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Research articles

Clinical experimental study of GoldMag® immunochromatography in high sensitive C reactive protein detection from whole blood and plasma

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ABSTRACT

Objective: To investigate the consistency between GoldMag® immunochromatography and immune scatter turbidimetry in hs-CRP detection.
Methods: The data of hs-CRP were collected from 78 hospitalized patients, and these patients were divided into fingertip blood group and the venous blood group according to the different blood collection methods. The whole blood and plasma level of hs-CRP was detected by GoldMag® immunochromatography and immune scatter turbidimetry. Correlation analysis was carried out to compare the data of hs-CRP obtained by different approaches.
Results: In both fingertip blood group and venous blood group, the concentration of hs-CRP determined by GoldMag® immunochromatography were positively correlated with those found with immune scatter turbidimetry. There was no significant difference in the level of hs-CRP provided by these two different approaches. ($P > 0.05$).
Conclusion: GoldMag® immunochromatographic assay has good uniformity with immune scatter turbidimetry Scatter Turbidimetric Method for hs-CRP detection. Based on the double antibody sandwich method, antibody labeled GoldMag nanoparticles capture hs-CRP from whole blood or plasma and aggregate in test line of test strip. The magnetic intensity of agglomerated GoldMag® nanoparticles collected is measured by magnetic quantitative immunoassay analyzer. The concentration of hs-CRP can be obtained according to the standard curve between concentration and magnetic intensity. GoldMag® immunochromatography is served as an easy operation, high accuracy and stable method.

1. Introduction

C-reactive protein (CRP) is a non-specific marker of a systemic inflammatory response. It has been used clinically as early as in the 1940s and 1960s. CRP is a compound that react with pneumococcal C polysaccharides to from complexes [1]. The acute phase reaction protein, CRP has a variety of biological functions and is involved in a variety of physiological and pathophysiological processes. In acute inflammation, infection, and tissue damage, CRP is mainly produced by the liver under the stimulation of cytokines (such as interleukin-6, tumor necrosis factor alpha). It is also produced, but in much lower quantities, in a number of extra-hepatic sites including: neurons, kidney, immune cells, coronary artery smoot muscle cells and atherosclerotic lesions. The half-life of CRP in the blood is around 19 h. Its concentration depends mainly on its production in the liver [2]. Values for CRP below 8 mg/L indicate that no acute infection is present. In case of such infections values may raise to 200 mg/L. In case of chronic inflammation CRP values raise insufficiently to be measured by the traditional methods. High sensitivity C-reactive protein (hs-CRP) has reference ranges below 2 mg/L. Methods used in clinical routine lack sufficient sensitivity for measuring these values. Nevertheless, they have a high predictive value. For example, patients with values < 2.0 mg/L have a significantly lower risk to develop cardiovascular disease than patients with values > 2.0 mg/L. New methods were therefore developed for measuring CRP with higher sensitivity.

In the eraly stage, hs-CRP was mainly measured by enzyme-linked immunosorbent assays. In recent years, immunoturbidimetry, latex-enhanced immunoturbidimetry, and immunolumine- scence techniques have been used to increase the sensitivity of detection. The detection limit is 0.005 to 0.10 mg/L [3]. This makes the measurement of low

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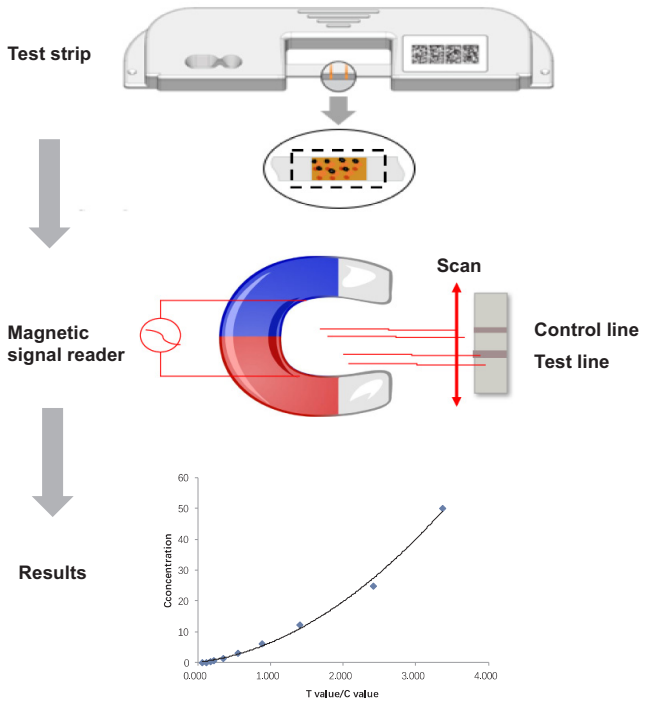

Scheme 1. Schematic illustration of the configuration and measurement principle of GoldMag immunochromatography.

