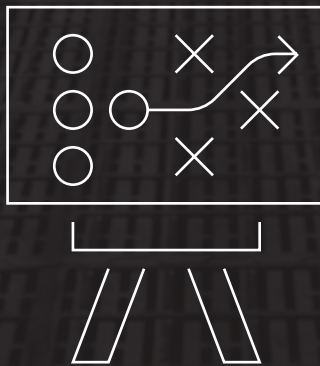
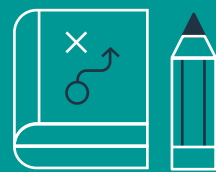


IAV-S STABILIZATION AND ELIMINATION

# INFLUENZA PLAYBOOK

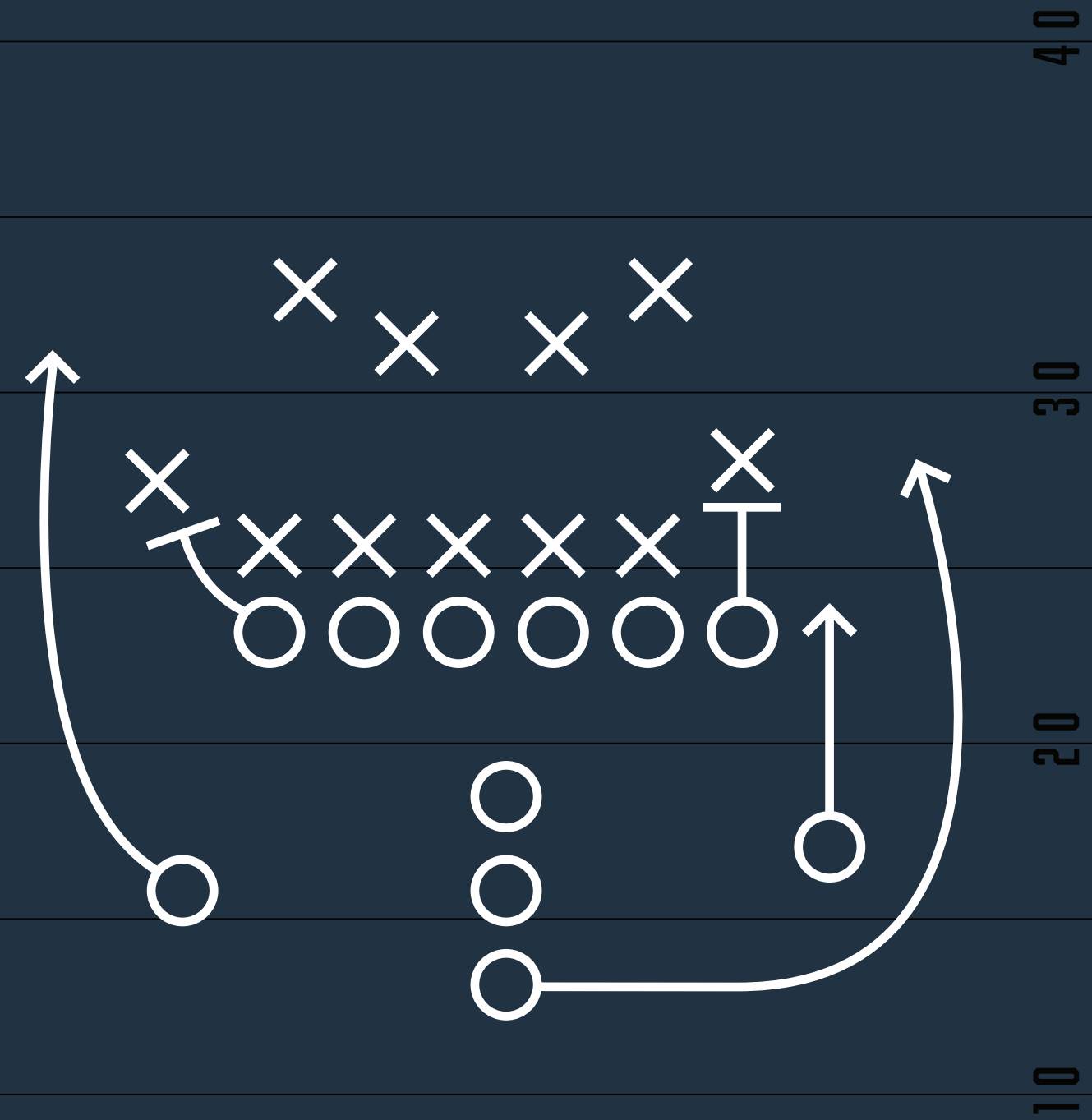


**STUDY YOUR OPPONENT.  
BUILD A GAME PLAN.**



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# INTRODUCTION

Influenza A virus in swine (IAV-S) is an RNA virus with a wide array of mammalian and avian hosts. Multiple strains of H3N2, H1N1 and H1N2 are presently endemic in the U.S. swine herd. Infection with IAV-S can result in high fevers, coughing, sneezing, poor feed intake and damage to the respiratory epithelium, which results in increased susceptibility to bacterial co-infections and pneumonia. IAV-S is an important cofactor in the porcine respiratory disease complex (PRDC), where it causes the greatest economic impact.

# Economic Impacts of IAV-S

A 2024 study in which IAV-S was eliminated from five sow farms reported that the nursery average daily gain (ADG) was improved by 36.6 grams per day, mortality was reduced by 0.45% and treatment costs were reduced by \$0.27 per pig following farm elimination of IAV-S.<sup>1</sup> Nursery mortality was reduced by 2%, ADG was improved by 123 grams per day and feed conversion was reduced by 0.26 in a 1,200-head sow farm where IAV-S was eliminated.<sup>2</sup>

Comparison of IAV-S negative and IAV-S positive flows within the same system reported for the IAV-S negative flows a 0.1 pound per day improvement in ADG in both nursery and finishing phases, while feed conversion was improved by 0.2 lb. in the nursery and 0.1 lb. in the finisher phase in IAV-S negative flows.<sup>3</sup>

Finishing closeouts collected from 2007-2011 from an integrated pork production system showed that the diagnosis of IAV-S reduced ADG by 0.04 lbs. per day and increased percent mortality, culls and tail-enders by 1.87%. **The overall economic impact of IAV-S was determined to be \$3.23 loss per pig.**<sup>4</sup>

Finally, nursery pigs vaccinated with SEQUIVITY® IAV-S NA at 3 days and 3 weeks of age and challenged with a virulent strain of H1N1 at approximately 5 weeks of age were 2 lbs. heavier than unvaccinated controls at 10 weeks of age.<sup>5</sup> In weaned pigs, there is an additive effect on the disease cost incurred by IAV-S when co-infections (i.e., PRRSV) are involved, making early nursery IAV-S infections costly.



The impacts of IAV-S on producers and veterinarians can be summarized as “frustration, cost on productivity, increased medication costs and decreased quality of piglets at weaning. We will make a positive impact on public health by eliminating IAV-S from more farms within the swine industry.”

**DR. MONTSE TORREMORELL**

## ELIMINATING COFACTORS IMPROVES ALL ASPECTS OF PRODUCTION.

Given the significant production impact of IAV-S during the growing stage, there has been an increased effort to eliminate IAV-S from breeding herds with the goal to wean IAV-S negative pigs. Pigs free from IAV-S during the growout period will have one less cofactor should they become infected with PRRSV or *Mycoplasma hyopneumoniae*, thereby reducing the chance of developing severe pneumonia. Prevention of respiratory disease or decreasing the impact of PRDC will also reduce the use of antibiotics, improve livability, sustainability and animal welfare.



“My goal is to always wean IAV-S negative pigs. They are better able to transition onto feed, resist secondary bacterial and viral diseases, improve feed intake and ADG in the nursery phase and reduce the number of substandard pigs and mortalities.”

**DR. BOB THOMPSON**

Reducing the number of IAV-S infections in swine herds may also reduce the chances of viral reassortment, which can result in the emergence of new virulent strains. Additionally, reducing viral replication may decrease the emergence of new strains through antigenic drift.

Production of IAV-S negative weaned piglets is attainable for many farms provided that the multiple complexities of IAV-S transmission, vaccine usage, production and pig flow at the farm and/or production system level are taken into consideration when designing an effective stabilization or elimination protocol.



“A IAV-S negative weaned pig adds \$5 to 10 per head value, depending on the market at the time. It also creates happier pork producers since there is less cough and secondary disease.”

