RESEARCH ARTICLE

Development of a Lateral Flow Kit for Detection of IgG and IgM Antibodies Against Rift Valley Fever Virus in Sheep

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Abstract

Rift Valley Fever (RVF) is a viral zoonosis that primarily affects animals but also has the capacity to infect humans. The disease also results in significant economic losses due to death and abortion among RVF-infected livestock. A lateral flow device (LFD) was developed for the rapid detection of IgG and IgM anti-RVF antibodies in infected and vaccinated ovine sera. The conjugated RVF antigen with nano-gold was used as a detector antigen and was laid on a conjugated pad. The staphylococcal protein A was used as the capture complex (antibodies IgG-antigen conjugated with nanogold) at the first test line of device and anti-IgM antibodies were used as the capture complex (antibodies IgM-antigen conjugated with nanogold) at the second test line of device. When compared with RVF specific ELISA, The sensitivity, specificity, and accuracy of developed LFD for detection of IgG were found to be 88.2, 96.9 and 94.0%, respectively, while the same for detection of IgM was found to be 100, 96, and 96%, respectively.

Keywords: Lateral flow device, Nanogold, Rift valley fever, Sheep.

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Introduction

Rift Valley fever (RVF) virus is a member of the genus Phlebovirus and the family Bunyaviridae which has been isolated from more than 30 mosquito species (EFSA, 2005). In the past few decades, its occurrence has been expanded from East Africa across the sub-Saharan region to North Africa and the Arabian Peninsula (Ahmad, 2000). RVF outbreaks in domesticated animals are often communicated to humans due to occupational exposure and vector-borne transmission. Infected food, fomites, and aerial exposures represent other modes of transmission for the disease. Sheep and cattle act as amplifier hosts for the virus.

The OIE prescribes a virus neutralization (VN) test for the detection of the RVF virus due to its high specificity and widespread species applicability. Further, ELISAs and other serological tests can also detect specific IgM or rising titers. Antibodies are detectable in domestic ruminants 4-5 days post-infection(OIE, 2014).

ELISAs and VN test can detect antibodies to the RVF virus. Virus neutralization tests that require live viruses are generally not recommended outside endemic regions or in laboratories not capable of biocontainment (Van Vuren and Paweska, 2010), as it is very expensive, time-consuming, and needs technical expertise. Therefore, the need for a simple, sensitive, rapid, and reliable test for the detection of anti-RVFV antibody that is sufficient to be applied on a large scale of animals is essentially required. Among the test candidates nominated to achieve this goal, immunochromatographic lateral flow assay is a suitable option for the detection of IgG and IgM anti-RVF antibodies in sera of infected and vaccinated ovine samples.

The lateral flow assay is among the most widely used techniques for detection of microbial analyte in clinical specimens such as *Staphylococcus aureus* (Shyu, *et al.,* 2010), *Salmonella enteritidis* (Shaimaa *et al.,* 2018), Salmonella antibodies (Ibrahim, 2017), Tuberculosis antibodies (IgG and IgM) (Ben-selma *et al.,* 2011), Swine fever virus antibodies (Xuewu *et al.,* 2012), Influenza viruses (Miyagawa *et al.,* 2012), Dengue virus antibodies (IgG + IgM) (Moorthy *et al.,* 2009), Rabies virus (Hualei *et al.,* 2010) and HIV in human (Shon *et al.,* 2009). The lateral flow immunochromatographic technique is a simple strip or device assays, which gain more and more popularity as a rapid diagnostic method that can be used for direct diagnosis at the production line or in the field. The present research aimed to develop an LFD for the rapid detection of IgM and IgG against RVF virus in one step to detect the phase of infection or vaccination with consideration of the case history.

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Materials and methods

Molecular Identification of RVFV

The RVF virus master seed was procured from central laboratory for evaluation of veterinary biologics, Abbasia, Cairo.RVFZH501. The viral RNA extraction was carried out using Easy Pure Viral DNA / RNA Kit, Transgen, China. RT-PCR was performed by the protocol described by Sall *et al*. (2001). For the RT-PCR step for nonstructural protein, NSca (5-'CCT-TAA-CCT-CTA-ATC-AAC-3') and NSng (5'-TA-TCATGG-ATT-ACT-TTC-C-3') primers were used and the thermo-cycler run at 45°C for 30 minutes: 1 cycle; 95°C for 2 minutes: 1 cycle; 94°C for 30 seconds, 44°C for 30 seconds, 72°C for 1 minute: 40 cycles; 72°C for 5 minutes: 1 cycle. After electrophoresis, a positive result was indicated by the presence of an 810 bp.

