Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Development of a colloidal gold immunochromatographic test strip for detection of lymphocystis disease virus in fish

X.Z. Sheng, J.L. Song and W.B. Zhan

Laboratory of Pathology and Immunology of Aquatic Animals, Ocean University of China, Qingdao, China

Keywords
colloidal gold, immunochromatographic test strip, lymphocystis disease virus, monoclonal antibody.

Correspondence
Wenbin Zhan, Laboratory of Pathology and Immunology of Aquatic Animals, Ocean University of China, 5 Yushan Road, Qingdao 266003, China. E-mail: wbzhan@ouc.edu.cn

2012/0959: received 25 May 2012, revised 2 July 2012 and accepted 3 July 2012

doi:10.1111/j.1365-2672.2012.05389.x

Abstract

Aims: To develop a gold immunochromatographic test strip for on-site rapid detection of lymphocystis disease virus (LCDV).

Methods and Results: Monodispersional colloidal gold and gold-labelled anti-LCDV monoclonal antibody (McAb) 2D11 were prepared and characterized by UV-visible spectroscopy and transmission electron microscopy. Gold-labelled probe was used as the detection antibody, and goat anti-mouse IgG at the control line and anti-LCDV McAb 1A8 at the test line of the test strip served as the capture antibody. The positive results could be easily judged by the presence of a red test line with naked eye within 10 min. The test strip, in good agreement with enzyme-linked immunosorbent assay and dot-blotting in sensitivity and LCDV detection, gave a detection limit of 1 μg ml^{-1} of LCDV and was stable for 6 months at room temperature and 12 months at 4°C.

Conclusions: The test strip was specific, simple and convenient for rapid detection of LCDV presenting good stability and reproducibility.

Significance and Impact of the Study: This ready-to-use test strip allows on-site rapid detection of LCDV in fish without the requirement of specialized equipments and professional personnel, which could augment the practical application for diagnosis of LCDV even in disadvantage areas.

Introduction

Lymphocystis disease, caused by lymphocystis disease virus (LCDV), is a chronic viral disease characterized by the appearance of pearl-like nodules consisting of hypertrophied dermal cells in fish skin, gills, fins and even the internal organs (Sheng *et al.* 2007; Cano *et al.* 2009). It has a worldwide geographical distribution and has been detected in more than 140 species of freshwater, estuarine and marine fishes (Wolf 1988). Lymphocystis disease is rarely fatal; however, the diseased fish exhibiting deformation cannot be commercialized and are more susceptible to secondary infection by other micro-organisms resulting in a great economic loss in aquaculture (Iwamoto *et al.* 2002; Kvitt *et al.* 2008). In addition, water contamination, oxygen depletion and nutrition deficiency in fish culture may activate virus latent in asymptomatic carriers and lead to a disease occurrence (Cano *et al.* 2006). In this case, it is imperative for LCDV surveillance in aquaculture systems to control viral dissemination.

Various laboratory methods are available for the diagnosis of LCDV, such as indirect immunofluorescence assay, indirect dot-blot immunoenzymatic assay, flow cytometry based on cell culture inoculation, immunoblot, PCR, multiplex PCR and in situ hybridization techniques (García-Rosado *et al.* 2002; Cano *et al.* 2006, 2007, 2009; Kitamura *et al.* 2006). Generally, these diagnostic approaches have respective merits in terms of accuracy, sensitivity, safety and cost. However, their performances are restricted to laboratory mainly because of the requirement of highly trained personnel and specialized equipments.

Gold immunochromatographic assay (GICA), a technique based on the specific antigen–antibody immunoreactions, is a highly useful tool in diagnostics, as the assay results are directly visible to the naked eye without the requirement of specialized equipments and complicated handling procedures, providing convenience for rapid testing. These characteristics enable it feasible and accessible for on-site detection of antigens (Wang and Zhan

2006). This study demonstrated the development of a GICA-based test strip as a supplementary technique for rapid detection of LCDV in fish. The test strip utilized two monoclonal antibodies (McAbs) of distinct specificity, one LCDV-specific McAb 1A8 immobilized on nitrocellulose (NC) membrane as the capture antibody and another LCDV-specific McAb 2D11 labelled with colloid gold particles as the detection antibody. The performance of assay was very simple and the test could be completed within 10 min.

Materials and methods

Samples

Flounders (*Paralichthys olivaceus*) were collected from a farm located in Qingdao, Shandong province of China, and subjected to PCR to ensure LCDV free as described previously (Zhan *et al.* 2010). LCDV-infected flounders were from another farm in Shandong province, and flounders suspected of LCDV infection were from Hebei province of China.

