A NEW METHOD OF ANTIBODY DETECTION BY THE INDIRECT IMMUNOPEROXIDASE PLAQUE STAINING (IIPS) METHOD

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Most of the available tests to detect antibodies for viral diseases in livestock depend on a single parameter or indicator, i.e. the degree of hemolysis in the complement fixation test, a precipitin line of identity in the Ouchterlony double diffusion test, radioactivity in the radioimmunoassay, the degree of color formation in the enzyme-linked immunosorbent assay (ELISA), etc. On the other hand, the indirect immunofluorescence (IIF) test has two indicators to serve in determining the test results. In a positive reaction, the infected cells fluoresce and display antigen distribution typical for that particular virus. Furthermore, the test serum contains antibodies to the cells per se, the uninfected cells will also fluoresce. Thus, it is possible to virtually distinguish positive from false-positive reactions, and much confusion can be avoided in interpreting the result. Although the IIF test is more accurate and reliable than the other methods of detecting viral antibodies, it is not readily adaptable to large scale surveys. We have developed an immunoperoxidase plaque staining (IIPS) method that has all of the superior features of IIF but is more efficient, and the results can be read with the unaided eye under ordinary light. Thus far, we have evaluated the method by comparing it with the IIF test for its ability to detect antibodies to African swine fever (ASF) virus.

A total of 1,176 sera, including pre-inoculation sera, was collected from swine experimentally infected with five ASF virus isolates. One hundred forty-two sera collected from the Dominican Republic were also included in this study. All 1,318 sera were tested in IIPS and IIF tests.

For the IIF test, coverslip cultures of Vero cells infected with Lisbon'60 virus were prepared as previously described. The ovine anti-swine IgG ($OASI_g$) antibody precipitated by one-third saturation with ammonium sulfate and conjugated to FITC was used.

Ovine anti-swine-IgG antibody for making the appropriate conjugates for the IIPS test was prepared as follows: Swine IgG was purified by chromatography on a DEAE cellulose column equilibrated and eluted with 0.04 M Tris-phosphate buffer solution (pH 8.6). Each sheep received a total of 10 mg of swine-IgG in complete Freund's adjuvant subcutaneously injected in four sites. A booster injection with the same dosage of IgG in incomplete Freund's adjuvant was given after 7 days and the sheep were exanguinated 14 days later. The $OASI_g$ was purified from antiserum by affinity chromotography with swine IgG conjugated to Sepharose-4B-CNBr as an immunoadsorbent. The immunoadsorbent was suspended in ovine antiserum and agitated with a rocking motion on a shaker at 33C for two hours. After a thorough washing with PBS until the final wash solution had an optical density of 0 at 280 nm in a spectrophotometer, the antibody was eluted by suspending the immunoadsor-bent-antibody complex in a solution of 0.5 M NaCl in 0.1 M glycine-HCl buffer, pH 3.0 (GBS). After shaking for 30 minutes on the shaker at 33C, the entire suspension was poured into a glass column equipped with a sintered glass plate at the bottom. The eluent from the column was collected in fractions, and the column was further washed by the GBS until the effluent no longer absorbed at 280 nm. The protein solution was neutralized with 1 N NaOH solution and were precipitated by adding an equal volume of a saturated aqueous solution of ammonium sulfate and pelleted by centrifugation. The protein pellet was dissolved in a minimum volume of distilled water and dialysed against running tap water. Precipitates were removed by centrifugation at 800 xG. The supernatant was dialysed against 1,000 volumes of PBS and frozen at -20 C until used. The purified OASIgG was conjugated to horseradish peroxidase according to the method of Nakane and Kawaoi.

Confluent monolayers of Vero cells grown in Costar cell culture dishes with 24 or 96 well-clusters and infected with the Vero cell-adapted Lisbon'60 isolate of ASF virus served as antigen for the IIPPS test. The virus inoculum was adjusted so that each well contained approximately 150 or 60 plaques, respectively, under an agarose overlay (0.7%), after 5 days of incubation in a humidified 5% CO_2 atmosphere at 37 C. After the agarose overlay was removed and the cell sheet dried with a stream of warm air, the cell sheet was fixed with dry methanol for 10 minutes and dried. Finally, the plates were sealed with tape and stored at -70 C until used. The antigenicity after 8 months' storage (the longest period tested) equaled that of freshly prepared antigen plates.

For performing the IIPS test, antigen plates taken from the freezer were warmed and dried before tape was removed in order to prevent the cell sheets from detaching from the plastic wall during washing. Three-tenths milliliter (24-well-plate) or 0.05 ml (96-well-plate) of undiluted test sera was applied to the respective wells of the antigen plates and the plates were left at room temperature for 30 minutes. The serum was shaken from the plates, and the residual serum was removed by pressing the well-mouths firmly against clean paper towels. Wells were rinsed twice with 2% saline solution, and then filled with saline that was left at room temperature for 10 minutes before it was decanted. Three-tenths milliliter or 0.05 ml of HPO-conjugate, the optimal concentration of which was predetermined, was placed into each well, and the plates were kept at room temperature for 30 minutes. Wells were washed and 0.5 ml or 0.1 ml of 3,3'-diaminobenzidine (1 mg/ml) in 0.01% H_2O_2 saline solution was placed into each well. The reaction was stopped after 30 minutes by rinsing the plates once with tap water and drying them.

Wells containing dark brown-colored plaques were recorded as positive. When the HPO-conjugate prepared with the purified antibody was used, the background (uninfected area) was colorless during the 30-minute reaction period even when extensively hemolysed sera were tested.

The IIPS and IIF tests had 96% agreement in detecting sera containing antibody; and either test had about 2% more positives than the other test. Thus, both tests had comparable sensitivities and specificities. As to the efficiency of the IIPPS test, a trained technician is able to process up to 400 sera a day without difficulty.

Although ASF virus and antisera were used in developing the IIPS test, the test can no doubt be utilized for any other viruses that can be adapted and grown in cell cultures, regardless of the cytopathic or non-cytopathic nature of the viruses.

Selected references: Hess, W.R.: Monogr. Virol, 1971, 9:1; Pan, I.C., DeBoer, C.J., and Hess, W.R.: Can. J. Comp. Med., 1972, 36:309; Pan, I.C., Trautman, R., Hess, W.R., DeBoer, C.J., Tessler, J., Ordas, A., Botija, C.S., Ovejero, H., and Botija, Maria Carmen: Am. J. Vet. Res., 1974, 35:784; Bool, P.H., Ordas, A., and Sanchez Botija, C.: Bull. Off. Internat. Epizoot., 1969, 72:819; Nakane, P.K., and Kawaoi, J.A.: J. Histochem. Cytochem., 1974, 22:1084; Pan, I.C., Shimizu, M. and Hess, W.R.: Am. J. Vet. Res., 1978, 39 (3):491.