Title: A Plan for Obtaining More Accurate and Specific Results on PRRSV Serological Tests When Using Commercial ELISA's - **NPB# 01-007**

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Date Received: 6/61/2002

I. Abstract

Attaining and maintaining negative herd status for PRRSV is now a major goal for many producers. To achieve such health status, it is important to count on a reliable PRRSV serological test. There is one commercial kit for PRRS serology (manufactured by Idexx labs) which is extensively used worldwide. The Idexx assay consistently presents the inconvenience of exhibiting a high rate (2 % or more) of false positive animals which translates into a significant incidence of isolated reactors (singletons) in herds that should otherwise test negative. The occurrence of these singletons poses a significant problem for the certification and commercialization of pigs. In the last few years alternative serological ELISA kits became commercially available outside the US.

The goal of this project was to assess whether two new foreign tests could detect PRRSV infections with good level of specificity and sensitivity. We were interested in testing if these assays could be used as alternative sero-tests to confirm the real serologic status of the PRRSV singletons that are frequently obtained through the use of the Idexx test. To this end, we used a large collection of sera of previously known PRRSV reactivity. Neither the Biovet (Canada) or the HIPRA (Spain) tests showed sufficient sensitivity of detection of infected animals in order to confidently allow their use in lieu of the Idexx test. Our conclusion is that for the time being the Idexx test continues to be the sole acceptable commercial ELISA test for PRRSV certification. Singletons can still be ruled out by the complementary use of indirect fluorescent assays.

II. Introduction:

The effective control and eradication of veterinary infectious diseases is based on an accurate identification of infected animals. To that end, the serological detection of all the infected animals in a herd has always been central to this process. The development of serologic ELISA commercially available kits has constituted a significant

These research results were submitted in fulfillment of checkoff funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer reviewed

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advancement in the diagnosis of infectious diseases. The ELISA test most commonly used for PRRSV serological diagnosis (Idexx PRRSV ELISA, Portland, ME) consists of an indirect ELISA that employs two types of antigens combined on the same plate: 1) native proteins of the North-American PRRSV prototype strain together with 2) a genetic engineered antigen of the Lelystad (European) prototype strain. The Idexx ELISA kit has been widely used in the U.S. and overseas, being , in many places of the world, perhaps the only diagnostic tool or procedure for PRRSV that is available for official testing in that country or region. Some distinct features of this test are: 1) its ability to detect a serologic response to either (European or North American) PRRSV serotype,(an essential feature now that both PRRSV types may be present in US farms), 2) a "Sample-to-Positive" (S/P) ratio that allows a semi-quantification of the antibody content in a serum sample and 3) a relatively slow sero-conversion, which may require up to three weeks post-infection to become established. After an animal has sero-converted, the Idexx test is very consistent and follows closely the kinetics of appearance, peaking and disappearance of circulating antibodies. Therefore, essentially no false negatives are commonly observed with the Idexx test, other than those represented by animals that have not yet sero-converted but that have been recently exposed to PRRSV , or those animals in which, after a long post-infection period of seropositivity, the antibody level decays to below the cut-off value.

A significant disadvantage presented by the Iddex test is the high incidence of false positives that may reach up to 2 % or more in a herd and which translates into a significant incidence of isolated reactors (a.k.a "singleton reactors or SR") in a whole herd that otherwise should test negative. This trend of the Idexx ELISA to produce a higher-than-average % of false positives was not much of an overall concern when positivity in herds was the prevailing pattern of ELISA scores around the country (i.e. during initial establishment and spread of PRRSV infections in the Midwest). However, now industry is organizing their own strategies to clean herds and segregate sero-negative herds. Likewise, more and more regions or entire countries are trying to prevent the introduction of PRRSV in their stocks and therefore a complete PRRSV-negative status is essential in national and international animal trade. As has also been the case with PRV, the occurrence of these SRs is a regulatory dilemma and a significant complication for the management of the herds that need to be tested for export or that are under a disease eradication program. In the case of either disease, little is known about the reason(s) for the occurrence of these SRs. It has been postulated that multiple immunizations with unrelated vaccines may be one of the reasons for this occurrence. In our laboratory we have observed that treatments known to cause a polyclonal stimulation of the lymphoid cells (i.e. inoculation of PRRSV-free pigs with attenuated Mycobacterium BCG) cause a certain percentage (up to 6 %) of the individuals in a given PRRSV-free pig population to develop a sizable S/P for Idexx PRRSV ELISA. While finding an explanation for the occurrence of SRs by ELISAs is important, even more so is finding diagnostic procedures alternative to the Idexx ELISA to ascertain the real infection status of these animals.

