ANNEXURE - 2.2 (pages 1 -8) [Chemical Tests]

S.no	Group of products, materials or items tested	Specific tests or types of tests performed	Specification, standard (method) or technique used	Range of testing/ Limit of detection	MU (±)
1.	Recombinant Product i. rh-Insulin inj. preparations of ; Regular, biphasic Isophane suspension, NPH, Lente	**High Performance Liquid Chromatography Reverse Phase HPLC** 3 HPLC Units in Room No: L-1015 (2 units) & L-1037 (1 unit)	Identification: human insulin peak matches with the Ref standard solution. 1. Certified Ref material : i. Human insulin ii. Porcine insulin iii. Bovine insulin 2. Human Insulin working standard calibrated against CRM.	0.16 mg/ml -4 mg/ ml for system suitability solutions and Resolution	Retention time: 20± 2min
			Potency as per USP claim i. NLT 95% and NMT 105% of the potency stated on the label ii. NLT 90% and NMT 110% of the amount of Insulin stated on the label as per EP/BP / IP claim iii. L±5% of the ratio (L is the % soluble insulin human stated on the product label. iv. NMT 2.5% of total insulin content 1.Certified Ref material : i. Human insulin 2. Human Insulin working standard calibrated against CRM	1.6 mg/ ml – 4 mg/ml	Retention time: 20± 2min - The 95% limit of the assigned unitage of certified Ref. Std. - Working std: 80-120% of the peak area of Human Insulin.
			Related proteins i. NMT 2% in Insulin human ii. A-21 desamido insulin is NMT 5% of the total area of peaks. iii. Sum of areas due to Insulin and A-21 desamido insulin is NMT 6% of the total area of peaks 1.Certified Ref material : ii. Human insulin 2. Human Insulin working standard calibrated against CRM References: IP- 2007: Pg 97-99, 652-654, 1230-1235; EP 6.0: Pg 2137-2141, 2146-2149 USP 31: Pg 2403-2411	linearity solution 0.16 mg/ml-4mg/ml & Resolution (R)	

The tests given are as per the requirements given in pharmacopoeia or other approved sources for each biological product to be tested. The Monographs are mentioned in Pharmacopoeia –European, British, US, Indian and International.

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Contd. 1.	Recombinant Product ii. rh-Insulin Glargine inj	**High Performance Liquid Chromatography Reverse Phase HPLC** 3 HPLC Units in Room No: L-1015 (2 units) & L-1037 (1 unit)	Identification : insulin Glargine peak matches with the Ref standard solution Potency as per manufacturer claim : NLT 95% and NMT 105% of the potency stated on the label Related proteins • Largest single related proteins ≤ 0.5% • Sum of related proteins < 1.5% 1. Certified Ref material : i. Human insulin ii. Porcine insulin iii. Bovine insulin 2. Manufacturer' Insulin Glargine working standard Reference: Insulin Glargine Manufacturer Protocol	0.6mg/ml 1.5mg/ml	Retention time of sample=± 3% Retention time of Ref. Std. (18- 23 min.)
	iii. Human insulin bulk	-DO-	Chromatogram obtained with test and ref. solutions are qualitatively similar. Identify the peaks in Ref solutions due to digest fragments I, II and III 1. Certified Ref material : • Human insulin • Porcine insulin • Bovine insulin 2. Staphylococcus aureus strain V8 protease References: IP- 2007: Pg 97-99, 652-654, 1230-1235 EP 6.0: Pg 2137-2141, 2146-2149 USP 31: Pg 2403-2411	0.2% w/V solution of substance under examination (EP/IP)	25^0C± 1^0C of incubation

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2.	Recombinant Product i. rh-Insulin inj	**High Performance Liquid Chromatography Reverse Phase HPLC** **Size exclusion (Gel permeation)** 4 Units in Room No: L-1015(2) , L-1037, L-1011	NMT 1.7%: to NMT 3%: for different insulin preparations Human Insulin working standard exposed for 10 days	Resolution for 4.0 mg/ml of Insulin containing more than 0.4% HMWP	Not applicable
	ii. rh-Insulin Glargine inj ,	-DO-	≤ 0.3% of HMWP Insulin Glargine working standard exposed for 5 days References: IP- 2007: Pg 97-99, 652-654, 1230-1235 EP 6.0: Pg 2137-2141, 2146-2149 USP 31: Pg 2403-2411 & Insulin Glargine Manufacturer Protocol		
	Blood Product iii. Albumin-5%, 20%, 25%	-DO-	The area of the peak due to polymers and aggregates is not greater than 10 percent of the total area of the chromatogram (corresponding to about 5 % of polymers & aggregates). In-house control / Human albumin Sigma References: BP- 2004	Not applicable	Not applicable

