

impurities is not more than 5%; and the *Test solution* shows a major peak at approximately 37 minutes.

<141> PROTEIN—BIOLOGICAL ADEQUACY TEST

This test is intended for the evaluation of the biological adequacy, as an index to the completeness of the mixture of amino acids contained, of Protein Hydrolysate Injection.

Depletion Diet—

	Parts by Weight
Dextrin	83.9
Corn Oil	9.0
Salt Mixture	4.0
Agar	2.0
Cod Liver Oil	1.0
Choline Chloride	0.15
Inositol	0.10
Calcium Pantothenate	0.002
Niacinamide	0.0015
Riboflavin	0.0003
Pyridoxine	0.00025
Thiamine	0.0002
p-Aminobenzoic Acid	0.0002
Folic Acid	0.0002
Menadione	0.0002
Biotin	0.00002

Salt Mixture—Prepare the salt mixture specified in the *Depletion Diet* as follows:

Sodium Chloride	139.3 g
Potassium Biphosphate	389.0 g
Magnesium Sulfate, Anhydrous	57.3 g
Calcium Carbonate	381.4 g
Ferrous Sulfate	27.0 g
Manganese Sulfate	4.01 g
Potassium Iodide	0.79 g
Zinc Sulfate	0.548 g
Cupric Sulfate	0.477 g
Cobaltous Chloride	0.023 g

Place a portion of the weighed quantity of sodium chloride in a suitable mortar and add, with grinding, the potassium iodide. Set aside the mixture, and mix in a similar manner all the other salts with the remainder of the sodium chloride, adding finally the previously mixed sodium chloride and potassium iodide. Reduce the entire mixture to a fine powder (see *Powder Fineness* <811>).

Control Nitrogen Supplement Mixture—Place 50 g of calcium caseinate and 46 g of anhydrous dextrose in a beaker, add sufficient water to make a paste, and finally add 1000 mL of water. Heat the solution between 70° and 82° for 5 minutes with stirring, and cool. Determine nitrogen on an aliquot using *Nitrogen Determination—Method I* or *Method II* <461>. Store in a refrigerator. Mix before removing portions for analysis or use.

Depletion and Control Periods—Select a group of not less than six male rats 2 to 4 months of age and each

weighing between 190 g and 225 g. Place the rats in individual cages with free access to water and the *Depletion Diet* for 12 days. Weigh the depleted rats, and discard any rat that weighs more than 90% of its starting weight.

For the next 3 days substitute as drinking water the *Control Nitrogen Supplement Mixture* in a quantity equivalent to 0.12 g of nitrogen per rat per day, diluted with water to 20 mL, and offered at the same time each morning either in a dish suitable for preventing spillage or in a reservoir fitted with a drinking tube. Remove all drinking water from the cages of the depleted rats during each feeding, and return it after the supplement has been consumed or is removed. On the third day, weigh each rat. Discard any rats that have not consumed all of the *Control Nitrogen Supplement Mixture*.

For the next 3 days, replace the *Control Nitrogen Supplement Mixture* with water ad libitum, and continue the rats on the *Depletion Diet*. Weigh the rats, and discard any that have not lost weight since the previous weighing.

Procedure—Assemble not less than six rats that have completed the depletion and control periods. For 5 days maintain the assembled rats on the *Depletion Diet* with a daily supplement of 20 mL, accurately measured, of a solution containing the Protein Hydrolysate Injection in an amount equivalent to 0.12 g of nitrogen offered each morning in the same way as the *Control Nitrogen Supplement Mixture* was offered previously. Withhold water for at least 2 hours prior to offering the supplement and for 4 hours afterward. Then if the supplement has been consumed, offer water ad libitum.

On the afternoon of the fifth day, weigh each rat, and compare the respective final and starting weights. Not fewer than 80% of the group of rats used gain weight or maintain their weight during the test.

