temperatures: 50°, 70°, and 121°. Therefore, the class designation of a plastic must be accompanied by an indication of the temperature of extraction (e.g., IV-121°, which represents a class IV plastic extracted at 121°, or I-50°, which represents a class I plastic extracted at 50°).

Plastics may be classified as USP Plastic Classes I–VI only on the basis of the response criteria prescribed in *Table 1*.

[1] USP High-Density Polyethylene RS.

Table 1. Classification of Plastics

Plastic Classes[a]						Tests to be Conducted			
I	II	III	IV	V	VI	Test Material	Animal	Dose	Procedure[b]
x	x	x	x	x	x	Extract of *Sample* in *Sodium Chloride Injection*	Mouse	50 mL/kg	A (iv)
x	x	x	x	x	x		Rabbit	0.2 mL/animal at each of 10 sites	B
	x	x	x	x	x	Extract of *Sample* in *1 in 20 Solution of Alcohol in Sodium Chloride Injection*	Mouse	50 mL/kg	A (iv)
	x	x	x	x	x		Rabbit	0.2 mL/animal at each of 10 sites	B
		x		x	x	Extract of *Sample* in *Polyethylene Glycol 400*	Mouse	10 g/kg	A (ip)
				x	x		Rabbit	0.2 mL/animal at each of 10 sites	B
		x	x	x	x	Extract of *Sample* in *Vegetable Oil*	Mouse	50 mL/kg	A (ip)
			x	x	x		Rabbit	0.2 mL/animal at each of 10 sites	B
			x		x	Implant strips of *Sample*	Rabbit	4 strips/animal	C
			▲x		x	Implant *Sample*	Rat	2 *Samples*/animal	C▲*USP35*

[a] Tests required for each class are indicated by "x" in appropriate columns.

[b] Legend: A (ip)—Systemic Injection Test (intraperitoneal); A (iv)—Systemic Injection Test (intravenous); B—Intracutaneous Test (intracutaneous); C—Implantation Test (intramuscular ▲or subcutaneous▲*USP35* implantation).

This classification does not apply to plastics that are intended for use as containers for oral or topical products, or that may be used as an integral part of a drug formulation. *Table 1* does not apply to natural elastomers, which are to be tested in Sodium Chloride Injection and vegetable oils only.

The *Systemic Injection Test* and the *Intracutaneous Test* are designed to determine the systemic and local, respectively, biological responses of animals to plastics and other polymers by the single-dose injection of specific extracts prepared from a *Sample*. The *Implantation Test* is designed to evaluate the reaction of living tissue to the plastic and other polymers by the implantation of the *Sample* itself into animal tissue. The proper preparation and placement of the specimens under aseptic conditions are important in the conduct of the *Implantation Test*.

These tests are designed for application to plastics and other polymers in the condition in which they are used. If the material is to be exposed to any cleansing or sterilization process prior to its end-use, then the tests are to be conducted on a *Sample* prepared from a specimen preconditioned by the same processing.

Factors such as material composition, processing and cleaning procedures, contacting media, inks, adhesives, absorption, adsorption and permeability of preservatives, and conditions of storage may also affect the suitability of a material for a specific use. Evaluation of such factors should be made by appropriate additional specific tests to determine the suitability of a material for its intended use.

USP Reference Standards ⟨11⟩—USP High-Density Polyethylene RS.

Extracting Media—

SODIUM CHLORIDE INJECTION (see monograph). Use Sodium Chloride Injection containing 0.9% of NaCl.

1 IN 20 SOLUTION OF ALCOHOL IN SODIUM CHLORIDE INJECTION.

POLYETHYLENE GLYCOL 400 (see monograph).

VEGETABLE OIL— Use freshly refined Sesame Oil (see monograph) or Cottonseed Oil (see monograph) or other suitable vegetable oils.

DRUG PRODUCT VEHICLE (where applicable).

WATER FOR INJECTION (see monograph).

