

# Influence of Heparin on Fibrinogen Assay by Clauss Method

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**Abstract:** *Objective:* To investigate the effect of heparin on fibrinogen detection by Clauss method. *Methods:* A normal plasma pool (NPP) of 20 healthy people was prepared with 3.2% sodium citrate anticoagulant. For experimental group 1, samples containing different concentrations of heparin were prepared. For experimental group 2, samples of experimental group 1 were diluted twice. For experimental group 3, samples of experimental group 1 were diluted 4 times. For the control group, samples of normal saline with the same volume as heparin in experimental group 1 were prepared. The fibrinogen contents of experimental group 1, experimental group 2, experimental group 3, and control group were detected by Clauss method and prothrombin time (PT)-derived method, and the fibrinogen detection results of different groups were analyzed. *Results:* The trend of fibrinogen detected by Clauss method and PT-derived method in experimental group 1 was different; there was significant difference between the results of experimental group 1 and the control group ( $P < 0.05$ ); there was no significant difference between the results of experimental group 2 and the control group ( $P > 0.05$ ); there was no significant difference between the results of experimental group 3 and the control group ( $P > 0.05$ ); there was no significant difference between the results of experimental group 2 and experimental group 3 ( $P > 0.05$ ); the relative deviation between experimental group 1 and the control group was higher in high-concentration heparin sample. *Conclusion:* Heparin affects fibrinogen detection by Clauss method, and the effect can be reduced by sample dilution.

**Keywords:** Heparin; Clauss method; PT-derived method; Fibrinogen

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## 1. Introduction

Heparin is often used in the treatment of venous thrombosis, arterial thrombosis, and disseminated intravascular coagulation (DIC) caused by various factors <sup>[1]</sup>, as well as in the prevention of thrombosis after major surgery and thrombosis recurrence. It is the first choice of anticoagulant in cardiac surgery and vascular surgery. Activated partial thromboplastin time (APTT) is usually monitored 6 hours after administration of heparin, and it is generally prolonged to 1.5–2.0 times the baseline level. Heparinized patients should be monitored with whole-blood clotting time (CT) or activated clotting time (ACT).

Fibrinogen detection is one of the commonly used coagulation function tests in clinical practice. Fibrinogen can be detected by various methods, including Clauss method, prothrombin time (PT)-derived method, immunoturbidimetry, etc. <sup>[2,3]</sup>. Clauss method is based on solidification time and is widely used in laboratories. The PT-derived method is a fibrinogen detection method based on the change of absorbance when PT is measured. The change of absorbance of the reaction system during PT detection is positively correlated with the concentration of fibrinogen <sup>[4]</sup>. Immunoturbidimetry is a quantitative test based on

antigen-antibody reaction. However, it is not a routine test in clinical laboratories. The detection of fibrinogen by Clauss method is affected by direct thrombin inhibitors, such as bivalirudin, and dabigatran. In addition, Zhang demonstrated that argatroban can also affect the detection of fibrinogen by Clauss method [5]. The effect of heparin on fibrinogen detection is unclear. This study might help clinicians understand the effect of heparin on fibrinogen, so as to better evaluate the status of patients. This study provides some reference to the understanding of the effects of other drugs on fibrinogen.

## **2. Materials and methods**

### **2.1. General information**

The blood of 20 healthy people was collected into 3.2% sodium citrate anticoagulant to prepare a normal plasma pool (NPP). Supernatant was extracted after centrifugation at 1500 g for 15 min. Experimental group 1 was prepared by adding NPP and heparin (heparin sodium for injection, Ma'anshan Fengyuan Pharmaceutical Co., Ltd.) to make 0 U/mL, 0.25 U/mL, 0.5 U/mL, 0.75 U/mL, 1 U/mL, 1.25 U/mL, 1.5 U/mL, 1.75 U/mL, 2 U/mL, 3 U/mL, and 4 U/mL heparin samples. Experimental group 2 was prepared with the sample of experimental group 1 after 2-fold dilution, experimental group 3 was prepared with the sample of experimental group 1 after 4-fold dilution, and the sample prepared by replacing the heparin used in the experimental group with saline was assumed as the control group.

### **2.2. Fibrinogen detection**

#### **2.2.1. Experimental equipment**

The equipment used in the experiment was the ACL TOP 750 automatic coagulation analyzer (Werfen, Instrumentation Laboratory, MA, USA).

#### **2.2.2. Experimental reagents**

The reagents used were Fibrinogen-C XL (Werfen, Instrumentation Laboratory, MA, USA) and RecombiPlasTin 2G (Werfen, Instrumentation Laboratory, MA, USA).