In the case of Pseudorabies, an alleviation for the problem caused by the SRs is jointly provided by molecular diagnosis and by the biology of the disease itself. The vast majority if not all of the latent infections by PRV will become established in precise tissue sites of the animals that can be investigated by PCR (upon necropsy) with exquisite sensitivity. In the case of PRRSV, we still lack that certainty. We know that PCR will be highly sensitive to detect the PRRSV while this virus remains in the blood for a period that sometimes lasts up to several weeks post-exposure. Beyond that point, we know that the infectious PRRSV will remain in the lymphoid tissues for periods that

can last up to 5 months PI , but there is still some controversy about which would be the most consistent site to molecularly ascertain the presence of PRRSV upon necropsy. The most appropriate procedure for those cases in which reaching a decision at the herd level justifies the killing of the SR, is the bioassay of a pool of tissues collected upon necropsy, which is conducted by inoculation in a naive pig. This method has the intrinsic limitation of the extensive time involved to confirm the infectious status of the animal donating the tissues (up to one month). The bioassay applied to SR cases works under the assumption that the only biologically significant persistence of PRRSV seems to occur in the form of replicating, infectious virus, which seems to be confirmed by our most recent research about persistence.

Regarding serological analysis of SRs, the approaches currently used are : 1) to retest the animal and its in-contacts with the Idexx ELISA for either possible disappearance of the erratic signal or , alternatively, evidence for an active infection that is being transmitted to other contact animals, and 2) to test the sample by an alternative serologic assay. In the latter respect the indirect immunofluorescence has been the only alternative method available to the diagnostic laboratories in the US. However, the indirect immunofluorescence has intrinsic limitations imposed by its impracticality and the subjectivity presented by the fact that its end-point score has to be reached by human eye. Certainly the availability of other enzyme immunoassays that could be as easily standardized as the Idexx ELISA but that would be based on different antigenic principles than the Idexx ELISA would be an ideal avenue to explore in their application to the PRRSV SR situation.

Two alternative new commercial PRRSV ELISAs have been recently released in foreign countries: The Bio-Vet Competitive PRRSV ELISA (Quebec, Canada) and the CIVTEST SUIS of Hipra SRL (Barcelona Spain). These two tests are based on different antigen (HIPRA: native proteins of PRRSV European prototype, BIOVET: E.coli-expressed nucleocapside of a NorthAmerican Strain) and/or format (BIOVET is a competitive ELISA based on the reactivity of a highly conserved epitope throughout North-American strains).

III Objectives:

1) to comparatively ascertain the sensitivity and specificity of 3 commercial PRRSV serologic tests (Idexx, HIPRA and Biovet) by comparing their performance against the same bank of sera of defined origin and with previously characterized reactivity.
2) to test the simultaneous reactivity of singleton reactors (SR) sera in the three immunoassays so as to compare their ability to rule out the false positives without sacrificing sensitivity or specificity.

IV. Procedures

We have evaluated a total of 278 serum samples for their content in Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) antibodies, using three commercial ELISA kits: the Idexx PRRSV ELISA, the BioVet Competitive PRRSV ELISA (Quebec, Canada) and the CIVTEST SUIS of Hipra SRL (Barcelona Spain). The 278 samples used for our comparison included 206 field samples and 72 serums from pigs experimentally infected with different US strains of PRRSV. The field samples included 111 serums from presumptive PRRSV-negative animals and 92 PRRSV-

positive animals. Most of these PRRSV-positive field samples corresponded to farms known to be endemically infected with PRRSV. The positive field samples included a large number of mid-range and weak positive samples, including serums from young pigs carrying different levels of maternal antibodies. Idexx laboratories provided 26 additional samples that were obtained from animals infected with European strains of PRRSV.