NOTE—The Sesame Oil or Cottonseed Oil or other suitable vegetable oil meets the following additional requirements. Obtain, if possible, freshly refined oil. Use three properly prepared animals, and inject the oil intracutaneously in a dose of 0.2 mL into each of 10 sites per animal, and observe the animals at 24, 48, and 72 hours following injection. Rate the observations at each site on the numerical scale indicated in *Table 2*. For the 3 rabbits (30 injection sites), at any observation time, the average response for erythema is not greater than 0.5 and for edema is not greater than 1.0, and no site shows a tissue reaction larger than 10 mm in overall diameter. The residue of oil at the injection site should not be misinterpreted as edema. Edematous tissue blanches when gentle pressure is applied.

Table 2. Evaluation of Skin Reactions

Erythema and Eschar Formation	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4
Edema Formation[a]	**Score**
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

[a] Excludes noninflammatory (mechanical) edema from the blank or extraction fluid.

Apparatus—The apparatus for the tests includes the following.

AUTOCLAVE—Use an autoclave capable of maintaining a temperature of 121 ± 2.0°, equipped with a thermometer, a pressure gauge, a vent cock, a rack adequate to accommodate the test containers above the water level, and a water cooling system that will allow for cooling of the test containers to about, but not below, 20° immediately following the heating cycle.

OVEN—Use an oven, preferably a forced-circulation model, that will maintain operating temperatures of 50° or 70° within ±2°.

EXTRACTION CONTAINERS—Use only containers, such as ampuls or screw-cap culture test tubes, of Type I glass. If used, culture test tubes are closed with screw caps having suitable elastomeric liners. The exposed surface of the elastomeric liner is completely protected with an inert solid disk 0.05 mm to 0.075 mm in thickness. A suitable disk may be fabricated from a polytef resin.

Preparation of Apparatus—Cleanse all glassware thoroughly with chromic acid cleansing mixture, or if necessary with hot nitric acid, followed by prolonged rinsing with water. Clean cutting utensils by an appropriate method (e.g., successive cleaning with acetone and methylene chloride) prior to use in subdividing a specimen. Clean all other equipment by thorough scrubbing with a suitable detergent and prolonged rinsing with water.

Render containers and equipment used for extraction, and in transfer and administration of test material, sterile and dry by a suitable process. [NOTE—If ethylene oxide is used as the sterilizing agent, allow adequate time for complete degassing.]

Procedure—

PREPARATION OF SAMPLE—Both the *Systemic Injection Test* and the *Intracutaneous Test* may be performed using the same extract, if desired, or separate extracts may be made for each test. Select and subdivide into portions a *Sample* of the size indicated in *Table 3*. Remove particulate matter, such as lint and free particles, by treating each subdivided *Sample* or *Negative Control* as follows: place the *Sample* into a clean, glass-stoppered, 100-mL graduated cylinder of Type I glass, and add about 70 mL of *Water for Injection*. Agitate for about 30 seconds, and drain off the water. Repeat this step, and dry those pieces prepared for the extraction with *Vegetable Oil* in an oven at a temperature not exceeding 50°. [NOTE—Do not clean the *Sample* with a dry or wet cloth or by rinsing or washing with an organic solvent, surfactant, etc.]

Table 3. Surface Area of Specimen To Be Used[a]

Form of Material	Thickness	Amount of Sample for each 20 mL of Extracting Medium	Subdivided into
Film or sheet	<0.5 mm	Equivalent of 120 cm^2 total surface area (both sides combined)	Strips of about 5 × 0.3 cm
	0.5–1 mm	Equivalent of 60 cm^2 total surface area (both sides combined)	
Tubing	<0.5 mm (wall)	Length (in cm) = 120 cm^2/(sum of ID and OD circumferences)	Sections of about 5 × 0.3 cm
	0.5–1 mm (wall)	Length (in cm) = 60 cm^2/(sum of ID and OD circumferences)	
Slabs, tubing, and molded items	>1 mm	Equivalent of 60 cm^2 total surface area (all exposed surfaces combined)	Pieces up to about 5 × 0.3 cm
Elastomers	>1 mm	Equivalent of 25 cm^2 total surface area (all exposed surfaces combined)	Do not subdivide[b]

[a] When surface area cannot be determined due to the configuration of the specimen, use 0.1 g of elastomer or 0.2 g of plastic or other polymers for every 1 mL of extracting fluid.
[b] Molded elastomeric closures are tested intact.

