Terminology for classifying swine herds by porcine reproductive and respiratory syndrome virus status

Derald J. Holtkamp, DVM, MS; Dale D. Polson, DVM, PhD; Montserrat Torremorell, DVM, PhD; and committee members
Bob Morrison, DVM, PhD, MBA (chair); Dyneah M. Classen, DVM; Lisa Becton, DVM; Steve Henry, DVM; Max T. Rodibaugh, DVM;
Raymond R. Rowland, PhD; Harry Snelson, DVM; Barb Straw, DVM, PhD; Paul Yeske, DVM, MS; Jeff Zimmerman, DVM, PhD

Summary

Standardized terminology for the porcine reproductive and respiratory syndrome virus (PRRSV) status of swine herds is necessary to facilitate communication between veterinarians, swine producers, genetic companies, and other industry participants. It is also required for implementation of regional and national efforts towards PRRSV control and elimination. The purpose of this paper is to provide a herd classification system for describing the PRRSV status of herds, based upon a set of definitions reflecting the biology and ecology of PRRSV. The herd classification system was developed by a definitions committee formed jointly by the American Association of Swine Veterinarians (AASV) and the United States Department of Agriculture PRRS-Coordinated Agricultural Project, and was approved by the AASV Board of Directors on March 9, 2010. The committee included veterinarians from private practice and industry, researchers, and representatives from AASV and the National Pork Board.

Breeding herds, with or without growing pigs on the same premises, are categorized as Positive Unstable (Category I), Positive Stable (Category II), Provisional Negative (Category III), or Negative (Category IV) on the basis of herd shedding and exposure status. Growing-pig herds are categorized as Positive or Negative. Recommended testing procedures and decision rules for herd classification are detailed.

Resumen - Terminología para clasificar los hatos de cerdos en relación a su estatus frente al virus del síndrome reproductivo y respiratorio porcino

La terminología estandarizada para determinar el estatus de los hatos de cerdos frente al virus del síndrome reproductivo y respiratorio porcino (PRRS por sus siglas en inglés) es necesaria para facilitar la comunicación entre veterinarios, productores porcinos, compañías genéticas, y otros participantes de la industria. También es necesaria para la implementación de los esfuerzos nacionales y regionales dirigidos a controlar y eliminar el virus del PRRS. El propósito de este artículo es proveer un sistema de clasificación para describir el estatus de PRRS basado en un conjunto de definiciones que reflejan la biología y la ecología del virus del PRRS. El sistema de clasificación de hatos desarrollado por un comité de definiciones formado por la Asociación Americana de Veterinarios Especialistas en Cerdos (AASV por sus siglas en inglés) y el Proyecto Agrícola Coordinado del PRRS del Departamento de Agricultura de los Estados Unidos, fue aprobado por el Consejo Directivo de la AASV el 9 de Marzo de 2010. El comité incluyó veterinarios de práctica privada y de la industria, investigadores, así como representantes de la AASV y del Consejo Nacional de Porcicultores.

Las piaras, con ó sin cerdos de crecimiento en las mismas instalaciones, se catalogan como Positiva Inestable (Categoría I), Positiva Estable (Categoría II), Provisional Negativa (Categoría III), ó Negativa (Categoría IV) en base a la excreción del hato y el estatus de exposición. Los hatos de cerdos en crecimiento se catalogan como Positivo ó Negativo. En el artículo se ofrecen detalles sobre las estrategias de muestreo y las reglas de decisión para la clasificación de hatos.

DJH, JZ: Iowa State University, Ames, Iowa.

DDP: Boehringer Ingelheim Vetmedica, Inc, St Joseph, Missouri.

MT, BM: University of Minnesota, St Paul, Minnesota.

DMC: Carthage Veterinary Services, Carthage, Illinois.

LB: National Pork Board, Johnston, Iowa.

SH: Abilene Animal Hospital, Abilene, Kansas.

MTR: Swine Health Services LLC, Frankfort, Indiana.

RRR: Kansas State University, Manhattan, Kansas.

HS: American Association of Swine Veterinarians, Burgaw, North Carolina.

BS: Michigan State University, East Lansing, Michigan.

PY: Swine Vet Center, St Peter, Minnesota.

Corresponding author: Dr Derald Holtkamp, Iowa State University, 2233 Lloyd Veterinary Medical Center, Ames, IA 50011; Tel: 515-294-9611; Fax: 515-294-1072; E-mail: holtkamp@iastate.edu.

