Development of a Lateral Flow Immunoassay Test Strip for the Rapid Detection of Rubella Virus

Truong Quoc Phong[1], Nguyen Thi Anh Hoa[1], Ngo Thu Huong[2], Pham Huong Giang[1], Khuat Huu Thanh[1]*

[1] *School of Biotechnology and Food Technology, Hanoi University of Science and Technology*

[2] *Center for Research and Production of Vaccines and Biologicals*

Received: August 16, 2017; Accepted: June 25, 2018

Abstract

This study was designed to construct a lateral flow immunoassay test strip for rapid detection of rubella virus. Rabbit anti-rubella antibody and pig anti-rubella antibody were successfully purified from sera of rabbit and pig infected with rubella virus. The yields of IgG affinity purification were 1.74 mg/ml and 3.23 mg/ml serum, respectively. Suitable conditions for conjugation of antibody and gold nanoparticles (AuNPs) were determined: 0.5 µg rabbit anti-rubella antibody was conjugated with 10 µl of AuNPs at pH 10, 37°C for 90 min. Similarly, suitable conditions for immobilization of pig anti-rubella antibody on the nitrocellulose were 3 µg of antibody, nitrocellulose membrane of Pall Vivid 170, immobilizing buffer of 20 mM sodium borate plus 2% sucrose, at 37°C for 30 min. The complete test strip was successfully constructed for rapid detection of rubella virus.

Keywords: Rubella virus, lateral flow immunoassay, rapid detection

1. Introduction

Rubella is an infectious disease caused by rubella virus and has a worldwide distribution [1]. Rubella disease usually expresses mild symptoms including a low-grade fever, sore throat and a rash with starting on the face and spreading to the rest of the body. If a woman is infected by rubella virus while she is pregnant, rubella virus can transmitted through the placenta to the fetus. Rubella can cause a miscarriage or congenital defects in a developing baby such as chronic cardiac disease, hepatosplenomegaly growth retardation, malformations, congenital deafness and other severe anomalies [2, 3].

Rubella is spread through contact with nose or throat secretions of an infected person. This may result from airborne droplet spread, direct contact with an infected person or indirect contact with freshly infected articles. Rubella mostly affects children, adolescents and young adults.

Rubella virus is the only member of the Rubivirus genus of the Togavirus family. The rubella virus is roughly spherical with a diameter of 60-70 nm. Rubella virus contains a single-stranded positive sense RNA genome. The virus contains three structural proteins surrounding RNA. Two envelope proteins (E1, E2) are glycoproteins projecting from the virus to form surface spikes. E1 protein is the dominant surface molecule and associated with neutralizing and haemagglutinating epitopes [4].

The rubella virus can be found in the blood between 5 and 7 days after infection as it spreads throughout the body. Clinical diagnosis of rubella is difficult and unreliable, as approximately 50% of infected patients are subclinical and only be detected by laboratory confirmation [5, 6]. Laboratory diagnosis can be made by testing blood or saliva samples [7]. Rubella virus infection can be detected by several methods such as RNA detection by RT-PCR, immunological test (IgG and IgM detection) or direct detection of rubella virus antigen by ELISA. These methods require a well-established laboratory and trained technicians, whereas suspected patients of rubella need to be rapidly screened at the local clinical units. Development of a lateral flow test strip could help to provide a suitable tool for rapid detection of rubella virus from suspected patients.

2. Materials and Methods

2.1. Materials

Standard rubella virus sample (live-attenuated vaccine strain), serum samples from rubella infected rabbits and pigs were from POLYVAC – Ministry of Health. Materials for generating the lateral flow strip were purchased from Shanghai JY-Biotech. The types of conjugate pad, sample pad and absorbent pad were JC-J2, GL-b02, and H5076, respectively. Protein A – Sepharose 4B conjugate was purchased from

* Corresponding author: Tel: (+84) 988793468
Email: phong.truongquoc@hust.edu.vn

Invitrogen (USA). Chemicals for IgGs purification, SDS-PAGE and buffers for the lateral flow immunoassay were purchased from Sigma-Aldrich (USA), Merck (Germany), Thermo Scientific (USA). Anti-rabbit IgG antibody was purchased from Arista Biologicals (USA). Gold nanoparticle solution was purchased from Sigma-Aldrich (USA).

