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Effects of different specimen pretreatment methods on the measurement of thyrotropin stimulating hormone in serum

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Abstract. – OBJECTIVE: The pre-analysis processing method of serum samples plays a very important role in the assurance of the quality of the entire test and the accuracy of the results. This study illustrates the importance of pretreatment methods of serum samples for the test results by comparing the effects of different pretreatment methods on the measurement of thyroid stimulating hormone (TSH) concentration in serum.

SUBJECTS AND METHODS: In this study, the concentrations of TSH of 37 patients' serum, which were treated in six different ways including the reverse mixing times after blood collection, clotting time and conditions, centrifugal speed and time, were detected on Automatic Chemiluminescence Immunoassay Analyzer, and a comparative analysis of the different results was performed.

RESULTS: For serum samples containing coagulants, the test results were significantly affected if the samples were not reversed mixing after collection. The abnormal results would be obtained with insufficient coagulation time, low reaction temperature, low centrifuge speed and insufficient centrifugation time.

CONCLUSIONS: The pre-analysis processing of serum samples is the beginning of the entire inspection process. The quality of the entire inspection will not be guaranteed if the pre-analysis processing method is irregular. Therefore, clinical laboratories should pay more attention to the pretreatment process of samples to ensure the quality of the entire inspection process.

Key Words:
Pretreatment of serum samples, Irregular treatment, Thyroid stimulating hormone, Auto-Lumo A2000Plus, Inspection quality.

Introduction

The quality of treatment for patients depends on the quality of useful information used by doctors in diagnosis. With the rapid development of clinical laboratory medicine, clinicians have become increasingly dependent on the accuracy of clinical test results. From collecting the patient's blood to reporting the results to the clinician, the inspection process can be divided into three stages: pre-analytical, analytical, and post-analytical. The pre-analysis stage is the entire process from the doctor's inquiry to the completion of sample collection and preparation for analysis. The analysis phase includes test of actual sample, while the post-analysis phase mainly includes the examination of abnormal results, reporting the results to the clinician, and sample preservation. In general, the emphasis of the quality control always focuses on the analysis phase in the clinical laboratories. However, it has been reported that the laboratory problems of about 60% came up in the pre-analysis phase[1], while the interference factors from the patient's body, such as endogenous interference in the specimen, including heterotopic antibody, autoimmune antibody, rheumatoid factor have a strong interference effect on the test analysis[2,3]. These factors cannot be controlled during clinical laboratory testing, but they should be considered in terms of the interference on abnormal results. On the other hand, improper handling of specimens, including insufficiency of blood clotting and insufficient specimen volume of serum specimens, can be reduced by standardized operating procedures in the process of clinical laboratory test[4-9].

In the pretreatment of serum specimen analysis, the factors affecting the quality control of the test mainly include the following: first, the suitability of the sampling vessel. Bowen et al[10] reported statistical differences in 17 serum markers detected using BD glass tubes versus SST tubes on DPC IMMULITE®2500 Total Triiodothyronine (TT3) (p<0.003). The differences in progesterone,

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total triiodothyronine, cortisol, thyroid binding globulin, thyroid total protein and insulin in glass and SST tubes were greater than 10%. The study indicated that high concentration of silicon-based surfactant was a potential source of interference in the immunoassay of this experiment. The authors suggest that a possible mechanism for interference is that the surfactant desorbs the antibody from the solid phase in the TT3 test[10], resulting in a pseudo increase in TT3 concentration test. Second, the differences arise from the process of the pretreatment on serum specimen, including environmental control, temperature control equipment, centrifugal conditions, collection time of each blood sample, as well as the differences in processing conditions that occur in different clinical laboratories and blood samples are often overlooked when abnormal results are presented and analyzed. The Clinical and Laboratory Standards Institute (CLSI) published guidelines on the handling of laboratory blood samples in document CLSI H18-A4[11], which recommended that the time for general items after collecting and before centrifugation should not exceed two hours. For all tubes containing additives except sodium citrate, the mixture should be gently reversed 5-10 times. At room temperature (22°C to 25°C), spontaneous and complete coagulation usually occurs within 30 to 60 minutes. If the patient receives anticoagulant therapy, the coagulation time will be prolonged, and frozen specimens (2°C to 8°C) will also delay coagulation. If insufficient time has been given to solidify the specimen, the formation of potential fibrin can cause problems with many instrumental systems, which may lead to the incorrect results. In addition, the centrifugal condition should refer to the manufacturer's documentation. Coagulation time and centrifugation conditions may not be able to fully unify and standardize in clinical laboratories, especially for some emergency tests, but the insufficient control of coagulation time and centrifugation conditions may easily lead to abnormal result[12].