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Résumé - Terminologie pour classifier les troupeaux porcins en fonction de leur statut par rapport au virus du syndrome reproducteur et respiratoire porcin

Une terminologie standardisée pour identifier le statut des troupeaux porcins en regard du virus de syndrome reproducteur et respiratoire porcin (PRRS) est nécessaire pour faciliter la communication entre les vétérinaires, les producteurs porcins, les compagnies de génétique, et les autres

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participants de l'industrie porcine. Cela est aussi requis pour la mise en place d'efforts régionaux et nationaux visant la maîtrise et l'élimination du virus du PRRS. Le but de cet article est de fournir un système de classification des troupeaux pour décrire le statut viral par rapport au PRRS, basé sur une série de définitions qui reflètent la biologie et l'écologie du virus du PRRS. Le système de classification des troupeaux, développé par un comité des définitions formé conjointement par l'Association américaine des vétérinaires porcins (AASV) et le Projet agricole coordonné-PRRS du Département de l'agriculture des États-Unis, a été approuvé par le Bureau de direction de l'AASV le 9 mars 2010. Le comité était formé de vétérinaires provenant de la pratique privée, de l'industrie, et de chercheurs, de même que de représentants de l'AASV et du Conseil national du porc.

Les troupeaux de reproducteurs, avec ou sans porcs en croissance sur le même site, sont catégorisés en tant que Positif Instable (Catégorie I), Positif Stable (Catégorie II), Négatif Provisoire (Catégorie III), ou Négatif (Catégorie IV) sur la base de l'excrétion par le troupeau et le statut de l'exposition. Les troupeaux de porcs en croissance sont catégorisés en tant que Positif ou Négatif. Les procédures recommandées pour les épreuves et les règles de décision pour la classification des troupeaux sont détaillées.

V eterinarians and researchers spend much time thinking about and communicating about porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV). However, the swine industry has yet to specify any standardized and clearly defined nomenclature for describing the PRRSV status of herds. Standardized terminology related to PRRSV and the PRRSV status of herds is necessary to facilitate communications between veterinarians, swine producers, genetic companies, and other industry participants. Likewise, a standardized system for herd classification based upon a set of related definitions is required for implementation of regional and national efforts towards PRRSV control, elimination, or both.

The purpose of this paper is to provide a herd classification system for describing the PRRSV status of herds based upon a set of definitions that reflect the biology and ecology of PRRSV as it is understood today.

This system was developed by a definitions committee formed jointly by the American Association of Swine Veterinarians (AASV) and the United States Department of Agriculture (USDA) PRRS-Coordinated Agricultural Project (PRRS-CAP) and was approved by the AASV Board of Directors on March 9, 2010. The committee included veterinarians from private practice and industry, researchers, and representatives from AASV and the National Pork Board.

Classification of herds

For breeding herds, with or without growing pigs on the same premises (herd and premises defined in Table 1), there are four categories: Positive Unstable (Category I), Positive Stable (Category II), Provisional Negative (Category III), and Negative (Category IV) (Figure 1 and Table 2). Category II is further divided into two subcategories: Positive Stable (II-A) and Positive Stable Undergoing Elimination (II-B). Premises with only growing pigs are categorized simply as Positive or Negative (Tables 3 and 4).

Herd classification for PRRSV is based on determining both shedding and exposure status of the herd (Figure 1). Testing methods to determine shedding include direct detection of the virus by polymerase chain reaction (PCR) or virus isolation. Polymerase chain reaction is the preferred testing method. Exposure is determined by antibody testing: enzyme-linked immunosorbent assay (ELISA), immunofluorescent antibody (IFA), or immunoperoxidase monolayer assay (IPMA). ELISA is the preferred testing method. Classification is determined by monitoring the PRRSV status of specific subpopulations in a herd. For the purpose of classifying herds, the relevant subpopulations are adult breeding animals, weaning-age pigs, breeding replacement animals, and growing pigs (post weaning). Testing is based upon representative sampling of the population.