Preparation of RVF Antigen

RVF virus ZH501 strain was propagated in Baby Hamster Kidney (BHK-21) and/or Vero cells for three successive passages with a final titre 10^8 $TCID_{50}$/mL. RVF virus seed was tested for bacterial and extraneous viral contaminants. RVFV was inactivated using 0.0001 M of Binary ethylamine (BEI) (Eman, 1995). Then over action of BEI was stopped by sterile 20% sodium thiosulphate solution. Samples from the inactivated virus were checked for residual infectivity; by inoculation on BHK-21 cell and in baby mice by intracerebral route. Absence of cytopathic effect on cell culture and absence of mortality in baby mice were indications of optimum inactivation process.

Preparation of Specific Rabbit Polyclonal Antibodies Against RVF Virus

It was done by the method described by Shaimaa *et al*. (2018). The serum containing rabbit polyclonal antibody specific to the RVF antigen was separated and tested by the Serum neutralization test (SNT). The prepared antibodies can screen any RVFV strains as they have good correlations with all antigenic characters.

Purification of IgG from Rabbit Polyclonal Antibodies using Caprylic Acid

Fifty rabbit sera were centrifuged at 12000 rpm for 30 minutes and removed the pellet. Double the serum volume of 0.06 M sodium acetate buffer pH 4.6 was added in a beaker and placed on a magnetic stirrer. 4.04 mL of caprylic acid was added slowly drop by drop while stirring at room temperature for 30 min and then centrifuged at 12000 rpm for 20 min. The supernatant was retained, and the pellet was discarded. The supernatant was dialyzed against PBS buffer at 4˚C overnight with two or three buffer changes; finally, the concentration of purified IgG was estimated by spectrophotometer (Elke *et al.,* 2008).

Preparation of Colloidal Gold Nanoparticles in 40 nm Diameter

One ml solution of 1% (m/v) sodium citrate was added to 100 ml boiling deionized water. When the mixture was heated to boiling again, one ml solution of 1% (m/v) $HAuC_{l4}$ was added rapidly by constant stirring. After the change in color of the solution (wine red in about 2 min), it was boiled for another 10 min. After cooling, deionized water was added until the volume reached to 100 mL. The obtained gold colloidal was supplemented with 0.02%(m/v) sodium azide and stored at 4°C. The particle diameter was checked with a scanning range 400-600 nm using a spectrophotometer (Kong *et al.,* 2017).

Conjugation of the Purified RVFV Glycoprotein with Colloidal Gold Nanoparticles

The purified RVFV glucoprotein was obtained from SinoBiological, China (Cat: 40338-V085B) with a concentration of 100µg/mL. Firstly colloidal gold nanoparticles were adjusted to pH 8.2 by using 0.02 M K_2CO_3. Half ml of RVFV glucoprotein was added to 50 ml of adjusted CG then gently mixed for 10 min. One ml of bovine serum albumin 10% was added for blocking with gently stirring for another 15 min and centrifuged at 10000 rpm for 30 min. The conjugated colloidal gold nanoparticles with RVFV glucoprotein was suspended in 1 mL conjugated diluted buffer (20 mM Tris containing 1% (w/v) BSA, 3% (w/v) sucrose and 0.02 % (w/v) sodium azide) and stored at 4°C (Ibrahim *et al.,* 2017).

Preparation of LFD

The lateral flow device was prepared as per the methods described by Shaimaa *et al*. (2018) and Moorthy *et al*. (2009)

Sample pad was glass fibered and pretreated with sample pad treated solution, pH 8.5 (purified water containing 3.81% (w/v) Borax, 1%(w/v) polyvinylpyrrolidone, 2% (w/v) titron X 100, 0.1% (w/v) casein sodium salt, 0.5% (w/v) sodium cholate, 0.15% (w/v) SDS, 0.02% (w/v) sodium azide) then dried at 37°C.

Conjugation pad was also glass fibered and pretreated with conjugation treated solution, pH 7.4 (20 mM PBS containing 2% (w/v) BSA, 2.5% (w/v) sucrose, 0.3% (w/v) polyvinylpyrrolidone, 1% (w/v) titron X100 and 0.02% (w/v) sodium azide) then dried at 37°C and kept in dry condition. Then it was saturated with conjugated colloidal gold nanoparticles with RVFV glucoprotein, finally dried at 37°C for 1hrand kept in dry condition.

