Latex Agglutination Method for Determination of von Willebrand Factor Antigen in Patients with Bleeding Disorders

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Abstract. Measurement of the level of von Willebrand factor antigen (VWF:Ag) in human plasma is usually carried out in a specialized laboratory in routine clinical practice and is carried out by the ELISA method which takes a long time or immunoturbidimetry which is less sensitive and specific. The purpose of this study was to qualitatively determine the von Willebrand factor (VWF) antigen in only a maximum of 5 min using the latex agglutination method. The research design is an exploratory laboratory experiment. This study was conducted in a cross sectional manner in patients with bleeding disorders with prolonged bleeding time and healthy individuals. The research stage is to prepare a VWF polyclonal antibody from Invitrogen Ref. Pa5–16634 with Lot No. XE3587846A in 0.1M MES{2-(N-morpholino) ethane-sulfonic acid buffer, 0.9% sodium chloride, pH 6, to be sensitized to Carboxyl latex, 4% w/v 1 m. Latex particles after passive adsorption, polyclonal antibodies stick to the latex surface. The patient's citrate blood sample is taken which will be compared with the normal individual's citrate blood. The results showed agglutination in patients with bleeding disorders and no agglutination in normal individuals. In conclusion, latex reagent containing polyclonal VWF can be used as a diagnostic tool to determine VWF antigen in patients with bleeding disorders.

Keywords: von willebrand factor antigen · latex agglutination method · bleeding disorders

1 Introduction

Von Willebrand Factor (VWF) was first identified as an adhesive glycoprotein involved in hemostasis by Zimmermann in 1971. Since then, VWF has been shown to play an important role in platelet adhesion, platelet binding to collagen and protection of factor VIII. Recent studies implicate VWF as a regulator of angiogenesis, smooth muscle cell proliferation, tumor cell metastasis and crosstalk in the immune system [3].

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When a blood vessel is cut, the power in the blood flow increases and changes its character. The dark side of this power causes bleeding and death. However, von Willebrand factor (VWF), with assistance from the circulatory system and platelets, harnesses the same power to form a hemostatic plug [4]. Functions of power and VWF are very closely intertwined, thus restoring them [8].

Immunoassays play an important role in clinical laboratories. Enzyme-linked immunosorbent assay (ELISA) and latex agglutination immunoassay technologies are currently the most widely used, while Luminescent Oxygen Channeling Immunoassay (LOCI®) and other chemiluminescence-based immunoassays are emerging technologies for coagulation laboratories. However, not all immunoassay technologies used are compatible with coagulation laboratory workflow requirements, and not all technologies are suitable for the detection or quantification of every marker [1].

The current application of the method is a rapid, simple, and inexpensive test for the detection of VWF by the latex agglutination method, thereby helping in improving clinical management in resource-limited settings [2].

Latex immuno-agglutination assays continue to be widely used in biology and medicine to detect small amounts of desired antibodies or antigens in body fluid samples. The physical adsorption of proteins on the surface of latex particles, of particular relevance to immunoglobulins, was analyzed with particular attention to factors influencing adsorption, such as medium condition (pH and ionic strength), surface characteristics (type and amount of charge), or hydrophobicity. Functional latex will differ for covalent bonding, as well as the corresponding chemical reactions. Techniques for detecting and measuring immunoreactions are briefly summarized, including visual observation, light scattering, turbidimetry, nephelometry, and angular anisotropy. Finally, some of the colloidal stability problems of this latex test were analyzed, as well as the formation of different solutions [6].

Based on the explanation above, it is necessary to conduct research with the following steps, to make the composition of latex particles suitable for passive adsorption with VWF antibodies. Then do the adsorption of polyclonal antibody VWF from Invitrogen to latex particles. The final stage is a challenge test with plasma citrate of bleeding patients with the criteria for prolonged Bleeding time, and normal individuals.

2 Material and Methods

2.1 Materials

VWF Polyclonal Antibody Invitrogen, Carboxyl Latex, 4% w/v 1 μm Invitrogen, Buffer MES,0,025 M, pH 6, Pierce BCA Micro Protein Determination Kit, Wash Buffer (Phosphate-Buffered Saline (PBS), 0,1 M, pH 7,2 [9].

2.2 Determining the Quantity of Antibodies and Latex

For 1 m Carboxyl Latex Beads at a concentration of 1% solids, this reaction can be easily increased or decreased as needed. The following equation can be used to calculate the

optimal number of antibodies. If you want to label 5 mg of antibody using 1 m particles, you need 100 mg of particles with the equation:

$$Weight\ of\ Ab = \frac{Weight\ og\ Ab\ for\ 1\ \mu m\ particle}{particle\ diameter\ in\ \mu m}$$

For 100 mg of 0.3 m Carboxyl Latex particles, it requires:

$$16,7\,mg = \frac{5}{0,3}$$

For 100 mg of 4.3 m Carboxyl particles, it requires:

$$1.2\,mg = \frac{5}{4,3}$$

2.3 Latex Preparation

Pipette 2.5 ml (40 mg/ml) of latex microspheres and dilute with 10 ml of MES buffer. Centrifugation of the mixture to precipitate particles: ~ 3,000g for ~ 20 min. Discard the supernatant and disperse the pellet again in 10 ml of MES buffer. Centrifugation again and remove the supernatant from the particles. Resuspend the pellets in 5 ml MES buffer, making sure to completely suspend the microsphere particles. Latex suspension is now approx. 2% solids (ie ~ 20 mg/ml).

