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Anticoagulant Heparin Solution

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click www.uspnf.com/rb-anticoagulant-heparin-sol-20230331.

DEFINITION

Anticoagulant Heparin Solution is a sterile solution of Heparin Sodium in Sodium Chloride Injection. Its potency is NLT 90.0% and NMT 110.0% of the potency stated on the label in terms of USP Heparin Units. It contains NLT 0.85% and NMT 0.95% of sodium chloride (NaCl). It may be buffered. It contains no antimicrobial agents.

Prepare Anticoagulant Heparin Solution as follows.

Heparin Sodium	75,000 Units
Sodium Chloride Injection, sufficient quantity to make	1000 mL

Add the Heparin Sodium, in solid form or in solution, to the Sodium Chloride Injection, mix, filter if necessary, and sterilize.

ASSAY

• **Anti-Factor IIa Potency**

pH 8.4 buffer: Dissolve 6.10 g of tris(hydroxymeth yl)aminomethane, 10.20 g of sodium chloride, 2.80 g of edetate sodium, and, if suitable, between 0 and 10.00 g of *polyethylene glycol 6000* and/or 2.00 g of bovine serum albumin in 800 mL of water. [Note—2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin.] Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 mL.

Antithrombin solution: Reconstitute a vial of antithrombin (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a solution of 5 Antithrombin IU/mL. Dilute this solution with *pH 8.4 buffer* to obtain a solution having a concentration of 0.125 Antithrombin IU/mL.

Thrombin human solution: Reconstitute thrombin human (factor IIa) (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to give 20 Thrombin IU/mL, and dilute with *pH 8.4 buffer* to obtain a solution having a concentration of 5 Thrombin IU/mL. [Note—The thrombin should have a specific activity of NLT 750 IU/mg.]

Chromogenic substrate solution: Prepare a solution of a suitable chromogenic thrombin substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a concentration of 1.25 mM.

Stopping solution: 20% (v/v) solution of acetic acid

Standard solutions: Reconstitute the entire contents of an ampule of USP Heparin Sodium for Assays RS with water, and dilute with *pH 8.4 buffer* to obtain at least four dilutions in the concentration range between 0.005 and 0.03 USP Heparin Unit/mL.

Sample solutions: Proceed as directed for *Standard solutions* to obtain concentrations of Anticoagulant Heparin Solution similar to those obtained for the *Standard solutions*.

Analysis

[Note—The procedure can also be performed using alternative platforms.]

For each dilution of the *Standard solutions* and the *Sample solutions*, at least duplicate samples should be tested. Label a suitable number of tubes, depending on the number of replicates to be tested. For example, if five blanks are to be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, T3, and T4 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, and S4 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [Note—Treat the tubes in the order B1, S1, S2, S3, S4, B2, T1, T2, T3, T4, B3, T1, T2, T3, T4, B4, S1, S2, S3, S4, B5.] Note that after each addition of a reagent, the incubation mixture should be mixed without allowing bubbles to form. Add twice the volume (100–200 µL) of *Antithrombin solution* to each tube containing one volume (50–100 µL) of either the *pH 8.4 buffer* or an appropriate dilution of the *Standard solutions* or the *Sample solutions*. Mix, but do not allow bubbles to form. Incubate at 37°

for at least 1 min. Add to each tube 25–50 µL of *Thrombin human solution*, and incubate for at least 1 min. Add 50–100 µL of *Chromogenic substrate solution*. Please note that all reagents, *Standard solutions*, and *Sample solutions* should be prewarmed to 37° just before use. Two different types of measurements can be recorded:

1. Endpoint measurement: Stop the reaction after at least 1 min with 50–100 µL of *Stopping solution*. Measure the absorbance of each solution at 405 nm using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩). The RSD over the blank readings is less than 10%.
2. Kinetic measurement: Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* ⟨857⟩). Calculate the change in absorbance/min (ΔOD/min). The blanks for kinetic measurement are also expressed as ΔOD/min and should give the highest values because they are carried out in the absence of heparin. The RSD over the blank readings is less than 10%.

Calculations: The statistical models for *Slope ratio assay* or *Parallel-line assay* can be used, depending on which model best describes the correlation between concentration and response.

Parallel-line assay: For each series, calculate the regression of the absorbance or change in absorbance/min against log concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Anticoagulant Heparin Solution in USP Units/mL using statistical methods for parallel-line assays.

Slope ratio assay: For each series, calculate the regression of the log absorbance or the log change in absorbance/min against concentrations of the *Standard solutions* and the *Sample solutions*, and calculate the potency of Anticoagulant Heparin Solution in USP Units/mL using statistical methods for slope ratio assays.

Acceptance criteria: 90.0%–110.0% of the potency stated on the label in terms of USP Heparin Units.

• **Sodium Chloride**

Sample solution: Solution and potassium chromate TS (5:1)

Analysis: Titrate with 0.1 N silver nitrate VS. Each mL of 0.1 N silver nitrate VS is equivalent to 5.844 mg of NaCl.

SPECIFIC TESTS

• **pH** ⟨791⟩**:** Between 5.0 and 7.5

• **Bacterial Endotoxins Test** ⟨85⟩**:** It contains NMT 2.5 USP Endotoxin Units/mL.

• **Injections and Implanted Drug Products** ⟨1⟩**:** Meets the requirements

ADDITIONAL REQUIREMENTS

Change to read:

• **Packaging and Storage:** Preserve in single-dose containers, of colorless, transparent, ▲preferably▲ (RB 1-Apr-2023) Type I or Type II glass, or of a suitable plastic material (see *Medical Devices—Bacterial Endotoxin and Pyrogen Tests* ⟨161⟩).

• **Labeling:** Label it in terms of USP Heparin Units, and to indicate the number of mL of Solution required per 100 mL of whole blood.

• **USP Reference Standards** ⟨11⟩

USP Heparin Sodium for Assays RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ANTICOAGULANT HEPARIN SOLUTION	Rebecca C. Potts Associate Scientific Liaison	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
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