Pearl-like nodules on the body surface of LCDV-infected flounder were isolated, and LCDV purification was carried out following the methods described by Cheng *et al.* (2006). Skin and fins from flounder suspected of LCDV infection were sampled and vigorously homogenized in PBS at a ratio of 10% (w/v) on ice, and the supernatant fluids were collected as test solution and used for LCDV detection.

Production and purification of anti-LCDV McAbs

Hybridomas secreting anti-LCDV McAb 1A8 and 2D11 (Cheng *et al.* 2006), stored in liquid nitrogen, were revived by quickly warming in a water bath at 37°C. Each 8-week-old BALB/c female mouse was injected with 0·5 ml of liquid paraffin intraperitoneally and induced for 7 days, and then 1×10^6 of McAb 1A8 and 2D11 hybridoma clones were injected into the peritoneal cavity of BALB/c mouse, respectively. Ten days later, the mice ascites were harvested and centrifuged at 800 ***g*** for 15 min, and anti-LCDV McAbs 1A8 and 2D11 were separated from the ascitic fluids by the method of salting out with caprylic acid–ammonium sulfate as described by Ogden and Leung (1988) and stored at −80°C until use.

Synthesis and identification of colloidal gold

Colloidal gold was produced through controlled reduction of chloroauric acid following the procedures described by Frens (1973) with slight modifications. Briefly, 100 ml of 0·01% (m v^{-1}) chloroauric acid ($HAuCl_4$) (China) was heated to boiling for 2 min in microwave oven (Galanz Group, Guangdong, China; 700 W), and 2·0 ml of 1% (m v^{-1}) sodium citrate solution was then added rapidly to the boiling chloroauric acid solution and reduced the heat to medium–low (280 W) for another 3 min. The colloidal gold suspension was allowed to cool down to room temperature and stored at 4°C.

Optimal amount of McAb conjugated by colloidal gold

The purified McAb 2D11 was dialysed against distilled water overnight at 4°C and subsequently centrifuged at 15 000 ***g*** for 30 min to get a clear supernatant for conjugation. The pH of colloidal gold was adjusted to 8·2 with 0·01 mol l^{-1} K_2CO_3 solution according to the pre-experiment. Various amounts of McAb 2D11 (0, 5, 10, 15, 20, 25 and 30 μg) were added to 1 ml of colloidal gold at room temperature, respectively, and incubated for 10 min with shaking, followed by addition of 100 μl of 10% NaCl solution and shaking for 10 min. Two hours later, the absorbance of each solution was measured spectrophotometrically at 520 nm, and the amount of protein showing maximum absorbance was confirmed as the minimum amount of McAb needed to prevent the colloidal gold from aggregating. The optimal amount of McAb used for conjugation was 20% excess of the minimum amount of McAb.

Preparation of colloidal gold–McAb probe

The purified McAb 2D11 (180 μg) was added to 10 ml of dispersed colloidal gold solution and agitated for 30 min. After coating, the reaction mixture was further stabilized by adding 2·5 ml of 10% bovine serum albumin (BSA) (Sigma-Aldrich Co. LLC, USA) with moderate stirring for 20 min and then stored overnight at 4°C. Following the colloidal gold–McAb probe centrifuged at 30 000 ***g*** for 30 min at 4°C, the supernatant was removed and the resulting precipitate was resuspended in 0·01 mol l^{-1} PBS containing 1% BSA and then centrifuged again to remove free unconjugated antibody. The pellet was resuspended in 1 ml storage buffer (0·01 mol l^{-1} PBS containing 1% BSA, 5% sucrose, 0·5% Tween-20 and 0·02% NaN_3) and stored at 4°C prior to use.

Transmission electron microscopy

The average diameter and dispersion of colloidal gold particles and colloidal gold–McAb probe were estimated by transmission electron microscopy (TEM). A drop of colloidal gold solution or colloidal gold–McAb conjugates

was dropped on a carbon-coated TEM copper grid and allowed to air dry for 15 min, and then colloidal gold particles were observed by TEM, while colloidal gold–McAb conjugates were measured after negative staining with uranyl acetate.

UV-visible spectroscopy

The colloidal gold solution and formation of colloidal gold–McAb probe were scanned by UV-visible (UV-vis) spectroscopy (200–700 nm) using a double-beam spectrophotometer. The colloidal gold solution was monitored for both conjugation and aggregation by measuring gold solution with: no antibody, immediately after addition of anti-LCDV McAb 2D11, and after centrifugation and resuspension of the colloidal gold–McAb conjugates in storage buffer.