PRRS virus shedding status is classified as negative, uncertain, or positive. A negative shedding status means that the available evidence supports the absence of viral shedding in the herd. A positive shedding status is supported by diagnostic and clinical evidence of viral shedding and transmission in the herd. A positive shedding status is also assumed for all herds and situations when information on the actual herd status is lacking. On the other hand, an uncertain shedding status is used when diagnostic data in tested herds is available to suggest a negative shedding status, but without sufficient confidence to support a negative shedding

status, usually because of lack of power in the sampling and testing strategy. An uncertain shedding status is considered transitory in the progress towards eliminating PRRSV from an infected herd.

Exposure status is classified as negative or positive. A negative exposure status means there is evidence to support the absence of prior exposure to the PRRSV in the population, assessed by the absence of antibodies to the virus in the samples tested.

Classification of breeding herds
Positive Unstable (Category I) breeding herds have positive shedding and exposure statuses. It is also the default category when herd shedding and exposure statuses have not been confirmed and when herds have not been tested. Herds going through a clinical PRRS outbreak and those with chronically recurring shedding of virus will fall into Category I.

Positive Stable (Category II) breeding herds have an uncertain shedding status and positive exposure status. Absence of clinical signs of PRRS in the breeding-herd population and confirmation of a sustained lack of detectable viremia in sampled weaning-age pigs (and growing pigs if present) for a minimum of 90 days is required. This classification requires a minimum of four consecutive negative PCR herd tests in weaning-age pigs sampled every 30 days or more frequently. "Weaning age" is defined as 7 days before and 3 days after weaning, regardless of the age of the pigs (Table 1). The exposure status of the breeding herd remains positive. The possibility that animals may still be infected and later shed the virus cannot be ruled out. A distinction is made between Category II breeding herds that are not undergoing PRRSV elimination (Category II-A) and those that are (Category II-B) (Table 2). A breeding herd is undergoing elimination if it has initiated an elimination procedure, as defined in Table 1. An elimination procedure begins when the last seropositive breeding replacements are introduced or when the last intentional exposure to any live PRRSV, wild-type or any vaccine (live or killed or both), occurs in the herd, whichever is later.

Confidence that the weaning-age pigs and growing pigs moving from Category II breeding herds are not shedding PRRSV increases over time if the breeding herd is undergoing PRRSV elimination, since steps are being taken to eliminate viral shedding

Table 1: Definitions relevant to herd classification for PRRSV

Term	Definition
Premises	Buildings or areas containing pigs at contiguous locations with common employees, management, or both.
Herd	The population of animals at a defined premises.
Internal multiplication and closed premises	A breeding herd where all replacement breeding stock are born and reared on the premises.
Internal multiplication and closed herd	A breeding herd where replacements are born on the premises but are reared at other premises before returning as replacements.
Elimination procedure	Any procedure used to eliminate the PRRSV from a swine herd. Ceasing the entry of PRRSV-positive animals and discontinuing intentional exposure to live virus are necessary elements of all elimination procedures. There are three broad categories of elimination procedures.
Complete depopulation and repopulation	Complete removal of all animals from a premises which are cleaned and sanitized prior to reintroduction of PRRSV-negative breeding replacements. Applies to both breeding herds and growing-pig herds.
Herd rollover	Any elimination procedure that relies upon cessation of viral shedding in the population and removal of previously infected animals, with subsequent introduction of negative breeding replacements. The premises are not completely depopulated in a herd rollover. Typically, herd rollovers involve herd closure (as defined below).
Incomplete depopulation	Any elimination procedure for a growing-pig herd that relies upon cessation of viral shedding in the population, with subsequent introduction of negative animals and removal of previously infected animals. The premises are not completely depopulated in an incomplete depopulation. Applies to growing-pig herds only.
Initiation of elimination	For breeding herds, initiation of elimination begins when the last seropositive breeding replacements are introduced or when the last intentional exposure to any live PRRSV occurs in the herd, whichever is later. For growing-pig herds, initiation of elimination begins when the last positive pigs are introduced.
Herd closure	A procedure where no breeding replacements, positive or negative, are allowed to enter the breeding herd to facilitate cessation of viral shedding in the population. The closure is usually maintained for at least 6 months. Typically done in herds that are eliminating the PRRSV by herd rollover.
Herd test	The evaluation of a herd based on diagnostic testing of a sample of (or all) animals in the herd and decision rules to classify the herd as positive or negative.
Weaning-age pigs	Pigs within 7 days before or 3 days after weaning, regardless of age.
Negative breeding replacement	For premises that are entering external replacements sourced from other breeding herds, including those with internal multiplication and closed herds, or internal multiplication and closed premises that temporarily introduce replacements from outside sources, confirmation that replacements are antibody-negative and not infected requires both of the following conditions: • Replacements must originate from Category III or IV breeding-herd premises; • Diagnostic confirmation that negative breeding replacements are seronegative by ELISA and negative for viremia by polymerase chain reaction (PCR) just prior to entry into the breeding herd (see Table 6). For premises that are entering internal replacements, confirmation that replacements are antibody-negative and not infected requires the following condition: • Diagnostic confirmation that negative breeding replacements are seronegative by ELISA and negative for viremia by PCR just prior to entry into the breeding herd (see Table 6).