Nitrocellulose (NC) membrane: The dispenser **(**Iso flow) was used to dispense three lines on the NC membrane (30 mm × 300 mm). The staphylococcal protein A (Sigma, Louis, MO, USA) (0.5mg/ml) was dispensed at the test line 1 (1 µL/1 cm line) while anti-ovine IgM (0.1 mg/mL) was dispensed at the test line 2 (1 µL/1 cm line). The purified specific rabbit anti-RVF antibodies (0.1 mg/mL) was dispensed at the control line (1 µL/1 cm line). The distance between the lines was 3 mm. The loaded NC membrane was dried at 37°C for 2 hrs and kept in dry condition.

The treated sample pad, treated conjugation pad, loaded NC membrane, and absorption pad was stick down on a PVC card. After that, the collected PVC card was cut into 3

mm width test-strips by using an automated cutter machine (Figures 1 and 2).

Principle for Lateral Flow Device Test

If ovine serum-containing RVF IgG is applied to a sample pad, it will rapidly wet through to the conjugate pad and will bind with it. The conjugated colloidal gold nanoparticles with RVFV glucoprotein (conjugation complex I) would be solubilized. This component begins to move with the sample flow front up the nitrocellulose membrane. The conjugation complex passes over the test line1 to which the captured staphylococcal protein A is immobilized, which has the ability to catch any ovine IgG, forming the test line1 complex (immobilized staphylococcal protein A with conjugation complex I), a red band in the test line 1.

If ovine serum-containing RVF IgM is applied to a sample pad, it will rapidly wet through to the conjugate pad and will bind with it. The conjugated colloidal gold nanoparticles with RVFV glycoprotein (conjugation complex II) would be solubilized; this component begins to move with the sample flow front up the nitrocellulose membrane. The conjugation complex II passes over the test line 2 to which the captured anti-ovine IgM is immobilized, which has the ability to catch any ovine IgM, forming the test line 2 complex (immobilized anti-ovine IgM with conjugation complex II) a red band in the test line 2.

In case the ovine serum sample contained both IgG and IgM, the two way of the flow was observed, and both test lines formed a red band. The excess of the conjugated colloidal gold nanoparticles with RVFV glycoprotein was trapped by the control zone (containing rabbit anti-RVF antibodies), forming a control line complex (immobilized rabbit anti-RVF antibodies with conjugated colloidal gold nanoparticles with FVFV glycoprotein), a red band in the control line. So if the ovine serum sample gave two red (test 1 and control line) it was considered IgG positive, while two red lines (test line 2 and control line) was considered IgM positive, in case of the three test line (test line 1, 2 and control line) it was considered IgG and IgM positive, but it gave one test line at control line it was considered IgG and IgM negative. If no band developed at the control line, the test was invalid (Figure 2).

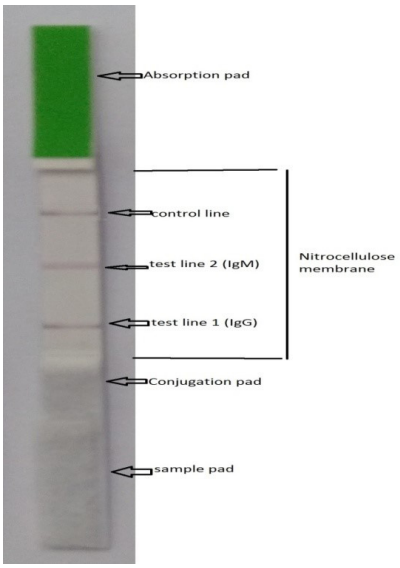

Figure 1: Lateral flow device components

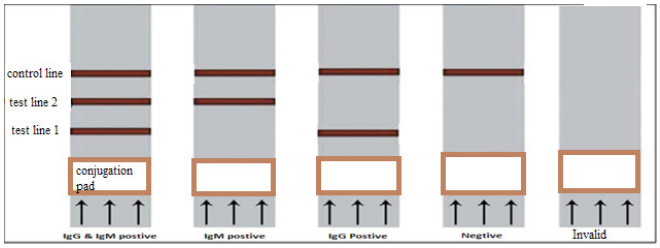

Figure 2: Interpretations of the LFD for detection of IgG and IgM in ovine sera against RVF virus

Conjugation pad: Saturated with conjugated colloidal gold nanoparticles with RVFV glycoprotein, Test line 1: The staphylococcal protein A, Test line 2: Immobilized anti-ovine IgM, Control line: rabbit anti-RVF antibodies.