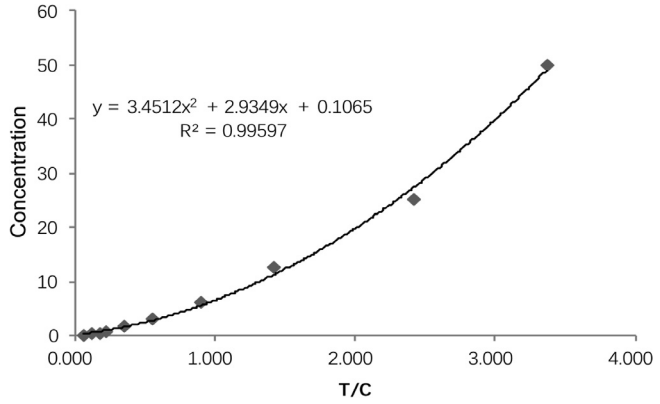

Fig. 1. Correlation between magnetic signal value on the T line and concentration of hs-CRP.

concentrations of CRP (eg 0.15 to 10 mg/L) more accurate. However, there are certain differences in results when hs-CRP is measured with different methods. The US Centers for Disease Control and Prevention and the World Health Organization have established relevant reference standards for the detection of hs-CRP [4]. As outline above, both hs-CRP and CRP measure the same protein, but the sensitivity of the methods and the range in whichthe results are linear are different. The serum CRP of healthy people is extremely small and remains constant in the human body for a long period of time, regardless of the season and the circadian rhythm. Therefore, a slight change in CRP can indicate changes in the body's physiological state. At present, hs-CRP is increasingly used in the prediction of atherosclerosis and cardiovascular-related diseases, metabolic syndrome, etc.

Immunochromatography test strip is a widely-used point of care test system. It has the advantages of lower cost, convenience, less time, high sensitivity and specificity. Based on the principle of immunochromatography, GoldMag® immunochromatography using Fe_3O_4/Au core/shell nanoparticles as carriers that not only conjugate with antibody without influence of activity, but also provide magnetic signal for quantification of test compound. As described in Scheme 1, Upon addition of samples (containing hs-CRP) onto the adjacent sample pad, the conjugates are consequently released into the migrating fluidic serum, the sandwich complex formed on the test line (T line), the GoldMag nanoparticles were detected by magnetic signal reader to obtain the intensity for this peak. The control line (C line) where rabbit IgG pre-immobilized was also read by the magnetic signal acquisition. In present study, we performed a correlation analysis between the results of hs-CRP determination by gold-magnetic particle immunochromatography and the common method based on turbidimetry.

2. Methods

2.1. Samples collection

In this prospective study, 161 patients were randomly assigned to a hospital for hs-CRP testing, regardless of disease type or test value. According to the method for blood collection, they were divided into the finger-tip blood group and the venous blood group. The finger-tip blood group consisted of 37 males and 41 females, aged from 6 months to 100 years. The venous blood group consisted of 46 males and 37 females, aged from 25 to 99 years.

2.2. The detection method

The venous blood or the capillary blood was taken from the patients. Hemolytic samples or samples with lipid induced turbidity were rejected. The hs-CRP Detection Kit produced by Xi'an Gold Magnetic Nano Biotechnology Co, Ltd. was employed. The test, gold-magnetic particle immunochromatography, uses nano-gold magnetic particles as a marker carrier and is based on a double antibody sandwich method. The detection marker is fixed in the detection area. The test compound is captured by the antibody labeled GoldMag® nanoparticles which is aggregated on the detection line. Because the intensity of the magnetic signal, caused by the gold magnetic particles, is proportional to the concentration of the marker contained in the sample, the magnetic gold particles collected at the detection line can be measured by a quantitative magnetic immunoassay analyzer (The magnetic field is about 750 gauss). The concentration of the sample can be measured by comparison with a standard curve. The data obtained were compared with those obtained with a C-reactive protein assay kit produced by Shenzhen Guosai Biotechnology. This latter kit is based on measurement of turbidity. The principle of this method is as follows: a soluble antigen reacts with specific antibodies to form insoluble complexes. When light passes through the reaction suspension the concentration of the resulting suspended particles can be measured with a nephelometer.