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction

in the herd undergoing elimination. These steps will include closure of the herd to introductions of breeding replacements and may also include restrictions on cross-fostering and initiation of whole-herd exposure to live virus or vaccination. All herds that are undergoing PRRSV elimination by herd rollover will meet the criteria to be classified as Category II-B at some point during the elimination process. Category II-A is the goal for herds that are trying to control, rather than eliminate, the virus.

Category II is meaningful for managing PRRSV both in a production setting and in regional or national elimination programs. In the context of managing PRRSV in a production setting, the absence of viremia is significant for management of pig flow and expectations about improved reproductive and growing-pig performance. In the context of a regional or national elimination program, the distinction between Positive Unstable and Positive Stable breeding herds and those undergoing elimination versus those that are not may have value for deciding where and when to move pigs.

Provisional Negative (Category III) breeding herds have a negative shedding status. For a herd to be classified as Category III, sustained introduction of negative breeding replacements ("negative breeding replacement" defined in Table 1) without their seroconversion to the PRRSV is required. Lack of seroconversion in introduced animals is considered sufficient evidence to confirm that PRRSV is no longer being transmitted in the herd. The negative breeding replacements must have been in contact with previously positive animals and remain seronegative by ELISA for a minimum of 60 days after entry into the breeding herd (Table 5). Some adult breeding animals in the herd may still have antibodies to the PRRSV due to prior infections. If growing pigs are present at the same premises, confirmation of a negative exposure status in that subpopulation is also required. The recommended diagnostic protocol to confirm that the negative breeding replacements are negative for antibodies and negative for viremia prior to entry into the breeding herd is presented in Table 6. Category III herds include those intentionally pursuing elimination via herd rollover by entering negative breeding replacements.

Negative (Category IV) breeding herds have a negative shedding and exposure status.

Figure 1: Breeding-herd classification for porcine reproductive and respiratory syndrome virus (PRRSV) according to shedding and exposure status.

Herd category	Shedding status	Exposure status
Positive Unstable (I)	Positive	Positive
Positive Stable (II-A)	Uncertain	Positive
Positive Stable (II-B) (Undergoing Elimination)	Uncertain – undergoing elimination	Positive
Provisional Negative (III)	Negative	Positive
Negative (IV)	Negative	Negative

Confirmation of the negative exposure status of Category IV breeding herds varies depending on how the herd is established negative. For herds established negative by herd rollover, diagnostic information is required to confirm the negative exposure status of adult breeding animals, but information from production records may also be used in conjunction with diagnostic information to confirm negative status. The recommended protocol is presented in Table 7. Production records may be used to identify the breeding animals on the inventory list just before the first negative breeding replacements are introduced: this is the population of animals that must have been removed. When the current inventory contains no adult breeding animals that were on that list, the herd has been completely "rolled over" once and may be classified as Category IV. Alternatively, a negative exposure status may be confirmed with a negative herd test based upon sampling adult breeding animals to confirm that they are seronegative (Table 8) after a minimum of 1 year from the start of Category III status. Individual animal records are not required for the alternative criterion. If growing pigs are present at the same premises, confirmation of a negative exposure status in that subpopulation is also required.

Category IV also encompasses new premises startups populated with negative breeding replacements or premises that were completely depopulated and repopulated with negative breeding replacements that have remained seronegative. For these herds, a negative ELISA herd test in adult breeding animals is required at least 30 days after the premises are populated to define the negative exposure status of the herd (Table 9).

