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 (IF) 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 confounding can be avoided in interpreting the result. Although the IF 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 (IPS) method that has all of the superior features of IF 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 IF test and 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 from the same swine were also included in this study. All 1,176 sera were tested in IF and IPS tests.

For the IF test, coverslip cultures of vero cells infected with fowlpox 60 virus were prepared as previously described. The anti-swine IgG (CASL) antibody precipitated by one-tenth saturation with ammonium sulfate and conjugated to fluorescein is used.

The anti-swine IgG antibody for making the appropriate conjugate for the IPS test was prepared as follows: Swine IgG was purified by chromatography on a DEAE-cellulose column equilibrated and eluted with 0.04 M meta-phosphate buffer solution (pH 6.1). 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 7 days after the first injection. After 1 day, the sheep were bled and the sera were examined 14 days later. The Ouchterlony method was used to determine the identity of the IgG isolated from swine IgG conjugated to sheep sera. The reaction was carried out in 3% agarose gel. A dried section of a swine IgG was mixed with the same IgG in a 1:1 ratio and was spotted on a blotted paper at 33°C for 2 hours. A blotter soaked with PBS until the final wash solution had an optical density of 200 units at 280 nm in a spectrophotometer. The antibody was eluted by suspending the immunoperoxidase-anti-body complex in a solution of 0.5 M NaCl in 0.1 M glycine-HCl buffer, pH 3.0 (GHB). After elution for 30 minutes on the shaker at 37°C, the antibody 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 with PBS until the effluent no longer absorbed at 280 nm. The protein solution was neutralized with 1 N NaOH solution and was precipitated by adding an equal volume of a saturated aqueous solution of ammonium sulfate and pelleted by centrifugation. The protein precipitate was dissolved in a minimum volume of distilled water and dialyzed against running tap water. Precipitates were removed by centrifugation at 800 rpm. The solution was filtered through 1,000 volumes of PBS and frozen at -20°C until used. The purified

Confluent monolayers of vero cells grown in Costar cell culture dishes with 24 or 96 well-plates and infected with the vero cell-adapted Listeria 60 isolate of ASF virus served as antigen for the IPS test. The virus inoculum was adjusted so that each well contained approximately 940 or 60 plates, respectively, under an aqueous overlay (0.7%), after 5 days of incubation in a humified 5% CO2 atmosphere at 37°C. After the agarose overlay was removed and the cell sheets dried with a stream of warm air, the cell sheets were fixed with dry methanol for 10 minutes and dried. Finally, the plates were sealed with tape and stained at -70°C until used. The antigenicity after 4 months storage (the longest period tested) equaled that of freshly prepared antigen plates.

For preparing the IPS test, antigen plates taken from the freezer were washed and dried before tape was removed in order to prevent the tape from detaching from the plastic wall during washing. Three-times milliliter (24- or 96-well) plates of uninfected test sera were applied to the respective wells of the antigen plates and the plates were left at room temperature for 90 minutes. The serum was then shaken from the plates, and the residual serum was removed by pressing the well-membrane firmly against clean paper towels. Wells were rinsed twice with 2 ml distilled water, and then filled with saline that was left at room temperature for 10 minutes before it was decanted. Three-times milliliter (24- or 96-well) plates of H2O-conjugate, the optimal concentration of which was determined, was placed into each well, and the plates were kept at room temperature for 30 minutes. Wells were washed 3 times with 0.5 ml of 0.1% Triton X-100 in 0.05 M NaCO3 saline solution was placed into each well. The reaction was stopped after 10 minutes by rinsing the plates once with tap water and drying them.

Wells containing dark brown-colored plaques were recorded as positive. When the H2O-conjugate prepared with the purified antibody was used, the background (uninfected area) was colorless during the 30-minute reaction period even when extensively hemolyzed sera were tested.

The IPS and IF test results were 95% agreement in detecting sera containing antibodies; and other tests had about 74% more positives than the other test. Thus, both tests had comparable sensitivities and specificities. To test the efficiency of the technique, a trained technician in able to process up to 40 sera a day without difficulty.

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