2.2. Methods

2.2.1. Construction of lateral flow test strip

Lateral flow test strip for rubella detection was designed including a sample pad connecting with a conjugate pad (rabbit anti-rubella virus antibody conjugated with gold nanoparticles), then a nitrocellulose membrane with immobilized pig anti-rubella virus IgG and goat anti-rabbit IgG antibody at test and control line respectively.

2.2.2. Purification of IgG from serum

Procedure for IgG purification was performed according to the instruction of Protein A – Sepharose kit using AKTA FPLC system (GE Healthcare, USA). Briefly, the column packed with Protein A – Sepharose bead (1 ml) was equilibrated 20 volumes of 50 mM Tris-HCl buffer, pH 7.2. Serum sample (1 ml) was loaded onto the column followed by washing with 20 volume of column. The IgG protein was eluted out of column by adding 0.1 M glycine pH 2.7 and neutralized by 0.1 M Tris-HCl pH 9.5. The amount of IgG protein was quantified by Bradford assay.

2.2.3. Conjugation of IgG and gold nanoparticles

Conjugation of IgG with colloidal gold nanoparticles and conjugate pad preparation were performed according to Zhang et al., (2009) [8]. Briefly, the pH of the colloidal gold nanoparticles suspension is adjusted to approximately 9.0. The certain amount of IgG protein is added to the colloidal solution and incubated at room temperature for 1 hour. Empty space on the surface of gold nanoparticles is blocked by BSA for 15 min at room temperature. Unbound IgG is removed by centrifugation and washing twice with 20 mM sodium borate containing 1% BSA. Finally, the conjugate pellet is suspended in 20 mM sodium borate and loaded on the wick pad followed by drying at 37^{o}C for 30 min in air drying oven to generate the conjugate pad.

2.2.4. Preparation of IgG-immobilized membrane

The antibody solution is dispended onto the membrane at 1.5 µl/cm. The blotted membrane then is dried at 37^{o}C in an air drying oven. The nitrocellulose membrane is cut into pieces with the width of 3 mm.

3. Results and Discussion

3.1. Purification of anti-rubella virus IgG

Protein A, a 42 kDa surface protein from *Staphylococcus aureus* can bind specifically to Fc regions of several mammalian immunoglobulins and thus commonly used as an affinity absorbent to purify antibody from serum. In this work, anti-rubella virus IgGs from sera of rabbit and pig was purified by affinity chromatography using protein A – Sepharose 4B conjugate beads. With rabbit IgG purification, the chromatography showed the presence of two peaks on the chromatographic chart with retention times of 2.03 and 30.09 min, respectively (Fig. 1).

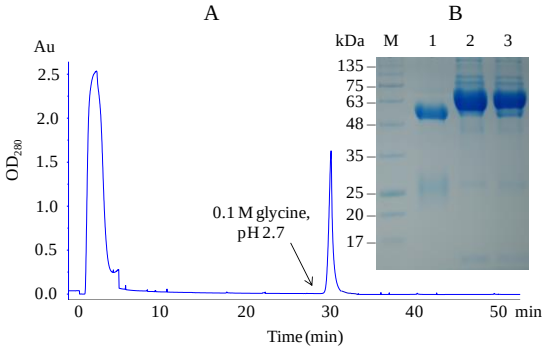

Fig.1. The chromatographic chart of rabbit anti-rubella virus IgG affinity purification using Protein A – sepharose bead (A) and protein profile (B). Lane M, protein marker (Intron, Korea); lane 1, eluted IgG fraction (peak 2); lane 2, flow through (peak 1); lane 3, whole serum.

The first peak was present just after loading sample (2.03 min) indicating that proteins in this peak were unbound proteins. The second peak appeared after adding eluent agent (glycine, pH 2.7) implicating that proteins in this peak were protein A specific binding IgGs. The linkages between protein A and IgGs are weak bonds therefore they will be broken under low pH condition to release IgGs in the eluent fraction. The protein profiles of serum sample, unbound fraction and eluent fraction were analyzed by SDS-PAGE. The obtained results showed the presence of two significant bands with size of around 50 and 25 kDa on the electrophoresis pattern (Fig 1B). These bands were predicted as the IgG heavy chain (50 kDa) and light chain (25 kDa), respectively. Observed results demonstrated that the rabbit IgG was successfully purified from serum using affinity chromatography. The purity of IgG extract was 99% and the efficiency of IgG purification was 1.74 mg/ml serum.

