n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Hermetic containers. Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Isophane Insulin Injection (Aqueous Suspension)

イソフェンインスリン水性懸濁注射液

Isophane Insulin Injection (Aqueous Suspension) is an aqueous suspension for injection. It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.01 mg and not more than 0.04 mg of zinc (Zn: 65.39) for each labeled 100 Units.

When Sodium Chloride is used in the preparation of Isophane Insulin Injection (Aqueous Suspension), this should be stated on the label.

Method of preparation Prepare as directed under Injections, with Insulin and Protamine Sulfate. To each 100 mL of Isophane Insulin Injection (Aqueous Suspension) add either 0.38 to 0.63 g of Dibasic Sodium Phosphate, 1.4 to 1.8 g of Concentrated Glycerin, 0.15 to 0.17 g of Cresol, and 0.06 to 0.07 g of Phenol, or 0.38 to 0.63 g of Dibasic Sodium Phosphate, 0.42 to 0.45 g of Sodium Chloride, 0.7 to 0.9 g of Concentrated Glycerin, and 0.18 to 0.22 g of Cresol.

Description Isophane Insulin Injection (Aqueous Suspension) is a white aqueous suspension. When allowed to stand, it separates into a white precipitate and colorless supernatant liquid, and the precipitate returns easily to the suspension state on gentle shaking.

When examined microscopically, the precipitate mostly consists of fine, oblong crystals of 5 to 30 μm in major axis, and does not contain amorphous substances or large aggregates.

Identification Proceed as directed in the Identification under Insulin Zinc Protamine Injection (Aqueous Suspension).

pH 7.0 - 7.4

Purity **(1)** Protein—Perform the test as directed under the Nitrogen Determination: not exceeding 0.85 mg of nitrogen (N: 14.01) is found for each labeled 100 Units.

(2) Isophane ratio—(i) Buffer solution A: Dissolve 2.0 g of anhydrous disodium hydrogenphosphate, 16 g of glycerin, 1.6 g of *m*-cresol, and 0.65 g of phenol in water to make exactly 200 mL.

(ii) Buffer solution B: Dissolve 2.0 g of anhydrous disodium hydrogenphosphate, 4.35 g of sodium chloride, 8.0 g of glycerin, and 2.0 g of *m*-cresol in water to make exactly 200 mL.

(iii) Insulin solution: Weigh accurately 1000 Units of Insulin Reference Standard, dissolve in 1.5 mL of diluted hydrochloric acid (1 in 360), and add 5.0 mL of buffer solution A and water to make 20 mL. Adjust the pH to 7.2 with dilute hydrochloric acid or sodium hydroxide TS. The solution is clear. Dilute with water to make exactly 25 mL. The solution is clear, and the pH is between 7.1 and 7.4. When it is stated on the label that Sodium Chloride is used in the preparation, use 5.0 mL of buffer solution B instead of buffer solution A in the above procedure.

(iv) Protamine solution: Weigh accurately 50 mg of Protamine Sulfate Reference Standard, and dissolve in 2 mL of buffer solution A and water to make 8 mL. Adjust the pH to 7.2 with dilute hydrochloric acid or sodium hydroxide TS, and dilute with water to exactly 10 mL. The solution is clear, and the pH is between 7.1 and 7.4. When it is stated on the label that Sodium Chloride is used in the preparation, use 2 mL of buffer solution B instead of buffer solution A in the above procedure.

(v) Procedure: When Isophane Insulin Injection (Aqueous Suspension) contains 40 Units per ml, centrifuge a portion of the suspension, measure exactly two 10-mL portions of the supernatant liquid in two tubes A and B, respectively, add exactly 1 mL of the insulin solution to tube A, and 1 mL of the protamine solution to tube B, mix the contents of each tube, allow to stand for 10 minutes, and determine the turbidity of each mixture by using a photometer or a nephelometer: the turbidity of the mixture in tube B is not greater than that in tube A. When Isophane Insulin Injection (Aqueous Suspension) contains 80 Units per ml, measure exactly 5 mL of the supernatant liquid, and proceed in the same manner.

Assay **(1)** Insulin—To Isophane Insulin Injection (Aqueous Suspension) add diluted hydrochloric acid (1 in 100) to adjust pH to about 2.5, and proceed with the clear solution as directed in the Assay under Insulin Injection.

(2) Zinc—Pipet a volume of Isophane Insulin Injection (Aqueous Suspension), equivalent to about 400 Units according to the labeled Units, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make exactly 100 mL, dilute, if necessary, with water to contain 0.6 to 10 μg of zinc (Zn: 65.39) per mL, and use this solution as the sample solution. Separately, pipet a volume of Standard Zinc Solution for the Atomic Absorption Spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc (Zn: 65.39) per mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution according to the Atomic Absorption Spectrophotometry under the following conditions, and determine the amount of zinc in the sample solution using the analytical curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene gas
Supporting gas—Air
Lamp: Zinc hollow-cathode lamp
Wavelength: 213.9 nm

Containers and storage Containers—Hermetic containers.
Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Insulin Zinc Injection (Aqueous Suspension)

インスリン亜鉛水性懸濁注射液

Insulin Zinc Injection (Aqueous Suspension) is an aqueous suspension for injection. It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.20 mg and not more than 0.30 mg of zinc (Zn: 65.39) for each labeled 100 Units.