PREPARATION OF EXTRACTS—Place a properly prepared *Sample* to be tested in an extraction container, and add 20 mL of the appropriate extracting medium. Repeat these directions for each extracting medium required for testing. Also, prepare one 20-mL blank of each medium for parallel injections and comparisons. Extract by heating in an autoclave at 121° for 60 minutes, in an oven at 70° for 24 hours, or at 50° for 72 hours. Allow adequate time for the liquid within the container to reach the extraction temperature. [NOTE—The extraction conditions should not in any instance cause physical changes such as fusion or melting of the *Sample* pieces, which result in a decrease in the available surface area. A slight adherence of the pieces can be tolerated. Always add the cleaned pieces individually to the extracting medium. If culture tubes are used for autoclave extractions with *Vegetable Oil*, seal screw caps adequately with pressure-sensitive tape.]

Cool to about room temperature but not below 20°, shake vigorously for several minutes, and decant each extract immediately, using aseptic precautions, into a dry, sterile vessel. Store the extracts at a temperature between 20° and 30°, and do not use for tests after 24 hours. Of importance are the contact of the extracting medium with the available surface area of the plastic and the time and temperature during extraction, the proper cooling, agitation, and decanting process, and the aseptic handling and storage of the extracts following extraction.

SYSTEMIC INJECTION TEST

This test is designed to evaluate systemic responses to the extracts of materials under test following injection into mice.

Test Animals—Use healthy, not previously used albino mice weighing between 17 and 23 g. For each test group use only mice of the same source. Allow water and food, commonly used for laboratory animals and of known composition, ad libitum.

Procedure—[NOTE—Agitate each extract vigorously prior to withdrawal of injection doses to ensure even distribution of the extracted matter. However, visible particulates should not be injected intravenously.] Inject each of the five mice in a test group with the *Sample* or the *Blank* as outlined in *Table 4*, except to dilute each g of the extract of the *Sample* prepared with *Polyethylene Glycol 400*, and the corresponding *Blank*, with 4.1 volumes of *Sodium Chloride Injection* to obtain a solution having a concentration of about 200 mg of polyethylene glycol per mL.

Table 4. Injection Procedure—Systemic Injection Test

Extract or Blank	Dose per kg	Route[a]	Injection Rate (μL per second)
Sodium Chloride Injection	50 mL	IV	100
1 in 20 solution of Alcohol in Sodium Chloride Injection	50 mL	IV	100
Polyethylene Glycol 400	10 g	IP	—
Drug product vehicle (where applicable)	50 mL	IV	100
	50 mL	IP	—
Vegetable Oil	50 mL	IP	—

[a] IV = intravenous (aqueous sample and blank); IP = intraperitoneal (oleaginous sample and blank).

Observe the animals immediately after injection, again 4 hours after injection, and then at least at 24, 48, and 72 hours. If during the observation period none of the animals treated with the extract of the *Sample* shows a significantly greater biological reactivity than the animals treated with the *Blank*, the *Sample* meets the requirements of this test. If two or more mice die, or if abnormal behavior such as convulsions or prostration occurs in two or more mice, or if a body weight loss greater than 2 g occurs in three or more mice, the *Sample* does not meet the requirements of the test. If any animals treated with the *Sample* show only slight signs of biological reactivity, and not more than one animal shows gross symptoms of biological reactivity or dies, repeat the test using groups of 10 mice. On the repeat test, all 10 animals treated with the *Sample* show no significant biological reactivity above the *Blank* animals during the observation period.

INTRACUTANEOUS TEST

This test is designed to evaluate local responses to the extracts of materials under test following intracutaneous injection into rabbits.