Classification of growing-pig herds

Premises with growing pigs only are classified as either positive or negative (Table 10). Positive herds have a positive shedding status or a positive exposure status or both. It is also the default category when diagnostic information is inadequate to classify a herd as negative. Negative herds have a negative shedding status and a negative exposure status (Table 11).

Supporting material to define shedding and exposure status

The criteria for defining negative or uncertain shedding status or negative exposure status are based upon observable, objective, and quantifiable measures. The objective evidence includes diagnostic results and information from production records, both of which should be routinely collected. Absence of clinical signs consistent with PRRS can also be used to support the herd-status classification, but clinical signs alone are not sufficient.

Diagnostic evidence is based upon periodic herd tests. The definition for herd tests (Table 1) is adapted from the definition proposed by Christensen and Gardner.[1] When possible, pooling of samples to increase sample size, frequency of testing, or both, and targeted sampling of subpopulations to improve herd-level sensitivity, are recommended. For the purpose of establishing positive versus negative herd status using currently available ELISAs, and until a differential diagnostic assay and related differentiable vaccine becomes available, no clear distinction is possible between diagnostic results that are positive due to live or killed vaccine usage versus those that are positive

Table 2: Criteria for and summary of supporting evidence required for breeding-herd classification for PRRSV

Herd category	Criteria	Supporting evidence required
Positive Unstable (I)	Any virus detected on the site along with clinical signs consistent with PRRS. Herds that do not meet the criteria for any of the other categories (II through IV) are Category I by default.	None required. Non-tested herds are Category I by default. Detection of virus in any tissue and presence of clinical signs would confirm status.
Positive Stable (II-A)	Category II starts after a 90-day period of sustained lack of viremia in weaning-age pigs and no clinical signs of PRRS in the breeding herd. Herd has not initiated an elimination program.	Test serum from weaning-age pigs by PCR.* No positive results over a 90-day period (four consecutive negative herd tests sampling every 30 days or more frequently) and no clinical signs consistent with PRRS observed in breeding herd.
Positive Stable Undergoing Elimination (II-B)	Category II starts after a 90-day period of sustained lack of viremia in weaning-age pigs and no clinical signs of PRRS in the breeding herd. Herd has initiated an elimination program and intends to become Negative.	Test serum from weaning-age pigs by PCR.* No positive results over a 90-day period (four consecutive negative herd tests sampling every 30 days or more frequently) and no clinical signs consistent with PRRS observed in breeding herd.
Provisional Negative (III)	Category III starts 60 days after negative breeding replacements are first introduced during a herd rollover with diagnostic evidence that they remain uninfected.	Test serum from negative breeding replacements by ELISA.† No positive results, after ruling out false-positives, at least 60 days after the initial introduction of negative breeding replacements.
	If growing pigs are present at the same premises, a confirmation of negative exposure status in that subpopulation is also required.	Test serum from growing pigs by ELISA.† No positive results, after ruling out false-positives.
Negative (IV)	For herd rollovers, Category IV starts when all previously infected animals have been removed from the herd.	Test serum from adult breeding animals by ELISA.† No positive results, after ruling out false-positives, subsequent to completion of rollover. Confirmed by breeding-animal inventory lists from production records.
	Alternatively, Category IV starts 1 year after the herd was classified as Category III if all animals in the herd are seronegative by ELISA.	Test serum from adult breeding animals by ELISA.† No positive results, after ruling out false-positives, 1 year after the herd was classified as Category III. Individual animal records are not required for the alternative criteria.
	For herds established Negative as a new startup or by complete depopulation and repopulation.	Test serum from adult breeding animals by ELISA.† No positive results, after ruling out false-positives, at least 30 days after population of premises with negative breeding replacements.
	If growing pigs are present at the same premises, confirmation of a negative exposure status in that subpopulation is also required.	Test serum from growing pigs by ELISA.† No positive results, after ruling out false-positives.

* Other virus-detection or antigen-detection tests may be used.

† Other antibody-detection tests may be used.

PRRS(V) = porcine reproductive and respiratory syndrome (virus); PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay

due to wild-type virus exposure; thus, no distinction is made.