Method of preparation Prepare as directed under Injections, with Insulin and Zinc Chloride. It contains 0.15 to 0.17 g of Sodium Acetate, 0.65 to 0.75 g of Sodium Chloride and 0.09 to 0.11 g of Methyl Parahydroxybenzoate for each 100 mL of Insulin Zinc Injection (Aqueous Suspension).

Description Insulin Zinc Injection (Aqueous Suspension) is a white suspension. When allowed to stand, it separates into a white precipitate and a colorless supernatant liquid, and it readily becomes a suspension again on gentle shaking.

When it is examined microscopically, the majority of the particles in the suspension are crystals, the dimension of which is 10 to 40 μm. The rest is amorphous and does not exceed 2 μm in dimension.

Purity Dissolved insulin—Perform the following test with a clear liquid obtained by centrifuging Insulin Zinc Injection (Aqueous Suspension): not more than 2.5% of the labeled units is found.

Use the clear liquid of Insulin Zinc Injection (Aqueous Suspension) as the sample solution. Prepare the standard solution having a concentration of 2.5% of the labeled units of Insulin Zinc Injection (Aqueous Suspension by proceeding as directed in the Assay (iv) under Insulin Injection. Divide the healthy rabbits weighing not less than 1.8 kg, fasted for not less than 14 hours before injection, into 2 equal groups of not less than 3. Inject subcutaneously an amount of the standard solution or the sample solution equivalent to 0.3 units per kg of body mass to the animals of each group. Collect blood before and 1 hour and 2.5 hours after injection, then proceed as directed in the Assay (viii) under Insulin Injection, and calculate the ratio of the average blood sugar level of 1 hour and 2.5 hours after to that of before injection of each animal: the mean value for the group injected the sample solution is not less than that for the group injected the standard solution.

Nitrogen content Perform the test as directed under the Nitrogen Determination: the amount of nitrogen (N: 14.01) is not less than 0.50 mg and not more than 0.64 mg for each labeled 100 Unites.

Assay **(1)** Insulin—Proceed as directed in the Assay under Insulin Injection with the clear liquid obtained from Insulin Zinc Injection (Aqueous Suspension) by adjusting the pH to about 2.5 with diluted hydrochloric acid (1 in 100).

(2) Zinc—Measure exactly a volume of Insulin Zinc Injection (Aqueous Suspension), equivalent to about 400 Units according to the labeled units, add 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient water to make exactly 200 mL, then dilute with water to contain 0.6 to 1.0 μg of zinc (Zn: 65.39) per mL, and use this solution as the sample solution. Separately, pipet a volume of Standard Zinc Solution for atomic absorption spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc (Zn: 65.39) per mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under the Atomic Absorption Spectrophotometry according to the following conditions, and determine the amount of zinc in the sample solution using the calibration curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene gas
Supporting gas—Air
Lamp: Zinc hollow-cathode lamp
Wavelength: 213.9 nm

(3) Crystalline insulin—Measure exactly a volume of Insulin Zinc Injection (Aqueous Suspension), equivalent to about 600 Units according to the labeled units, centrifuge, discard the supernatant liquid, suspend the residue in 5 mL of water, add 10 mL of sodium acetate-acetone TS, shake for 3 minutes, and centrifuge. Discard the supernatant liquid, and repeat the above treatment on the residue. Wash down the residue into a Kjeldahl flask with 15 mL of sulfuric acid, and perform the test as directed under the Nitrogen Determination: the amount of nitrogen (N: 14.01) is not less than 55% and not more than 70% of the total nitrogen content. Calculate the total nitrogen content for insulin Units of the sample taken from the values of nitrogen obtained in the Nitrogen content. The amount of nitrogen (N: 14.01) is not less than 55% and not more than 70% of the total nitrogen content.

Containers and storage Containers—Hermetic containers.
Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Amorphous Insulin Zinc Injection (Aqueous Suspension)

無晶性インスリン亜鉛水性懸濁注射液

Amorphous Insulin Zinc Injection (Aqueous Suspension) is an aqueous suspension for injection. It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.12 mg and not more than 0.30 mg of zinc (Zn: 65.39) for each labeled 100 Units.

Method of preparation Prepare as directed under Injections, with Insulin and Zinc Chloride. Each 100 mL of Amorphous Insulin Zinc Injection (Aqueous Suspension) contains 0.15 to 0.17 g of Sodium Acetate, 0.65 to 0.75 g of Sodium Chloride, and 0.09 to 0.11 g of Methyl Parahydroxybenzoate.

Description Amorphous Insulin Zinc Injection (Aqueous