2000

USP 24

THE UNITED STATES PHARMACOPEIA

NF 19

THE NATIONAL FORMULARY

By authority of the United States Pharmacopeial Convention, Inc., meeting at Washington, D.C., March 9–12, 1995. Prepared by the Committee of Revision and published by the Board of Trustees

Official from January 1, 2000

UNITED STATES PHARMACOPEIAL CONVENTION, INC.
12601 Twinbrook Parkway, Rockville, MD 20852

NOTICE AND WARNING

Concerning U.S. Patent or Trademark Rights

The inclusion in the Pharmacopeia or in the National Formulary of a monograph on any drug in respect to which patent or trademark rights may exist shall not be deemed, and is not intended as, a grant of, or authority to exercise, any right or privilege protected by such patent or trademark. All such rights and privileges are vested in the patent or trademark owner, and no other person may exercise the same without express permission, authority, or license secured from such patent or trademark owner.

Concerning Use of USP or NF Text

Attention is called to the fact that USP and NF text is fully copyrighted. Authors and others wishing to use portions of the text should request permission to do so from the Secretary of the USPC Board of Trustees.

ISSN 0195-7996
ISBN 1-889788-03-1

Printed by National Publishing, Philadelphia, PA

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 3 years after date of issue from manufacturer's cold storage (5°, 3 years).

Labeling—Label it to state that passive immunization with Immune Globulin modifies hepatitis A, prevents or modifies measles, and provides replacement therapy in persons having hypo– or agammaglobulinemia, that it is not standardized with respect to antibody titers against hepatitis B surface antigen and that it should be used for prophylaxis of viral hepatitis type B only when the specific Immune Globulin is not available, that it may be of benefit in women who have been exposed to rubella in the first trimester of pregnancy but who would not consider a therapeutic abortion, and that it may be used in immunosuppressed patients for passive immunization against varicella if the specific Immune Globulin is not available. Label it also to state that it is not indicated for routine prophylaxis or treatment of rubella, poliomyelitis or mumps, or for allergy or asthma in patients who have normal levels of immunoglobulin, that the plasma units from which it has been derived have been tested and found non-reactive for hepatitis B surface antigen, and that it should not be administered intravenously but be given intramuscularly, preferably in the gluteal region.

Rh_o (D) Immune Globulin

» Rh_o (D) Immune Globulin conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, nonpyrogenic solution of globulins derived from human blood plasma containing antibody to the erythrocyte factor Rh_o (D). It contains not less than 10 g and not more than 18 g of protein per 100 mL, not less than 90.0 percent of which is gamma globulin. It has a potency, determined by a suitable method, not less than that of the U. S. Reference Rh_o (D) Immune Globulin. It contains 0.3 *M* glycine as a stabilizing agent and contains a suitable preservative.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—The expiration date is not later than 6 months from the date of issue from manufacturer's cold storage, or not later than 1 year from the date of manufacture, as indicated on the label.

Anti–Human Globulin Serum

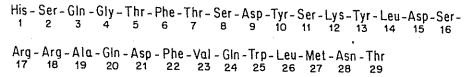
» Anti–Human Globulin Serum conforms to the regulations of the FDA concerning biologics (see *Biologics* ⟨1041⟩). It is a sterile, liquid preparation of serum produced by immunizing lower animals such as rabbits or goats with human serum or plasma, or with selected human plasma proteins. It is free from agglutinins and from hemolysins to non-sensitized human red cells of all blood groups. It contains a suitable antimicrobial specific for immunoglobulin IgG, and at least the C3d component of human complement (for use in the direct antiglobulin test, it contains this Anti-C3d and Anti-IgG activity) and which may be artificially colored green; (2) a reagent containing antibodies only against immunoglobulin IgG (not heavy chain specific) intended for use in the indirect antiglobulin test, and which may be artificially colored green; and (3) reagents containing antibodies specific for individual or selected components of human complement, such as Anti-C3, and Anti-C3b-C3d-C4, or a single class of immunoglobulins, such as Anti-IgG (heavy chain specific), used only to identify plasma components coated on the surface of red blood cells. Anti–Human Globulin Serums containing Anti-IgG meet the requirements of the test for potency, in parallel with the U. S. Reference Anti–Human Globulin (Anti-IgG) Serum (at a 1:4 dilution) when tested with red cells suspended in isotonic saline sensitized with decreasing amounts of non-agglutinating Anti-D (Anti-Rh_o) serum, and with cells sensitized in the same manner with an immunoglobulin IgG Anti-Fy^a serum of similar potency. Anti–Human Globulin Serum containing one or more Anti-complement components meets the requirements of the tests for potency in giving a 2+ agglutination reaction (i.e., agglutinated cells dislodged into many small clumps of equal size) by the low-ionic sucrose or sucrose-trypsin procedures when tested as recommended in the labeling. Anti–Human Globulin Serum containing Anti-3Cd activity meets the requirements for stability, by potency testing of representative lots every 3 months during the dating period.

