A Single Radial Haemolysis Technique for Rapid Diagnosis of Goat Pox Diseases

Short Communication

Sadri*[1], R., Masoudi, S.[2], Kargar, R.,[3] Khedmati, K.,[3]Varshovi, H.[1] and Haghighi, S.[1]

1. Viral Animal Vaccine Production & Research Dept., Razi Vaccine & Serum Research Institute, P.O. Box 11365-1558, Tehran, Iran

2. Poultry Vaccines Production & Research Dept., Razi Institute

3. Viral Animal Diseases Research & Diagnosis Dept., Razi Institute

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Summary

For detection of goat pox antibodies, a single radial haemolysis (SRH) technique using soluble antigens (SAgs) of the virus was developed. In comparison with CIE, ELISA and AGP as diagnostic tests, SRH is a rapid, simple, and reproducible, time efficient and inexpensive test. The plates of test result could be kept for two months at 4°C. Results of SRH test showed only 20% difference with serum neutralization test, as the golden test.

Key words: single radial haemolysis, poxvirus, soluble antigen, antibody, SN

Introduction

Pox disease is one of the goat viral diseases with high mortality rate. Many clinical symptoms of pox are similar to other viral diseases. For rapid diagnosis of goat pox infection and confirmation serological tests including CIE (Sharma *et al* 1988), ELISA (Rao *et al* 1996) and AGP (Bhambani & krishnamurthy 1963, Pandey & Singh 1972) are used. Among them CIE and ELISA need high expert with expensive materials and devices. AGP technique is much simpler to perform, and has greatly improved accuracy. However, it is less sensitive than the other techniques. Single radial haemolysis (SRH) test has been used to estimate the

* Author for correspondence. E.mail: roya@rvsri.com

haemolytic activity of mouse IgG antibody (Weiler *et al* 1965) and to measure mouse 19S anti-red blood cell antibody (Hiramoto *et al* 1971). SRH test is successfully used for detection of Newcastle disease, rinderpest and influenza viruses (Russell *et al* 1975, Majiyagbe *et al* 1965). We report the establishment of SRH test for detection of goat pox antibodies using soluble antigens (SAgs) of goat poxvirus instead of whole virus. The SAgs were extracted from pox lesion suspension by concentrating and purifying the suspension using PEG and Arcton 113. The different parameters, which affected the sensitivity of SRH test including concentration of antigen, diffusion time and volume of serum, were considered.

Materials and Methods

Preparation of pox virus SAgs. SAgs of goat pox virus were purified according to Isloor and Negi (1995) and, Pandey and Singh (1972). A 10 % suspension of goat pox scabs was prepared in chilled phosphate buffered saline (PBS). The suspension was homogenized and its supernatant was purified by fluorocarbon (Arcton 113; $CFCl_2$, CFCL and PEG) treatment and centrifuged at 2000-2300rpm at 4°C for 1h. It was stored at –20°C after adding 500IU/ml penicillin and 25mg/ml streptomycin.

Preparation of hyperimmune sera. Antisera against SAgs were prepared in 8-9 mon-old adult rabbits by four successive injections of 1ml purified SAg. SAg was emulsified with equal part of Freund's complete adjuvant for the first injection (intramuscularly) and with Freund's incomplete adjuvant for the second and third injections (intramuscularly). For the last administration, SAg was injected intravenously without adding any adjuvant. The injections were given on days 0, 14, 21 and 35. The rabbits were bled 10 days after the last injection and the sera were collected, pooled, aliquoted and stored at –20°C according to Rao *et al* 1996, Sarmbyal & Singh 1978, Sharma & Dhanda 1969.

Complement. Fresh serum of guinea pig was used as complement.

Red blood cell (RBC). Sheep RBCs were collected in anticoagulant (Alsever's solution) in aseptic condition and stored at 4°C. According to Russell *et al* (1975) the cells were washed three times in PBS, pH7.2 before use.

Coating of RBCs with SAgs. For coating of RBCs, 0.5ml of packed RBC was mixed with 1ml SAg, 1ml chromium chloride hexahydride (Cl_3Cr, $6H_2O$)(1/400), and 2ml veronal buffer pH7.0. The mixture was shaken at room temperature for 15-20min. Then 10ml of chilled physiological saline was added to the mixture for stopping the reaction. The coated RBC was washed three times in veronal buffer and packed at 1500rpm for 10min. The packed RBC was suspended in 2ml veronal buffer, pH7.0.

Gel preparation. A 1% concentration of agarose in distilled water was made up, incorporating 0.1% sodium azide. After cooling of agarose to 45°C, 1.2ml of RBCs suspension and 1.2ml of complement were added to 24ml of it. The mixture was poured at once into a plate and allowed to set. Then 3mm diameter wells were punched in the gel. Each well was filled with 10µl of serum and the gels were stored in a moist box at 37°C for 2-3h to allow the completion of diffusion. Storing of plate overnight at 25°C resulted in the formation of lysis zones around those wells which were filled with sera having antibody to the SAgs.