Preparation of immunochromatographic test strip

The test strip consisted of four components assembled together on a plastic backing: the colloidal gold–McAb conjugate pad, NC membranes, sample pad and absorption pad. Colloidal gold–McAb conjugate pad was prepared by spraying colloidal gold-labelled McAb 2D11 on a glass fibre and dried at room temperature. Purified McAb 1A8 at 1 mg ml^{-1} and goat anti-mouse IgG (Sigma) at 0·5 mg ml^{-1} in 0·01 mol l^{-1} PBS were dispensed on NC membrane (Whatman Inc., Clifton, USA) with 5–8-μm pore size to form test line and control line, respectively. The distance between the two lines was about 5 mm. The membrane was then air dried, blocked with 2·5% BSA in 0·01 mol l^{-1} PBS at 37°C for 1 h and washed three times with PBST (0·01 mol l^{-1} PBS containing 0·05% Tween-20) and dried again at 37°C for 1 h. For test strip assembly, the NC membrane was pasted onto the centre of the plastic plate and covered with the colloidal gold–McAb conjugate pad and the absorption pad on each side. The sample pad receiving liquid sample to be analysed was attached to the other side of colloidal gold–McAb conjugate pad (Fig. 1). Then, they were cut into 3-mm-wide pieces and stored desiccated at 4°C.

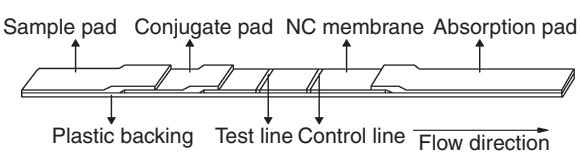

Figure 1 Schematic diagram of a test strip (cross section) showing several components.

Lymphocystis disease virus detection by immunochromatographic strip test

Flounder suspected of LCDV infection were detected by the developed immunochromatographic strip. About 100 μl of gill or fin supernatant fluids was pipetted onto the sample pad, or the sample pad of test strip was immersed into the test solution of which the surface must be lower than the conjugate pad, allowing the test solution to flow by chromatography through the NC membrane to the other end. The result was observed by naked eye within 10 min, and the presence of two red lines in control line and test line region indicated a positive reaction denoting the sample containing LCDV, while the presence of one red line in control line region suggested the absence of LCDV in the sample or below the limit of detection for the strip. If the red control line did not appear, the test was invalid.

The indirect enzyme-linked immunosorbent assay (ELISA) and dot-blotting as control methods were also performed on these supernatant fluids. LCDV-infected flounder and LCDV-free founder were used as positive and negative controls, respectively.

For ELISA, the 96-well EIA plates (Costar, Corning, NY, USA) were coated overnight at 4°C with serial dilutions of purified LCDV (100 μl per well) and blocked with 3% BSA at 37°C for 1 h. After washing the wells three times with PBST, the anti-LCDV McAb was added to the ELISA plates and held at 37°C for 1 h and then washed three times with PBST. Goat anti-mouse immunoglobulin conjugated with AP (1 : 20 000) (Sigma) was applied and incubated at 37°C for 1 h and washed three times with PBST. Finally, the wells were incubated with freshly prepared pNPP (*p*-Nitrophenyl phosphate) substrate solution for 15 min, and the absorbance of the reaction product was measured at 405 nm with a Versa Max automated microplate reader.

For dot-blotting, serial dilutions of purified LCDV were made (4 μl) and the resultant LCDV was spotted onto NC membrane and air dried. The NC membrane was blocked with 10% BSA at 37°C for 1 h and washed three times with PBST. Next, the anti-LCDV McAb was added and held at 37°C for 1 h and then washed three times with PBST, followed by addition of goat anti-mouse immunoglobulin conjugated with AP (1 : 20 000) and incubation at 37°C for 1 h. After three washes with PBST, the colour reaction was developed with NBT-BCIP (nitro-blue tetrazonium chloridez—5-bromo-4-chloro-3'-indolyphosphate *p*-toluidine salt) substrate solution for 15 min in the dark as described by Zhan *et al.*(2004).

Sensitivity and specificity of immunochromatographic strip

Purified LCDV was diluted with PBS to protein concentrations of 50, 10, 5, 1 and 0·5 μg ml^{-1} and used as test solution for the sensitivity, and the last dilution that yielded an observable positive result was regarded as the detection limit of immunochromatographic test strip. The diluted test solution was also used for ELISA and dot-blotting simultaneously as described above, and the tissue homogenate supernatant of LCDV-free founder served as negative controls.

To determine specificity of the test strip for LCDV detection, gill homogenates of LCDV-infected *P. olivaceus*, infectious hematopoietic necrosis virus (IHNV)-infected *P. olivaceus* and turbot reddish body iridovirus (TRBIV)-infected *Scophthalmus maximus* (Qingdao, China) were detected using the strip. Purified LCDV served as positive controls.

Reproducibility and stability of immunochromatographic strip

Positive and negative samples of LCDV were tested three times to determine the reproducibility of test strips. The same batch or three different batches of test strips were applied.

