



# i-STAT 1 SYSTEM TECHNICAL BULLETIN

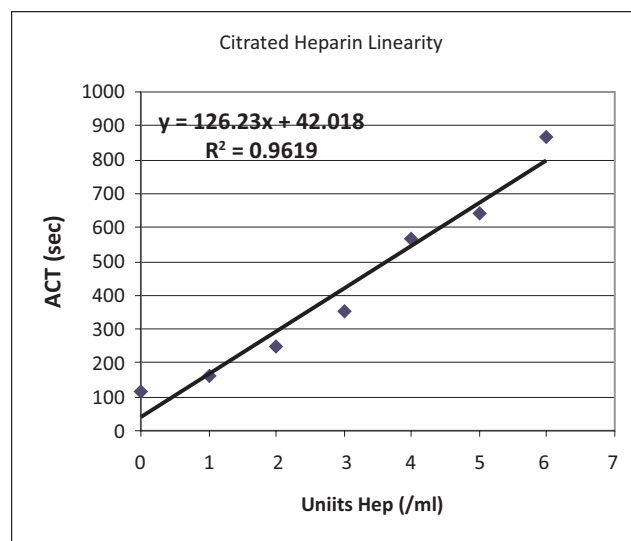
## i-STAT Celite<sup>®</sup> ACT\* and i-STAT Kaolin\* ACT Heparin Linearity Procedure

### INTRODUCTION

The i-STAT Celite ACT and the i-STAT Kaolin ACT tests are intended for *in vitro* diagnostic use. This test can be performed at the bedside using venous or arterial whole blood. The i-STAT Celite ACT is commonly used for heparin anticoagulation monitoring for adults during cardiopulmonary bypass (CPB) surgery and percutaneous transluminal coronary angioplasty (PTCA). The i-STAT Kaolin ACT is used for heparin anticoagulation monitoring during cardiopulmonary bypass (CPB) surgery, and can be used in the presence of aprotinin. The i-STAT ACT tests can be performed using the i-STAT Portable Clinical Analyzer or i-STAT 1 Analyzer. The reportable range of the i-STAT ACT test is from 50-1000 seconds.

The i-STAT ACT tests have demonstrated linearity between 0.0 and 6.0 units of heparin in blood samples from normal, healthy volunteers.

An *in vitro* heparin sensitivity curve was generated by adding increasing amounts of heparin to aliquots of normal donor blood (See graph below which serves as an example only. Each patient demonstrates a unique dose-response curve.)



\* Not all cartridges are available in all regions. Check with your local representative for availability in specific markets.

If an attempt is being made to reproduce and demonstrate the manufacturer's claims as they relate to the linearity and sensitivity of an ACT test, the following procedure may be used. An *in vitro* laboratory assay of heparin sensitivity is a universally accepted method of evaluating the ACT assay performance. An acceptable degree of linearity in a heparin dose-response sensitivity curve is an indication of ACT performance validation. Heparin sensitivity curves are generated using either citrated or fresh donor whole blood, where incremental concentrations of heparin are added to aliquots of the blood specimen. The i-STAT ACT tests can be performed using these specimens.

When performing the procedure on an i-STAT Analyzer, run the samples in the Patient Mode, as there are too many levels to run them in the Calibration Verification Mode.

## LINEARITY PROCEDURE FOR USING CITRATED WHOLE BLOOD

### Materials

- i-STAT Celite ACT cartridges or i-STAT Kaolin ACT cartridge (14). Note: Not all cartridges are available in all regions. Check with your local representative for availability in specific markets.
- Plastic test tubes, no additives (7)
- Large collection tube for heparin dilution, 10 mL minimum, no additives (1)
- Large plastic collection tube for blood pooling, 10 mL minimum, no additives (1)
- 1,000 units/mL USP Heparin (beef lung or porcine)
- Isotonic Saline (9.0 mL)
- 0.025M Calcium Chloride
- Precision pipettes (1,000 µL)
- 3.2 or 3.8% Sodium Citrate evacuated blood collection tubes (blue top) for 9mL collection (i.e., 2 x 4.5mL tubes).

### Procedure

**Note:** *Although clinical testing utilizes fresh whole blood exclusively, for the purposes of the linearity assessment, citrated whole blood can be substituted.*

**Note:** *When using a citrated whole blood source clotting times may be slightly higher than when using fresh whole blood.*

1. Obtain 14 i-STAT ACT cartridges and two i-STAT Analyzers of the same model.
2. Using a standard pharmaceutical heparin preparation (either beef or porcine derived material from any manufacturer), dilute the heparin using saline to a concentration of 100 units/mL of total volume. This can be accomplished by adding 9.0 mL of saline to 1.0 mL of standard USP heparin supplied at 1,000 units/mL.
3. Label seven (7) plastic test tubes in the following manner: "A", "B", "C", "D", "E", "F", and "G". Dispense the following quantities of the diluted heparin into the respective test tubes. The final concentration of heparin in the plastic test tubes after the addition of blood and calcium can be found in the table below.