Table 1
Comparison of hs-CRP value from different sampling and detection methods.

Sample type	n	immune scatter turbidimetry CRP (mg/L)	GoldMag® immunochromatography hs-CRP (mg/L)	K-M value	P value	Z value	P value
Fingertip blood	78	7.66 ± 7.65	Whole blood8.75 ± 8.88	0.165	< 0.001	−0.503	0.615
			Plasma 8.14 ± 8.73	0.171	< 0.001	−0.177	0.859
Venous blood	83	13.12 ± 13.49	Whole blood12.84 ± 13.53	0.176	< 0.001	−0.594	0.552
			Plasma 11.81 ± 12.68	0.177	< 0.001	−1.019	0.308

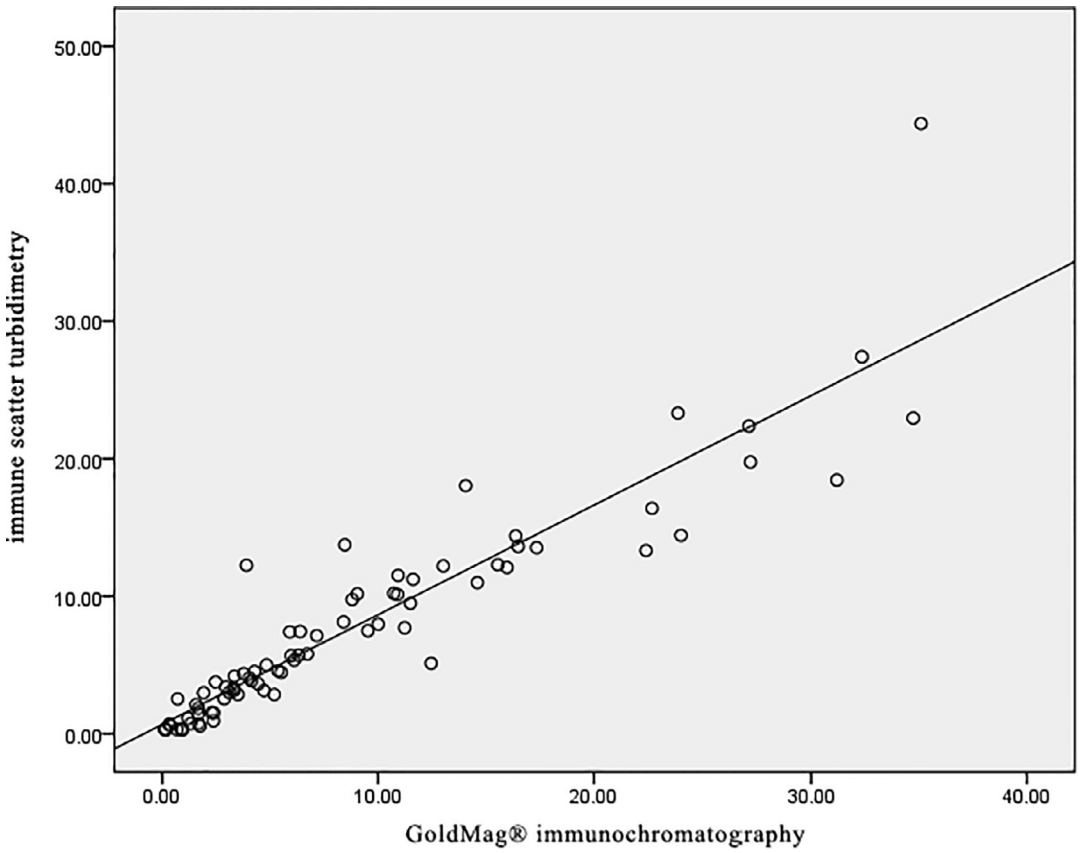

Fig. 2. Correlation analysis of values for hs-CRP measured in fingertip blood with the GoldMag® immunochromatography and immune scatter turbidimetry method (r = 0.925).