Packaging and storage—Preserve at a temperature between 2° and 8°.

Expiration date—Its expiration date is not later than 1 year after the date of issue from manufacturer's cold storage (5°, 1 year; or 0°, 2 years).

Labeling—Label it to state the animal source of the product. Label it also to state the specific antibody activities present; to state the application for which the reagent is intended; to include a cautionary statement that it does not contain antibodies to immunoglobulins or that it does not contain antibodies to complement components, wherever and whichever is applicable; and to state that it is for in vitro diagnostic use. [NOTE—The lettering on the label of the general-purpose polyspecific reagent is black on a white background. The label of all other Anti–Human Globulin Serum containers is in white lettering on a black background.]

Glucagon



$C_{153}H_{225}N_{43}O_{49}S$ 3482.82

Glucagon (pig).

Glucagon [*16941-32-5*].

the blood. It is obtained from porcine and bovine pancreas glands.

Packaging and storage—Preserve in tight, glass containers, under nitrogen, in a refrigerator.

USP Reference standards ⟨11⟩—*USP Glucagon RS. USP Dextrose RS.*

Identification—The retention time of the major peak in the chromatogram of the *Test solution* obtained as directed in the test for *Chromatographic purity* corresponds to that in the chromatogram of the *Standard solution.*

Chromatographic purity—

Mobile phase—Prepare a solution containing 9.8 g of monobasic sodium phosphate and 170 mg of cysteine in 710 mL of water. Prepare a filtered and degassed mixture of this solution and 290 mL of acetonitrile, and adjust with phosphoric acid to a pH of 2.6. Make adjustments, if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Standard solution—Dissolve an accurately weighed quantity of USP Glucagon RS in 0.01 *N* hydrochloric acid to obtain a solution having a known concentration of about 1 mg per mL.

Test solution—Dissolve an accurately weighed quantity of Glucagon in 0.01 *N* hydrochloric acid to obtain a solution containing about 1 mg per mL.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains packing L7 with a 150 Å pore size. The column temperature is maintained at 35° and the flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the chromatograms as directed for *Procedure:* the resolution, *R*, is not less than 1.8; the retention time for the major glucagon peak is between 16 and 20 minutes; and the tailing factor is not more than 2.0.

Procedure—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the peak responses. Calculate the percentage of each impurity in the portion of Glucagon taken by the formula:

$$100(r_i / r_t),$$

in which r_i is the peak response for an individual impurity, and r_t is the sum of the responses of all of the peaks: not more than 2.5% of any individual impurity is found, and not more than 10.0% of total impurities is found.

Water, *Method I* ⟨921⟩: not more than 10.0%.

Residue on ignition ⟨281⟩: not more than 2.5%.

Nitrogen content, *Method II* ⟨461⟩: between 16.0% and 18.5%, calculated on the anhydrous basis.

Zinc content ⟨591⟩: not more than 0.05%.

Assay—

NOTE—All buffers have a final pH of 7.4, unless otherwise indicated.

Hepatocyte preparation—

CALCIUM-FREE PERFUSION BUFFER WITH DEXTROSE—Prepare a solution containing, in each liter, 7.92 g of sodium chloride, 0.35 g of potassium chloride, 1.80 g of dextrose, 0.15 g of edetic acid, and 2.38 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid. Oxygenate prior to circulation.