**DR. CAMERON SCHMITT**

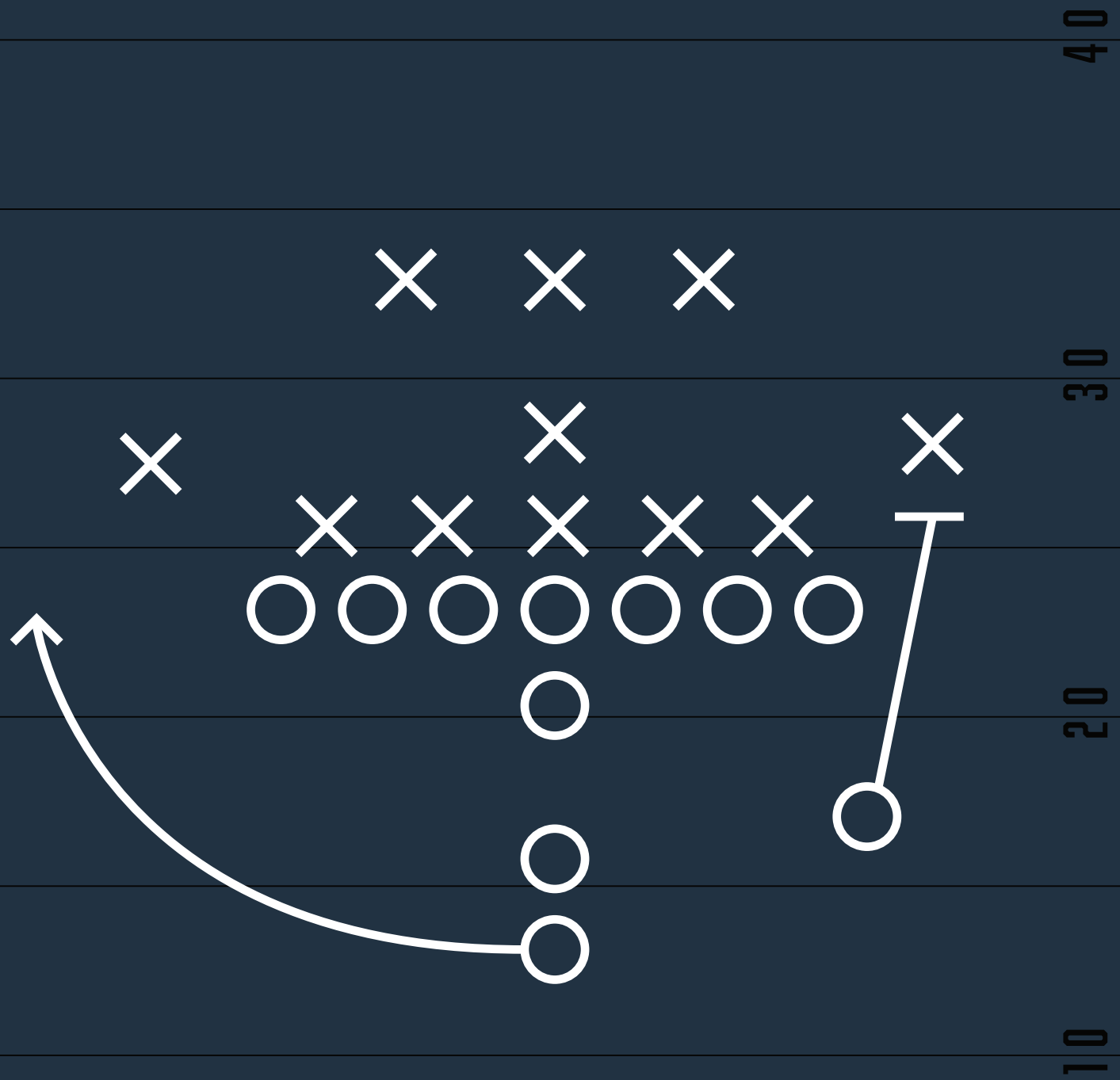




# DRIVEN BY PREVENTION

Merck Animal Health has spent many years working with customers to create effective IAV-S stabilization and elimination programs for breeding herds to improve animal welfare, sustainability and economic returns. This playbook is intended to share this information with the goal of improving the efforts of swine producers and veterinarians to control and eliminate IAV-S.





# IAV-S BREEDING HERD CLASSIFICATION SYSTEM

To establish an IAV-S status goal for the breeding herd, it is first necessary to classify the current IAV-S status of the farm. Swine herds can fall into four broad categories with regard to IAV-S: “**Unstable,**” “**Stable,**” “**Provisionally Negative**” and “**Negative.**” These categories are differentiated by the presence of virus (polymerase chain reaction or PCR) and serological status of the herd.

# IAV-S BREEDING HERD CLASSIFICATION SYSTEM

Summary of breeding herd IAV-S classifications based on diagnostic monitoring history and clinical signs of the herd.

Herd Category	UNSTABLE	STABLE (WEAN PIG NEGATIVE)	PROVISIONALLY NEGATIVE	NEGATIVE-VACCINATED	NEGATIVE-UNVACCINATED/NAIVE
PCR-Positive Samples - Farrowing	+	-	-	-	-
PCR-Positive Samples - Whole Herd	+	+	-	-	-
Clinical Signs - Farrowing	+/-	-	-	-	-
Clinical Signs - Whole Herd	+/-	+/-	-	-	-
HA-Positive Sera	+	+	+/-	+/-*	-
NA-Positive Sera	+	+	+/-	+/-*	-
NP-Positive Sera	+	+	+/-	+/-*	-

\*Negative when DIVA capable vaccine used. May be positive if whole inactivated virus vaccine is used and will require negative serology from sentinel pigs to confirm. (See Section 9 for more details.)

## UNSTABLE

An “unstable” breeding herd is one in which the virus can be detected via PCR (sample types include lung tissue, nasal swabs, nasal wipes, oral fluids, udder wipes or other appropriate sample types) in the farrowing house (dams and/or piglets). Virus will often be detected from other areas of the sow farm including the gilt developer unit (GDU), gestation, weaned pig holding areas and/or any on-site nurseries or finishers.

Unstable breeding herds can be further subdivided into categories based on the timeframe since infection. During the acute phase (epidemic) of the herd’s infection following a new IAV-S strain introduction, clinical signs may be present, and there is typically a high prevalence of the population shedding virus. Farms that continue to circulate virus in a subpopulation of susceptible animals after the initial infection are classified as endemically infected (chronic phase) and may or may not have respiratory clinical signs due to existing immunity and decreased viral circulation. Additionally, some IAV-S strains (i.e., pandemic H1N1 strains) may not elicit pronounced clinical signs in individual pigs or in populations of pigs even during the acute outbreak.

Most herds seeking a stabilization program fall into the unstable category, and the stabilization program recommended is the same regardless of whether they are in the acute or chronic phase of the herd infection.

There are two options for unstable herds: to become either “stable” or “provisionally negative.” Both of these classifications result in the production of IAV-S negative weaned pigs. The difference lies in the ability of the farm to eliminate IAV-S from the whole herd and source IAV-S negative replacement animals.

## STABLE

If the farm can only control the virus in the farrowing house and/or cannot obtain IAV-S negative replacement animals, it may become “stable.” A stable farm may have IAV-S circulating in other portions of the farm (e.g., GDU, gestation, on-site nursery and finisher barns) but has had at least 3 consecutive months of IAV-S PCR-negative testing of respiratory secretions from the due-to-wean (DTW) suckling pigs.

This category has a moderate risk of returning to an unstable status if diligent biosecurity practices, diagnostic monitoring and maintenance vaccination programs are not maintained. (See Section 9 for more details.) Stable herds will maintain serum antibodies from previous infections and/or vaccination.

## PROVISIONALLY NEGATIVE

If the farm can eliminate IAV-S from the entire breeding herd and has access to seronegative replacements that are IAV-S PCR negative, it can be designated as “provisionally negative.” In order to obtain the classification of “provisionally negative,” there must be 3 consecutive months of IAV-S PCR negative testing from all areas of the farm. (See Section 9 for more details.)

“If the producer can get to this stage, the whole farm will perform better. Gilts and sows breed in a timely manner, gilt retention is better and lactation feed intake and return to estrus are all efficient.”

**DR. BOB THOMPSON**



## NEGATIVE

If the provisionally negative breeding herd remains free of detectable virus and avoids further virus introduction for at least 1 year, antibodies should wane and the herd will become virus and antibody negative. This breeding herd would then be classified “negative.” (See **Section 9** for more details.)



**“Achieving negative IAV-S status of the herd provides the highest value to producers. It is amazing how performance is improved when pigs are negative and stay negative.”**

***DR. CAMERON SCHMITT***

It is important to recognize that herds containing animals never exposed to virus (through natural infection or vaccine) would be PCR- and seronegative and would be considered naive to IAV-S and would fall under the negative category. Breeding herds that become negative yet elect to discontinue the use of IAV-S vaccination on the farm would be classified as “negative-unvaccinated”.

## NEGATIVE-VACCINATED

Given the high seasonal risk of introduction from humans as well as the high prevalence of IAV-S circulating in certain geographical regions within the U.S., many herds will choose to continue vaccination after becoming provisionally negative. Herds that become negative and decide to maintain a regular IAV-S vaccination program will be classified as “negative-vaccinated.”



**“One of the most critical aspects to executing a successful IAV-S elimination program is getting all employees to commit and to follow the biosecurity rules.”**

***DR. BOB THOMPSON***

The methods to serologically monitor vaccinated animals will differ depending on the type of IAV-S vaccine used [whole inactivated virus (WIV) or subunit (HA or NA)].

For example, if a breeding herd uses a DIVA capable HA IAV-S vaccine, the herd can be reclassified as negative-vaccinated when they are unable to detect circulating IAV-S virus via PCR testing from all areas of the farm for one year, and negative serology results on both the neuraminidase inhibition (NI) assay and the nucleoprotein (NP) ELISA. The hemagglutinin inhibition (HI) assay would remain positive due to the specificity of the HA vaccination strategy.