The amount of scattered light is proportional to the concentration of a specific protein in the test sample. The instrument will automatically calculate the concentration of a specific protein in the sample in our case the patient's whole blood/plasma hs-CRP.

2.3. Statistical processing

SPSS 17.0 statistical software was used for statistical analysis. All data were tested for normality. The calculated data were expressed as the mean ± standard deviation. Normal distributed data was evaluated by T test. abnormal distributed data was evaluated by Wilcoxon Signed rank sum test Correlation test between two variables was carried out by linear correlation analysis with correlation coefficient r. Samples were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Establishment of standard curve

Before quantifying the amount of hs-CRP in samples through GoldMag® immunochromatography, a standard curve was established by using hs-CRP standards. As shown in Fig. 1, a correlation between magnetic signal value of hs-CRP-GoldMag nanoparticles on the detection line and the concentration of hs-CRP was observed. R^2 values was 0.996.

3.2. hs-CRP concentration in fingertip blood group

1) By means of two methodologies, statistical analysis was performed on the hs-CRP values of finger-tip blood (whole blood/plasma) measurements in 78 patients. The samples did not meet the normal distribution. The K-M value was 0.165/0.171, and the P value was < 0.001; Using the Wilcoxon signed rank sum test, Z = −0.503/−0.177, P = 0.615/0.859, and the P values were all > 0.05. Therefore, there was no statistical difference in the values as measured by the immune scatter turbidimetry method and the GoldMag® immunochromatography method, neither in whole blood nor in plasma (Table 1).
2) Analysis of the data of the determination of hs-CRP in whole blood/plasma with the immune scatter turbidimetry method and the GoldMag® immunochromatography method. From Figs. 2 and 3, we can see that the results of the hs-CRP assays using both methods are positively correlated, both in whole blood and in plasma. The linear regression equation for the determination of hs-CRP is Y = 0.797X + 0.687/Y = 0.812X + 1.054; r^2 = 0.855/ 0.857; r = 0.925/0.962.

3.3. Venous blood group

1) Statistical analysis was performed of the data of the determination of hs-CRP of venous blood of 83 patients, using whole blood and plasma, with the immune scatter turbidimetry method and with the GoldMag® immunochromatography method. The samples did not meet the normal distribution. The K-M value was 0.176/0.177, and the P values were both < 0.001. Using the Wilcoxon signed rank sum test, Z = −0.594/−1.019, P = 0.552/0.308, all P values are > 0.05. It is therefore considered that there is no statistical difference between the results obtained with the two methods, neither in blood nor in plasma (Table 1);
2) Analysis was performed of the correlation of the results of measuring hs-CRP using whole blood and plasma with both methods. From Figs. 4 and 5, we can see that the outcome of the hs-CRP measurements with both methods was positively correlated. For the calculation of the linear regression of hs-CRP data we used the