COLLAGENASE BUFFER—Prepare a solution containing, in each liter, 3.62 g of sodium chloride, 23.83 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 0.35 g of potassium chloride, 0.52 g of calcium chloride, and 1.8 g of dextrose. Adjust to a pH of 7.6, and oxygenate. Immediately before perfusion, dissolve a quantity of collagenase in this solution to obtain a concentration of 0.03% to 0.05%.

WASH BUFFER—Prepare a solution containing, in each liter, 7.92 g of sodium chloride, 0.35 g of potassium chloride, 0.15 g of edetic acid, 2.38 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 2.2 g of calcium chloride, and 0.12 g of magnesium sulfate.

INCUBATION BUFFER—Prepare a solution containing, in each liter, 7.92 g of sodium chloride, 0.35 g of potassium chloride, 0.15 g of edetic acid, 11.915 g of *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, and 1% bovine serum albumin (BSA). Adjust to a pH of 7.5.

TEST ANIMALS—Male Sprague-Dawley rats are maintained on a standard rat chow diet and freely given water. On the morning of the test, select a healthy rat weighing approximately 300 g, and administer 100 Units of *Heparin Sodium* subcutaneously.

PROCEDURE—Anesthetize the rat with an appropriate gas anesthetic. Open the abdominal cavity and isolate the portal vein. Insert an angiocatheter connected to a perfusion pump, and tie into the portal vein at the general location of the lienal branch. Start the perfusion (25 mL per minute) in situ with *Calcium-free perfusion buffer with dextrose*, equilibrated with oxygen, at a temperature of 37°. As the liver enlarges, cut the inferior vena cava to allow pressure equilibrium. [NOTE—About 300 mL of the perfusate is needed to clear the liver of red blood cells at a flow rate of 40 to 50 mL per minute.] Then circulate *Collagenase buffer* at a flow rate of 40 to 50 mL per minute for about 10 minutes. The exact concentration of collagenase (within the range of 0.03% to 0.05%) is determined empirically for each lot of enzyme. The concentration of collagenase is that necessary to cause consistently a breakdown of the liver about 10 minutes after initial entry of the *Collagenase buffer* into the liver. When the liver significantly increases in size, changes color and consistency, and starts to leak perfusate out of the lobes, change the system to the oxygenated prewarmed *Wash buffer.* About 100 mL of *Wash buffer* is needed to wash the liver of collagenase at a flow rate of 25 mL per minute. Surgically remove the liver from the animal and place in a prewarmed tray containing oxygenated *Wash buffer* (37°). Gently comb the liver with a stainless steel, fine-toothed comb to free the hepatocytes. Wash the hepatocytes with *Wash buffer*, and filter through cheesecloth (or a 150-µm mesh polyethylene net) into a plastic beaker. Centrifuge the cell suspension for about 2 minutes at 40 × *g* to form a loosely packed pellet. Discard the supernatant liquid, and resuspend the pellet in *Wash buffer.* Repeat the washing procedure twice for a total of three washes. Resuspend the final pellet in 100 to 200 mL of *Incubation buffer* depending on cell yield. [NOTE—If the *Assay* procedure is interrupted, cool the cells by collecting the cells in a beaker placed in ice. The cells are washed with ice cold *Wash buffer* and stored on ice until ready for use. At that point the cells are pelleted once more and resuspended in ice cold *Incubation buffer.*]

SUITABILITY—The concentrations of cells may vary due to the collagenase activity and the viability of the hepatocytes. To check cell viability and to determine viable cell concentration, dilute duplicate 100-µL aliquots of cell suspension with 400 µL of *Wash buffer* and 500 µL of isotonic 0.4% trypan blue. The aliquots are counted in a hemocytometer. The cells are suspended in *Incubation buffer* to obtain a cell concentration of 4×10^6 per mL. Count several distinct fields: the viability is greater than 90%. [NOTE—Viable cells are those cells that exclude the trypan blue.]

Negative control solution—Prepare a solution containing 0.5% bovine serum albumin in sterile water.

