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# Demonstration of Heparin Reversal with Protamine Administration Using an Automated Protamine Dose Assay: A Comparison of Two Methods

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## Abstract

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**Systemic heparinization is required in procedures utilizing extracorporeal circulation. Once the procedure is complete, heparinization must be reversed. The drug used to neutralize heparin is protamine sulfate. The complications associated with protamine administration are well documented. An accurate and safe method of determining optimal dose is a clinically desirable tool.**

**A comparison study of 317 patients was performed using two different methods of protamine dose determinations. The first method utilized the classic Bull heparin dose-activated clotting time (ACT) curve determined prior to bypass. The second consisted of a protamine titration curve constructed using a Hemochron CA510 tube containing diatomaceous earth and a PDA699 tube containing a known amount of lyophilized protamine. The intercept of the protamine titration curve with the baseline ACT was multiplied by patient fluid volume to determine protamine dose. All ACT determinations utilized an automated technique.**

**A comparison of the two methods showed that 95% of the sample ( $p < 0.001$ ) returned to an ACT baseline with a decreased protamine administration using the protamine titration assay.**

## Introduction

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Systemic heparinization is required in procedures utilizing extracorporeal circulation to inhibit the coagulation process which occurs when blood comes in contact with the extracorporeal circuit. Once this procedure is complete, heparinization must be reversed. The drug used to neutralize this heparin is protamine sulfate.<sup>1</sup>

Protamine is an amino acid composed of 67% arginine resulting in a highly alkaline polycationic compound. The multiple positive charges on protamine associate with the negatively charged phosphate groups on DNA to form the nucleoprotamine.<sup>2</sup> The protamine protein has two active sites; one neutralizes heparin and the other exerts a mild anticoagulant effect. When protamine is administered in the presence of heparin, which is strongly acidic, the two bind to form an inactive stable salt. Once the point of complete heparinization reversal has been reached, a plateau is observed where neither drug has an anticoagulating effect.<sup>3</sup> Continuation of protamine administration beyond this plateau stage could result in anticoagulation. Protamine administration has also been associated with such side effects as:

- pulmonary vasoconstriction
- systemic hypotension
- bleeding
- cardiac decompensation.<sup>4</sup>

Therefore, it is important to determine accurate protamine infusion doses.

There are several methods available to the perfusionist to determine the protamine sulfate dose. They fall into two main categories. One is the use of an indexed clotting time to determine heparin activity. This includes the Lee-White clotting time and the activated clotting time (ACT). The other main category for determination of heparin activity is the use of heparin and protamine dose assays (PDA). These methods include the Hepcon Method<sup>a</sup> and Hemochron<sup>b</sup> Protamine Dose Assay.

A common application of the activated clotting time is the use of an automated ACT determination to obtain a classic Bull Dose Response Curve.<sup>6</sup> A baseline ACT is determined. After heparin administration another

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ACT test is conducted. Then the patient's heparin concentration is multiplied by the patient's kilogram body weight and by an index factor to obtain a protamine dose for heparin neutralization. This method was used as the basis for a comparison of the techniques with the automated protamine dose assay.

## Materials and Methods

The method used at the Johns Hopkins Hospital is: prior to patient heparinization, a 2.5 ml blood sample was drawn and 2.0 ml introduced into an ACT test tube to determine the patient's baseline clotting time. The heparin dose was calculated utilizing a 300 units heparin/kg protocol. Patients were heparinized to a target ACT of 480 seconds. A two-point dose - response curve was constructed and subsequent maintenance heparin doses were calculated from this curve. Additional heparin was administered as needed to maintain the desired ACT extension.

Prior to patient removal from cardiopulmonary bypass, a protamine dose-response neutralization assay was performed. This was accomplished by obtaining a 5 ml blood sample from the cardiopulmonary bypass circuit. Two simultaneous ACT's were performed by addition of 2.0 ml of blood to one CA510 (black-top) test tube and 2.0 ml blood sample to the PDA699 (grey-top) test tube. If the ACTs during bypass were in excess of 600 seconds, the PDA700 (blue-top) test tube was used instead of the grey-top tube. The black-top tube contained 0 mcg/ml protamine, the grey-top tube contained 15 mcg/ml protamine and the blue tube contained 20 mcg/ml.

An adjustment for the change in patient blood volume during extracorporeal circulation was necessary. The adjusted blood volume was calculated by addition of the patient blood volume, which was determined from a nomogram, to the net volume change during bypass, fluid intake minus fluid remaining in the circuit.