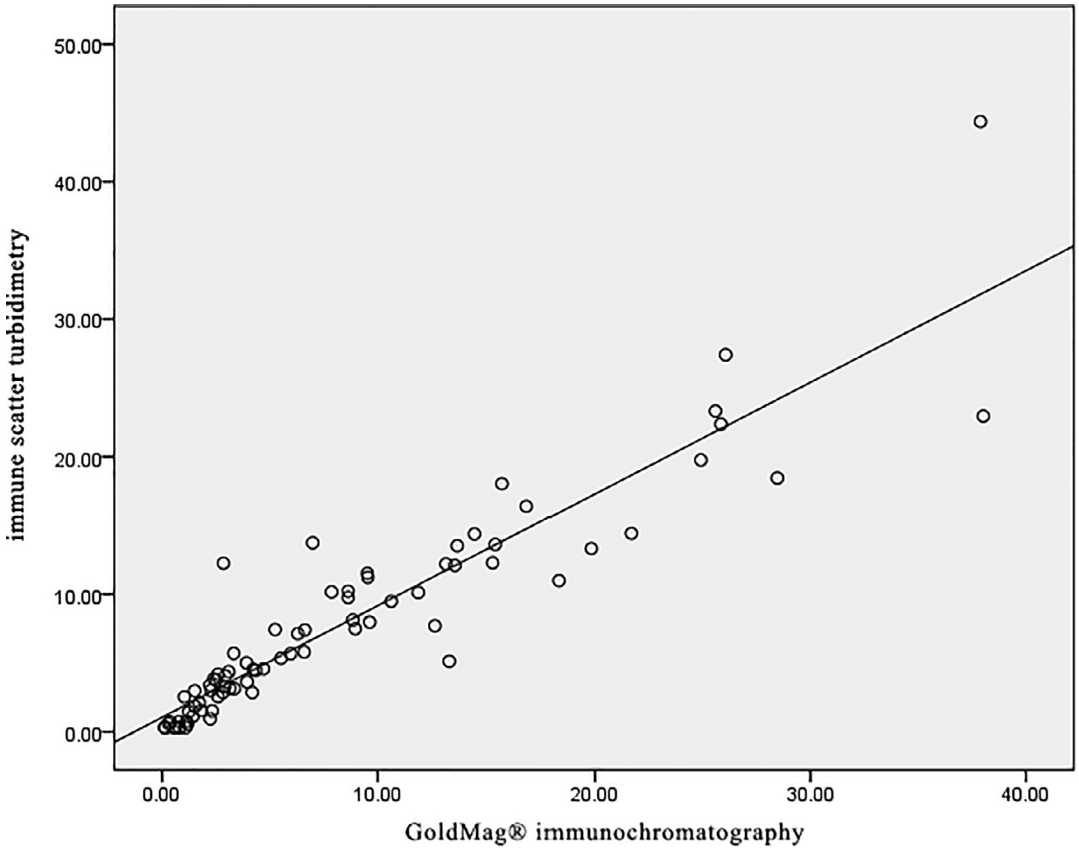

Fig. 3. Correlation analysis of the values for hs-CRP measured in plasma isolated from fingertip-blood competition and using the GoldMag® immunochromatography and immune scatter turbidimetry method (r = 0.962).

equation: Y = 0.975X + 0.607/Y = 1.034X + 0.915; r^2 = 0.956/0.944; r = 0.978/0.972.

4. Conclusion

CRP is an acute-phase protein and one of the important markers of acute infection in the clinic [5]. In the case of an infection the liver cells overexpress CRP and secrete it to the blood. It can adhere to the surface of the pathogen, activate the immune response and kill the pathogen. The level of CRP can increase rapidly in all kinds of acute infections, tissue injury, malignant tumor and surgical trauma. When the lesion is healed, the level of CRP drops to normal again. Therefore, CRP is a reliable and sensitive index for acute inflammatory response [6]. Generally, the neonatal serum CRP level is below 2 mg/L while for normal children and adults the serum CRP level is below 10 mg/L [7]. Race, sex, age, obesity, pregnancy and other factors, including a selective polymorphism of the CRP gene can affect the level of CRP [8,9]. All kinds of bacterial infections can increase the CRP level. A level of 10 ~ 99 mg/L CRP suggests the presence of a focal or superficial infection. Values above 100 mg/L suggests septicemia or an invasive infection [10].

In the past decade, CRP, especially hs-CRP draw great attention in the studies of atherosclerosis prediction, cardiovascular related diseases and metabolic syndrome [11]. The combined application of hs-CRP and multiple clinical indicators has become an important research direction [12]. The application of hs-CRP has been expanded from diagnosis of infectious diseases to the prediction and monitoring of cardiovascular diseases [13]. A large number of studies have confirmed that the increase of hs-CRP is an independent risk factor for cardiovascular disease caused by chronic inflammation, and the detection of hs-CRP level has great clinical value for the treatment and prognosis of cardiovascular disease [14]. Ping et al. found that the pathogenesis of hypertension of pregnancy was closely related to hs-CRP level [15]. The level of serum hs-CRP significantly increased in pregnant women with hypertensive disorder. Chen *et al* found that serum hs-CRP levels in patients with metabolic syndrome were significantly higher than those found with health people ($P < 0.05$), which suggest inflammatory response in the patients with metabolic syndrome [16] of the change of hs-CRP level reflect the relationship between progressive inflammation and long-term cardiovascular risk events. Combined with blood lipid and other related indicators, hs-CRP will play a vital role in clinical diagnosis. It is obvious that hs-CRP detection is very important and has been widely used for early diagnosis and diagnosis of various disease [17]. In this study, the correlation between two methods, hs-CRP detection, gold magnetic particle immunochromatography and scattering turbidimetry, has been analyzed. The results showed that there was no significant difference in the detection level of hs-CRP in whole blood/plasma samples between the fingertip blood group and venous blood group ($P > 0.05$). The results of consistency analysis of whole blood/plasma hs-CRP levels between turbidimetry and gold magnetic particle immunochromatography showed that R^2 for fingertip blood group was 0.855/0.857 and R^2 for venous blood group was 0.956/0.944. It indicates that these two detection methods had good correlation and hs-CRP detection performance.