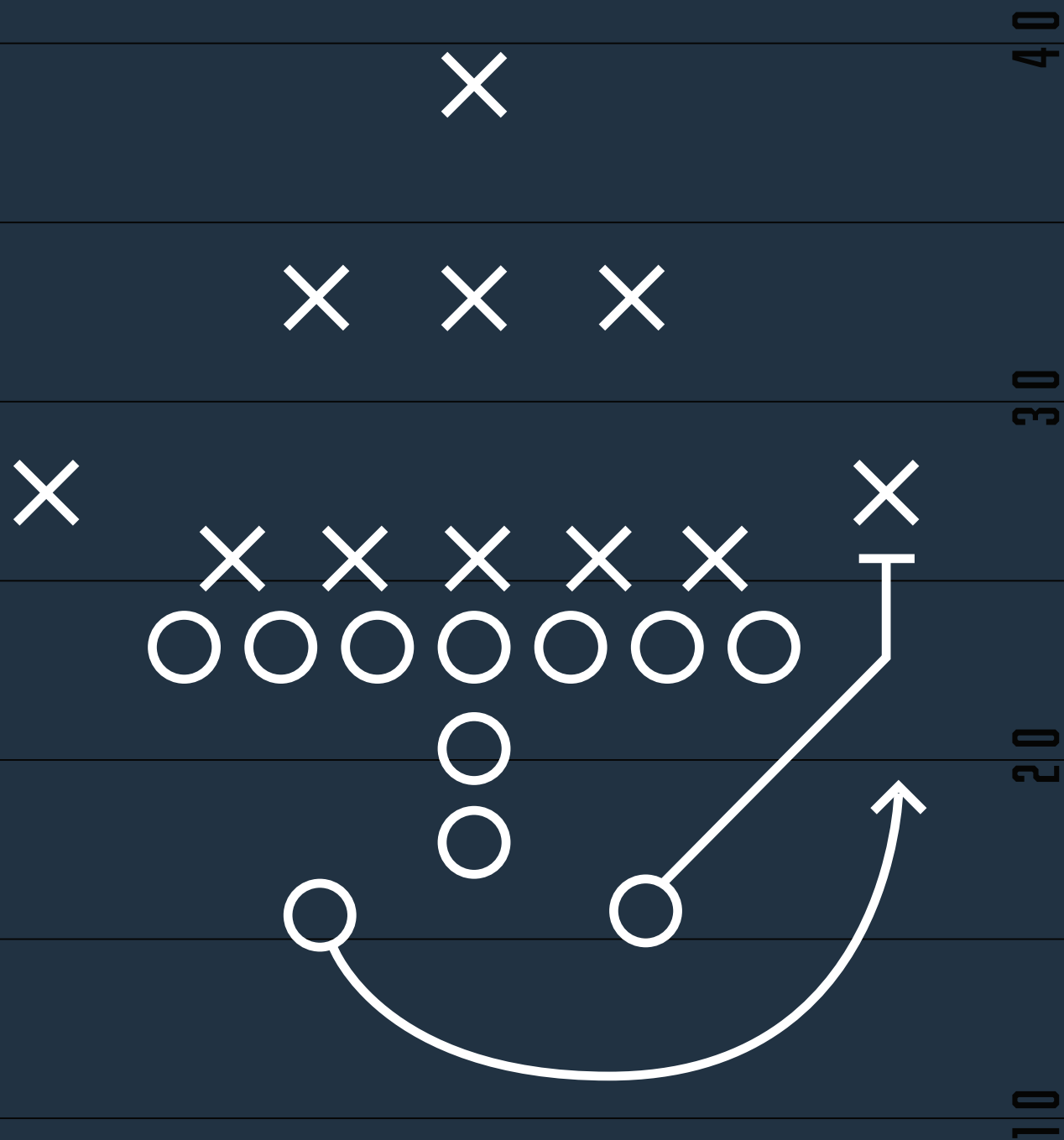
For this situation, it is recommended that serum samples should be collected from 30 DTW suckling pigs, 30 sows from each gestation and breeding barn (sampled across parities) and 30 of the oldest gilts in GDU. (See **Section 9** for more details.)

If a breeding herd uses a whole inactivated virus (WIV) IAV-S vaccine, the PCR requirements for confirming the absence of circulating virus on farm remain the same to classify as negative-vaccinated. However, the serology testing will require the introduction of at least 45 IAV-S naive sentinel gilts (never exposed or vaccinated, PCR- and antibody-negative) who remain unvaccinated for IAV-S and are confirmed seronegative (by HI, NI and NP) for a minimum of 6 months after introduction into the farm.

Farms using DIVA capable vaccines may either choose to monitor replacement gilts with multiple serological IAV-S assays or may also choose to use negative sentinels as well.







## PLANNING AND GOAL SETTING

Understanding the farm's IAV-S clinical and vaccination history, as well as the potential risks for IAV-S introduction and circulation, are key in developing overarching goals for IAV-S control in the farm. These goals will help determine the correct IAV-S stabilization or elimination plan for the herd.

There is a form in **Appendix A** that can be completed to help the team understand the current IAV-S situation and best plan for either IAV-S stabilization or elimination at the level of the farm/flow or production system. A herd's IAV-S history and current IAV-S status (which IAV-S strains are present and where virus shedding is occurring) help determine the appropriate IAV-S control goal and pathway for the herd. Farm layout and animal and people movements are critical to determine if enhanced biosecurity steps are needed to reach the specific classification goal of the herd.

## COMMON PRODUCTION PRACTICES THAT MAY THWART A SUCCESSFUL IAV-S STABILIZATION PROGRAM

Many factors are at play when determining the chances of achieving a stabilization or elimination goal. The dynamics of each breeding herd must be carefully considered before attempting an IAV-S stabilization or elimination program.

Once the history and farm information are gathered, an IAV-S goal for the herd can be established and planning can begin.

This manual provides sections centered around the critical components of IAV-S stabilization programs: biosecurity, planning and execution, vaccine antigen selection and vaccination programs and diagnostics.

When it comes to IAV-S stabilization plans, there are individual sections outlining the implementation procedures for the desired IAV-S classification that have been selected for the target herd. If the goal is elimination, refer to the sections on “provisionally negative” and “negative.”

If it is not possible to become “provisionally negative,” which typically hinges on the ability to source IAV-S negative replacement gilts, there is a separate section that outlines a plan for those wanting to achieve “stable” status.

### FACTORS WITH A HIGH RISK OF IAV-S STABILIZATION FAILURE:

Vaccine used is a poor match (homology, key sites, genetic clade) with herd strain(s)

Farrowing house rooms cannot be run all-in-all-out (AIAO) by airspace

Nurse sows, bump weans and cross-fostering after 24 hours cannot be stopped

The farm holds back litters in farrowing or has on-site nurseries or holding areas with older pigs and is unable to stop these practices temporarily.

For those attempting a whole-herd elimination: If breeding animals cannot be fully vaccinated before arrival or be shedding IAV-S upon arrival.

### FACTORS WITH A MODERATE RISK OF IAV-S STABILIZATION FAILURE:

Multiple IAV-S strains (genetic clades) present on farm

For those attempting a whole-herd elimination: GDU is IAV-S positive and is continuous flow in one airspace (gilts >10 weeks of age)

Unable to change/wash boots, change gloves and coveralls between farrowing rooms and other parts of farm

For those attempting a whole-herd elimination: Heat-check boars move regularly between GDU and general population gestation

# THREE PHASES

## OF IAV-S STABILIZATION PROGRAM

### WEEKS



## PHASE 1

### WEEKS 1-6

**Phase 1** is weeks 1-6 after the start of mass vaccination of the sow herd when IAV-S circulation will still be present and can be detected on the farm.