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3.	Recombinant Product i. rh-Insulin inj ii. rh-Insulin Glargine	**ATOMIC ABSORPTION SPECTROMETER** 1 unit in room No: A028	i. rh-Insulin inj R, NPH, Biphasic isophane NMT 40 µg /100 IU : total Zn-Lente formulation 0.12 -0.25 mg /100 IU : Zn in soln- Lente formulation 20% & 65% of total Zn : iii. rh-Insulin Glargine 27.0- 33.0µg /ml Zinc standard solution -500- 1000ppm from Sigma References: IP- 2007: Pg 97-99, 652-654, 1230-1235 EP 6.0: Pg 2137-2141, 2146-2149 USP 31: Pg 2403-2411 & Insulin Glargine Manufacturer Protocol	0.25µg/ml to 2.0µg/ml for Zinc Std . sensitivity as: 0.018mg/ml for Zn by Atomic absorption at λ 213.9	Not applicable
	Blood Product iii. Albumin -5%, 20%, 25%	-DO-	Contains NLT 95% and NMT 105 % of the contents of Na and K stated on label which are in any case NMT 160 millimoles of Na per litre and 2 millimoles of K per litre. Na^+ / K^+ standard -Perkin Elmer	**Na^+ and K^+:** 0.5– 2ppm of Na+ / K+ Standard	
4.	Blood Product i. Albumin-5%, 20%, 25%	**PROTEIN QUANTIFICATION** 3 units in room No: L-1036, L-1011, L-1037	Contains not less than 95% and not more than 105 % of the stated amount of protein References: IP- 2007	In-house controls / Human albumin sigma/ Human immunoglobulin sigma	10 - 60 mg
5.	Enzymes & Hormones i. Streptokinase bulk ii. Streptokinase inj.	**STREPTODORNASE ACTIVITY** Room No: L-1037	Absorbance of test solution should be NMT 50% of Reference solution i. Streptokinase & Streptodornase Ref: IP2007	1, 50, 000 IU/mL	Not applicable
6.	Enzymes & Hormones i. Streptokinase bulk ii. Streptokinase inj.	**STREPTOLYSIN ACTIVITY** Room No: L-1037	Absorbance of test solution should be NMT 50% of the reference solution Anti-Streptolysin –'O' Reference reagent Ref: IP2007	5, 00, 000 IU/ 0.5 mL	Not applicable

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7.	Enzymes & Hormones i. Streptokinase bulk ii. Streptokinase inj.	**CHROMOGENIC METHOD** Room No: L-1037	The estimated potency NLT 90% & NMT 111% of stated potency Streptokinase -3rd international std Ref: IP2007	0.3 IU/mL – 2.4 IU/mL	Not applicable
8.	Blood Product i. Albumin - 5%, 20%, 25%	**STABILITY** Room No: L- 1037 , L- 1011	The content of the final container remain unchanged, as determined by visual inspection, after heating at 57°C for 50 hours when compared to its control consisting of a sample from the same lot which has not undergone this heating References: IP- 2007	Not applicable	
9.	Recombinant Product i. rh-Insulin inj ii. rh-Insulin Glargine inj	**PARTICULATE MATTER** • Particle measuring system a) Stage micrometer – microscope a) 1 unit in room No; L-1012 and b) 1 unit in room No: L-1036	≥10 µm size particles: NMT 6000/ vial ≥25 µm size particles: NMT 600/ vial *(Particle count other than Insulin crystals)* ≥10 µm size particles: NMT 3000/ vial ≥25 µm size particles: NMT 300/ vial *(Particle count other than Insulin crystals)* References: IP- 2007: Pg 97-99, 652-654, 1230-1235 EP 6.0: Pg 2137-2141, 2146-2149 USP 31: Pg 2403-2411 & Insulin Glargine Manufacturer Protocol	≥ 10 µm to ≥ 25 µm ≥ 10 µm to ≥ 25 µm	Not applicable
	Enzymes & Hormones iii. Streptokinase inj.	-DO- Room No: L-1037	On visual observation free of particles. Ref: IP2007	Not applicable	Not applicable