For stability, test strips were stored at room temperature and 4°C for up to 12 months and tested by positive and negative samples at 30-day intervals.

Results

Characterization of colloidal gold particles and colloidal gold–McAb probe

Transmission electron microscopy imaging

After gold nanoparticle formation, the colloidal gold solution had a ruby-red colour. Under TEM, the colloidal gold particles were well dispersed and uniform in size and shape with an average diameter of approximately 20 nm (Fig. 2). There was no obvious difference in the size distribution of colloidal gold before and after conjugation. Upon conjugation, TEM image showed a thin white layer, called 'halo' effect, surrounding the surface of the nanoparticles indicating coating of the gold with McAb 2D11 (Fig. 3), which was not present prior to treatment.

UV-visible spectra

Spectra of the colloidal gold solution without antibody, immediately after the addition of antibody and after centrifugation and resuspension of the colloidal gold–McAb conjugate, were recorded (Fig. 4). An absorbance peak at 520 nm in curve a, with a high symmetry and narrow width, was observed owing to surface plasmon resonance of colloidal gold particles, indicating that the prepared colloidal gold was monodisperse and had narrow size distribution. Upon addition of antibody (curve b), the surface resonance band shifted a little into the red

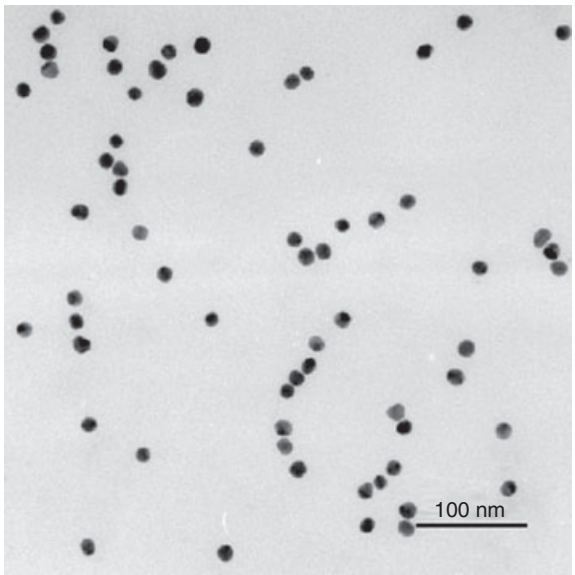

Figure 2 The colloidal gold particles micrograph observed by transmission electron microscopy.

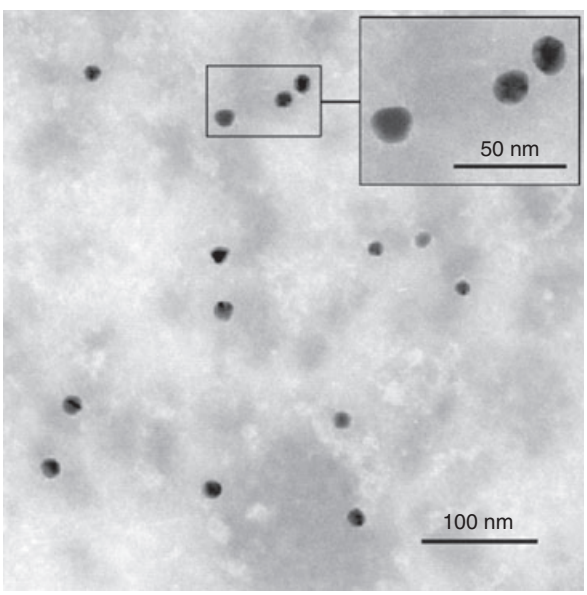

Figure 3 The colloidal gold–monoclonal antibody (McAb) probe micrograph observed by transmission electron microscopy after negative staining with uranyl acetate, a thin white 'halo' layer surrounding the surface of the nanoparticles indicating coating with the anti-lymphocystis disease virus McAb (insert area at higher magnification)

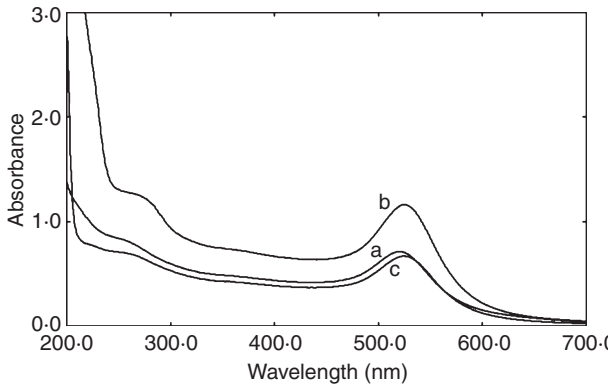

Figure 4 UV-visible spectra of colloidal gold and colloidal gold–monoclonal antibody (McAb) probe. Curve a, colloidal gold solution; curve b, the colloidal gold–McAb probe immediately after addition of anti-lymphocystis disease virus McAb 2D11 to colloidal gold solution; Curve c, the colloidal gold–McAb probe after centrifugation and resuspension.