Thyroid stimulating hormone is secreted by the anterior pituitary gland, which can promote the proliferation of thyroid follicular epithelial cells, and the synthesis and release of thyroid hormone. TSH is a sensitive indicator of thyroid function, and a subtle change in free thyroid concentration will lead to a significant adjustment of TSH concentration in the opposite direction[13]. Therefore, the detection of thyroid stimulating hormone in serum is particularly important. In this study, to further study the influence of serum sample pretreatment on the detection results, 37 serum samples treated with different conditions, which included the times of reverse mixing, coagulation time and conditions, centrifugal speed and time, were compared. The purpose of this study was to research the influence of different treatment conditions on test results of TSH in serum, and to indicate the importance of sample pretreatment in the whole test process.

Subjects and Methods

Research Subjects

The subjects were clinical patients in The Fifth Affiliated Hospital of Zhengzhou University, and 37 clinical specimens were collected in total.

Research Methods

The subjects maintained an empty stomach before blood collection, and the venous blood of each sample was evenly divided into six tubes containing coagulant. According to different pretreatment methods, the samples were labeled as group A, B, C, D, E and F. Serum samples were pretreated according to different treatment methods (such as reversed mixing times, coagulation time and conditions, centrifugal speed, and time) as shown in Table I, and then, the TSH concentration in serum was detected.

Experimental Apparatus

Auto-Lumo A2000Plus and thyroid stimulating hormone detection kit (magnetic particle chemiluminescence), batch number: 20191106.

Statistical Analysis

Passing-Bablok regression analysis was used to compare the results between the non-standard treatment group B-F and the standard treatment group A, and 95% confidence intervals for slope and intercept were calculated based on the standard error of regression. Bland-Altman plots were used to evaluate the difference in results between the non-standard treatment group B-F and the standard treatment group A. MedCalc statistical software V19 was used for statistical analysis.

Results

Regression Analysis of Test Results

The TSH concentrations of 37 clinical specimens were measured in parallel by using the

Table I. Different pretreatment methods of serum samples.

Group	Reverse mixing times after blood collection (times)	Coagulation time of blood (min)	Coagulation temperature (°C)	Centrifugal speed (g)	Centrifugation time (min)
A	8	30	25±2	1500	10
B	0	30	25±2	1500	10
C	8	30	15±2	1500	10
D	8	10	25±2	1500	10
E	8	30	25±2	800	10
F	8	30	25±2	1500	5

non-standard sample pretreatment method B-F and the recommended pretreatment method A on the Auto-Lumo A2000plus instrument for Passing-Bablok regression analysis of specimen detection results. The results (Figure 1) showed that there was no significant difference between treatment group B-F and treatment group A from statistical analysis. However, it is worth noting that the slope of treatment group B relative to the standard treatment group A is 0.906 compared with the C-F group, and the relative change trend of detection value is the largest (Figure 1A). This result indicated that TSH in serum was greatly affected by the treatment mode of non-standard treatment in group B ($p < 0.0001$). In addition, regression analysis was conducted between treatment group C-F and treatment group A, and the slope was between 0.95-1.01, without significant bias (Figure 1).