Shedding status can be measured by direct detection of the virus by PCR and virus isolation. Polymerase chain reaction is the most commonly used agent-based detection technique, and therefore recommendations in this document are based on PCR testing, although other agent-detection methods could also be appropriate. The PCR test on serum detects viral genetic material and is relied upon to define shedding status. A negative result indicates the absence of viremia, but negative PCR results do not necessarily rule out the possibility that the animal is infected[2] or shedding.[3] However, monitoring serum from weaning-age pigs by PCR can be used as an indirect measure to monitor vertical transmission as well as horizontal sow-to-piglet and piglet-to-piglet

Table 3: Criteria for and summary of supporting evidence required for growing-pig herd classification for PRRSV

Herd category	Criteria	Supporting evidence required
Positive	Any virus detected on the site, along with clinical signs consistent with PRRS. Herds that do not meet the criteria for Negative are Positive by default.	None required. Non-tested herds are Category I by default. Detection of virus in any tissue and presence of clinical signs would confirm status.
Negative	None positive by ELISA after ruling out false-positives	Test serum from growing pigs by ELISA. No positive results, after ruling out false-positives, and no clinical signs consistent with PRRS observed in growing pigs.

PRRS(V) = porcine reproductive and respiratory syndrome (virus)

transmission in breeding herds.[4] Negative PCR tests on weaning-age pigs alone are not sufficient to establish a negative shedding status for a breeding herd. The ideal method for detecting shedding and transmission of virus in the breeding herd involves use of sentinel animals.

Prior exposure to the PRRSV is indicated by the presence of antibodies to the virus and is measured by tests such as ELISA, IFA, and IPMA. Commercial ELISA tests are the preferred methods to detect antibodies, and this document focuses on their use. Antibody levels are detectable in serum within 14 days[5] after infection and may wane over time to a level that is no longer detectable in individual animals. A positive ELISA test indicates previous exposure. Because serological tests are rarely 100% specific, some false-positives are to be expected. To rule out false-positives, serial testing using another antibody-based test of greater specificity, such as the IFA, can be used.[6,7] ELISA testing of serum is used to define exposure status as well as to confirm the seronegative status of negative breeding replacements. This is necessary for them to serve as sentinels to confirm the negative shedding status in Category III breeding herds.

For all herd tests that involve sampling, the sample size should be based on the target prevalence and desired confidence in detecting a positive.[8] Confirmation of a negative

or uncertain shedding status and negative exposure status of a previously positive herd will typically be sought when a herd is transitioning from positive to negative and the expected prevalence of positive animals is low. Furthermore, individual test sensitivity is potentially lowest in the transition from positive to negative when the concentrations of analytes (ie, PRRSV antibodies or viral RNA) are likely lowest in the course of infection. Therefore, a conservative sampling approach should be used to define the shedding and exposure status of a herd.[9]

Testing strategies should be modified as new methodologies are developed. For example the use of oral fluids offers great potential to test populations and should be considered in the future.[10] New technologies in antibody and antigen detection should also be evaluated as they are developed.

Discussion

An accepted set of terms and herd classifications is required to facilitate regional and national efforts to eliminate PRRSV. Furthermore, the availability of standardized nomenclature should facilitate communications among researchers, veterinarians, producers, genetic companies, and other industry participants. Standardized nomenclature will facilitate better contractual and business arrangements, especially those between genetic and commercial production

companies and agreements between producers that offer premiums for weaned pigs from breeding-herd sources with a specific PRRSV status. Researchers writing proposals and papers and making presentations may save time otherwise spent defining terms related to PRRSV, and standardized definitions will facilitate comparison of field-based research results by making clearer the conditions under which research trials are conducted.

Among the committee, definition of the Positive Stable category (II) was a contentious issue. The value of including this category, accounting for differences between herds pursuing elimination versus those pursuing control, the criteria for defining the status, and the meaning of the category were all fiercely debated. The literature does not support the proposal that negative PCR tests on weaning-age pigs constitutes sufficient evidence that a breeding herd is not infected with PRRSV. Without the benefit of monitoring a population of sentinel animals, it is not possible to establish the shedding status of breeding herds with sufficient certainty. However, anecdotal evidence from the field supports the notion that herds classified as Positive Stable (II) have better reproductive and growing-pig performance than herds that are Positive Unstable (I). Being Positive Stable (II) is the goal for breeding herds that are trying to control the virus. In the context of area or national elimination efforts, subdivision of category II into II-A and II-B is important to convey the likely differences in the risk of current or future shedding of virus by animals in these herds.

The standard herd classification scheme for the PRRSV status of herds and related definitions proposed here are consistent with the biology and ecology of PRRSV as it is understood currently. As new information becomes available, these definitions may need to be reviewed.