direction as a result of colloidal gold–McAb interaction and a peak at approximately 280 nm in the conjugation system arose. After centrifugation and resuspension of colloidal gold–McAb probe, two absorption bands was observed at 524 and 280 nm as shown in curve c, suggesting that the antibody was present on the gold surface. The intensity of the surface resonance band and protein absorbance band at 280 nm decreased because of the reduction of colloidal gold concentration and removal of the unconjugated McAb molecules in solution.

Optimal amount of McAb for colloidal gold conjugate

Various amounts of anti-LCDV McAb 2D11 were added to colloidal gold solution. The minimum amount of McAb required for conjugation with colloidal gold particles was determined based on the measured absorbance at 520 nm, showing a maximum absorbance at approximately 15 μg of McAb (Fig. 5). To ensure complete reaction with colloidal gold particles, the optimal amount of McAb 2D11 was chosen to be 120% of the minimum amount of McAb.

Detection limit of immunochromatographic strip and LCDV detection

In test solution containing LCDV, the virus would bind to colloidal gold-conjugated McAb 2D11, and the resulting complex would be captured by the McAb 1A8 at the test line to give a red band. The unbound McAb conjugated with colloidal gold moved across the test line to be captured by the goat anti-mouse IgG and form a red band at the control line. Immunochromatographic strip test (ICT) showed that the serial dilutions of purified

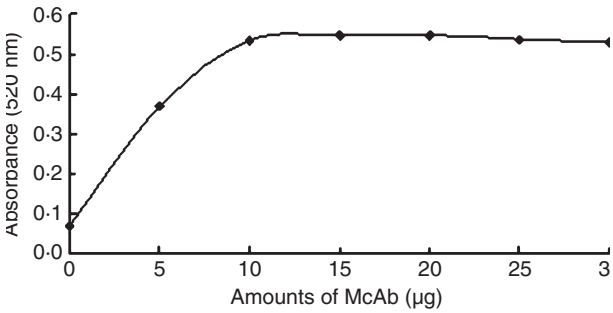

Figure 5 Absorbance of colloidal gold conjugates measured at 520 nm with various amounts of anti-lymphocystis disease virus monoclonal antibody (0–30 μg).

LCDV at protein concentrations of 1, 5, 10 and 50 μg ml^{-1} yielded positive results, so the detection limit of test strip was 1 μg ml^{-1} (Fig. 6). The same serial virus dilutions were examined by ELISA and dot-blotting simultaneously, and the sensitivity of ELISA (Fig. 7) and dot-blotting (Fig. 8) was found to be 1 μg ml^{-1}.

Samples from flounder *P. olivaceus* suspected of LCDV infection were tested positive by ICT, which were in agreement with the detection results of ELISA and dot-blotting (data not shown).

Specificity of immunochromatographic strip

Cross-reactivity studies using gill homogenates of LCDV-infected *P. olivaceus*, IHNV-infected *P. olivaceus* and

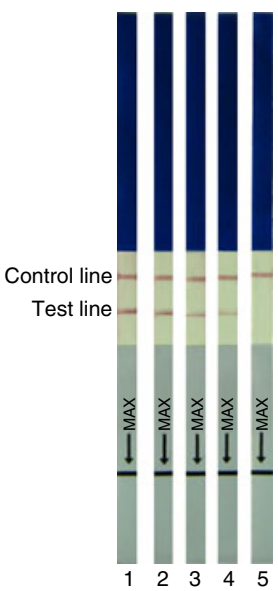

Figure 6 Immunochromatographic strip test results of purified lymphocystis disease virus (LCDV) showed positive at protein concentrations of 50, 10, 5 and 1 μg/ml (1–4), using the tissue homogenate supernatant of LCDV-free founder as negative control (5).