Sample Difference Analysis

Statistical analysis was conducted on the deviation of the test results of specimens in the treatment group B-F and group A that was greater than 20%. The statistical results were shown in Table II. The results showed that there were 9 cases in the treatment group B-F, and the deviation was greater than 20% compared with A, among which there were 8 cases in the pretreatment group B, and results with a deviation greater than 20% were all lower results.

Discussion

This study found that when TSH concentration in serum was detected on the Auto-Lumo A2000Plus platform, the pretreatment of serum samples would affect the detection results, and the influence degree was different in treatment conditions, including the number of reversed mixing after blood collection, coagulation time and conditions, centrifugation speed and time. Among them, the reverse mixing of blood vessels after blood collection had the greatest impact on the detection results of TSH concentration in serum, showing a holistic bias. Significant deviation between the test values of some samples and the control values occurred in the detection of coagulation time, coagulation temperature, centrifugal speed, and centrifugal time. In addition, both the overall bias in these non-standard sample pretreatment methods and the evident deviation in individual samples tended to be lower than the control values. TSH detection reagent is the reaction principle of double-antibody sandwich method, which may be theoretically interfered by endogenous factors in serum samples, such as heterotopic antibodies and rheumatoid factors. In fact, interference factors had nothing to do with the pre-analytical treatment of specimens in this study. Koch et al[12] found a pseudo elevation of troponin I (TNI) during use of BD rapid coagulation tubes on the Roche Cobas E411 platform. The paper suggested that the pseudo elevation may be caused by fibrin residues in plasma. In addition, Dimeski et al[13] showed that troponin I was pseudo elevated when heparin lithium plasma tubes were used in Beckman Access 2 and DXI800 platforms. The interference mechanism proposed by Er et al[14] is that the antibody binds to the fibrin in non-specific way, or the indicator enzyme may be physically captured by the fibrin in the isolated matrix. Dimeski et al[13] believe that fibrin is unlikely to cause false positive results in TNI, because both antibodies (capture and signal) must bind to the fibrin and remain in the reaction cup after washing to cause a false elevation. If antibodies and serum samples in combination with the non-specific fibrous protein, Nosanchuk et al[15] think it would lead to Abbott AxYSM analyzer pseudo elevated cTnI test result. Roberts et al[16] use Dade Stratus II immune analysis method in incomplete clotting serum specimens also found the same phenomenon; however, it can solve this problem by using