Table 4: Recommended protocol to assess PRRSV shedding status of weaning-age pigs for Category II-A or II-B breeding herds

	Requirements
Test(s) performed	Polymerase chain reaction
Animals tested	Weaning-age pigs*
Specimen(s) collected	Serum (blood, notch/swab, tail/swab)
Sampling or whole-herd testing (every animal in population)	Sampling
If sampling:	
Targeted subpopulation (if any) sampled	Light-weight males from gilt litters may increase sensitivity (optional)
Systematic sampling procedure	One pig per litter, both pig and litters selected randomly
Minimum number of samples per herd test	30 samples, determined by target prevalence to be detected of 10%, and 95% confidence level, for any population size
Pooling strategies (if any)	Pools of five
Procedures to rule out false-positives	None
Minimum number of periodic herd tests	Minimum of four to account for variation in prevalence and increase confidence of finding positives if present
Frequency of herd tests (minimum frequency)	Every 30 days or more frequently to confirm status Frequency to reconfirm status after initial tests depends upon reason for classifying herd
Decision rules that classify the herd	None positive over a 90-day period (four consecutive negative herd tests if sampling every 30 days); no clinical signs in breeding herd

*   Weaning-age pigs defined as 7 days before and 3 days after weaning regardless of age.
PRRSV = porcine reproductive and respiratory syndrome virus

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*Non-refereed references.

Table 5: Recommended protocol to assess PRRSV exposure status of negative breeding replacement for Category III breeding herds

	Requirements
Test(s) performed	ELISA
Animals tested	Negative breeding replacements (negative when entered; serve as sentinels)
Specimen(s) collected	Serum
Sampling or whole-herd testing (every animal in population)	Sampling
If sampling:	
Targeted subpopulation (if any) sampled	None
Systematic sampling procedure	Random sample from multiple ages of breeding replacements
Minimum number of samples per herd test	60 samples, determined by target prevalence to be detected of 5%, and 95% confidence level, for any population size
Pooling strategies (if any)	None
Procedures to rule out false-positives	For positive and suspect samples, retest by ELISA and perform IFA and PCR; resample population if still positive
Minimum number of periodic herd tests	One
Frequency of herd tests (minimum frequency)	One at least 60 days after initial introduction of negative breeding replacements
	Frequency to reconfirm status after initial test depends upon reason for classifying herd
Decision rules that classify the herd	None positive after ruling out false-positives

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; IFA = immunofluorescent antibody; PCR = polymerase chain reaction

Table 6: Recommended protocol to assess PRRSV status of breeding replacements on the basis of serological status (by ELISA testing) and viremia (by PCR testing) prior to entry into breeding herds

	Requirements
Test(s) performed	ELISA and PCR
Animals tested	Negative breeding replacements in quarantine prior to entry into breeding herd
Specimen(s) collected	Serum
Sampling or whole-herd testing (every animal in population)	Sampling
If sampling:	
Targeted subpopulation (if any) sampled	None
Systematic sampling procedure	Random sample
Minimum number of samples per herd test	60 samples
	Determined by target prevalence to be detected of 5%, and 95% confidence level, for any population size
Pooling strategies (if any)	None for ELISA; pools of five for PCR
Minimum number of periodic herd tests	Two recommended
Procedures to rule out false-positives	For positive and suspect samples, retest by ELISA and perform IFA and PCR; resample if still positive
Frequency of herd tests (minimum frequency)	One immediately after entry into quarantine, ELISA only
	One just prior to exiting quarantine and entry into breeding herd, ELISA and PCR
Decision rules that classify the herd	None positive after ruling out false-positives

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; IFA = immunofluorescent antibody

Table 7: Recommended protocol to assess PRRSV exposure status of adult breeding animals for Category IV herds established negative by herd rollover using production records to confirm that all previously infected animals have been removed from the breeding herd