Similarly, pig anti-rubella virus IgG was also purified by the affinity chromatography using protein A - sepharose bead. The similar results were observed

and showed in the Fig. 2. The efficiency of IgG purification was 3.23 mg/ml serum and purity was 91.9%.

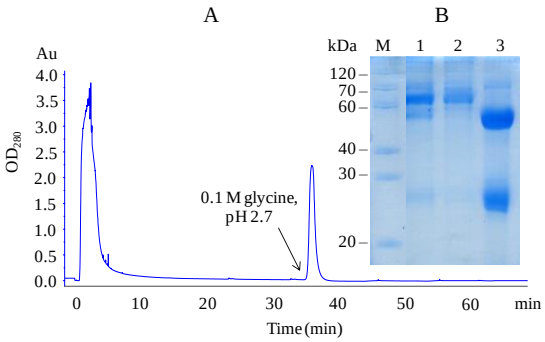

Fig.2. The chromatographic chart of pig anti-rubella virus IgG affinity purification using Protein A – sepharose bead (A) and protein profile (B). Lane M, protein marker (Genscript, USA); lane 1, whole serum; lane 2, flow through (peak 1); lane 3, eluted IgG fraction (peak 2).

3.2. Conjugation of antibody with colloidal gold nanoparticles (AuNP)

3.2.1. Determination of suitable pH for conjugation of IgG and AuNP

The antibody – AuNP conjugate is one of the most critical components of LFA test strip. Conjugation of antibody and AuNP can be maintained by physical interactions including (a) ionic attraction between the negatively charged gold particles and the positively charged antibody, (b) hydrophobic attraction between the antibody and the gold surface and (c) coordinate covalent bond between the gold conducting electrons and sulfur atoms of the antibody (linkers). For conjugation of antibodies to the gold particles, both covalent and non-covalent immobilization modes have been used [9]. In general, a protein maximally absorbs on the surface of gold nanoparticles at the isoelectric point (pI) of protein or 0.5 pH units higher [8]. In this study, the polyclonal antibodies were nonspecifically absorbed onto gold nanoparticles. The appropriate pH of antibody adsorption was determined to be approximately 10 (Fig. 3) and could be used for next experiments.

3.2.2. Determination of suitable IgG amount for conjugating with AuNP

The number of antibody molecules absorbed on the surface of AuNP is depended on the amount of used antibody. The optimal amount of antibody may be application-dependent. Therefore, it is necessary to optimize the amount of antibody for conjugation with AuNP. To determine optimal amount of conjugation antibody, several dilutions of antibody were added into a given amount of colloidal gold nanoparticles. The color of mixtures was gradually changed from brilliant red to dark with the decrease of antibody concentration. In principle, the highest dilution of the antibody solution with no change of color implicates the optimal antibody amount for colloidal gold labeling. In this study, the result showed that no change of color at antibody amount of 0.5 µg (Fig. 4a). The result of test strip also showed that a significant signal band appeared with 0.5 µg of antibody for conjugation while no signal was observed with 0.1 µg antibody (Fig. 4b). Obtained results indicated that the suitable amount of antibody was determined as 0.5 µg/10 µl (OD = 10) of AuNP.

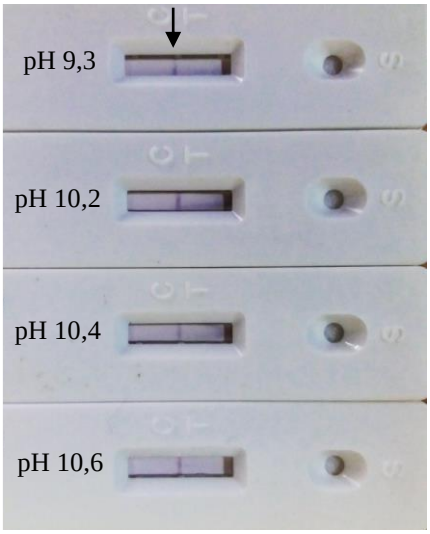

Fig.3. Conjugation of IgG and AuNP at different pHs of 9,3, 10,2, 10,4 and 10,6. The signal band is indicated by arrow.