NLT: not less than **NMT: not more than**

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10.	Blood Product i. Albumin-5%, 20%, 25%	**GEL ELECTROPHORESIS** Room No: L 1011	No impurity (any band other than the main band) in the electropherogram obtained with the test solution may be more intense than the other impurity band obtained with the reference solution In-house control/ Human albumin sigma References: IP- 2007	0.025µg – 2.5µg protein	
11.	Enzymes & Hormones i. Streptokinase bulk, ii. Streptokinase inj. iii. Heparin inj	**pH DETERMINATION** Room No: L-1037	6.8-7.5 -do- 5.5-8.0 Standard pH buffer : pH 4.0, 7.0, 9.0 Ref: IP2007	pH 4.0-9.0	Not applicable
	Blood Product iv. Albumin-5%, 20%, 25%	**-DO-**	6.7-7.3 Standard pH buffer 7.0/ in-house controls References: IP- 2007	pH 1-14	
	Recombinant Product v. rh-Insulin inj vi. rh-Insulin Glargine inj	**-DO-**	6.8-7.8 Regular 6.9-7.5 Biphasic Isophane, Isophane, NPH, Lente 3.5- 4.5 Insulin Glargine Standard pH buffer 4.0, 7.0 and 9.0 References: IP- 2007: Pg 97-99, 652-654, 1230-1235 EP 6.0: Pg 2137-2141, 2146-2149 USP 31: Pg 2403-2411 & Insulin Glargine Manufacturer Protocol	pH 1-14	Not applicable

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12.	Enzymes & Hormones i. Streptokinase inj.	**COLOR**	Clear solution Ref: IP2007	Not applicable	Not applicable
	Blood Grouping Reagents ii. Anti- A	**-DO-** Ref: IP2007 & Transfusion medicine technical manual, 2003.	Artificially colored blue or blue-green	Not Applicable	Not Applicable
	iii. Anti-B		Artificially colored yellow		
	iv. Anti-A,B		Artificially colored or colorless		
	v. Anti-D (IgM)		Yellowish or colorless		
	vi. Anti-D(IgM + IgG)				
	vii. Anti-D(IgG)				
	viii. Anti-A_1 (Lectin)	**-DO-**	---		
	ix. Anti-H (Lectin)				
	Recombinant Product x. rh-Insulin inj	**-DO-**	A colorless liquid, Free from turbidity and foreign matter, during storage, traces of a very fine sediment may be deposited	For rh- Insulin Regular formulation	Not applicable
			A white suspension, Which on standing deposits a white sediment and leaves a colorless or almost colorless supernatant liquid, the sediment is readily resuspended by gently shaking. References: IP- 2007: Pg 97-99, 652-654, 1230-1235 EP 6.0: Pg 2137-2141, 2146-2149 USP 31: Pg 2403-2411	Other than Regular formulation	
	xi. rh-Insulin Glargine inj		Colorless to almost colorless solution Reference: Insulin Glargine Manufacturer Protocol	For rh- Insulin Glargine	
13.	Recombinant Product i. rh-Insulin inj ii. rh-Insulin Glargine inj	**CLARITY**	A liquid is considered clear if its clarity is the same as that of water, solvent used for preparing the solution being examined and opalescence is NMT that of OS1. References: IP- 2007: Pg 97-99, 652-654, 1230-1235 EP 6.0: Pg 2137-2141, 2146-2149 USP 31: Pg 2403-2411 & Insulin Glargine Manufacturer Protocol	For rh- Insulin Regular formulations and Insulin Glargine inj.	Equivalent to turbidity standard I of EP out of 4 opalescence standard

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14.	Blood Grouping Reagents i. Anti- A	**PHYSICAL APPEARANCE**	Clear, no turbidity, no precipitate, no particles or no gel formation on visual inspection. Ref: IP2007 & Transfusion medicine technical manual, 2003.	Not Applicable	Not Applicable
	ii. Anti-B				
	iii. Anti-A,B				
	iv. Anti-D (IgM)				
	v. Anti-D(IgM + IgG)				
	vi. Anti-D(IgG)				
	vii. Anti-A_1 (Lectin)				
	viii. Anti-H (Lectin)				