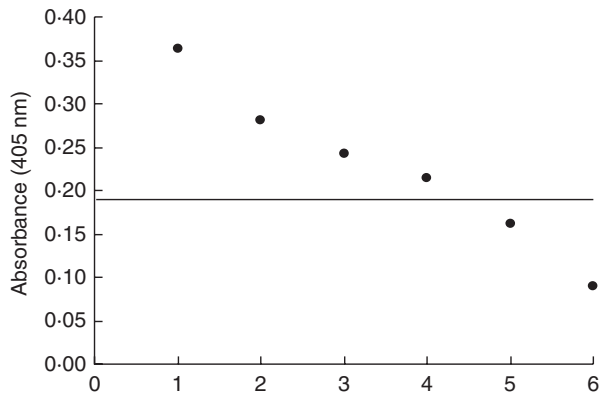

Figure 7 Result of enzyme-linked immunosorbent assay for lymphocystis disease virus (LCDV) detection. Purified LCDV at protein concentrations of 50, 10, 5, 1 and 0·5 μg/ml (1–5) were tested. Absorbance values twice higher than the background level reactivity on negative samples (6) were considered to be positive. The dashed line (- - -) indicated the visual detection limit.

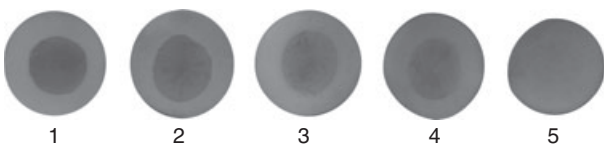

Figure 8 Result of dot-blotting for lymphocystis disease virus (LCDV) detection. Purified LCDV at the protein concentrations of 50, 10, 5, 1 μg/ml gave positive results (1–4), showing a detection limit of 1 μg/ml (4). Tissue homogenate supernatant of LCDV-free founder was used as negative control (5).

TRBIV-infected *S. maximus* showed that positive signals were observed in the positive control and LCDV-infected *P. olivaceus*, but not in IHNV, TRBIV samples.

Reproducibility and stability of immunochromatographic strip

Lymphocystis disease virus viral suspensions and the LCDV-free flounder samples tested three times with the same batch of immunochromatographic test strips showed the same positive or negative results, respectively. Similar results were obtained for the three different batches of test strips.

The test strips were stable for at least 6 months at room temperature and 12 months at 4°C without changes in sensitivity (data not shown).

Discussion

Gold immunochromatographic assay is a technology combining immunology with chromatography which has been developed for several years and applied increasingly in immunoanalysis diagnosis (Sithigorngul *et al.* 2007; Cui *et al.* 2008; Zhao *et al.* 2010). The quality of colloidal gold particles plays a key role in the development of a GICA-based one step lateral-flow strip for antigen analysis. Appropriate size and uniformity of the colloidal gold particles can result in good performance and yield more tests per litre (Carney *et al.* 2006). The size and hence the property of the gold nanoparticles can be controlled through the use of different reducing agents and the speed of reduction (Frens 1973; Morrow *et al.* 2009). In this study, colloidal gold in ruby-red colour was synthesized with the reduction of chloroauric acid by sodium citrate at pH 8·2. As shown in TEM images, the gold nanoparticles were well dispersed and uniform in size with diameter of approximately 20 nm, revealing they were of good quality and aggregate free. Additionally, the stability of gold–McAb conjugates is important for desirable biological reactivity and optimal performance of test strip (Thobhani *et al.* 2010). Our research showed that the developed step strip was stable for 6 months at room temperature and 12 months at 4°C without changes in sensitivity for LCDV detection, suggesting that the synthesized colloidal gold–McAb conjugates were stable which ensured the quality, stability and reproducibility of batches of test strip.

UV-visible spectroscopy is a powerful characterization tool, which can be used to monitor the conjugation of nanoparticles and indicate adsorption of proteins onto gold with a change in absorbance peak for the nanoparticles, and the intensity and position of spectrum peak of colloidal gold solution are related to the dispersity of nanoparticles (Thobhani *et al.* 2010). In the colloidal gold solution without antibody, an absorbance peak at 520 nm with high symmetry and narrow width was observed, showing that gold particles were monodisperse and had similar size, which was consistent with the results of TEM. Upon addition of antibody, the surface resonance band shifted a little suggesting the McAb conjugation, and a peak at approximately 280 nm arose for the absorbance from the tyrosine and tryptophan residues in the protein of McAb (Gole *et al.* 2002). UV-vis provided physical confirmation that the anti-LCDV McAb had bound to the nanoparticles with well dispersity and size uniformity.