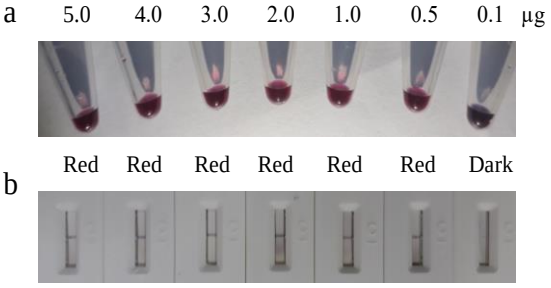

Fig.4. Optimization of antibody amount for conjugation with gold nanoparticles. (a) Color presentation of mixtures between several amounts of antibody and gold nanoparticles. (b) The test strips for rubella detection using conjugate pads with different amounts of antibody

3.2.3. Determination of incubation temperature

Incubation temperature can affect the absorbent efficiency of antibody on the surface of gold nanoparticles. In this experiment, conjugation of

antibody and gold nanoparticle was performed at three different temperatures of 4ºC, 25 ºC and 37 ºC. The result showed that the highest signal was observed at condition of 37 ºC incubation (Fig.5). Therefore this temperature could be chosen for further experiment.

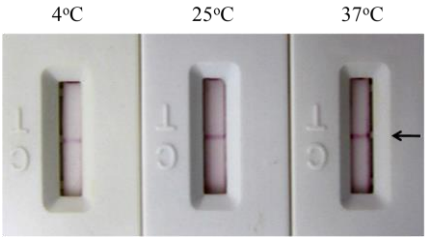

Fig.5. Incubation temperature for conjugation of antibody and gold nanoparticle. The signal band is indicated by arrow.

3.2.4. Determination of incubation time for conjugation of antibody and gold nanoparticle

In principle, absorbent efficiency of antibody on the surface of gold nanoparticle is proportional to time of incubation. However, long time incubation also affects the activity and stability of antibody. A range of incubation times of 30, 60, 90, 120 and 150 min. Obtained results (Fig. 6) indicated that the increase of incubation time resulted in the increase of the conjugation efficiency of antibody and gold nanoparticles. The signal at 90 min incubation was no significant difference when compared with longer incubation. Therefore, incubation time of 90 min was selected for next experiment.

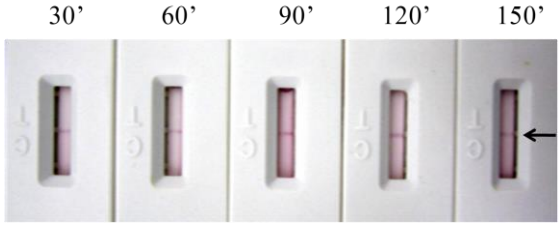

Fig.6. Incubation time for conjugation of antibody and gold nanoparticles. The signal band is indicated by arrow.

3.3. Immobilization of antibody on the nitrocellulose membrane

The membrane is also probably one of the single most important materials used in a lateral flow test strip. The membrane permits the migration of the complex of antigen – antibody conjugated AuNP to reach the lanes of immobilized antibody on the membrane.

3.3.1. Types of nitrocellulose membrane

Physical and chemical attributes of the membrane affect its capillary flow properties. The capillary properties in turn affect reagent deposition, sensitivity, specificity and test line consistency [10]. Two types of membrane were tested in this study and showed that a significant signal band appeared on the nitrocellulose membrane of Vivid 170 from Pall, while there was a slight signal band in the Whatman AE99 membrane (Fig. 7).

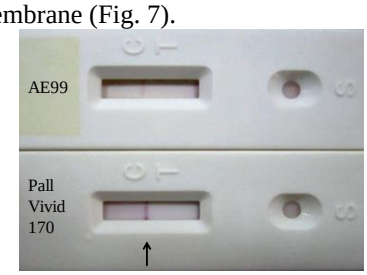

Fig. 7. Different types of the membrane for lateral flow test strip. Signal band is in dicated by arrow.