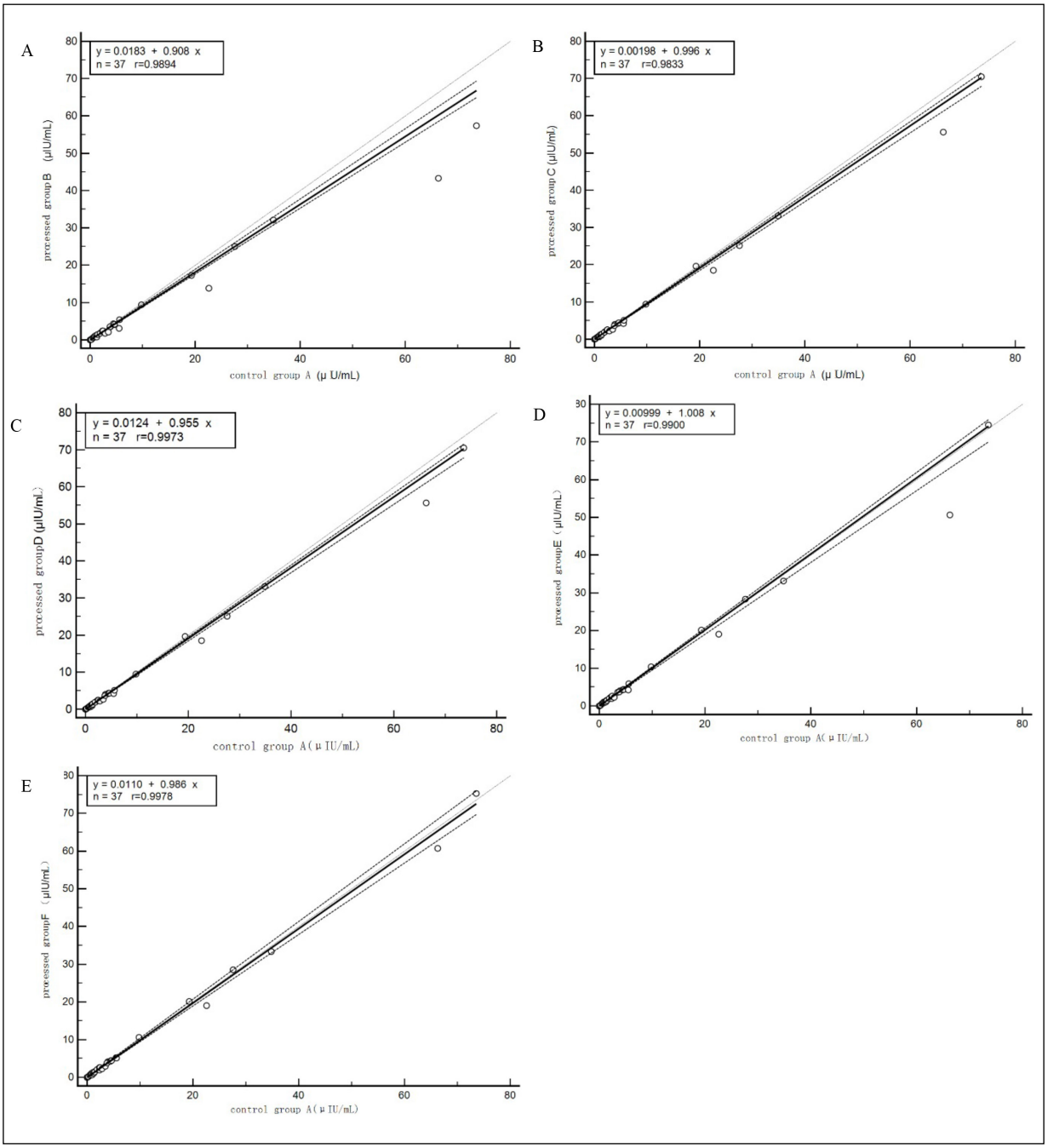

Figure 1. TSH test results regression analysis about pretreatment group B-F and pretreatment group A.

plasma samples for determination of troponin. In the subsequent study of Kazmierczak et al[17], false positive results of troponin I on Abbott Axsym platform were found to be correlated with fibrin interference. The authors suggest that it usually takes up to 20 minutes for non-anticoagulant blood to clot. If the specimen is contaminated with heparin and other reasons, fibrin may interfere with the detection system.

Fibrin is a potential interference substance commonly seen in clinical laboratories[18-20], and the fibrin residue usually emerges in the collection and post-collection processing of blood samples. The presence of fibrin in the form of visible clots may cause pin blockage in the sample needle on the automatic analyzer. If the fibrin is present in the more subtle form of invisible microfibers, it will cause abnormalities in the test results. Al-

Table II. Non-standard pretreatment sample difference analysis.