The principle of magnetic quantitative immunoassay system is to use gold magnetic nanoparticles as a marker carrier. The gold magnetic nanoparticles showed good superparamagnetism at room temperature and were well dispersed in water with surface plasmon resonance absorption peak varied from 538 nm to 570 nm [18]. Based on the double antibody sandwich method, the test compound is fixed in the detection area. The magnetic intensity of the gold magnetic nanoparticles gathered at the detection line is measured by the magnetic quantitative immunoanalyzer. The concentration of test compound can be obtained refer to standard curve. This detection system improves signal

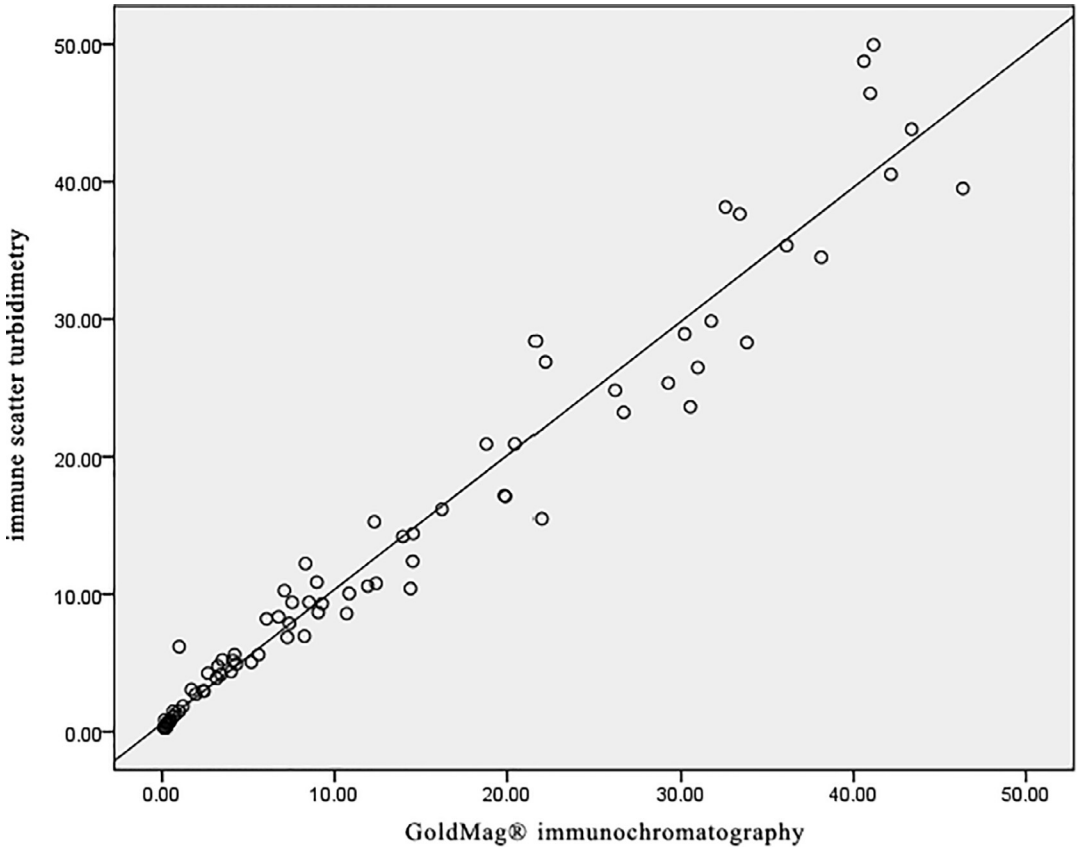

Fig. 4. Correlation analysis of hs-CRP in venous whole blood between GoldMag® immunochromatography and immune scatter turbidimetry (r = 0.978).