Incubation flasks—Use 25-mL conical flasks, the bottoms of which have been heated and pushed inward to form a conically raised center.

Standard preparations—Prepare two solutions by dissolving in each a vial of USP Glucagon RS in 0.01 *N* hydrochloric acid or other appropriate diluent to obtain a final concentration of 1.0 USP Glucagon Unit per mL. All dilutions thereafter are made using 0.5% BSA (w/v) in water. Dilute accurately measured volumes of each solution with *Negative control solution* to obtain six concentrations—200, 100, 50, 25, 12.5, and 6.25 micro-Units per mL—of each solution (*Standard preparations*). Pipet 0.2 mL of each *Standard preparation* into separate *Incubation flasks*. Pipet 0.2 mL of *Negative control solution* into each of 2 flasks (*Negative control solution 1* and *2*). Then add the hepatocytes into each of the eight flasks. [NOTE—Hepatocytes incubated with a saturating concentration of 500×10^{-6} USP Glucagon Units demonstrates at least 165%

Assay preparations—Using accurately weighed quantities of Glucagon, proceed as directed for *Standard preparations*.

D-*Glucose determination*—

STOCK STANDARD SOLUTION—Transfer 2.0 g of USP Dextrose RS, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with saturated benzoic solution to volume.

STANDARD SOLUTIONS—Transfer suitable quantities of *Stock standard solution* to three flasks and dilute with saturated benzoic acid solution to obtain known solutions having known concentrations of 0.5, 1.0, and 1.5 times the typical sample glucose concentration.

POTASSIUM FERROCYANIDE SOLUTION—Dissolve 1.25 g of trihydrate potassium ferrocyanide in 125 mL of *Sterile Water for Injection*.

SYSTEM SUITABILITY—Analyze the *Potassium ferrocyanide solution*, the *Standard solutions*, and five replicates of the middle *Standard solution*. Prepare a standard curve using the *Standard solutions* as directed for *Procedure:* the relative standard deviation of the standard curve is not more than 2.0%; the response of the *Potassium ferrocyanide solution* is not more than 30 mg per liter; and the relative standard deviation is not more than 2.0% for the replicate analyses of the middle *Standard solution*.

Procedure—Dispense 5 mL of *Hepatocytes preparation* into the special incubation flasks in sequence from high glucagon concentration to low glucagon concentration alternating the *Standard preparations* with the *Assay preparations*. The flasks are swirled in an orbiting water bath at 125 rpm at 30° for approximately 30 to 60 minutes. [NOTE—The exact incubation time must be determined to optimize the signal-to-noise.] Following incubation place 0.5- to 1.0-mL aliquots, in duplicate, from each incubation flask into labeled tubes, and centrifuge at 100 × *g*. Determine the percentage of glucose concentration in each flask's supernatant liquid.

To conform to the linear range of the instrument being used, it may be necessary to adjust by dilution each of the preparations. Use a glucose analyzer that has demonstrated appropriate specificity, accuracy, precision, and linear response over the range of concentrations being determined. [NOTE—A suitable analyzer may use an immobilized, oxidase–enzyme membrane or jacket-generating hydrogen peroxide, which is then detected at the electrode.] Perform the glucose analysis in the following sequence: *Negative control solution 1*, *Standard preparations*, *Assay preparations*, and *Negative control 2*. Determine the percentage of glucose against the *Negative control solution* for each preparation.

Calculations—

LINEARITY TEST—Use an analysis of variance (ANOVA) with one sample assayed against a standard, and using two replicates each, construct a table (see Table 1). Compare the value of the ratio $MSNL/MSRES_1$ to a critical value obtained from a table for an F distribution with $m - 2$ and $3m - 3$ degrees of freedom (df), where m is the number of dose levels for each preparation. If the ratio MSNL/MSRES does not indicate the presence of significant nonlinearity (ratio value is lower than the critical value), then proceed to the test for parallelism. If the ratio exceeds the critical value (significance level of 0.05), the nonlinearity is statistically significant and the test is repeated, discarding the results from either the highest or lowest dose of both the *Standard preparations* and the *Assay preparations* (four dose levels). If the ratio MSNL/MSRES does not indicate the presence of significant nonlinearity, then proceed to the test for parallelism.