### **2.3. Statistical analysis**

IBM SPSS 23.0 was used to process data. Paired sample *t*-test was used for comparison between groups, and the test level was  $\alpha = 0.05$ .  $P < 0.05$  was considered statistically significant.

### **2.4. Experimental procedure**

Fibrinogen in experimental group 1 was detected by Clauss method and PT-derived method, while fibrinogen in experimental group 2, experimental group 3, and the control group was detected by Clauss method; the results were recorded.

The relative deviation of fibrinogen between experimental group 1, experimental group 2, experimental group 3, and the control group was compared, respectively.

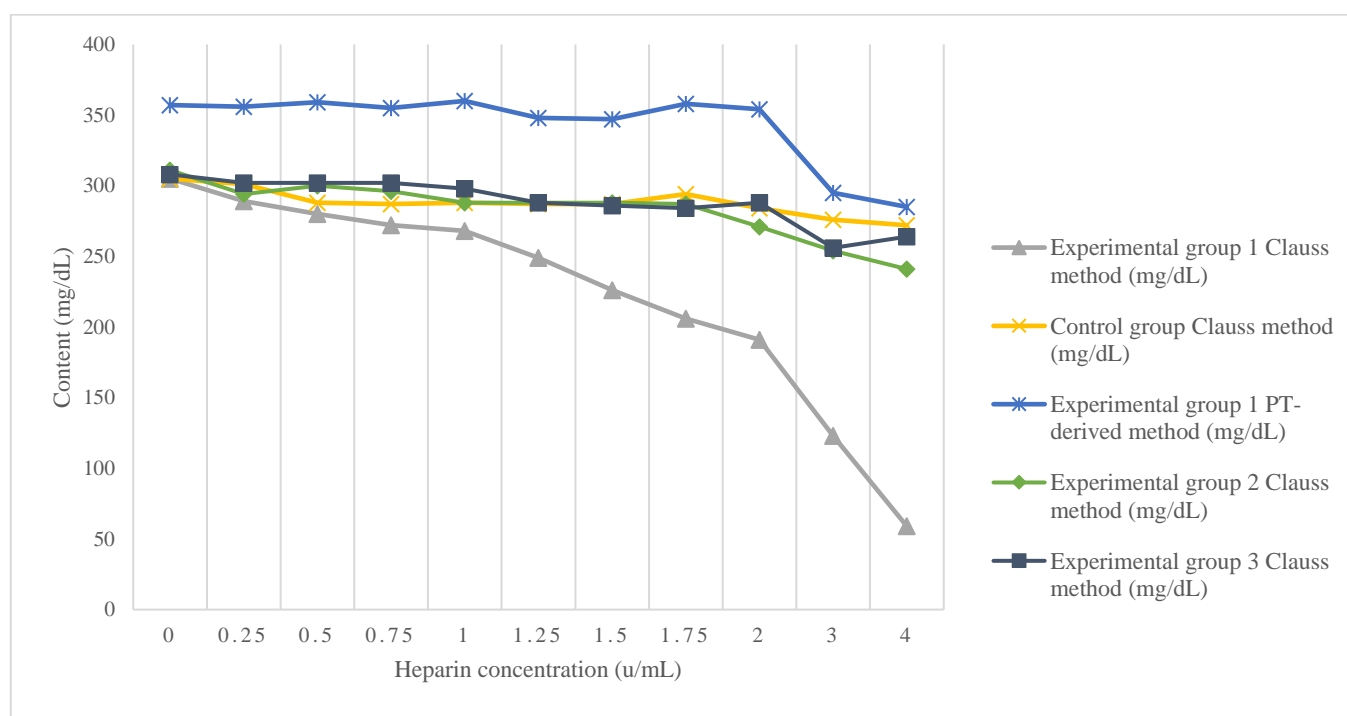
## **3. Results**

### **3.1. Trend of fibrinogen detected in the experimental groups and the control group**

The fibrinogen detected by Clauss method and PT-derived method in experimental group 1 and the fibrinogen detected by Clauss method in experimental group 2, experimental group 3, and the control group are shown in **Table 1**. The trend of fibrinogen detected by Clauss method in experimental group 1, experimental group 2, experimental group 3, and the control group as well as the trend of fibrinogen detected by PT-derived method in experimental group 1 are shown in **Figure 1**.