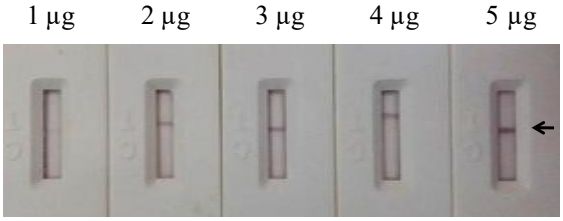

Fig.8. Immobilization of different antibody amount on the nitrocellulose membrane. The signal band is indicated by arrow.

3.3.2. Determination of suitable antibody amount for immobilization on the nitrocellulose membrane.

The loading capacity of a protein on a certain surface area depends on the protein compactness of structure and its effective diameter. For IgG protein, the approximate loading capacity is 1 µg/cm^2. Multiplying the loading capacity of IgG by the surface area ratio of the membrane (50-200) produces an approximate IgG binding capacity of 50-200 µg/cm^2. In a typical lateral flow test strip, the test line is around 0.03 cm^2, the amount of IgG that can be bound is 1.5 – 6 µg [10]. Therefore, the different amounts of pig anti-rubella antibody of 1, 2, 3, 4, 5 µg were sprayed on the nitrocellulose membrane to determine the appropriate antibody amount for generating the test line. The result showed that the intensity of the signal band was proportional with amount of IgG. A slight signal band was observed in case of 1 µg of IgG immobilized, while a significant signal band was observed at 3 µg antibody immobilized (Fig. 8). From obtained results, the appropriate amount of antibody for immobilization on the nitrocellulose membrane was chosen as 3 µg.

3.3.3. Antibody immobilizing buffer

The immobilizing buffer can affect the stability of immobilized antibody during storage and capillary

flow during test. In this study, four different buffers of IB1 (20 mM sodium borate), IB2 (20 mM sodium borate, 2% sucrose), IB3 (1X PBS pH 7.4), IB4 (1X PBS pH 7.4, 2% sucrose) were used to prepare antibody solution for immobilization. The result showed that all four conditions present the significant signal bands (Fig. 9a) and after 1 month (Fig. 9b). Obtained result indicated that antibody activity was still remained after 1 month storage when it was immobilized on the nitrocellulose membrane. However the appearance of the signal band was the earliest when antibody was immobilized in IB2.

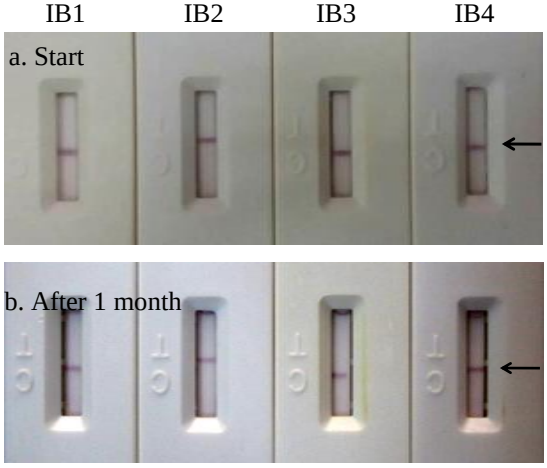

Fig. 9. Immobilization of antibody on the membrane in different buffers (IB1-IB4). Activity of immobilized antibody is checked at day 0 (a) and day 30 (b). Signal band is indicated by arrow.

3.3.4. Drying temperature of the nitrocellulose membrane for immobilization of antibody

After loading antibody, the membrane needs to be dried to immobilize antibody. The drying of membrane was performed at 4, 25^{o}C overnight and 37^{o}C for 30 min and resulted in the appearance of similar signals in the different drying temperatures (Fig. 10).

Drying of membrane at elevated temperature (37^{o}C) will take a short time (30 min) while it took longer time at lower temperature.

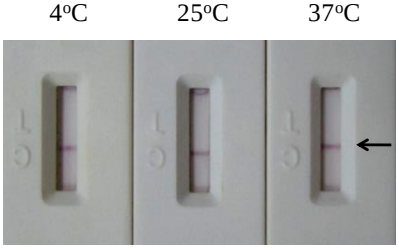

Fig. 10. Drying of the membrane at different temperatures for immobilizing antibody. Signal band is indicated by arrow.