A: Test is invalid, B: Positive (+ve) IgG and IgM, C:Positive (+ve) IgM, D:Positive (+ve) IgG, E:Negative (-ve) Result.

Sensitivity and Specificity Test

Preparation of standard ovine serum against RVFV was done by vaccination of five sheep using a high dose (3X of recommended dose) of standard RVF vaccine (pre-evaluated and certified). The sheep were bleed at 30-day post-vaccination. Prepared standard positive serum of RVF was tested and diluted (1, 10^{-2}, 10^{-3}. 10^{-4}, 10^{-5}, and 10^{-6}) and determined the minimal concentration of antibodies that gave positive in prepared LFD by matching or comparing with detected ELISA.

The ovine positive serum for Pox and PPR were tested by LFD prepared kit for specificity.

Assessment of Validity of LFD for Diagnosis of Suspected Field Samples in Comparison with ELISA Kits

One hundred suspected infected and vaccinated sera samples were collected from sheep and tested with the new prepared LFD and the results were matched with a standard gold test (IgG &IgM antibody ELISA detected ID Screen RVF IgM and IgG), to determine the specificity, sensitivity, and accuracy of the newly prepared device.

Evaluation of validity of a Diagnostic assay

The evaluation was done according to Thrusfield (2007)

Results and Discussion

Molecular identification RT-PCR was carried out for nonstructural protein in the RVF virus, and the PCR product was run in gel electrophoreses, and its length detected was found to be 810 bp as shown in Figure 3.

The gold solution was reaching a peak of 528, which indicated that the particle size was 40nm (Figure 4).

The LFD could detect the IgG antibodies until the dilution 10^{-4}, as shown in Figure 5. The same dilutions (1, 10^{-2}, 10^{-3}. 10^{-4}, 10^{-5}, and 10^{-6}) were tested by ID screen Rift valley fever competition Multi-species. The kits were used according to instructions of the manufacturer (Kit interpretation: S = sample, N = Negative control, A suspect or negative, S/N value of ≤ 40% was considered to be positive). The ELISA kit could detect the IgG till the dilution 10^{-5}.

The LFD could detect the IgM antibodies until the dilution 10^{-3}, as shown in Figure 6. The same dilutions (1, 10^{-2}, 10^{-3}. 10^{-4} and 10^{-5}) were tested by ID screen Rift valley fever IgM capture. The ELISA kit could detect the IgM till the dilution 10^{-4}.

All the tested sera gave negative results in 2 two test lines (IgG and IgM). One hundred suspected ovine serum samples were collected and tested by LFD as well as IgG and IgM detection ELISA kits. The results were compared and analyzed to determine the sensitivity and specificity and accuracy (Table 1). When IgG LFD results were compared with IgG detected ELISA kit, the true positive (T+), false positive (F+), false negative (F–) and true negative (T–) were 30, 2, 4 and 64, respectively. While IgM LFD results when compared with IgM detection ELISA kit, the (T+), (F+), (F–), and (T–) were 0, 4, 0, and 96, respectively (Table 1).

Thus, we can conclude that the sensitivity, specificity, and accuracy of LFD for detection of IgG were 88.2 %, 96.9 %and 94 %, respectively, while for detection of IgM these were 100, 96, and 96%, respectively the sensitivity of IgM was 100% may be due to the minimal sample amount that gives positive IgM (Table 1).

The results of sensitivity, specificity and accuracy of LFD for detection of IgG and IgM when compared with IgG and IgM detection ELISA, those were agreed with Yang *et al*.(2011) who found the sensitivity and specificity of lateral flow test for detection of anti-FMD virus compared to anti-FMD ELISA kit as 100% and 99.1%, respectively. Oem *et al.* (2009) reported the sensitivity rate of 100% and a specificity of 98.8% of the

Figure 3: Gel electrophoresis for PCR product of RVF virus
Lane M: 100 bp DNA ladder (Fermentas), Line 1:Band at 810bp specific for RVF Virus, Line 2:No band, negative control