**Table 1.** Fibrinogen detected in experimental group 1, experimental group 2, experimental group 3. and the control group

|                            |  | Heparin concentration (U/mL) |      |     |      |     |      |     |      |     |     |     |
|----------------------------|--|------------------------------|------|-----|------|-----|------|-----|------|-----|-----|-----|
|                            |  | 0                            | 0.25 | 0.5 | 0.75 | 1   | 1.25 | 1.5 | 1.75 | 2   | 3   | 4   |
| Fibrinogen results (mg/dL) | Experimental group 1 Clauss method     | 305                          | 289  | 280 | 272  | 268 | 249  | 226 | 206  | 191 | 123 | 59  |
|                            | Control group Clauss method            | 305                          | 301  | 288 | 287  | 288 | 287  | 287 | 294  | 284 | 276 | 272 |
|                            | Experimental Group 1 PT-derived method | 357                          | 356  | 359 | 355  | 360 | 348  | 347 | 358  | 354 | 295 | 285 |
|                            | Experimental group 2 Clauss method     | 311                          | 294  | 300 | 296  | 288 | 288  | 288 | 287  | 271 | 254 | 241 |
|                            | Experimental group 3 Clauss method     | 308                          | 302  | 302 | 302  | 298 | 288  | 286 | 284  | 288 | 256 | 264 |



**Figure 1.** Trend of results of fibrinogen detection by Clauss method in experimental group 1, experimental group 2, experimental group 3, and the control group, as well as the trend of results of fibrinogen detection by PT-derived method in experimental group 1

### 3.2. Relative deviation of fibrinogen detected by Clauss method in the experimental groups and the control group

The fibrinogen detected by Clauss method in the experimental groups and the control group was statistically analyzed using paired sample *t*-test. The results are shown in **Table 2**. When comparing the results of experimental group 1 with those of the control group, *P* was less than 0.05, indicating that the difference was significant; when comparing the results of experiment group 3 with those of the control group, *P* was more than 0.05, indicating that the difference was insignificant; when comparing the results of experimental group 3 with those of the control group, *P* was more than 0.05, indicating that the difference was insignificant; when comparing the results of experimental group 2 with those of experimental group 3, *P* was more than 0.05, indicating that the difference was insignificant.

**Table 2.** Relative deviation of fibrinogen detected by Clauss method in the experimental groups and the control group

|                        |  | Heparin concentration (U/mL) |       |       |       |       |        |        |        |        |        |        |
|------------------------|--|------------------------------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|
|                        |  | 0                            | 0.25  | 0.5   | 0.75  | 1     | 1.25   | 1.5    | 1.75   | 2      | 3      | 4      |
| Relative deviation (%) | Experimental group 1 and control group | 0.00                         | -3.99 | -2.78 | -5.23 | -6.94 | -13.24 | -21.25 | -29.93 | -32.75 | -55.43 | -78.31 |
|                        | Experimental group 2 and control group | 1.97                         | -2.33 | 4.17  | 3.14  | 0.00  | 0.35   | 0.35   | -2.38  | -4.58  | -7.97  | -11.40 |
|                        | Experimental group 3 and control group | 0.98                         | 0.33  | 4.86  | 5.23  | 3.47  | 0.35   | -0.35  | -3.40  | 1.41   | -7.25  | -2.94  |

#### 4. Discussion

The research results showed that heparin has a certain influence on the detection of fibrinogen by Clauss method and the influence of heparin on the results can be weakened by 2-fold dilution and 4-fold dilution. The influence of heparin on the detection of fibrinogen by Clauss method is based on the principle of heparin action and the principle of the method itself. Heparin binds to antithrombin (AT), causing it to undergo conformational change and further forming a triplex complex with thrombin, thereby irreversibly inhibiting serine proteases with coagulation activity, including thrombin, and interfering with the clotting time. The working principle of Clauss method is that thrombin acts on the fibrinogen in the plasma and converts it into fibrin, and the plasma coagulates. The fibrinogen content in the plasma is negatively correlated with the clotting time, and the fibrinogen content can be obtained by comparing the test results with the standard curve prepared from the reference plasma [6]. The main component of the reaction reagent in Clauss method is thrombin, and heparin is an indirect thrombin inhibitor. When the sample contains a high concentration of heparin, it is believed that it will also inhibit the thrombin in the reagent, thus affecting the detection of fibrinogen to a certain extent. In this study, there was significant difference in the fibrinogen Clauss assay results between the experimental group and the control group, which demonstrated the above inference; the results of the experimental group were lower than those of the control group, indicating that heparin has a role in reducing fibrinogen Clauss assay results.

In addition, based on the trend of the results, with the increase in heparin concentration, especially when heparin concentration is above 1 U/mL, the graph showed a gradual downward trend. This trend was not apparent in the control, 2-fold dilution, 4-fold dilution, and PT-derived method groups until the concentration of heparin reached 2 U/mL.

By calculating the relative deviation between the experimental groups and the control group (Table 2), it can be seen that the relative deviation between experimental group 1 and the control group is within an acceptable range ( $\pm 10\%$ ) when the heparin concentration is 1 U/mL or below; the relative deviation between experimental group 2 and the control group is within an acceptable range when the heparin concentration is 3 U/mL and below; and the relative deviation between experimental group 3 and the control group is within an acceptable range when the heparin concentration is 4 U/mL and below. This shows that the influence of heparin on the results can be weakened by moderate dilution.