Test Animals—Select healthy, thin-skinned albino rabbits with fur that can be clipped closely and skin that is free from mechanical irritation or trauma. In handling the animals, avoid touching the injection sites during observation periods, except to discriminate between edema and an oil residue. [NOTE—Rabbits previously used in unrelated tests, such as the *Pyrogen Test* ⟨151⟩, and that have received the prescribed rest period, may be used for this test provided that they have clean, unblemished skin.]

Procedure—[NOTE—Agitate each extract vigorously prior to withdrawal of injection doses to ensure even distribution of the extracted matter.] On the day of the test, closely clip the fur on the animal's back on both sides of the spinal column over a sufficiently large test area. Avoid mechanical irritation and trauma. Remove loose hair by means of vacuum. If necessary, swab the skin lightly with diluted alcohol, and dry the skin prior to injection. More than one extract from a given material can be used per rabbit, if it is determined that the test results will not be affected. For each *Sample* use two animals and inject each intracutaneously, using one side of the animal for the *Sample* and the other side for the *Blank*, as outlined in *Table 5*. [NOTE—Dilute each g of the extract of the *Sample* prepared with *Polyethylene Glycol 400*, and the corresponding *Blank*, with 7.4 volumes of *Sodium Chloride Injection* to obtain a solution having a concentration of about 120 mg of polyethylene glycol per mL.]

Table 5. Intracutaneous Test

Extract or Blank	Number of Sites (per animal)	Dose (µL per site)
Sample	5	200
Blank	5	200

Examine injection sites for evidence of any tissue reaction such as erythema, edema, and necrosis. Swab the skin lightly, if necessary, with diluted alcohol to facilitate reading of injection sites. Observe all animals at 24, 48, and 72 hours after injection. Rate the observations on a numerical scale for the extract of the *Sample* and for the *Blank*, using *Table 2*. Reclip the fur as necessary during the observation period. The average erythema and edema scores for *Sample* and *Blank* sites are determined at every scoring interval (24, 48, and 72 hours) for each rabbit. After the 72 hour scoring, all erythema scores plus edema scores are totalled separately for each *Sample* and *Blank*. Divide each of the totals by 12 (2 animals × 3 scoring periods × 2 scoring categories) to determine the overall mean score for each *Sample* versus each corresponding *Blank*. The requirements of the test are met if the difference between the *Sample* and the *Blank* mean score is 1.0 or less. If at any observation period the average reaction to the *Sample* is questionably greater than the average reaction to the *Blank*, repeat the test using three additional rabbits. The requirements of the test are met if the difference between the *Sample* and the *Blank* mean score is 1.0 or less.

Change to read:

IMPLANTATION TEST

The implantation test is designed for the evaluation of plastic materials and other polymeric materials in direct contact with living tissue. Of importance are the proper preparation of the implant strips and their proper implantation under aseptic conditions. ▲The intramuscular implantation test requires healthy adult rabbits. The test specimens are placed into needles as the delivery system for implantation. Although most materials lend themselves readily to this method, there are a number of materials that are unsuitable for intramuscular implantation. For materials with physical characteristics unsuitable for routine intramuscular implantation, the subcutaneous rat implantation model is a viable alternative.

Intramuscular Implantation in Rabbits

Prepare for implantation 8 strips of the *Sample* and 4 strips of USP High-Density Polyethylene RS. Each strip should measure not less than 10 × 1 mm. The edges of the strips should be as smooth as possible to avoid additional mechanical trauma upon implantation. Strips of the specified minimum size are implanted by means of a hypodermic needle (15- to 19-gauge) with intravenous point and a sterile trocar. Use either presterilized needles into which the sterile plastic strips are aseptically inserted, or insert each clean strip into a needle, the cannula and hub of which are protected with an appropriate cover, and then subjected to the appropriate sterilization procedure. [NOTE—Allow for proper degassing if agents such as ethylene oxide are used.]

Test Animals—▲*USP35*Select healthy, adult rabbits weighing not less than 2.5 kg, and with paravertebral muscles that are sufficiently large in size to allow for implantation of the test strips. Do not use any muscular tissue other than the paravertebral site. The animals must be anesthetized with a commonly used anesthetic agent to a degree deep enough to prevent muscular movements, such as twitching.