Many serological methods have been developed for rapid and sensitive detection and diagnosis of fish viruses according to immunological principles, such as ELISA, dot-blotting, immunofluorescence assay test (IFAT) and immunohistochemistry (Olesen *et al.* 1993; Lorenzo *et al.* 1996; Falk *et al.* 1998; Shieh and Chi 2005; Fenner *et al.* 2006), and McAbs against unique antigens or epitopes of antigens have been widely used (Zhou *et al.* 2006;

Reschova *et al.* 2007; Chen *et al.* 2008). As a highly effective and economical immunological tool, McAbs assure a reliable source of antibodies and often achieve greater specificity and sensitivity than polyclonal antibodies (Shi *et al.* 2003; Dixon and Longshaw 2005; Reschova *et al.* 2007). To avoid the possible cross-reactivity and low affinity of polyclonal antibodies, two clones of McAbs that recognized different surface epitopes of LCDV (Cheng *et al.* 2006) were chosen to develop the colloidal gold immunochromatographic test strip for LCDV detection of fish. The test strip gave a detection limit of 1 μg ml^{-1} of LCDV and was in good accordance with ELISA and dot-blotting in sensitivity and in LCDV detection. Moreover, ICT had several advantageous features such as simplicity, immediate result and the fact that it could be performed anywhere at any time without prior preparation of equipment. Therefore, the rapid ICT could augment the practical application for diagnosis of LCDV even in disadvantage areas and laboratories with limited technical expertise.

The affected fish with lymphocystis disease show typical pearl-like nodules on the skin and fins, and fibroblasts are proved to be the main target cells for LCDV replication (Cano *et al.* 2009). Thus, fish skin and fins were homogenized and used as test samples of suspected fish. Considering sensitivity of immunological methods is confined by viral extraction, suspected positive samples tested with the test strip may show negative results. It is necessary that the tiny infection samples should be further confirmed by cell culture, histological or molecular tests. Nevertheless, the strip test is still recommended for screening of subclinical infection or greater intensity infection of LCDV to differentiate from other pathogen infections.

Acknowledgements

This study was supported by National Natural Science Foundation of China (grant 31172429, grant 31072232) and the National Science and Technology Supporting Program (grant 2012BAD17B01).

References

Cano, I., Alonso, M.C., Garcia-Rosado, E., Saint-Jean, S.R., Castro, D. and Borrego, J.J. (2006) Detection of lymphocystis disease virus (LCDV) in asymptomatic cultured gilt-head seabream (*Sparus aurata,* L.) using an immunoblot technique. *Vet Microbiol* **113**, 137–141.

Cano, I., Ferro, P., Alonso, M.C., Bergmann, S.M., Romer-Oberdorfer, A., Garcia-Rosado, E., Castro, D. and Borrego, J.J. (2007) Development of molecular techniques for detection of lymphocystis disease virus in different marine fish species. *J Appl Microbiol* **102**, 32–40.

Cano, I., Ferro, P., Alonso, M.C., Sarasquete, C., Garcia-Rosado, E., Borrego, J.J. and Castro, D. (2009) Application of in situ detection techniques to determine the systemic condition of lymphocystis disease virus infection in cultured gilt-head seabream, *Sparus aurata* L. *J Fish Dis* **32**, 143–150.

Carney, J., Braven, H., Seal, J. and Whitworth, E. (2006) Present and future applications of gold in rapid assays. *IVD Technol* **12**, 41–49.

Chen, Z.Y., Liu, H., Li, Z.Q. and Zhang, Q.Y. (2008) Development and characterization of monoclonal antibodies to spring viraemia of carp virus. *Vet Immunol Immunopathol* **123**, 266–276.

Cheng, S.F., Zhan, W.B., Xing, J. and Sheng, X.Z. (2006) Development and characterization of monoclonal antibody to the lymphocystis disease virus of Japanese flounder *Paralichthys olivaceus* isolated from China. *J Virol Methods* **135**, 173–180.

Cui, S.J., Chen, C.M. and Tong, G.Z. (2008) A simple and rapid immunochromatographic strip test for monitoring antibodies to H5 subtype Avian Influenza Virus. *J Virol Methods* **152**, 102–105.

Dixon, P.F. and Longshaw, C.B. (2005) Assessment of commercial test kits for identification of spring viraemia of carp virus. *Dis Aquat Organ* **67**, 25–29.

Falk, K., Namork, E. and Dannevig, B.H. (1998) Characterization and applications of a monoclonal antibody against infectious salmon anaemia virus. *Dis Aquat Organ* **8**, 77–85.

Fenner, B.J., Du, Q., Goh, W., Thiagarajan, R., Chua, H.K. and Kwang, J. (2006) Detection of betanodavirus in juvenile barramundi, *Lates calcarifer* (Bloch), by antigen capture ELISA. *J Fish Dis* **29**, 423–432.

Frens, G. (1973) Preparation gold dispersions of varying particle size: controlled nucleation for the regulation of the particle size in monodiperse gold suspensions. *Nature (Phys Sci)* **241**, 20–22.

García-Rosado, E., Castro, D., Cano, I., Pérez-Prieto, S.I. and Borrego, J.J. (2002) Serological techniques for detection of lymphocystis virus in fish. *Aquat Living Resour* **15**, 179–185.