Fluid intake consisted of —pump prime  
—cardioplegia

Fluid output consisted of—volume remaining in  
extracorporeal circuit

Earlier experiences with this method had shown that other changes in patient fluid status did not significantly contribute to the final blood volume calculation.<sup>6</sup>

The adjusted blood volume was recorded in the appropriate space and the two ACT results on the protamine dose assay graph (Figure 1), the black on the 0 mcg/ml line (pt. B), and the grey tube result on the 15 mcg/ml line (pt. C). The baseline ACT was drawn along the axis (pt. A), then a line drawn from

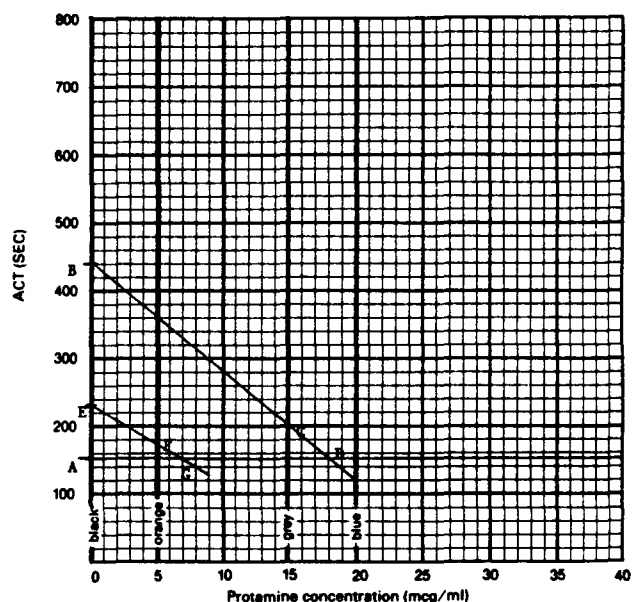


Figure 1. Graph of protamine dose determination utilizing the protamine dose assay.

pt. B to pt. C and extended through the baseline. At this intercept (pt. D), the protamine concentration in mcg/ml required to neutralize the patient's heparin load is determined.

On the worksheet, mcg/ml were converted to mg/ml. The patient's protamine dose was then determined by multiplying the protamine concentration by the adjusted blood volume. This was then multiplied by the index factor to give the infusion dose. The index factor was determined by dividing the unit protamine concentration (UPC) of the clinical protamine used by the UPC of the protamine used in the PDA. For the Johns Hopkins Hospital, the index factor was 1.0. The index factor is a ratio of relative reactivity of different protamines from different suppliers.

After the patient was removed from cardiopulmonary bypass, protamine was administered. Five minutes following completion of protamine infusion, a 5.0 ml blood sample was drawn. Two ACTs were simultaneously performed by addition of 2.0 ml blood to each of two ACT tubes, one black and one orange. (The orange tube contained 5 mcg/ml protamine.)

The ACTs were recorded and additional protamine was administered as required by the guidelines of the following chart (Figure 2):

If the ACT results indicated additional protamine administration was necessary, the calculations were repeated in a similar fashion. On the same graph, a second dose - response neutralization curve was constructed. The black top ACT result was plotted on the 0 mcg/ml line (pt. E), and the orange top ACT tube

BLACK TOP	ORANGE TOP	INTERPRETATION
at baseline	at baseline	no further infusion
at baseline	prolonged*	no further infusion
prolonged*	shortened from black top	additional protamine indicated, calculate dose from new curve and infuse
prolonged*	prolonged*	monitor for possible protamine excess or coagulopathy

\*Prolonged relative to baseline value

Figure 2. Chart used to determine if additional protamine dose administration was necessary.

result on the 5 mcg/ml line (pt. F). A line connecting the two points was extended to intersect the baseline value. The protamine concentration was converted to mg/ml and multiplied by patient blood volume. The required protamine dose was then infused. Five minutes post-infusion the ACT was retested to verify return to baseline status.

## Results

A comparison study between the conventional Bull ACT method and the protamine titration method was conducted. The patient sample size was 317. The population consisted of:

- orthotopic heart transplant (n = 6)
- coronary artery bypass grafting (n = 216)
- heart valve replacement (n = 35)
- combination bypass grafting and valve replacement (n = 21)
- aortic aneurysm repair (n = 8)
- composite graft (n = 8)
- redo valve replacement (n = 11)
- redo CAB (n = 11)

Using the Bull method, the mean protamine dose was 277 mg with a range of 85–650 mg. With the PDA method, mean protamine dose was 147 mg with a range of 60–300 mg. The calculated protamine dose using the PDA method was less in 95% of the patients in this study group (Figure 3).