## PHASE 2

### WEEKS 6-10

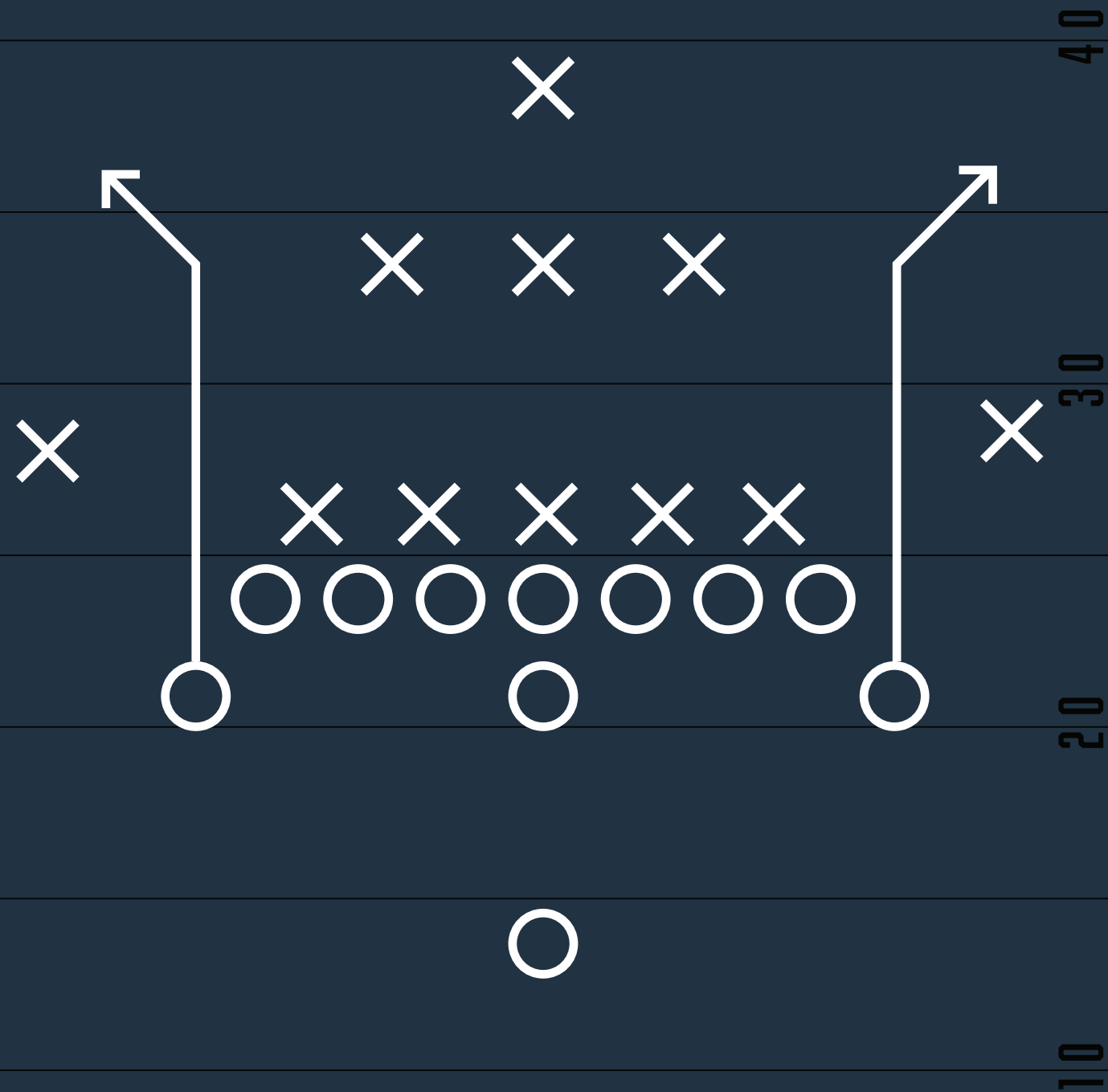
**Phase 2** is 6-10 weeks following mass vaccination of the herd and marks the time period when the farrowing house is expected to be populated with IAV-S negative piglets.



## PHASE 3

### WEEKS 10+

**Phase 3** begins after farrowing house diagnostics confirm active IAV-S circulation is no longer detectable and involves ongoing surveillance to confirm the status of the farm (expected stable).



# BIOSECURITY MEASURES FOR IAV-S STABILIZATION AND ELIMINATION

Enhanced biosecurity practices are a critical component of a successful IAV-S stabilization or elimination program. These practices are performed in conjunction with mass vaccination to help reduce the level of IAV-S transmission and infection occurring within the farm by animals, people, the environment and equipment. Biosecurity measures are centered on isolating positively infected pigs from negative ones.



# BIOSECURITY OF FARMS



**“People remain the biggest risk to new IAV introductions to pigs, and this can be difficult to control.”**

**DR. PHIL GAUGER**

It is important that all farm staff and visitors understand they can potentially infect the animals if they come into the farm sick with influenza. Therefore, it is recommended that people exhibiting clinical signs consistent with influenza (fever, cough, sore throat, runny or stuffy nose, muscle or body aches, headaches or fatigue) or have a confirmed diagnosis of influenza should not enter the farm while actively shedding virus. Farms wanting to take the utmost care to reduce the risk of IAV-S entering their herd should take the temperatures of staff/visitors prior to entering the farm and not permit entry of anyone exhibiting a fever (>100° F).

This practice is especially important on farms once an IAV-S stabilization/elimination program has started to reduce the risk of introducing new influenza strains into the farm. Some farms may take the extra precaution of requiring employees and visitors to wear an appropriately fitted N95 mask to help reduce the infection risk of IAV-S between people and pigs within the farm. Vaccination of farm personnel against influenza is another optional layer of protection to help limit

the introduction of new IAV-S strains into the herd. New employees should monitor themselves closely for clinical signs of infection as they are at risk of contracting IAV-S in unstable farms, given it is a zoonotic disease.

Since influenza can typically survive on surfaces for up to 48 hours, supplies entering the farm should be disinfected and allowed to dry in a segregated area (D&D room) before entering the farm.<sup>6</sup> Anyone disinfecting supplies should wear gloves and protective clothing (not clothes worn into the barns) and immediately remove and dispose of these when leaving the supply area.

It is critical to communicate what parts of the farm are actively circulating IAV-S throughout the whole stabilization timeline.

Ideally, staff from positive areas of the farm should not enter negative areas. If that is not possible, it is preferable for staff to start in negative areas before entering positive ones. If one must move from positive to negative areas, it is recommended to shower and change clothes. An example of this would be weaning days in the farrowing house when most staff must first handle weaned pigs, which would be suspected IAV-S-positive until 10 weeks after the stabilization program begins. After weaning is completed, all farm staff should shower and change into new coveralls and clean boots before working with the pigs in younger rooms in farrowing.

Additionally, hallways where the weaned pigs traveled should be cleaned and disinfected before they are trafficked again by pigs or people. If this cannot be done in other areas of the farm (i.e., GDU), a change of boots and coveralls with a hand wash/glove change station can be implemented. It is also recommended to put boot wash stations outside of positive areas to minimize the spread of IAV-S in the hallways. While water and soap help remove organic debris and possibly some viable virus from the skin, it is recommended to follow up with an alcohol-based hand sanitizer to ensure complete killing of the virus.



**“One of the most critical aspects to executing a successful IAS-S elimination program is getting all employees to commit and follow the biosecurity rules.”**

**DR. BOB THOMPSON**

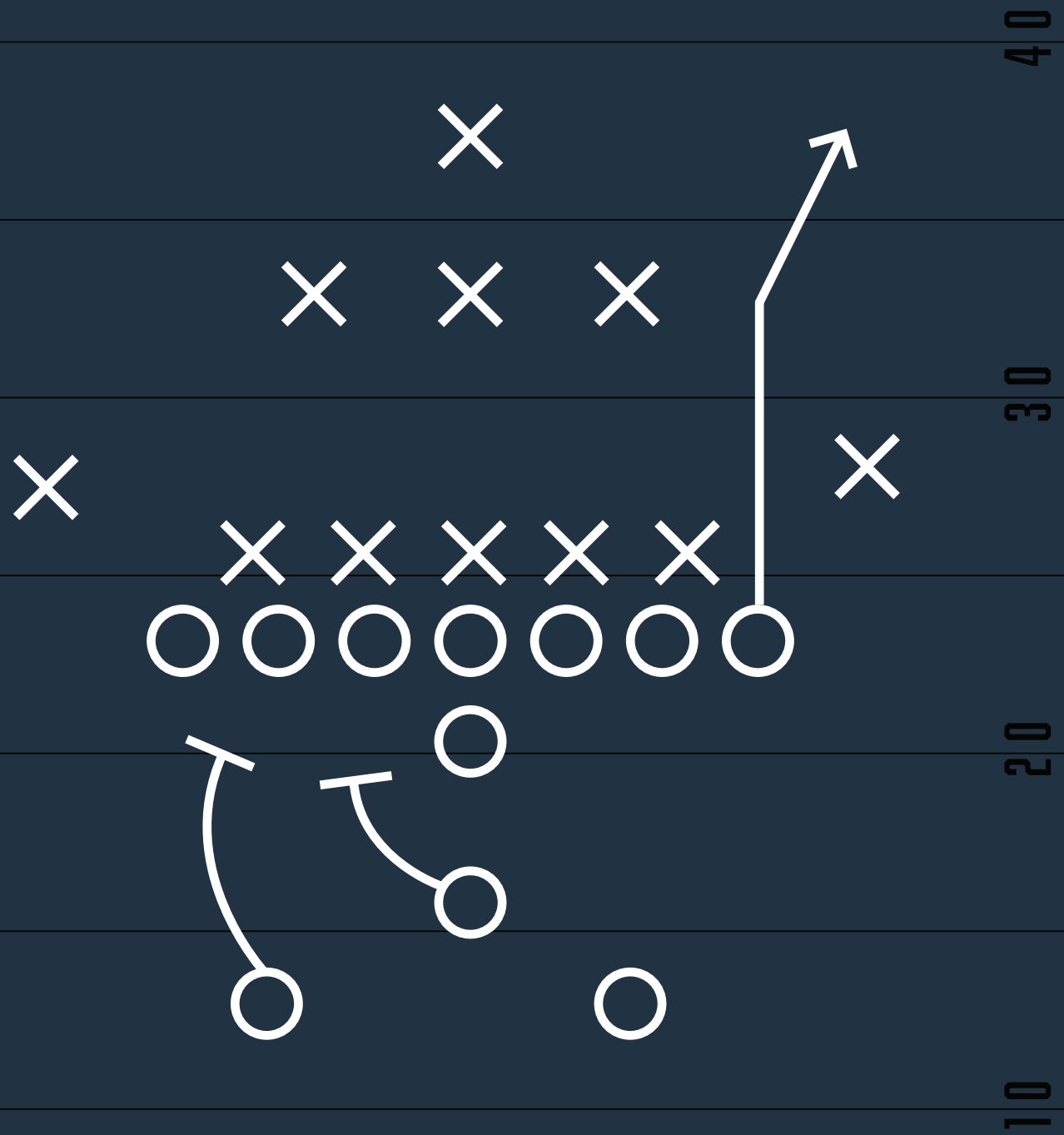
It is also important to recognize that common areas in the farm where farm staff cross over (i.e., office, break room, supply or medication rooms and the boot area) are potential spots where IAV-S might reside on surfaces. People handling pigs with IAV-S could transfer it to surfaces in these highly trafficked spaces where fellow employees could then pick it up and later expose an IAV-S-negative pig in another area of the farm.