Serum neutralization (SN). The SN test was done according to the standard procedure with some modifications (OIE, Manual 2000). Briefly, test sera are diluted 1/2 in Eagles/HEPES and inactivated at 56°C for 30min. Then $100TCID_{50\%}$ of virus is mixed with each serum and the mixtures were incubated for 1h at 37°C, Eagles medium containing 2% fetal calf serum and antibiotics was added to the cell culture and the cells were incubated at 37°C for 6 days. Using an inverted microscope, the monolayers were examined daily from day 3 for evidence of CPE.

Results and Discussion

In this method, RBCs are coupled with SAgs (when main is antibody detection) or antibody (when main is antigen detection). After 12h because of diffusion around

each well, lysed zone of RBCs were observed. The appearance of the zones of lysis is shown in figure 1. For each zone, two diameters at right angles were measured. Using 5% formalin or 0.1% sodiumazide treatment it is possible to store the gel for two months at 4°C in a moist atmosphere.

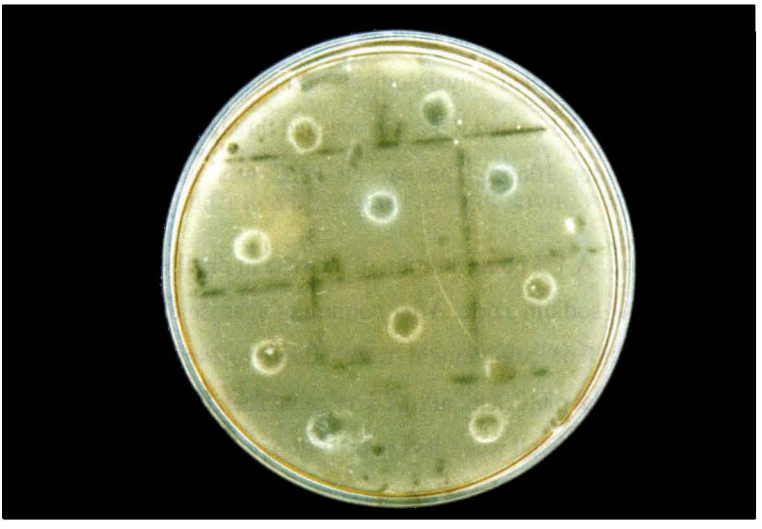

Figure 1. *The results of SRH test on ten field serum samples. The zone of lysis is observed around each well*

The parameters that affect the sensitivity of SRH test are concentration of antigen, diffusion time and volume of serum (Russell *et al* 1975). The first parameter is controlled both by the number of RBCs in the gel and the amount of virus which coats them. They found out that as the concentration of coated cells in the gel was reduced, the zone size increased but the contrast between lysed and unlysed areas of gel was diminished. We used 300µL of coated RBCs in 3ml agarose gel. The amount of antigen used to coat the RBC for routine use is a compromise, and was fixed at 1ml of SAgs and 0.5ml of RBC packed. Russell and his colleagues showed that the increment of virus concentration did not increased haemolysis zone and reduction in virus concentration increase the sensitivity of the technique. It seems

probable that at this concentration of antigens, most of the available receptors on the red cell surface have been saturated with antigens. The second parameter is the diffusion time, since diffusion of an antiserum through a gel is time-dependent; zone size should be measured at a fixed time after the start of diffusion or when the diffusion is substantially complete. In the experiment described, zone of lysis was measured after 18h. Increase this time up to 72h produced no significant increase in zone size .The third parameter is the volume of sera sample. The size of the zone produced depends on the absolute amount of antibody free to diffuse. Thus the increase in the volume of sera increases the size of the zone .In this work 3mm diameter wells were used. These wells conveniently hold 10μL of serum at a single filling and thus this technique is then capable of adequate sensitivity. It should be noted that the concentration of the complement is not critical. The complement dose not affect the size of the zones and only serves to make it visible by lysing the red blood cells which have antigen-antibody complexes at their surfaces.

To compare SRH with SN, as the golden test, both tests were done on a vaccinated flock with 80%-90% immunity. The comparison of SRH results with SN test is shown in table 1. According to the table the results of SRH showed only 20% difference with SN test.

Table 1. *Comparison of SN and SRH results on ten field sera*

No of serum	1	2	3	4	5	6	7	8	9	10
SN	+	+	+	+	+	+	+	+	+	+
SRH	+	-	+	-	+	+	+	+	+	+

The SRH technique has been used extensively with haemagglutinating viruses, especially the influenza virus group, to assay for antibody in serum samples (Russell *et al* 1975). The results obtained gave highly positive correlation between SRH and conventional HI and SN tests. However, the test has not been used extensively with

non-haemagglutination virus. Majiyagbe (1985) used the test for field evaluation of rinderpest antibody. Their results have shown that the SRH test is suitable for large scale surveys.

It appears to be particularly suitable to the screening of serum sample. Since the SRH technique is simple, rapid, and reproducible with accuracy, unaffected by non-specific inhibitor and dose not require expensive materials and devices in addition it is specific and sensitive. The sample doesn't require treatment before testing. The results maybe observed within 12-18h and the interpretation of the results is simple.

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