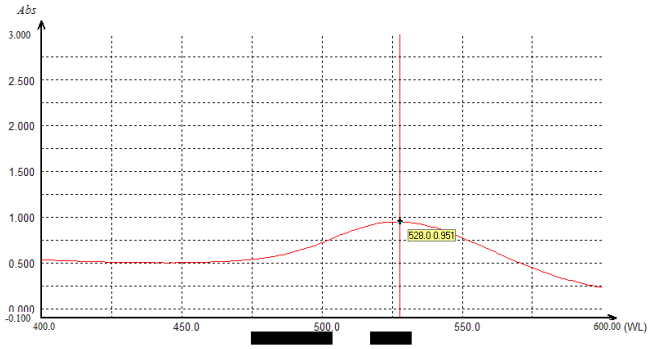

Figure 4: Spectrophotometer curve for 40nm colloidal gold nanoparticles.

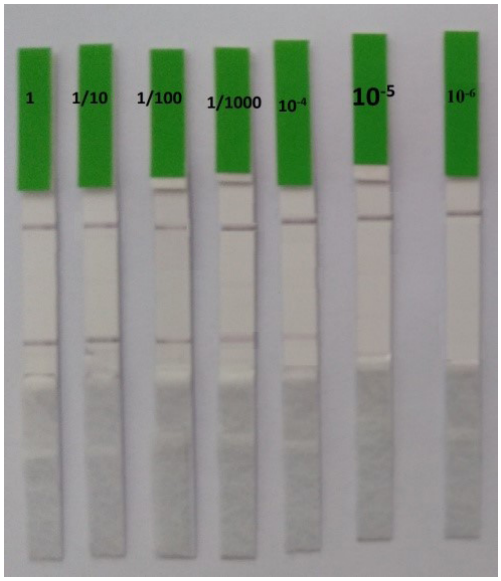

Figure 5: The Sensitivity of the ELISA kit in the detection of the IgG against RVFV.

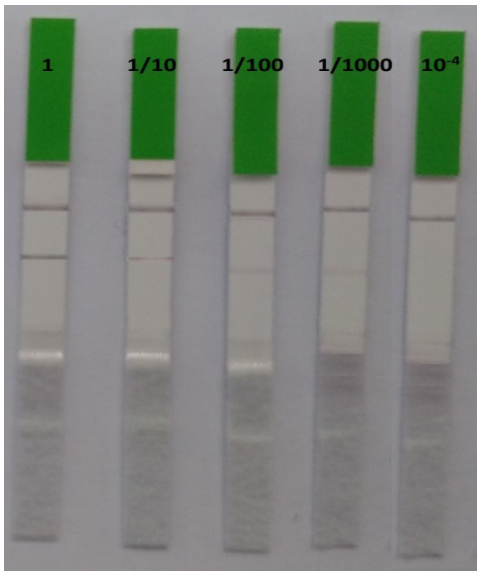

Figure 6: Sensitivity of the ELISA kit in the detection of the IgM against RVFV.

Table 1: Sensitivity, specificity and accuracy results of LFD for detection of IgG and IgM antibodies against RVFV

LFD		*ELISA*					
+ ve		*- ve*	*Total*		*Sensitivity test*	*Specificity test*	*Accuracy test*
IgG	+ ve	(T+) 30	(F+) 2	32	88.2%	96.9%	94%
	- ve	(F-) 4	(T-) 64	68			
	Total	34	66	100			
IgM	+ve	(T+) 0	(F+) 4	4	100%	96%	96%
	-ve	(F-)0	(T-) 96	96			
	Total	0	100	100			

lateral flow strips developed for the detection of serotype FMD compared to the ELISA anti-O FMD detection kit. The sensitivity, specificity, and accuracy of the prepared SE-Lateral flow kits reported were 94.4%, 90%, and 94%, respectively (Ibrahim *et al.,* 2017). Furthermore, Shiota *et al.* (2009) developed a lateral flow test for the detection of antibodies against the rabies virus and reported the sensitivity and specificity of 88.7% and 91.9%, respectively, as compared with ELISA.

The prepared LFD did not have the ability to differentiate between the infected and vaccinated animals; the same gave results positive at IgG test line 1. So we should know the case history of the tested animals while in case of the recent infection, the result is positive at IgM test line 2.

Conclusion

The prepared LFD is designed to identify in less than 5 minutes and in one step any of the IgM and IgG antibodies in sera against RVFV to detect the phase of infection or vaccination with consideration of the case history.

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