<151> PYROGEN TEST

The pyrogen test is designed to limit to an acceptable level the risks of febrile reaction in the patient to the administration, by injection, of the product concerned. The test involves measuring the rise in temperature of rabbits following the intravenous injection of a test solution and is designed for products that can be tolerated by the test rabbit in a dose not to exceed 10 mL per kg injected intravenously within a period of not more than 10 minutes. For products that require preliminary preparation or are subject to special conditions of administration, follow the additional directions given in the individual monograph or, in the case of antibiotics or biologics, the additional directions given in the federal regulations (see *Biologics* <1041>).

APPARATUS AND DILUENTS

Render the syringes, needles, and glassware free from pyrogens by heating at 250° for not less than 30 minutes or by any other suitable method. Treat all diluents and solutions for washing and rinsing of devices or parenteral injection assemblies in a manner that will assure that they are sterile and pyrogen-free. Periodically perform control pyrogen tests on representative portions of the diluents and solutions for washing or rinsing of the apparatus. Where Sodium Chloride Injection is specified as a diluent, use Injection containing 0.9 percent of NaCl.

TEMPERATURE RECORDING

Use an accurate temperature-sensing device such as a clinical thermometer, or thermistor probes or similar probes that have been calibrated to assure an accuracy of $\pm 0.1^\circ$ and have been tested to determine that a maximum reading is reached in less than 5 minutes. Insert the temperature-sensing probe into the rectum of the test rabbit to a depth of not less than 7.5 cm, and, after a period of time not less than that previously determined as sufficient, record the rabbit's body temperature.

TEST ANIMALS

Use healthy, mature rabbits. House the rabbits individually in an area of uniform temperature between 20° and 23° and free from disturbances likely to excite them. The temperature varies not more than $\pm 3^\circ$ from the selected temperature. Before using a rabbit for the first time in a pyrogen test, condition it not more than seven days before use by a sham test that includes all of the steps as directed for *Procedure* except injection. Do not use a rabbit for pyrogen testing more frequently than once every 48 hours, nor prior to 2 weeks following a maximum rise of its temperature of 0.6° or more while being subjected to the pyrogen test, or following its having been given a test specimen that was adjudged pyrogenic.

PROCEDURE

Perform the test in a separate area designated solely for pyrogen testing and under environmental conditions similar to those under which the animals are housed and free from disturbances likely to excite them. Withhold all food from the rabbits used during the period of the test. Access to water is allowed at all times, but may be restricted during the test. If rectal temperature-measuring probes remain inserted throughout the testing period, restrain the rabbits with light-fitting neck stocks that allow the rabbits to assume a natural resting posture. Not more than 30 minutes prior to the injection of the test dose, determine the "control temperature" of each rabbit: this is the base for the determination of any temperature increase resulting from the injection of a test solution. In any one group of test rabbits, use only those rabbits whose control temperatures do not vary by more than 1° from each other, and do not use any rabbit having a temperature exceeding 39.8° .

Unless otherwise specified in the individual monograph, inject into an ear vein of each of three rabbits 10 mL of the test solution per kg of body weight, completing each injection within 10 minutes after start of administration. The test solution is *either* the product, constituted if necessary as directed in the labeling, *or* the material under test treated as directed in the individual monograph and injected in the dose specified therein. For pyrogen testing of devices or injection assemblies, use washings or rinsings of the surfaces that come in contact with the parenterally administered material or with the injection site or internal tissues of the patient. Assure that all test solutions are protected from contamination. Perform the injection after warming the test solution to a temperature of $37 \pm 2^\circ$. Record the temperature at 30-minute intervals between 1 and 3 hours subsequent to the injection.

TEST INTERPRETATION AND CONTINUATION

Consider any temperature decreases as zero rise. If no rabbit shows an individual rise in temperature of 0.5° or

more above its respective control temperature, the product meets the requirements for the absence of pyrogens. If any rabbit shows an individual temperature rise of 0.5° or more, continue the test using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of 0.5° or more and if the sum of the eight individual maximum temperature rises does not exceed 3.3° , the material under examination meets the requirements for the absence of pyrogens.