V. Results

The Idexx test was able to detect 165/278 positive animals, while the Hipra test detected only 57/278 and the Biovet just 32/278. Particularly important are the results shown in table 1, which correspond to the performance of the 3 kits in a sub-group of serums of bonafide status that pertained to a battery check test developed by the NVSL/USDA/APHIS. The Idexx test detected 16/16 positive sera of the NVSL panel, while the Hipra test detected 11/16 and the Biovet test detected only 4/16 of the NVSL samples.

The trend of the results in the 278 sera would suggest that those serums with high content of antibodies (S/P ratios in the Idexx ELISA equal or higher than 1.1) tend to agree better among the 3 tests and be positively detected by the 3 tests, while those mid- and low-range positive serums (those with low content of antibodies, which are characterized Idexx kit S/P ratios between .0.4 and 1.0) tended to be missed by the Hipra and the Biovet tests. Interestingly, the Biovet test indicated positive status of PRRSV infection for four early serums not detected by the other two tests (four serum samples collected at 9 days after infection) We do not know the reason of the higher sensitivity of the Biovet kit in this particular case, but it may be related to the type of epitope recognized on the virion nucleocapside by the monoclonal antibody used in the composition of the kit. It is possible that the response to this part of the gp 5 protein is early in character. Likewise, the late PI serums tended to be consistently missed by the Biovet kit, indicating a lower preponderance of the epitope represented in the Biovet kit, later in the post-infection period.

VI. Conclusions

1) While the Idexx kit shows a consistent level of acceptable sensitivity, the Hipra and specially the Biovet kit present poor sensitivity.

2) The poor sensitivity of the Biovet and Hipra tests make uncertain the validity of a negative score obtained by these two tests. This renders the tests unacceptable for the confirmation of the serological status of individual animals in large sero-negative populations (singletons).

3) Based on our data, neither the Biovet nor the Hipra tests can replace the indirect immunofluorescence test in the verification of true serologic status of single Idexx reactors.

4) Only the Idexx test seems to effectively detect, with appropriate sensitivity, antibodies to the two major serotypes of PRRSV (US and European types). This is particularly important because there are many parts of North America where herds infected with both types of PRRSV seem to co-exist.

Table 1 Comparative Performance of the 3 tests in a check test panel 2000 obtained from NVSL, Ames, IA

SAMPLE	PIG ID	IDEXX S/P	HIPRA	BIOVET
1	PRRS (US) antiserum	1.017 Pos	51.95 Pos	2.87% Neg (-)
2	PRRS (US) antiserum	0.964 Pos	50.21 Pos	1.01% Neg
3	PRRS (US) antiserum Day 105	2.632 Pos	81.30 Pos	46.23% Pos
4	(katz) PRRS (US) antiserum	2.111 Pos	77.83 Pos	50.98% Pos
5	PRRS (US) antiserum Day 105	1.099 Pos	20.00 Neg	18.66% Neg
6	PRRS (US) antiserum	2.498 Pos	148.91 Pos	68.13% Pos
7	(Lelystad) 69 DPI	2.294 Pos	259.78 Pos	19.67% Neg
8	(US) Day 105	0.454 Pos	10.00 Neg	9.66% Neg (-)
9	PRRS negative	0.061 Neg	3.04 Neg	7.72% Neg (-)
10	PRRS (US) antiserum Day 105	2.218 Pos	53.48 Pos	35.11% Pos
11	PRRS (Lelystad) antiserum	2.643 Pos	362.17 Pos	18.95% Neg
12	PR negative serum	0.000 Neg	11.52 Neg	14.49% Neg (-)
13	PR negative serum	0.002 Neg	10.43 Neg	18.66% Neg
14	PRRS (US) antiserum	0.956 Pos	51.09 Pos	0.56% Neg (-)
15	PRRS (US) antiserum Day 105	1.225 Pos	18.04 Neg	13.54% Neg
16	PRRS (US) antiserum Day 105	1.204 Pos	19.13 Neg	5.97% Neg
17	PRRS negative	0.055 Neg	5.65 Neg	9.85% Neg (-)
18	PRRS (US) antiserum Day 105	1.365 Pos	20.43 Pos	2.28% Neg
19	PRRS (US) antiserum Day 105	1.385 Pos	19.78 Neg	2.68% Neg
20	(Lelystad) 69 DPI	2.597 Pos	236.09 Pos	16.14% Neg