Consequently, it is necessary to clean and disinfect these areas daily. It is also recommended to wipe down or disinfect all commonly touched surfaces daily on the farm (i.e., tables, counters, chairs and door handles). Additionally, staff should thoroughly wash their hands and arms with soap and water followed by an alcohol-based sanitizer after working with the animals. Boots should be removed and soiled clothing changed before entering common spaces with other employees.

Map all traffic patterns between positive and negative animals to ensure that they do not overlap. If areas overlap, it is important during Phase 2 to make sure they are disrupted or discontinued. To create traffic pattern rules, ask all farm employees about their regular daily traffic patterns on the farm. A map of this information should be included in the farm biosecurity plan.

Given the high amount of traffic in farm hallways, it is important to clean and disinfect hallways immediately after animal movements. Always double-check that the hallway is clean before bringing new groups of animals through the farm. It is possible that an adult sow could become infected in a dirty hallway in which IAV-S positive weaned pigs recently traveled.

Likewise, equipment can transfer influenza and facilitate entry of the virus within or around farms. Therefore, equipment should be cleaned and disinfected before moving it between different locations, rooms and barns.



# PROTOCOL TO ACHIEVE PROVISIONALLY NEGATIVE

This section outlines an 11-step protocol to move a herd from unstable to provisionally negative. The goal for this farm would be to eliminate all active IAV-S circulation from the herd and produce IAV-S negative weaned pigs.

Provisionally Negative

# PROTOCOL TO ACHIEVE PROVISIONALLY NEGATIVE

## STEP 1

Communicate the plan and obtain buy-in from all key constituents (farm staff, farm owner, manager and veterinarian).

## STEP 2

Assign an IAV-S project leader and an on-farm project foreperson.

## STEP 3

Assign a primary diagnostic contact who will coordinate diagnostic sample collections (supplies, schedule, shipping, dissemination of results). There should be a centralized assembly of diagnostic monitoring results.

## STEP 4

Generate a one-year timeline and review with everyone. This timeline will encompass the following phases:

### PHASE 1

#### WEEKS 1-6

**Phase 1** is weeks 1-6 after the initial mass vaccination of the sow herd when IAV-S circulation will still be present on farm.

### PHASE 2

#### WEEKS 6-10

**Phase 2** is 6-10 weeks after the beginning of mass vaccination, which marks when the farrowing house will be populated with IAV-S-negative piglets.

### PHASE 3

#### WEEKS 10+

**Phase 3** begins after 10 weeks post-vaccination when farrowing house diagnostics should confirm no active IAV-S circulation and involves ongoing surveillance testing and a decision to transition to negative-vaccinated or negative-unvaccinated.

## STEP 5

Set up weekly or biweekly calls with all key constituents (veterinarian, farm manager, etc.) to help ensure the plan is being followed. More frequent calls are usually necessary during the initial 4 months of the program. Frequent communication will allow the team to talk through any questions or issues that arise along the way and allows for a quick response.

## STEP 6

A second whole-herd sampling should be repeated at least 1-4 weeks before the stabilization program begins to confirm the circulation patterns and adjust the protocol accordingly (i.e., animal movement/flow). Use the sampling protocol based on the farm classification determined from the first sample collection and diagnostic results.

## STEP 7

Perform training for enhanced biosecurity and sanitation practices on the farm that includes biosecurity for people and animal traffic (outlined in **Section 4**). Enhanced biosecurity practices are most critical during Phase 2 of the project. These enhanced biosecurity measures are focused around the two critical areas of IAV-S circulation: the farrowing house and the GDU.

The objective of these additional practices is to prevent contamination of the newborn piglets by older pigs shedding virus and to prevent gilts and boars arriving from the GDU from contaminating older sows. Following double mass vaccination of the sow farm (Phase 2), suckling pigs will be born IAV-S negative, and the risk of IAV-S introduction to these animals is through contaminated people or equipment or older infected suckling pigs.

Many times, Phase 2 enhanced biosecurity is started during Phase 1 to ensure that all practices are well understood and to discover any potential implementation problems. This enhanced biosecurity protocol can only be relaxed after the farm has entered Phase 3.

# PROTOCOL TO ACHIEVE PROVISIONALLY NEGATIVE

## STEP 7 (CONTINUED)

### Enhanced Biosecurity in Farrowing During Weeks 6-10 (Phase 2):

1. Cross-fostering is allowed within the first 24 hours of life within the same room, or no cross-fostering is allowed at all.
2. Fallback piglets can be moved to a designated fallback litter within the same farrowing room if needed. Nurse sows should have their udders wiped down with chlorhexidine solution before receiving a new litter.
3. Wear gloves at all times in farrowing. Change gloves between litters.
4. Avoid stepping in and out of farrowing crates.
5. Processing equipment should be disinfected between each litter. Change processing needles between litters.
6. All suckling piglets must be weaned when they reach 21 days of age regardless of weight. Wean entire rooms and make sure to clean, disinfect and dry the room before reloading with sows. Do not hold any piglets back in farrowing crates or IAV-S positive nurseries.
7. Perform chores in farrowing house rooms from youngest to oldest. If possible, dedicate a subset of farrowing staff to newly born litters and/or IAV-S negative rooms. If it is necessary to visit younger IAV-S negative pigs after working with older suckling pigs, shower and change coveralls and boots beforehand.
8. When entering rooms with piglets from dams that have been double mass-vaccinated for IAV-S, use only clean boots, clean coveralls and new gloves.
9. Do not allow traffic patterns of negative animals to contact or cross those of positive animals.

In the farrowing house, it is expected for IAV-S circulation to continue through the first 9-10 weeks of the elimination program. After that, the goal is to, step-wise, remove or “walk” influenza out of the farm with each room of pigs weaned. It is critical to follow the sanitation and traffic guidelines to ensure IAV-S from older pigs is not carried back to infect rooms with younger piglets. Piglets are born negative to influenza, and the goal is to keep them that way.

## STEP 7 (CONTINUED)

### Enhanced Biosecurity in Gilt Developer Unit During Weeks 6-10 (Phase 2):

1. If diagnostics have detected the presence of IAV-S in the GDU, the ages and/or rooms must be identified as positive. It may take time for the virus to stop circulating in the GDU following mass vaccination, so monthly diagnostic surveillance is key to identifying positive areas. It might be beneficial to add signage on GDU room doors to alert employees to the room’s IAV-S status.
2. An employee should start with being freshly showered and donning clean coveralls and boots when beginning their work in the GDU.
3. Chore negative to positive (usually oldest to youngest).
4. A boot wash, change of clothes and hand wash/sanitization or glove change should be implemented after leaving positive rooms/ages.
5. In IAV-S positive rooms, change gloves between pens.
6. Heat check boars should be dedicated to working either the GDU or the sows (NOT BOTH). Ideally, GDU heat check boars are contained in their own area and in a location that is either within or nearest the GDU. GDU heat check boars traveling outside the GDU should not be given time to interact with mature sows on the farm.
7. Heat check boars should not be allowed to interact with positive rooms or ages. If they are put in a positive room, they should remain there until Phase 2 is concluded.
8. Shut the GDU down to all new arrivals until at least 1 month after the second mass vaccination and when IAV-S diagnostics confirm no IAV-S circulation in the GDU.
9. Do not allow traffic patterns of negative animals to contact or cross those of positive animals.

If the farm has onsite nursery pigs, it is advised to empty the nursery by the time the farm is consistently weaning IAV-S negative piglets, which should align with the conclusion of Phase 2. A bubble depopulation, repeated quarterly mass vaccinations and enhanced biosecurity procedures can be employed in finisher barns to ensure IAV-S negative pigs placed in these barns remain IAV-S negative.



## PROTOCOL TO ACHIEVE PROVISIONALLY NEGATIVE

### STEP 8

On day 1 of the IAV-S stabilization program, all adult animals (gilts, sows and boars) and all animals housed on the farm's site (gilts in GDU, nursery or finishing pigs) should receive one full dose of IAV-S vaccine. These same animals should be mass vaccinated again 3-4 weeks later. For additional protection to weaned piglets (maintain high maternally derived antibodies (MDA) levels), it is recommended to administer a pre-farrow IAV-S vaccine booster during Phase 2. After which, a quarterly mass vaccination program to mature adult animals in conjunction with routine replacement gilt and boar vaccination is recommended for at least the following year. One year of negative virus detection through a surveillance program should be achieved before reducing the frequency of IAV-S vaccination in the sow herd.

If replacement gilts are retained from the farm (internal multiplication), it is advised to vaccinate them with IAV-S vaccine as early as possible. This can be done with either a SEQUIVITY® IAV-S NA vaccine at 3 days and 3 weeks or an HA vaccine at 10 or more weeks of age when maternal antibodies are expected to wane. Serological testing of replacement gilts can help guide replacement gilt vaccination timing. (See **Section 9** for more details.)