	Requirements (using production records)
Test(s) performed	ELISA
Animals tested	Adult breeding animals
Specimen(s) collected	Serum
Sampling or whole-herd testing (every animal in population)	Sampling
If sampling:	
Targeted subpopulation (if any) sampled	None
Systematic sampling procedure (if any)	Random sample from multiple parities and stages of gestation and boars
Minimum number of samples per herd test	30 samples, determined by target prevalence to be detected of 10%, and 95% confidence level, for any population size
Pooling strategies (if any)	None
Procedures to rule out false-positives	For positive and suspect samples, retest by ELISA and perform IFA and PCR; resample if still positive
Minimum number of periodic herd tests	One
Frequency of herd tests (minimum frequency)	One test subsequent to confirmation that all previously infected animals have been removed from the breeding herd using the animal inventory lists from production records. Frequency to reassess status after initial test depends upon reason for classifying herd
Decision rules that classify the herd	None positive after ruling out false-positives

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; IFA = immunofluorescent antibody

Table 8: Recommended protocol to assess PRRSV exposure status of adult breeding animals for Category IV herds established negative by herd rollover when production records are not available or not used

	Requirements (not using production records)
Test(s) performed	ELISA
Animals tested	Adult breeding animals
Specimen(s) collected	Serum
Sampling or whole-herd testing (every animal in population)	Sampling
If sampling:	
Targeted subpopulation (if any) sampled	None
Systematic sampling procedure (if any)	Random sample from multiple parities and stages of gestation and boars
Minimum number of samples per herd test	60 samples, determined by target prevalence to be detected of 5%, and 95% confidence level, for any population size
Pooling strategies (if any)	None
Procedures to rule out false-positives	For positive and suspect samples, retest by ELISA and perform IFA and PCR; resample if still positive
Minimum number of periodic herd tests	One
Frequency of herd tests (minimum frequency)	One test after the herd has been Category III for a minimum of 1 year. Frequency to reassess status after initial test depends upon reason for classifying herd
Decision rules that classify the herd	None positive after ruling out false-positives

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; IFA = immunofluorescent antibody

Table 9: Recommended protocol to assess PRRSV exposure status of adult breeding animals for Category IV herds established negative as new premises startup or by complete depopulation and repopulation

	Requirements
Test(s) performed	ELISA
Animals tested	Adult breeding animals
Specimen(s) collected	Serum
Sampling or whole-herd testing (every animal in population)	Sampling
If sampling:	
Targeted subpopulation (if any) sampled	None
Systematic sampling procedure (if any)	Random sample from multiple parities and stages of gestation and boars
Minimum number of samples per herd test	30 samples, determined by target prevalence to be detected of 10%, and 95% confidence level, for any population size
Pooling strategies (if any)	None
Procedures to rule out false-positives	For positive and suspect samples, retest by ELISA and perform IFA and PCR; resample if still positive
Minimum number of periodic herd tests	One
Frequency of herd tests (minimum frequency)	One at least 30 days after population of premises with negative breeding replacements
	Frequency to reassess status after initial test depends upon reason for classifying herd
Decision rules that classify the herd	None positive after ruling out false-positives

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; IFA = immunofluorescent antibody

Table 10: Growing-pig herd classification for PRRSV when only growing pigs are on the premises (wean-to-feeder, wean-to-finish, and feeder-to-finish herds)

Herd category	Shedding status	Exposure status
Positive*	Positive	Positive
Negative	Negative	Negative

* Herd categorized as "Positive" if either the shedding status or the exposure status is positive.

PRRSV = porcine reproductive and respiratory syndrome virus

Table 11: Recommended protocol to assess PRRSV shedding and exposure status of growing pigs

	Requirements
Test(s) performed	ELISA
Animals tested	Growing pigs
Specimen(s) collected	Serum
Sampling or whole-herd testing (every animal in population)	Sampling
If sampling:	
Targeted subpopulation (if any) sampled	None
Systematic sampling procedure	Longitudinal: random sample from multiple age groups if present (3 weeks apart in nursery and 8 weeks apart in finisher)
Minimum number of samples per periodic herd test	30 samples per age group, determined by target prevalence to be detected of 10%, and 95% confidence level, for any population size
Pooling strategies (if any)	None
Procedures to rule out false-positives	Retest positives and suspects by ELISA and IFA, resample if still positive by ELISA
Minimum number of periodic herd tests	One
Frequency of periodic herd tests (minimum frequency)	Frequency to assess status will depend upon the reason for determining the shedding and exposure status
Decision rules that classify the herd	None positive after ruling out ELISA false-positives

PRRSV = porcine reproductive and respiratory syndrome virus; ELISA = enzyme-linked immunosorbent assay; IFA = immunofluorescent antibody