2.4 Antibody Absorption Protocol on Latex Particles

Antibodies were prepared in MES at 1mg/ml. To 5 ml of antibody solution in MES buffer (i.e. 5 mg Ab) added 5 ml of latex required for single layer. This order of addition will ensure the best coating of the particles with the least possible aggregation. Incubate the latex and antibody mixture at room temperature overnight. Centrifuge to separate antibody labeled latex particles from unbound proteins. Discard and store the supernatant for protein determination. Resuspend pellets in 10 ml of PBS. Centrifuge again to precipitate particles. Repeat the washing step two more times for a total of 3 washes. Resuspend the final latex in 10 ml of storage buffer giving a final concentration of 1% solids. Store at 4 °C until use.

2.5 Agglutination Test Protocol on Samples of Patients with Bleeding Disorders

Pipette 50 μL of plasma citrate patients with bleeding disorders and 50 μL of normal individuals, and 50 μL of physiological saline solution on a circular object glass, then 50 μL of latex reagent sensitized with VWF antibody was added to each circle. Then it was homogenized and rotated for 2 min, then the agglutination was observed under a microscope with a magnification of 100x.

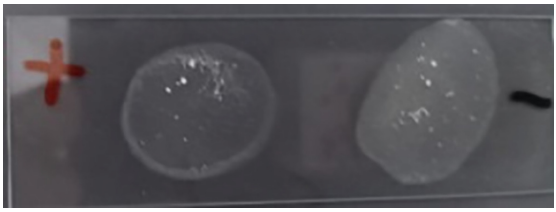

Fig. 1. Latex Agglutination Test in patients with bleeding disorders on the sign (+) and normal individuals on the sign (-).

3 Result and Discussion

3.1 Characteristics of Subjects

The subjects of the study were patients with bleeding disorders leading to VWF on screening were prolonged BT, normal PT, and normal/prolonged aPTT. The results of aPTT depended on plasma factor VIII levels. Hemoglobin (Hb) and platelets may be found to be normal/decreased, and the platelet count on the peripheral blood smear may be normal.

3.2 Characteristics Aglutinasi di atas Object Glass

The results of the latex agglutination test in patients with bleeding disorders with normal individuals, the following results were obtained,

The results on Fig. 1 Seen in the sign (+) positive VWF patient there is agglutination, on the edge of the circle and the solution is clear, whereas in the sign (-) negative VWF patient there appears no agglutination on the edge of the circle, and the solution looks cloudy (Figs. 2 and 3).

3.3 Characteristics Agglutination Under a Microscope with a Magnification of 100x

3.4 Discussion

According to Bolivar, the physical adsorption of proteins to the surface of latex particles is of particular relevance to immunoglobulins, with particular attention to factors affecting the adsorption of medium conditions such as pH and ion strength, surface characteristics of various types and amounts of charge, or hydrophobicity [7].

The results of this study were compared with studies of reproducibility, stability, linearity, limit of detection, interference, and comparison of methods carried out by evaluating the performance of this test with a strong correlated immunoturbidimetric reference test (Spearman's rho $= 0.946$, $P < 0.001$, $n = 132$). Evaluation studies indicate that the latex agglutination method is suitable as a rapid test tool for the estimation of VWF:Ag levels in various clinical conditions associated with high and low VWF:Ag levels [5].

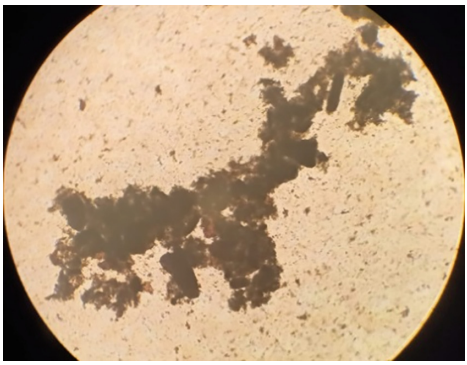

Fig. 2. Latex Agglutination Test in a bleeding disorder patient with 100x magnification, latex clots are seen.

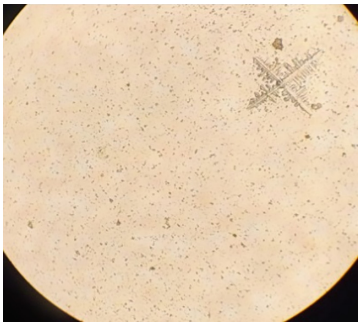

Fig. 3. Latex Agglutination Test in normal individuals with a magnification of 100x, visible no visible lumps of latex.

4 Conclusion

The VWF antigen test from citrate plasma of patients with bleeding disorders using VWF polyclonal antibodies that are absorbed into Carboxyl particles can give an agglutination reaction, so that in the future this latex reagent can be used to detect VWF in patients with bleeding disorders.

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