RADIOACTIVE PHARMACEUTICALS

Test Dose for Preformulated, Ready-to-Use Products Labeled with Radioactivity

AGGREGATED ALBUMIN and OTHER PARTICLE-CONTAINING PRODUCTS

For the rabbit pyrogen test, dilute the product with Sodium Chloride Injection to not less than 100 μCi per mL, and inject a dose of 3 mL per kg of body weight into each rabbit.

OTHER PRODUCTS

Where Physical Half-life of Radionuclide Is Greater Than 1 Day—Calculate the maximum volume of the product that might be injected into a human subject. This calculation takes into account the maximum recommended radioactive dose of the product, in μCi , and the radioactive assay, in μCi per mL, of the product at its expiration date or time. Using this information, calculate the maximum volume dose per kg to a 70-kg human subject.

For the rabbit pyrogen test, inject a minimum of 10 times this dose per kg of body weight into each rabbit. If necessary, dilute with Sodium Chloride Injection. The total injected volume per rabbit is not less than 1 mL and not more than 10 mL of solution.

Where Physical Half-life of Radionuclide Is Less Than 1 Day—For products labeled with radionuclides having a half-life of less than 1 day, the dosage calculations are identical to those described in the first paragraph under *Other Products*. These products may be released for distribution prior to completion of the rabbit pyrogen test, but such test shall be initiated at not more than 36 hours after release.

Test Dose for Pharmaceutical Constituents or Reagents to Be Labeled

The following test dose requirements pertain to reagents that are to be labeled or constituted prior to use by the direct addition of radioactive solutions such as Sodium Pertechnetate Tc 99m Injection, i.e., "cold kits".

Assume that the entire contents of the vial of nonradioactive reagent will be injected into a 70-kg human subject, or that $1/70$ of the total contents per kg will be injected. If the contents are dry, constitute with a measured volume of Sodium Chloride Injection.

For the rabbit pyrogen test, inject ($1/7$) of the vial contents per kg of body weight into each rabbit. The maximum dose per rabbit is the entire contents of a single vial. The total injected volume per rabbit is not less than 1 mL and not more than 10 mL of solution.

<161> TRANSFUSION AND INFUSION ASSEMBLIES AND SIMILAR MEDICAL DEVICES

The requirements apply to sterile and nonpyrogenic assemblies or devices in contact directly or indirectly with the cardiovascular system, the lymphatic system, or cerebrospinal fluid. This includes, but is not limited to, solution administration sets, extension sets, transfer sets, blood administration sets, intravenous catheters, implants extracorporeal oxygenator tubings and accessories, dialysers and dialysis tubing and accessories, heart valves, vascular grafts, intramuscular drug delivery catheters, and transfusion and infusion assemblies. These requirements do not apply to orthopedic products, latex gloves, or wound dressings.

Sterility—Proceed as directed for *Sterilized Devices* under *Sterility Tests* <71>.

Bacterial Endotoxins—Proceed as directed under *Bacterial Endotoxins Test* <85>.

For medical devices, the endotoxin limit is not more than 20.0 USP Endotoxin Units per device except that for those medical devices in contact with the cerebrospinal fluid the limit is not more than 2.15 USP Endotoxin Units per device.

A device that fails this test can be retested once by another *Bacterial Endotoxins* test. For devices that cannot be tested by the *Bacterial Endotoxins Test* <85> because of non-removable inhibition or enhancement, the *Pyrogen Test* <151> is applied.

Preparation of Devices—Select not less than 3 and not more than 10 devices. Rinse or soak the devices with LAL Reagent Water. The volume of rinsing or extracting solution may be adjusted for the size and configuration of the device.

For devices labeled “nonpyrogenic fluid pathway,” flush the fluid pathway with extracting fluid that has been heated to $37 \pm 1.0^\circ$, keeping the extracting fluid in contact with the relevant pathway for not less than 1 hour at controlled room temperature. Extracts may be combined, where appropriate. The endotoxin limit for the rinsing or extracting solution is calculated by the formula:

$$(K \times N)/(V)$$

where K is equal to the amount of endotoxin allowed per device, N is equal to the number of devices tested, and V is equal to the total volume of the extract or rinse. If the undiluted rinsing or extracting solution is unsuitable for the *Bacterial Endotoxins Test* <85>, repeat the inhibition or enhancement test after neutralization and removal of the interfering substances or after the solution has been diluted by a factor not exceeding the Maximum Valid Dilution. The Maximum Valid Dilution for devices is calculated by dividing the endotoxin limit by the labeled sensitivity λ of the LAL reagent used.