Please remember that following a mass vaccination of any swine must comply with the labeled vaccine withdrawal period. It is advised to coordinate the timing of cull loads before planned vaccination events.



**“Two whole-herd mass vaccinations staggered 30 days apart with partial herd closure and intense farrowing house biosecurity has been >90% successful in eradicating resident strains of influenza from herds our veterinarian team works with.”**

***DR. CAMERON SCHMITT***

### STEP 9

To pass from Phase 2 to Phase 3, three consecutive weeks of udder wipes (see **Appendix C**) or nasal swabs from late farrowing litters should be negative. If there is an onsite holding room, pigs housed within it should be sampled weekly with nasal swabs to confirm their IAV-S negative PCR status. All samples should be collected using the low-prevalence strategy. (See **Section 9** for recommended testing schematics.)

The GDU requires 3 negative monthly oral fluids from every 3 weeks of age using the low-prevalence sampling strategy.

### STEP 10

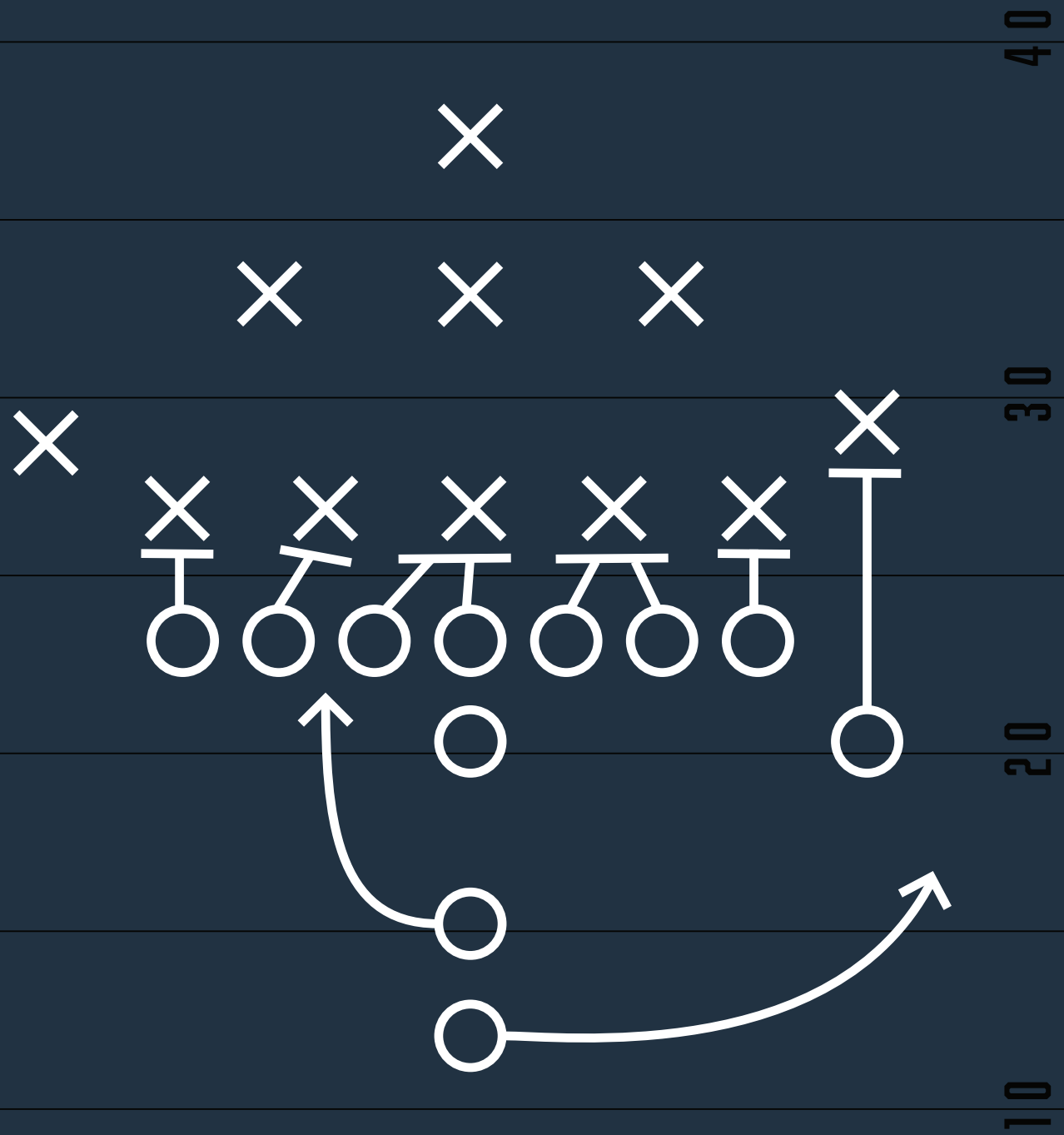
After reaching Phase 3, monitoring for virus must continue on a monthly basis. The farm should be tested at the low-prevalence level. Phase 3 concludes after 3 consecutive months of negative PCR testing across all sectors of the farm.

The farm is now classified as provisionally negative. All age groups of replacement gilts and boars should be tested with oral fluids 1-2 weeks before arrival to the sow farm to ensure no new introductions of virus, and any clinical signs of respiratory disease anywhere on the farm should be worked up. It would also be recommended to test the new gilt group by PCR within the first three days after arrival.

Any clinical signs of respiratory disease anywhere on the farm should be investigated with appropriate diagnostics using the high prevalence strategy.

### STEP 11

After 1 year of successfully remaining virus-free on all parts of the farm, the farm may decide to proceed to negative-unvaccinated or negative-vaccinated status.



# PROTOCOL TO ACHIEVE NEGATIVE

In order to move into the “negative” classification, the farm must satisfy two requirements:

1. It must achieve 1 year of no detectable virus by PCR using the low-prevalence sampling strategy in all areas of the farm (farrowing, holding areas, nursery, GDU and gestation).
2. It must confirm no additional IAV-S subclinical infection is occurring for 6 months by serologically monitoring animals. Serological monitoring can be done concurrently with the PCR monitoring, but it is recommended to start after residual IAV-S titers from the stabilization effort have declined.

Negative

# DECIDING TO BECOME NEGATIVE

Next, decide if the farm will remain vaccinated or not. New strains of virus can be carried by infected/shedding personnel, exposure to waterfowl or new animal introductions (i.e., replacement gilts or boars). Given the constant risk of IAV-S introduction on farms, it is highly recommended to continue to vaccinate the herd after eliminating all circulating IAV-S from the population.

Farms discontinuing vaccination altogether will be at a higher risk for clinical signs and production losses if a new virus enters the farm.



**“If a new IAV-S strain happens to enter a negative vaccinated herd, there will be less production loss in an unvaccinated negative herd.”**

**DR. BOB THOMPSON**

Farms choosing to discontinue IAV-S vaccination should be in low-risk geographies and be committed to practicing stringent biosecurity. These farms must have a source of IAV-S naive replacement animals. Pigs weaned off-site from these farms will be at risk for IAV-S and may need to be vaccinated. Some farms may continue to vaccinate their pigs with human seasonal IAV-S strains that have been shown to be carried by people into farms or with IAV-S strains that threaten the incoming gilt source.

## NEGATIVE VACCINATED FARMS

A vaccinated farm will continue to mass vaccinate quarterly or semiannually or switch to a pre-farrowing protocol to provide extra maternal antibodies to weaned piglets. The decision of vaccine frequency and timing is dependent on the IAV-S introduction risk of the farm and the risk of lateral infection of pigs weaned from the herd during the nursery and finisher phase. Quarterly mass vaccinations offer continuous protection to the herd, while semiannual vaccinations are often employed around seasonal risks. The pigs weaned off the farm may require active immunization to IAV-S depending on where they are relocated.

The farm that elects to continue vaccinating animals should understand that meeting the antibody requirement to move from provisionally negative to negative-vaccinated status depends on the type of vaccine used on the farm and will determine the specific antibody test required to accurately represent the status of the production system. Herds using a DIVA capable IAV-S vaccine (i.e., SEQUIVITY® technology) will be able to differentiate antibodies from wild-type infection versus those generated from vaccines (e.g., SEQUIVITY vaccines lack the nucleoprotein). Herds must bleed and test 30 DTW suckling pigs, 30 sows from each gestation and breeding barn (sampled across parities) and 30 of the oldest gilts in GDU.

If using a HA DIVA capable vaccine, all of the samples must be NI and negative.  
If using an NA DIVA vaccine, all samples must be negative for HI and NP.

However, herds using a WIV vaccine must enter at least 45 IAV-S naïve sentinel gilts who remain unvaccinated for IAV-S whose serology (HI, NI and NP) is confirmed negative for a minimum of 6 months after introduction into the farm. Unvaccinated IAV-S naïve replacement teaser boars can also be used as sentinels for serological monitoring.