Number	Test Value of pretreatment Group A (μIU/mL)	Test Value of pretreatment Group B (μIU/mL)	Value deviation between group B and group A	Test Value of pretreatment Group C (μIU/mL)	Value deviation between group C and group A	Test Value of pretreatment Group D (μIU/mL)	Value deviation between group D and group A	Test Value of pretreatment Group E (μIU/mL)	value deviation between group E and group A	Test Value of pretreatment Group F (μIU/mL)	Value deviation between group F and group A
P6	0.229	0.159	-30.6%	0.172	-24.9%	0.175	-23.4%	0.194	-15.2%	0.205	-10.3%
P11	0.924	0.951	3.0%	0.985	6.6%	0.655	-29.1%	0.875	-5.3%	0.689	-25.5%
P15	1.263	0.825	-34.7%	1.075	-14.9%	1.040	-17.7%	1.058	-16.2%	1.062	-15.9%
P22	2.797	1.752	-37.3%	2.414	-13.7%	2.270	-18.8%	2.323	-16.9%	2.334	-16.6%
P24	3.449	2.099	-39.2%	3.863	12.0%	2.653	-23.1%	3.664	6.2%	2.910	-15.6%
P29	5.477	3.122	-43.0%	4.327	-21.0%	4.208	-23.2%	4.284	-21.8%	5.306	-3.1%
P33	22.571	13.836	-38.7%	18.714	-17.1%	18.546	-17.8%	19.051	-15.6%	19.084	-15.5%
P36	66.280	43.314	-34.6%	47.570	-28.2%	55.626	-16.1%	50.657	-23.6%	60.730	-8.4%
P37	73.562	57.402	-22.0%	77.594	5.5%	70.506	-4.2%	74.519	1.3%	75.341	2.4%

though fibrinogen cannot be identified by naked eyes, it can directly affect the test results[21-24]. Unlike endogenous intruders, the interference of fibrin is usually non-repeatable, and disappears as the fibrin precipitates out of the sample. Specimen standardized handling during the pre-analysis phase can help reduce fibrin interference. In addition, the factors that affect the clotting process are also patient-related, and many conditions and treatments can affect the formation of clots. For example, pregnant women and patients on dialysis tend to have prolonged clotting time, and many patients on anticoagulant therapy also inhibit the process. Therefore, clinical laboratory staff should be aware of the above possibilities and obtain as much information as possible about the patient's diagnosis.

This study simulates several abnormal conditions of specimen status caused by non-standard specimen pre-processing methods in clinical laboratories. If the blood collection vessel is not reversed and mixed after blood collection, the coagulation additive on the inner wall is not completely in contact with samples, resulting in partial coagulation of the blood sample, while fibrinogen still exists in the serum after centrifugation, which will continue to react and interfere with the results during the detection process. The experimental results of this study indicated that TSH detection on the Auto-Lumo A2000Plus platform was greatly affected by this non-standard operation. In addition, this study also simulated the effects of insufficient coagulation time, coagulation reaction temperature too low, centrifugal speed, and centrifugation time too low on TSH detection in serum in clinical laboratory. The results of the study showed that the pretreatment conditions of the above serum samples did not produce an overall bias in the detection results, but the detection of some samples was abnormally low, and these samples were more concentrated in several treatment groups, however, the reasons for the abnormal results are still unknown.

Conclusions

As we all know, pre-analysis is particularly important for the detection process, and standardized specimen pretreatment is a prerequisite for the success of immunoassay. The previous study[25] provided standardized pretreatment recommendations through similar projects and experience. In this study, serum samples treated with different pretreatment methods were used to simulate the non-standard treatment methods of samples that might be encountered in clinical laboratories and screen the proportion of the influence of various factors on TSH test results, which provided early warnings for the standardization of sample pretreatment in clinical laboratories. On the other hand, when abnormal test results related to specimen pretreatment are found in the clinical laboratory, the effects of different treatment methods adopted in this study can be used as a reference to find out the causes of abnormal results.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Ethics Approval

The research contents and process of the project were reviewed by the Zhengzhou Railway Vocational & Technical College. The research work was approved in accordance with the international and national ethical requirements on biomedical research.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Availability of Data and Material

All data generated or analysed during this study are included in this published article.

Authors Contributions

X.D.J. conceived the study and guided to design experiments, Y.H.Z. performed experiments, X.D.J. and Y.P.Q. wrote the manuscript, and all authors have read, edited, and approved the manuscript.

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