3.4. Generation of complete lateral flow test strip

For generating the complete test strip, the test line (T-line) on the nitrocellulose membrane was probed with pig anti-rubella antibody, while the control line (C-line) was probed with goat anti-rabbit IgG antibody. The generated strip was test with both positive and negative samples. The result showed that there was the appearance of two signal bands at T-line and C-line positions after five minutes when tested with positive sample (containing rubella virus). When testing with negative sample, only one signal band was observed at C-line position (Fig. 11). This result implicated that the test strip could specifically detect rubella virus.

Fig. 11. Generation of complete lateral flow immunoassay test strip. Dashed arrow indicates the control line (C-line), line arrow indicates the test line (T-line)

The reproducibility of the generated test strip was determined by three times testing with positive and negative samples. The result showed that no change in intensity of the signal band was observed upon repeated testing of the sample (Fig. 12). Obtained results indicated that the test strip was successfully constructed for rapid detection of rubella virus.

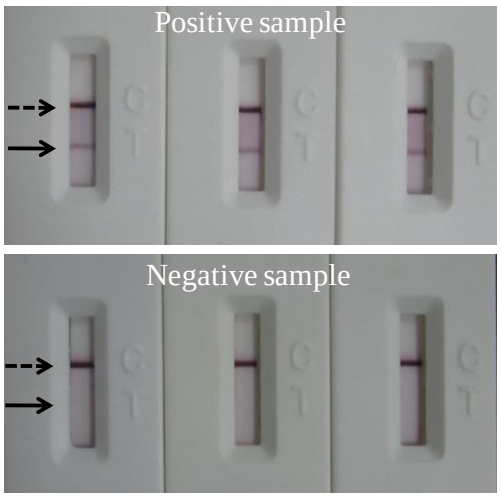

Fig. 13. The reproducibility of the generated test strip. Dashed arrow indicates the control line (C-line), line arrow indicates the test line (T-line)

4. Conclusion

Lateral flow immunoassay test strip is considered as the powerful tool for rapid detection of rubella virus. Determination of suitable conditions for generating a rapid test strip could help to produce an effective kit for rapid detection of rubella virus in serum or saliva sample of patients.

References

[1] WHO, Manual for the laboratory diagnosis of measles and rubella virus infection, WHO/IVB/07.01 (2007).

[2] J.M. Best, S. O'Shea, G. Tipples, N. Davies, S.M. Al-Khusaiby, A. Krause, L.M. Hesketh, L. Jin, G. Enders, Interpretation of rubella serology in pregnancy—pitfalls and problems, The British Medical Journal 325 (2002) 147-148.

[3] K.M. Thompson, E.A. Simons, K. Badizadegan, S.E. Reef, L.Z. Cooper, Characterization of the Risks of Adverse Outcomes Following Rubella Infection in Pregnancy, Risk analysis 36(7) (2016) 1315-1331.

[4] J.S. Wolinsky, Rubella, in Fields' Virology, B.N. Fields, D.M. Knipe, and P.M. Hawley, Editors. 1996, Raven Press: New York, 899-929.

[5] C. Vauloup-Fellous and L. Grangeot-Keros, Humoral Immune Response after Primary Rubella Virus Infection and after Vaccination, Clin. Vaccine Immunol. 14(5) (2007) 644-647.

[6] CDC, Epidemiology and Prevention of vaccine-preventable diseases, Chapter 20: Rubella, (2015) 325-340.

[7] S.A. de Oliveira, M.M. Siqueira, D.W.G. Brown, P. Litton, L.A.B. Camacho, S.T. Castro1 and B.J. Cohen, Diagnosis of rubella infection by detecting specific immunoglobulin M antibodies in saliva samples: a clinic-based study in Niterói, RJ, Brazil, Revista da Sociedade Brasileira de Medicina Tropical 33(4) (2000) 335-339.

[8] G. Zhang, J. Guo, X. Wang, Immunochromatographic lateral flow strip test, chapter 12 in A. Rasooly and K.E. Herold (eds), Methods in Molecular Biology: Biosensors and Biodetection, 504, Humana Press.

[9] M.H. Jazayeri, H.Amani, A.A. Pourfatollah, H. Pazoki-Toroudi, B. Sedighimoghaddam, Various methods of gold nanoparticles (GNPs) conjugation to antibodies, Sensing and Bio-Sensing Research 9 (2016) 17–22.

[10] Milipore. Rapid lateral flow test strips – Consideration for product development. Lit. No. TB500EN00EM (2013), Rev.C 12/13