Gole, A., Vyas, S., Phadtare, S., Lachke, A. and Sastry, M. (2002) Studies on the formation of bioconjugates of Endoglucanase with colloidal gold. *Colloid Surf B Biointerfaces* **25**, 129–138.

Iwamoto, R., Hasegawa, O., Lapatra, S. and Yoshimizu, M. (2002) Isolation and characterization of the Japanese flounder (*Paralichthys olivaceus*) lymphocystis disease virus. *J Aquat Anim Health* **14**, 114–123.

Kitamura, S.I., Jung, S.J. and Oh, M.J. (2006) Differentiation of lymphocystis disease virus genotype by multiplex PCR. *J Microbiol* **44**, 248–253.

Kvitt, H., Heinisch, G. and Diamant, A. (2008) Detection and phylogeny of *Lymphocystivirus* in sea bream *Sparus aurata*

based on the DNA polymerase gene and major capsid protein sequences. *Aquaculture* **275**, 58–63.

Lorenzo, G., Estepa, A. and Coll, J.M. (1996) Fast neutralization/immunoperoxidase assay for viral haemorrhagic septicemia with antinucleoprotein monoclonal antibody. *J Virol Methods* **58**, 16–21.

Morrow, B.J., Matijević, E. and Goia, D.V. (2009) Preparation and stabilization of monodisperse colloidal gold by reduction with aminodextran. *J Colloid Interface Sci* **335**, 62–69.

Ogden, J.R. and Leung, K. (1988) Purification of murine monoclonal antibodies by caprylic acid. *J Immunol Methods* **111**, 283–284.

Olesen, N.J., Lorenzen, N. and Jørgensen, P.E.V. (1993) Serological differences among isolates of viral haemorrhagic septicaemia virus detected by neutralising monoclonal and polyclonal antibodies. *Dis Aquat Organ* **16**, 163–170.

Reschova, S., Pokorova, D., Nevorankova, Z., Hulova, J. and Vesely, T. (2007) Detection of spring viraemia of carp virus (SVCV) with monoclonal antibodies. *Vet Med* **52**, 308–316.

Sheng, X.Z., Zhan, W.B., Xu, S.J. and Cheng, S.F. (2007) Histopathological observation of lymphocystis disease and lymphocystis disease virus (LCDV) detection in cultured diseased *Sebastes schlegeli*. *J Ocean Univ China* **6**, 378–382.

Shi, C., Wei, Q., Gin, K.Y. and Lam, T.J. (2003) Production and characterization of monoclonal antibodies to a grouper iridovirus. *J Virol Methods* **107**, 147–154.

Shieh, J.R. and Chi, S.C. (2005) Production of monoclonal antibodies against grouper nervous necrosis virus (GNNV) and development of an antigen capture ELISA. *Dis Aquat Organ* **63**, 53–60.

Sithigorngul, W., Rukpratanporn, S., Sittidilokratna, N., Pecharaburanin, N., Longyant, S., Chaivisuthangkura, P. and Sithigorngul, P. (2007) A convenient immunochromatographic test strip for rapid diagnosis of yellow head virus infection in shrimp. *J Virol Methods* **140**, 193–199.

Thobhani, S., Attree, S., Boyd, R., Kumarswami, N., Noble, J., Szymanski, M. and Porter, R.A. (2010) Bioconjugation and characterization of gold colloid-labelled proteins. *J Immunol Methods* **356**, 60–69.

Wang, X.J. and Zhan, W.B. (2006) Development of an immunochromatographic test to detect White Spot Syndrome Virus of shrimp. *Aquaculture* **255**, 196–200.

Wolf, K. (1988) *Fish Viruses and Fish Viral Diseases*. Ithaca, NY: Cornell University Press.

Zhan, W.B., Wang, X.J., Chen, J., Xing, J. and Fukuda, H. (2004) Elimination of shrimp endogenous alkaline phosphatase background and development of enzyme immunoassays for the detection of white spot syndrome virus (WSSV). *Aquaculture* **239**, 15–21.

Zhan, W.B., Li, Y.Q., Sheng, X.Z., Xing, J. and Tang, X.Q. (2010) Detection of lymphocystis disease virus in Japanese flounder *Paralichthys olivaceus* and other marine teleosts from northern China. *Chin J Oceanol Limnol* **28**, 1213–1220.

Zhao, X.H., He, X.W., Li, W.M., Liu, Y., Yang, L.S. and Wang, J.H. (2010) Development and evaluation of colloidal gold immunochromatographic strip for detection of *Escherichia coli* O157. *Afr J Microbiol Res* **4**, 663–670.

Zhou, G.Z., Li, Z.Q. and Zhang, Q.Y. (2006) Characterization and application of monoclonal antibodies against turbot (*Scophthalmus maximus*) rhabdovirus. *Viral Immunol* **19**, 637–645.