The PT-derived method is based on the change of absorbance when PT is measured. PT reagent contains a certain amount of heparin antagonist, which can theoretically antagonize heparin at a concentration of 1 U/mL. As observed in Figure 1, the trend of fibrinogen detected by PT-derived method decreased significantly when heparin was at a concentration of 2 U/mL. This may be attributed to the 1:8 ratio of sample to reagent in the final reaction system of ACL TOP 750, indicating that the sample was diluted to a certain extent and the concentration of heparin was reduced in the final reaction system. The

effect on the results of the PT-derived method was also reflected after the heparin concentration in the sample reached 2 U/mL. However, the results of the PT-derived method are for reference only. Shapiro *et al.* [7] used blood coagulation analyzers and supporting reagents from different manufacturers to detect fibrinogen and found that the fibrinogen Clauss results were significantly lower than the PT-derived results in 35 patients with hereditary dysfibrinogenemia. Miesbach *et al.* [6] reported that the fibrinogen detected by PT-derived method in 27 patients with inherited abnormal fibrinogen was approximately 5-fold higher than that detected by Clauss method, independent of the reagents and instruments used. In another study, the specificity and sensitivity of fibrinogen antigen/activity ratio (PT-derived result/Clauss result) greater than 1.43 were both found to be 100% for the diagnosis of hereditary dysfibrinogenemia [8]. The present study did not compare the PT-derived results with those of the control group but only observed the trend of fibrinogen PT-derived results in different concentrations of heparin samples. The effect on PT-derived method was smaller than that of Clauss method.

In the fibrinogen Clauss detection reaction process of the present study, the sample was first diluted 1:10, and thrombin was added. The ratio of the sample to the fibrinogen reagent containing thrombin was in fact 1:14. The thrombin content in Fibrinogen-C XL was 35 U/mL. We believe that when the sample is diluted and the same amount of thrombin is added, the ratio of thrombin to heparin will be higher than that without dilution, thereby weakening the effect of heparin. This statement is supported by the experimental results of this study. When comparing the results of experimental group 2 and the control group, *P* was more than 0.05, indicating that the difference was insignificant; and when comparing the results of experimental group 3 and the control group, *P* was more than 0.05, indicating that the difference was also insignificant. Therefore, when there is heparin interference in the sample, dilution should be considered. However, when comparing the results of experimental group 2 and experimental group 3, *P* was more than 0.05, indicating that the difference was not significant. This suggests that the dilution factor has no effect on the results, but the linearity of the instrument should be considered when diluting.

Molinaro *et al.* [9] and Zhang [5] showed that it is not only the drug concentration that affects the detection of fibrinogen, but also the thrombin concentration in the reagent. Laboratory testing personnel should have full understanding of the instruments and reagents used. The dearth of the present study is the failure to explore whether fibrinogen reagents containing high concentrations of thrombin will also be affected and whether the detection of fibrinogen by immunoturbidimetry is also affected by heparin. There have been reports on other thrombin-inhibiting anticoagulant drugs, such as argatroban, dabigatran, and bivalirux, that affect the detection of fibrinogen by Clauss method [5,9,10].

This study might help clinicians obtain more accurate detection results. Although there are now many anticoagulant drugs that have emerged, including indirect thrombin inhibitors (low molecular weight heparin, vitamin K antagonist warfarin, *etc.*), direct thrombin inhibitors (lepirudin, bivalirudin, argatroban, dabigatran, *etc.*), and factor Xa inhibitors (direct factor Xa inhibitor rivaroxaban, *etc.*; indirect factor Xa inhibitor fondaparinux sodium) [11], heparin still plays an irreplaceable role in the European Society of Cardiology (ESC) guidelines. Enoxaparin is recommended with high-quality evidence as an anticoagulant therapy in patients with non-ST-elevation acute coronary syndrome (NSTEMI-ACS) [12]. Therefore, it is of great significance to study the influence of heparin on the detection of common laboratory indicators by different methods. This study might prompt clinical testing of blood from heparinized patients or blood samples mixed with higher concentrations of heparin in which the test results may be affected to some extent. This study also provides solutions to laboratories when dealing with samples containing high concentrations of heparin. When laboratories encounter such samples, the validity of the results obtained by Clauss method should be inquired, and dilution should be considered to weaken the effect of heparin.

## 5. Conclusion

Heparin affects the detection of fibrinogen by Clauss method, and this effect can be reduced by diluting the sample.

## Disclosure statement

The authors declare no conflict of interest.

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