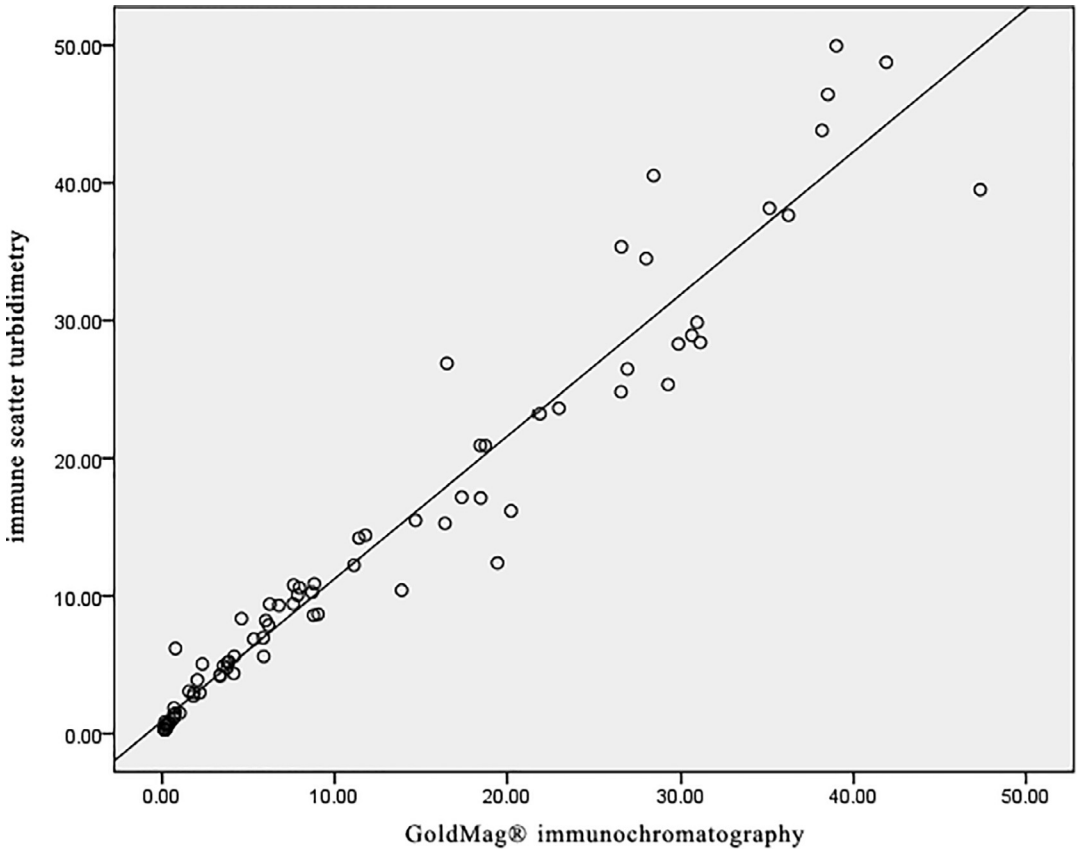

Fig. 5. Correlation analysis of hs-CRP concentration in venous plasma between GoldMag® immunochromatography and immune scatter turbidimetry (r = 0.972).

collection from 2-dimension to 3-dimension. The 3D collection of magnetic signals avoids the instability caused by the traditional heterogeneous reaction and the interference of the endogenous substances in the sample. At the same time, the test results can be retained for a long time, which effectively prevents false result caused by timeout detection and waste of reagent.

The GoldMag® immunochromatography not only has a great advantage in the methodology such as easy operation and visual interpretation, but also provide results more stable. The sensitivity can reach picogram level. There is no significant difference between the results of whole blood and plasma specimens. It is especially suitable for township hospitals and community hospitals where large medical devices were lacked. hs-CRP is more and more widely used in clinic. The detection of hs-CRP by gold magnetic particle immunochromatography can provide faster and more accurate results for clinical diagnosis.

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