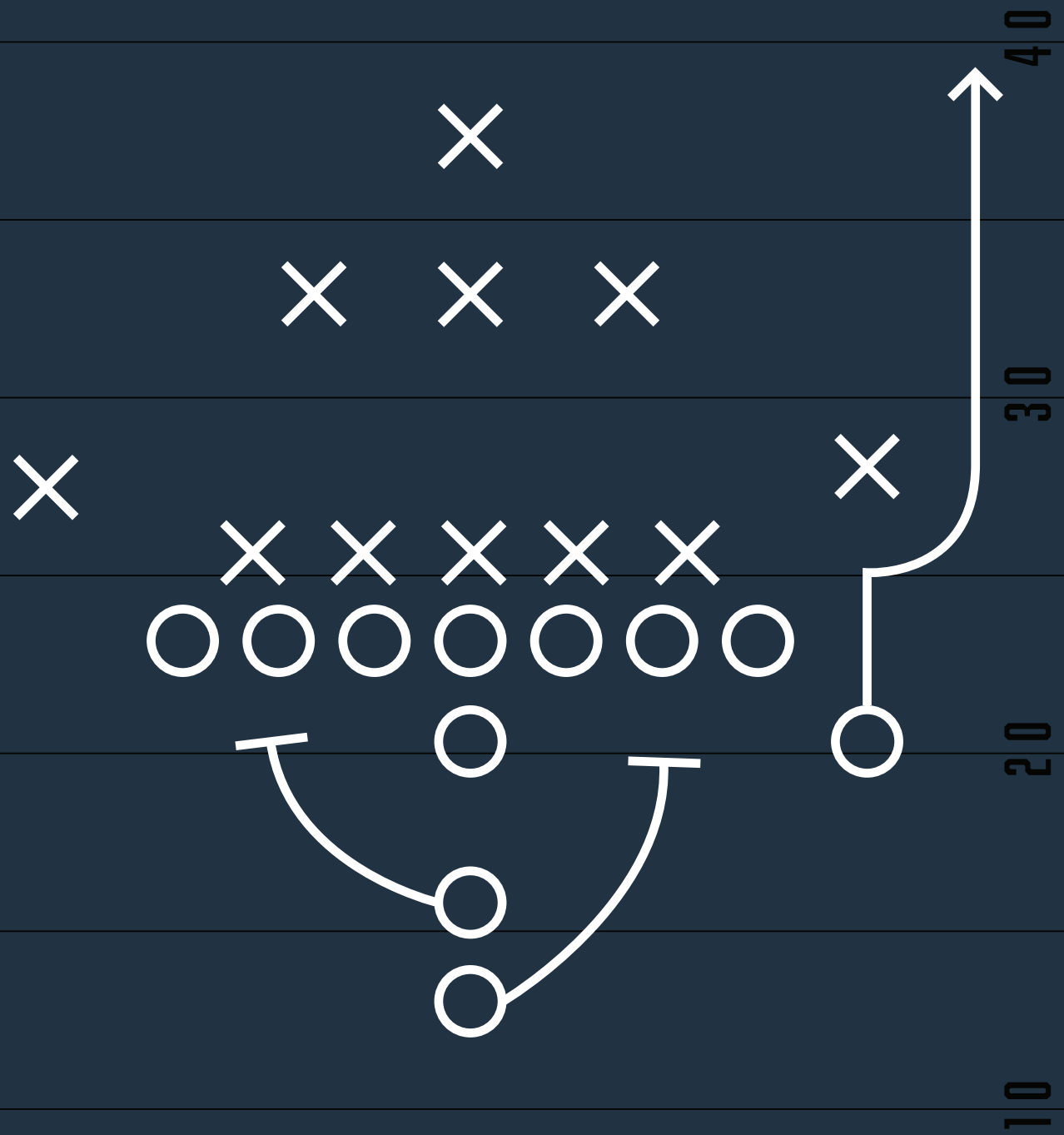


Finally, congratulations on all the hard efforts that have been done to get your farm to this ideal status.

Merck Animal Health is dedicated to helping support your IAV-S stabilization efforts. Please reach out to your Merck Animal Health sales and technical services partner to help you plan, execute and achieve your IAV-S farm goals.







# PROTOCOL TO ACHIEVE STABLE

This section outlines an 11-step protocol to move a herd from unstable to stable. The goal for this farm would be to produce IAV-S negative weaned pigs.



# PROTOCOL TO ACHIEVE STABLE

## STEP 1

Communicate the plan and obtain buy-in from all key constituents (workers, farm owner, manager and veterinarian).

## STEP 2

Assign an IAV-S project leader and a project foreperson to serve at the farm.

## STEP 3

Assign a primary diagnostic contact who will coordinate diagnostic sample collections (supplies, schedule, shipping, dissemination of results). There should be a centralized assembly of diagnostic monitoring results.

## STEP 4

Generate an eight-month timeline and review with everyone. A planning template is provided in **Appendix B**. This timeline will encompass the following phases:

## PHASE 1

**Phase 1** is weeks 1-6 after the initial mass vaccination of the sow herd.

## PHASE 2

**Phase 2** is 6-10 weeks after the beginning of mass vaccination. During the start of Phase 2, piglets from well-vaccinated dams are born and reach weaning age. Phase 2 can be officially concluded when there are three consecutive PCR negative weekly results (udder wipes, nasal swabs) from farrowing. Phase 2 testing can start at 8 weeks after the first mass vaccination.

## PHASE 3

**Phase 3** involves ongoing monthly farrowing house surveillance and ends with three consecutive months of PCR negative testing when the farm transitions to “stable.”

## STEP 5

Set up weekly or biweekly calls with all key constituents (veterinarian, farm manager, etc.) to help ensure the plan is being followed. More frequent calls are usually necessary during the initial 4 months of the program. Frequent communication allows the team to talk through any questions or issues that arise and allows for a quick response.

## STEP 6

Perform diagnostics to determine if the farrowing house and any on-site holding areas or hot nurseries are positive to IAV-S and to obtain sequences or isolates for vaccine manufacture. This may need to be done 12-16 weeks before the project begins to enable the proper timeline for vaccine manufacture. Additional nasal swabs and lung tissue samples may be needed if initial sampling identifies virus but fails to successfully sequence or isolate the virus.

## STEP 7

Perform training for enhanced biosecurity and sanitation practices on the farm that includes biosecurity for people and animal traffic (see Section 4 for more details). Enhanced biosecurity practices are most critical during Phase 2 of the project. The objective of these additional practices is to prevent contamination of the newborn piglets by older pigs shedding virus.

Following double mass vaccination of the sow farm (Phase 2), suckling pigs will be born IAV-S negative, and the risk of IAV-S introduction to these animals is through contaminated people or equipment or older infected suckling pigs. Many times, Phase 2 enhanced biosecurity is started during Phase 1 to ensure that all practices are well understood and to discover any potential implementation problems. This enhanced biosecurity protocol can only be relaxed after the farm has entered Phase 3.

## STEP 7 (CONTINUED)

### Enhanced Biosecurity in Farrowing During Weeks 6-10:

1. Cross-fostering is allowed within the first 24 hours of life within the same room or no cross-fostering is allowed at all.
2. Fallback piglets can be moved to a designated fallback litter within the same farrowing room if needed. Nurse sows should have their udders wiped down with chlorhexidine solution before receiving a new litter.
3. Wear gloves at all times in farrowing. Change gloves between litters.
4. Avoid stepping in and out of farrowing crates.
5. Processing equipment should be disinfected between each litter. Change processing needles between litters.
6. All piglets must be weaned when they reach 21 days of age regardless of weight. Wean entire rooms and make sure to clean, disinfect and dry the room before reloading with sows. Do not hold any piglets back in farrowing crates or hot nurseries.
7. Wean entire rooms and make sure to clean, disinfect and dry the room before reloading with sows. Do not hold any piglets back in farrowing crates or IAV-S positive nurseries.
8. Perform chores in farrowing house rooms from youngest to oldest. If possible, dedicate a subset of farrowing staff to newly born litters and/or IAV-S negative rooms. If it is necessary to visit younger IAV-S negative pigs after working with older suckling pigs, shower and change coveralls and boots beforehand.
9. When entering rooms with piglets from dams that have been double mass vaccinated for IAV-S, use only clean boots, clean coveralls and new gloves.
10. Do not allow traffic patterns of negative animals to contact or cross those of positive animals.

In the farrowing house, it is expected for IAV-S circulation to continue through the first 9-10 weeks of the elimination program. After that, the goal is to, step-wise, remove or “walk” influenza out of the farm with each room of pigs weaned. It is critical to follow the sanitation and traffic guidelines to ensure IAV-S from older pigs is not carried back to infect rooms with younger piglets. Piglets are born negative to influenza and the goal is to keep them that way.

If the farm has onsite nursery pigs, it is advised to empty the nursery by the time the farm is consistently weaning IAV-S negative piglets, which should align with the conclusion of Phase 2.

## STEP 8

On day 1 of the IAV-S elimination program, all adult animals (gilts, sows, boars) and all animals housed on the farm’s site (gilts in GDU, nursery or finishing pigs) should be mass-vaccinated, receiving 1 full dose of IAV-S vaccine. These same animals should be mass-vaccinated again 3-4 weeks later. For additional protection to weaned piglets (maintain high MDA levels), it is recommended to administer a pre-farrow IAV-S vaccine booster during Phase 2. After which, a quarterly mass vaccination program to mature adult animals in conjunction with routine replacement gilt and boar vaccination is recommended for at least 6 months.

Please remember that following a mass vaccination of any swine, the farm must comply with a 21-day vaccine withdrawal. It is advised to coordinate the timing of cull loads before planned vaccination events.

## STEP 9

To pass from Phase 2 to Phase 3, three consecutive weeks of udder wipes (see **Appendix C**) or nasal swabs from late farrowing litters should be negative. All samples should be taken using the low-prevalence strategy. It is recommended to not hold weaned pigs on farm until after Phase 3 is complete. If there is an onsite holding room used during Phase 3, it should be checked weekly with nasal swabs.