Pyrogen—For samples that cannot be tested by the *Bacterial Endotoxins Test* because of nonremovable inhibition or enhancement of the test, the *Pyrogen Test* <151> is applied. Select 10 devices, and obtain a pooled effluent, utilizing preparation methods appropriate to the device as directed for *Bacterial Endotoxins*, but with volumes of rinse or extraction fluid not to exceed 40 mL of sterile saline TS per device. The requirements of the *Pyrogen Test* <151> are met.

Other Requirements—The portions of medical devices that are made of plastics or other polymers meet the requirements specified for *Biological Tests—Plastics and Other Polymers* under *Containers—Plastics* <661>; those made of elastomers meet the requirements under *Elastomeric Closures for Injections* <381>. If a class designation for elastomers, plastics, or other polymers is needed, perform the appropriate in vivo

tests indicated in the general test chapter *Biological Reactivity Tests, In Vivo* <88>.

<171> VITAMIN B₁₂ ACTIVITY ASSAY

USP Reference Standards <11>—*USP Cyanocobalamin RS*.

Assay Preparation—Place a suitable quantity of the material to be assayed, previously reduced to a fine powder if necessary and accurately measured or weighed, in an appropriate vessel containing, for each g or mL of material taken, 25 mL of an aqueous extracting solution prepared just prior to use to contain, in each 100 mL, 1.29 g of disodium phosphate, 1.1 g of anhydrous citric acid, and 1.0 g of sodium metabisulfite. Autoclave the mixture at 121° for 10 minutes. Allow any undissolved particles of the extract to settle, and filter or centrifuge, if necessary. Dilute an aliquot of the clear solution with water so that the final test solution contains vitamin B₁₂ activity approximately equivalent to that of the *Standard Cyanocobalamin Solution* which is added to the assay tubes.

Standard Cyanocobalamin Stock Solution—To a suitable quantity of USP Cyanocobalamin RS, accurately weighed, add sufficient 25 percent alcohol to make a solution having a known concentration of 1.0 μ g of cyanocobalamin per mL. Store in a refrigerator.

Standard Cyanocobalamin Solution—Dilute a suitable volume of *Standard Cyanocobalamin Stock Solution* with water to a measured volume such that after the incubation period as described for *Procedure*, the difference in transmittance between the inoculated blank and the 5.0-mL level of the *Standard Cyanocobalamin Solution* is not less than that which corresponds to a difference of 1.25 mg in dried cell weight. This concentration usually falls between 0.01 ng and 0.04 ng per mL of *Standard Cyanocobalamin Solution*. Prepare a fresh standard solution for each assay.

Basal Medium Stock Solution—Prepare the medium according to the following formula and directions. A dehydrated mixture containing the same ingredients may be used provided that, when constituted as directed in the labeling, it yields a medium comparable to that obtained from the formula given herein.

Add the ingredients in the order listed, carefully dissolving the cystine and tryptophane in the hydrochloric acid before adding the next eight solutions in the resulting solution. Add 100 mL of water, mix, and dissolve the dextrose, sodium acetate, and ascorbic acid. Filter, if necessary, add the polysorbate 80 solution, adjust the solution to a pH between 5.5 and 6.0 with 1 N sodium hydroxide, and add purified water to make 250 mL.

L-Cystine	0.1 g
L-Tryptophane	0.05 g
1 N Hydrochloric Acid	10 mL
Adenine–Guanine–Uracil Solution	5 mL
Xanthine Solution	5 mL
Vitamin Solution I	10 mL
Vitamin Solution II	10 mL
Salt Solution A	5 mL
Salt Solution B	5 mL
Asparagine Solution	5 mL
Acid-hydrolyzed Casein Solution	25 mL
Dextrose, Anhydrous	10 g