Tube	Amount of Heparin (µL)	Final Heparin Concentration (units/mL)	Total Heparin Units
A	0	0	0
B	10	1.0	1.0
C	20	2.0	2.0
D	30	3.0	3.0
E	40	4.0	4.0
F	50	5.0	5.0
G	60	6.0	6.0

4. Obtain at least two 4.5 cc blue top (3.8% or 3.2% sodium citrate) tubes. Gently mix the tubes end to end 10 times. (Note: a total of 9.0 mL of citrated whole blood is needed and pooled in the larger collection tube)
5. Accurately dispense 0.70 mL of the citrated blood sample to each one of the seven test tubes prepared in step 3. (“A”, “B”, “C”, “D”, “E”, “F” and “G”). These are the tubes to which the heparin has previously been added. After adding the blood sample, gently mix the tubes by inversion.
6. Starting with test tube “A” add 0.30 mL of 0.025M Calcium Chloride to the tube. Mix thoroughly. (Do not add Calcium Chloride to the tube until ready to run the cartridge(s) for that heparin level.)
7. Immediately, use a plastic transfer pipet or a syringe to dispense the mixture into the sample well of 2 ACT cartridges. Begin the test.
8. Record the ACT results.
9. Repeat steps 6 through 9 for all tubes “B”, “C”, “D”, “E”, “F” and “G”.  
**Note:** Before testing tubes B – G, mix gently by inversion.
10. Record the clotting times and graph the results using, “Avg. ACT seconds” on the y-axis and “Heparin Concentration” (units/mL) on the x-axis.

### **Result Interpretation**

Inspection of the dose-response curve will identify a linear sensitivity response. Linearity is defined statistically by the correlation coefficient (r value) of the assay, which should be  $\geq 0.88$ .

#### **Notes:**

Due to the variability of heparin sensitivity, high levels may yield out of range high results. An intermediate amount of heparin can be used to perform linearity (e.g. 35  $\mu\text{L}$ ). The actual values obtained for a given heparin level will vary among donors. The heparin type (beef or porcine), manufacturer source and lot number of the heparin preparation will also affect results. The maximum concentration of heparin at which a donor’s blood will clot is dependent upon physiologic characteristics of the donor. Extremely elevated clotting times can be excluded from the analysis.

## **LINEARITY PROCEDURE FOR USING FRESH WHOLE BLOOD**

### **Materials**

- i-STAT Celite ACT or i-STAT Kaolin ACT cartridges (14). Note: Not all cartridges are available in all regions. Check with your local representative for availability in specific markets.
- Plastic test tubes, no additives (7)
- Large plastic collection tube for heparin dilution, 20 mL, no additives (1)
- 1,000 units/mL USP Heparin (beef lung or porcine)
- Isotonic Saline (9.0 mL)
- Precision pipettes (1,000  $\mu\text{L}$ )

## Procedure

1. Obtain 14 i-STAT ACT cartridges and at least two i-STAT Analyzers of the same model.
2. Using a standard pharmaceutical heparin preparation (either beef or porcine derived material from any manufacturer), dilute the heparin using saline to a concentration of 100 units/mL of total volume. This can be accomplished by adding 9.0 mL of saline to 1.0 mL of standard USP heparin supplied at 1,000 units/mL.
3. Label seven (7) plastic test tubes in the following manner: "A", "B", "C", "D", "E", "F" and "G".
4. Dispense the following quantities of the diluted heparin into the respective test tubes. The final concentration of heparin in the plastic test tubes after the addition of 1.0 mL fresh whole blood can be found in the table below.

Tube	Amount of Heparin (µL)	Final Heparin Concentration (units/mL)	Total Heparin Units
A	0	0	0
B	10	1.0	1.0
C	20	2.0	2.0
D	30	3.0	3.0
E	40	4.0	4.0
F	50	5.0	5.0
G	60	6.0	6.0

5. Using a butterfly needle and a 10 cc syringe, obtain 9.0 cc of fresh whole blood from a normal healthy donor who is not currently taking medications.
6. Accurately dispense 1.0 mL of the fresh whole blood sample to each of the seven (previously prepared) plastic test tubes A to G and gently mix by inversion.
7. Immediately using plastic transfer pipet or a syringe withdraw about 0.3 mL of the unheparinized blood from tube A and dispense into 2 ACT cartridges. Begin the test.
8. Record the ACT results.
9. Repeat steps 7-9 for blood samples "B", "C", "D", "E", "F" and "G".  
**Note:** Before testing tubes B – G, mix gently by inversion.
10. Record the clotting times and graph the results, using "Avg. ACT seconds" on the y-axis and "Heparin Concentration" (units/mL) on the x-axis.

## Result Interpretation

Inspection of the dose-response curve will identify a linear sensitivity response. Linearity is defined statistically by the correlation coefficient (r value) of the assay, which should be  $\geq 0.88$ .

**Note:** Due to variability of heparin sensitivity, high levels may yield out of range high results. An intermediate amount of heparin can be used to perform linearity (e.g. 35 µL). The actual values obtained will vary among donors. The heparin type (beef or porcine), manufacturer source and lot number of the heparin preparation will also affect results. The maximum concentration of heparin at which donor blood will clot is dependent upon physiologic characteristics of the donor. Extremely elevated clotting times can be excluded from the analysis.

# Heparin Linearity Data Collection Sheet

<b>Operator Name</b>	
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<b>Sample Type</b>	Citrated Whole Blood <input type="checkbox"/> Fresh Whole Blood <input type="checkbox"/>
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<b>Date</b>	<b>Facility Name</b>	
	<b>Analyzer Serial Number</b>	
	<b>i-STAT ACT Lot#</b>	

Hep Conc (U/ml)	Clotting Time (sec)		
	ACT 1 (sec)	ACT 2 (sec)	Average (sec)
0			
1			
2			
3			
4			
5			
6			

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