PARALLELISM TEST—Compare the ratio $MSNP/MSRES_2$ to a critical value obtained from an F distribution having 1 and $4m - 5$ df. If the ratio $MSNP/MSRES_2$ does not indicate the presence of significant nonparallelism, then the assay is considered valid. Use the appropriate dose levels for the estimation of the relative potency.

RELATIVE POTENCY—Calculate the relative potency, *R*, of the *Assay preparations* as compared to the *Standard preparations* as subsequent calculations, these doses are respectively represented by 1 through 5 as shown in the table below.

j	1	2	3	4	5
Dose	12.5	25	50	100	200
X_j	1.10	1.40	1.70	2.00	2.30

(2) To differentiate between the *Standard preparations* and the *Assay preparations* in the calculations, the subscript "i" will be used with i = 1 to designate the *Standard preparations* and i = 2 to designate the *Assay preparations*. Y_{ijk} will denote the glucose concentration associated with the k^{th} replicate of the j^{th} dose of the i^{th} preparation. For example, Y_{ijk} is the glucose concentration associated with the k^{th} replicate of the j^{th} dose of the appropriate *Standard preparation;* Y_{1ik} is the glucose concentration associated with the k^{th} replicate of dose 1 of the *Standard preparation* and Y_{21k} would denote the glucose concentration associated with the k^{th} replicate of dose 1 of the *Assay preparation*. Dose 1 represents a glucose dose of 12.5×10^{-6} USP Glucagon Units per mL. Finally, Y_{132} would represent the glucose concentration associated with the 2^{nd} replicate of dose 3 for the *Standard preparation*.

(3) Y_s and Y_t denote the average glucose concentrations for the *Standard preparation* and the *Assay preparation*, respectively.

(4) Calculate the least-squares slope estimate, b, for a linear regression relating the Y_{ijk}'s to the X_j's as follows: $b = S_{xy}/S_{xx}$ with S_{xy} and S_{xx} calculated using the equations in Table 2.

(5) The log potency, M, is calculated using $M = -1[(Y_s - Y_t)/b]$.

(6) R = antilog (M).

(7) Calculate the confidence limits (upper and lower) for the relative potency, R, using the value $s^2 = MSRES_3$ (see Table 1 and Table 2) as follows. Obtain t from a table for a t distribution having $4m - 4$ df. For the 95% limits, the t values can be obtained from Table 9 under *Design and Analysis of Biological Assays* ⟨111⟩.

[NOTE—For confidence limits having other probability levels (i.e., $100(1 - \alpha)$ %), the right tail t critical value having $\alpha/2$ area to its right is used.]

Calculate $g = t^2S^2/b^2S_{xx}$

$$\text{and } F = (ts/b)\sqrt{(1/m)(1 - g) + (M^2/S_{xx})},$$

and calculate $M_L = (M - F)/(1 - g)$

and $M_U = (M + F)/(1 - g)$

where M is the log potency and M_L and M_U are the log potency lower and upper confidence limits. The lower and upper confidence limits for the relative potency, R, are given by

$$RL = \text{antilog } (M_L)$$
$$RU = \text{antilog } (M_U)$$

It meets the requirements if the potency is between 0.9 to 1.3 USP Glucagon Units per mg, and the confidence interval width at P = 0.95 does not exceed 45% of the computed potency.

Table 1. ANOVA for the Rat Hepatocyte Assay for Glucagon.

Source	df	SS (Sum of Squares)	MS (Mean Square)
Preparations	1	SSPREP	MSPREP
Replicates	1	SSREP	MSREP
Linear Slope	1	SSLIN	MSLIN
$Residual_3$	4m-4	$SSRES_3$	$MSRES_3$
Nonparallelism	1	SSNP	MSNP
$Residual_2$	4m-5	$SSRES_2$	$MSRES_2$
Nonlinearity	m-2	SSNL	MSNL
$Residual_1$	3m-3	$SSRES_1$	$MSRES_1$
TOTAL	4m-1	SST	

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.