Procedure—Perform the test in a clean area. On the day of the test or up to 20 hours before testing, clip the fur of the animals on both sides of the spinal column. Remove loose hair by means of vacuum. Swab the skin lightly with diluted alcohol, and dry the skin prior to injection.

Implant four strips of the *Sample* into the paravertebral muscle on one side of the spine of each of two rabbits, 2.5–5 cm from the midline and parallel to the spinal column, and about 2.5 cm apart from each other. In a similar fashion implant two strips of USP High-Density Polyethylene RS in the opposite muscle of each animal. Insert a sterile stylet into the needle to hold the implant strip in the tissue while withdrawing the needle. If excessive bleeding is observed after implantation of a strip, place a duplicate strip at another site.

Keep the animals for a period of not less than 120 hours, and sacrifice them at the end of the observation period by administering an overdose of an anesthetic agent or other suitable agents. Allow sufficient time to elapse for the tissue to be cut without bleeding. Examine macroscopically the area of the tissue surrounding the center portion of each implant strip. Use a magnifying lens and auxiliary light source. Observe the *Sample* and *Control* implant sites for hemorrhage, necrosis, discolorations, and infections, and record the observations. Measure encapsulation, if present, by recording the width of the capsule (from the periphery of the space occupied by the implant *Control* or *Sample* to the periphery of the capsule) rounded to the nearest 0.1 mm. Score encapsulation according to *Table 6*.

Table 6. Evaluation of Encapsulation in the Implantation Test

Capsule Width	Score
None	0
Up to 0.5 mm	1
0.6–1.0 mm	2
1.1–2.0 mm	3
Greater than 2.0 mm	4

Calculate the differences between average scores for the *Sample* and *Control* sites. The requirements of the test are met if the difference does not exceed 1.0, or if the difference between the *Sample* and *Control* mean scores for more than one of the four implant sites does not exceed 1 for any implanted animal.

▲Subcutaneous Implantation in Rats

Prepare for implantation 10 sample specimens and 10 control specimens. The size and shape of the control specimens shall be as similar to that of the test specimens as practically possible. For example, specimens made of sheeting material shall be 10–12 mm in diameter and from 0.3 to 1 mm in thickness. The edges of the specimens should

be as smooth as possible to avoid additional mechanical trauma upon implantation.

Test Animals—Select healthy rats weighing between 225 and 350 g at the time of implantation.

Procedure—Perform the test in a clean area. Anesthetize the animal until a surgical plane is achieved. Clip the fur of the animals on both sides of the spinal column. Remove loose hair by means of vacuum. Clean the clipped area with povidone–iodine solution. Using aseptic technique, make two midline incisions (approximately 1.0 cm long) through the skin at the cranial and caudal regions on the dorsal surface. Using blunt dissection, separate the fascia connecting skin to muscle to form a pocket underneath the skin lateral to each side of the incision (base of pocket approximately 20 mm from the line of implant). Insert a sterile sample into each pocket, and close the incision with wound clips or sutures. Implant two test samples and two control samples in each of five rats. Keep the animals for a period of at least seven days, and sacrifice them at the end of the observation period by CO_2 induced hypoxia or administering an overdose of an anesthetic agent. Allow sufficient time to elapse for the tissue to be cut without bleeding. Cut the skin (dorsal surface) longitudinally and lay back. Carefully examine macroscopically the area of the tissue surrounding the implant. Cut the sample in half and remove for close examination of the tissue in direct contact with the sample. Use a magnifying lens and auxiliary light source, if appropriate. Observe the *Sample* and *Control* implant sites for hemorrhage, necrosis, discolorations, and infections, and record the observations. Measure encapsulation, if present, by recording the width of the capsule (from the periphery of the space occupied by the implant *Control* or *Sample* to the periphery of the capsule) rounded to the nearest 0.1 mm. Score encapsulation according to *Table 6*. Calculate the differences between average scores for the *Sample* and *Control* sites. The requirements of the test are met if the difference does not exceed 1.0.▲*USP35*

SAFETY TESTS—BIOLOGICALS

The safety test set forth here is intended to detect in an article any unexpected, unacceptable biological reactivity. This in vivo test is provided for the safety assessment of biologics (see *Biologics* ⟨1041⟩) and biotechnology-derived products.