Seventeen percent of the patients required additional protamine as determined by a second PDA utilizing the black and orange tubes. The mean additional dose was 35 mg with a range of 10–102 mg. The patients who received additional protamine as a result of the second PDA still received less than the original Bull calculated dose. The mean total dose of this group was 163 mg with a range of 80–260 mg.

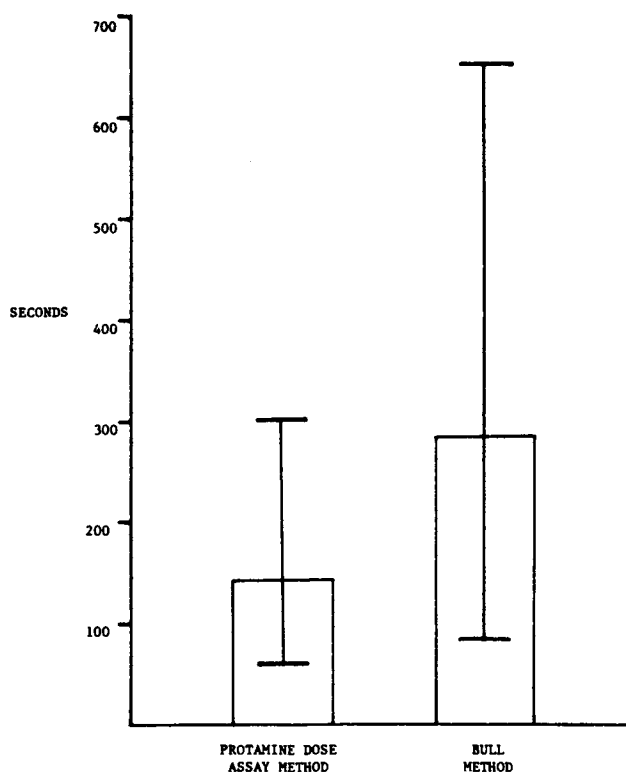


Figure 3. Graph demonstrating decrease in protamine administration utilizing the protamine assay.

## Discussion

All patients exhibited complete heparin reversal as determined by ACTs returning to patient's individual baseline. None of our patients exhibited a protamine excess as determined by the assay. The incidence of post-operative bleeding that required mediastinal reexploration was unchanged from the population that received a protamine dose determined from a Bull dose response curve prior to this PDA study. A change in the postoperative chest drainage volume was not appreciated in the group where a change in the protamine dose calculation methods was used. The decrease in protamine administered did not contribute to postoperative hemorrhage. When complete heparin reversal was confirmed by a return to baseline values and the existence of visible operative bleeding was noted, other coagulation parameters were examined and an appropriate treatment was instituted.

Protamine administration is a necessary treatment to reverse heparin in patients undergoing extracorporeal circulation. The inherent risks of this drug are many and can contribute to patient's morbidity and/or mortality. The ability to most accurately calculate the exact dose of protamine necessary for total heparin

neutralization is a clinically desirable tool. The PDA method has shown its utility in achieving this end. The data revealed a decrease in protamine administration utilizing the Protamine Dose Assay of 47% from the Bull calculated dose. Our results show that 95% of our sample returned to ACT baseline with a decreased protamine administration.

## References

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## Questions from the Audience

*Question:* Is the heparin or the protamine in the titration tubes of the same manufacturer that you use clinically?

*Answer:* One of the calculations that we use is the protamine is judged on a clinical basis and depending on the manufacturer, you may multiply the dosage by 1.0 or 1.2. Our protamine sample that we use in our institution has the same concentration as the basis for the test so we multiply 1.0.

*Frank LaDuca, Edison, NJ: Question:* It has been my good fortune to work with guys in his group while they were working with this assay and just have two short comments about the incidence of giving a second dose. Number 1, we tried to minimize the amount of protamine that was given and therefore by nature of any clinical assay, you are going to have a certain incidence which you have to give a second dose. Secondly, this is probably not a bad idea. There is a recent report in the *Journal of Thoracic and Cardiovascular Surgery* that shows that if you have a standard dose that you want to give, it is more effective in reducing blood heparin level to give it in two or equal doses as opposed to one large dose. Then my question to Dennis is, to let him finish this out, did you find in the OR that it was inconvenient to give a second dose?

*Answer:* No. It was no inconvenience at all.