Safety Test

Select five healthy mice not previously used for testing, weighing between 17 and 23 g, unless otherwise directed in the individual monograph or elsewhere in this chapter, and maintained on an adequate balanced diet. Prepare a test solution as directed in the individual monograph. Unless otherwise directed in the individual monograph or elsewhere in this chapter, inject intravenously a dose of 0.5 mL of the test solution into each of the mice, using a 26-gauge needle of suitable length, or of the length specified below as applicable. Observe the animals over the 48 hours following the injection. If, at the end of 48 hours, all of the animals survive and not more than one of the animals shows outward symptoms of a reaction not normally expected of the level of toxicity related to the article, the requirements of this test are met. If one or more animals die or if more than one of the animals shows signs of abnormal or untoward toxicity of the article under test, repeat the test using at least another 10 mice similar to those used in the initial test, but weighing 20 ± 1 g. In either case, if all of the animals survive for 48 hours and show no symptoms of a reaction indicative of an abnormal or undue level of toxicity of the article, the requirements of the test are met.

For biologics, perform the test according to the procedures prescribed in the *Federal Regulations* (see *Biologics* ⟨1041⟩), Section 610.11, using not less than two mice similar to those described above but weighing less than 22 g and not less than two healthy guinea pigs weighing less than 400 g. Unless otherwise directed in the individual monograph, for a liquid product or a freeze-dried product that has been constituted as directed in the labeling, inject a volume of 0.5 mL intraperitoneally into each mouse, and inject a volume of 5.0 mL intraperitoneally into each guinea pig. For freeze-dried products for which the volume of constitution is not indicated in the label, or for nonliquid products other than freeze-dried products, perform the test using the route of administration, test dose, and diluent approved by the Center for Biologics Evaluation and Research (FDA), on the basis of substantial evidence demonstrating that the test variation will assure sensitivity equal to or greater than that of the test described above. Observe the animals for a minimum observation period of 7 days. If all of the animals survive the test period, do not exhibit any response that is not specific for or expected from the product and that may indicate a difference in such product quality, and weigh no less at the end of the test period than at the time of injection, the requirements of the test are met. If the article fails to meet the requirements, the test may be repeated as in the initial test, in the one or both species in which the requirements were not met. If the animals fulfill the criteria specified for the initial test, the article meets the requirements of the test. If the article fails to meet the requirements after the first repeat test, and not less than 50% of the total number of animals of the species in which the requirements of the test were not met in the combined initial and first retests have survived, a second retest may be performed. Use twice the number of animals of the relevant species used in the initial test. If the animals fulfill the criteria specified for the initial test, the requirements of the test are met.

⟨90⟩ FETAL BOVINE SERUM—QUALITY ATTRIBUTES AND FUNCTIONALITY TESTS

PROCESSING

Fetal bovine serum (FBS) is the light-brown liquid fraction of clotted fetal bovine blood. It is depleted of cells, fibrin, and clotting factors. Although the complete composition of FBS is undefined, FBS contains high levels of growth factors and low levels of immunoglobulins. In addition, it contains other key ingredients that are essential in supporting proliferation of cells in culture. This product is used both in life science basic research and industrial manufacturing. FBS is a by-product of the meat industry and is collected from bovine fetuses removed from cattle found to be pregnant at slaughter. FBS is harvested from abattoirs that are inspected by the competent authority in the country of origin. Trained personnel following written and approved procedures should perform collection and processing. Blood is collected in a closed system in a dedicated area within the facility, and processed quickly to prevent hemolysis. The blood is allowed to clot and then typically is centrifuged in a refrigerated centrifuge to separate the serum from the other components. Serum typically is removed from the clot, transferred to labeled containers, and frozen. All manufacturers employ sterile filtration before final packaging. Additionally, gamma irradiation provides the highest assurance of the absence of viral activity. Gamma irradiation doses of