Any clinical signs of respiratory disease in the farrowing house should be investigated with appropriate samples and diagnostic testing. (See **Section 9** for diagnostic sampling recommendations.)

## STEP 10

After reaching Phase 3, monitoring for virus must continue, although at a lower frequency.

The farrowing house will need monthly testing of DTW piglets at the low-prevalence strategy.

## STEP 11

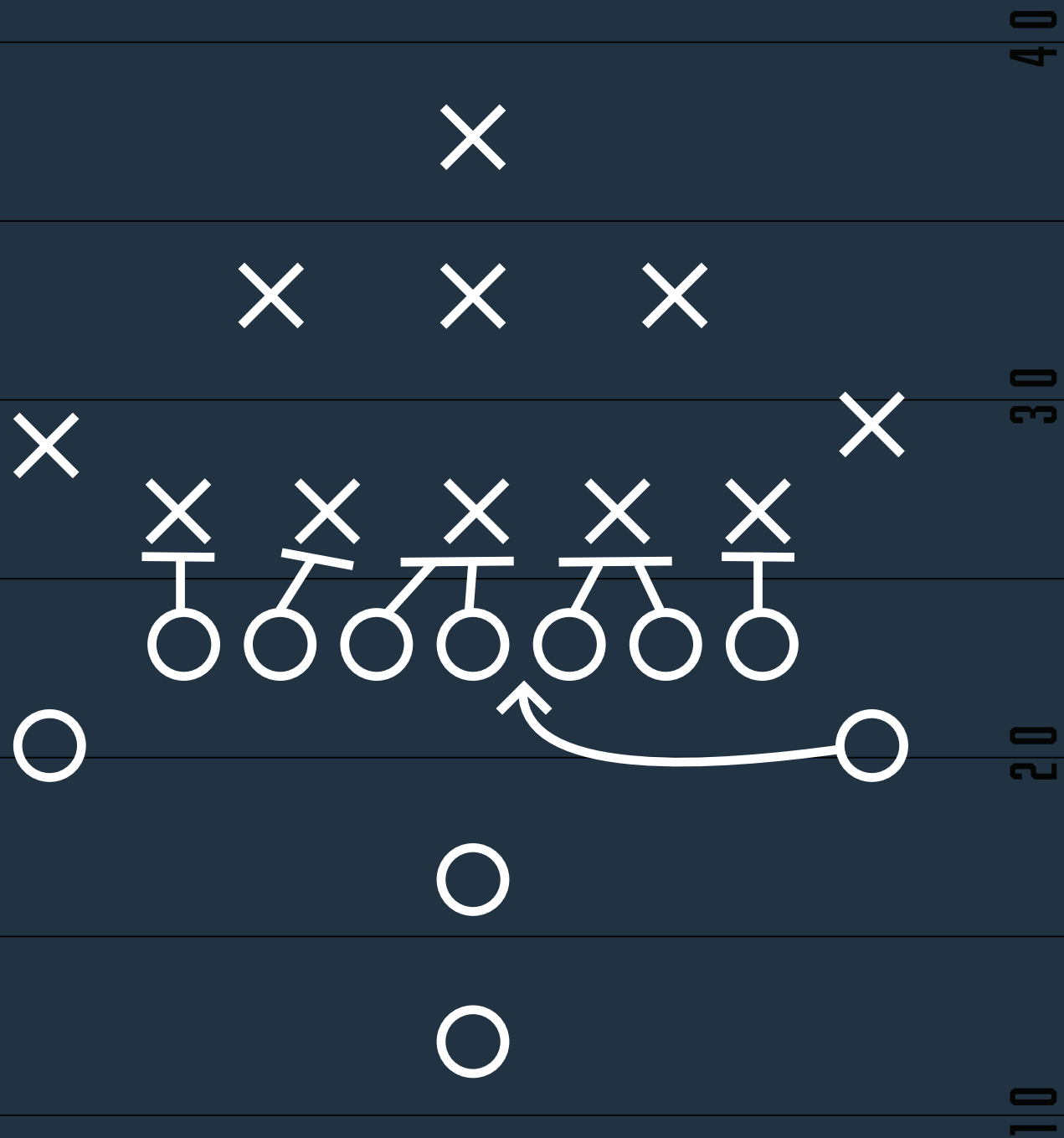
After three months of successfully producing virus negative weaned pigs, the farm may decide to continue with the current stable status and surveillance protocol or proceed to provisionally negative status. The herd may continue with quarterly mass vaccinations and/or transition to a pre-farrowing vaccination program if it wants to remain Stable. Some herds may reduce whole-herd vaccinations to a semiannual schedule (fall and spring). The vaccination maintenance schedule used should be tailored around limiting active IAV-S circulation within the herd.



Finally, congratulations on all the hard efforts that have been done to get your farm to this IAV-S status. Continue to monitor the IAV-S negative status. In the event the farm can address IAV-S circulation in other regions of the farm (e.g., GDU), consider the additional efforts to become “provisionally negative.”

Merck Animal Health is dedicated to helping support your IAV-S stabilization efforts.

Please reach out to your Merck Animal Health sales and technical services partner to help you plan, execute and achieve your IAV-S farm goals.



# VACCINATION PRINCIPLES

Vaccination is the cornerstone of a stabilization or elimination program. Although immunity after natural infection is expected to provide some level of protection, previous infections by themselves cannot be relied upon for adequate stabilization and elimination. This is due to three main reasons:

1. Sows and gilts have different levels of immunity and pass different levels of protection onto their piglets through colostrum. Reduced levels of immunity enable the virus to replicate in suckling piglets, often subclinically and undetected without diagnostics, and become endemic in the farrowing house.
2. As strains mutate or new strains come into the farm, immunity generated against endemic IAV-S does not ensure neutralizing immunity and protection against new strains.
3. The use of planned live-virus exposure, although used for other swine pathogens, should not be implemented with IAV-S because of zoonotic implications.



# VACCINATION PRINCIPLES

## Mass vaccination is the only way to create consistent immunity.

Mass vaccination is used to create consistent protective immunity to circulating strains for all animals at the same time. Sows and gilts with high antibody titers to the circulating farm strain will help ensure suckling piglets are adequately protected through maternally derived antibodies, thereby reducing or eliminating viral circulation in the farrowing house.

## Commercial WIV

Vaccines for IAV-S have been available for many years, starting in 1997 with MaxiVac-FLU®, a commercially licensed, whole inactivated virus product with an oil-in-water adjuvant. Since then, other commercial killed WIV vaccines have been launched. The disadvantage of IAV-S commercial WIV vaccines is the extended timeframe required to update vaccine strains when necessary to represent current circulating strains on farms. This timeframe is critical for influenza due to the propensity for field flu viruses to mutate and reassort. In addition, the high level of genetic variation with IAV-S makes it extremely difficult to produce a killed vaccine capable of providing the broad cross-protective immunity necessary for successful stabilization or elimination.

## Autogenous WIV

Autogenous WIV vaccines have been extensively used to provide immunity specific to the strains circulating on farms and have been used in successful stabilization programs. A major challenge for autogenous vaccine production, however, is maintaining relevant strains due to new strain introductions or when multiple IAV-S strains are present. This is further complicated by the need to isolate virus for vaccine production, which can be difficult with low-prevalence endemic strains. Even if virus is isolated, it may not be amenable to replication in cell culture to provide adequate quantities of antigen for vaccine production.

## The Risk of VAERD

WIV vaccine platforms (autogenous and commercial IAV-S vaccines) also introduce the risk of vaccine-associated enhanced respiratory disease (VAERD). VAERD is observed when swine vaccinated with an adjuvanted WIV vaccine are challenged with an antigenically mismatched, heterologous influenza virus within the same hemagglutinin (HA) subtype.

While the production impact of VAERD remains unknown, the clinical signs of VAERD include severe bronchointerstitial pneumonia with necrotizing bronchiolitis and peribronchiolar lymphocytic cuffing.<sup>7</sup> The severe pneumonia caused by a dysregulation in the cytokine response, characterized by increased innate proinflammatory cytokines and decreased anti-inflammatory responses, correlates with an influx of inflammatory cells, including macrophages and neutrophils, are the characteristics of VAERD. With VAERD, the respiratory disease elicited is often more significant in vaccinates than unvaccinated controls.

## Prescription Platform

The creation of prescription platform products (e.g., SEQUIVITY® with MDF) has enabled vaccines to be produced with only the sequence(s) of the gene of interest. In the case of prescription SEQUIVITY with MDF for IAV-S, this is the gene encoding the HA glycoprotein. Use of the gene sequence bypasses the need to propagate live virus for vaccine production. Antibodies against the HA prevent viral attachment to the host cell and, if homologous to the challenge virus, can be extremely effective in reducing lung lesions, clinical signs, replication and viral shedding.<sup>8</sup> Animals vaccinated with the HA gene in the prescription will not produce antibodies against the nucleoprotein (NP) or neuraminidase (NA) of IAV-S. Additionally, vaccines based exclusively on the HA have not been shown to cause VAERD.<sup>9</sup>

Prescription vaccines based on the HA gene allow farms to customize vaccines to include multiple strains present on the farm, as well as strains circulating in epidemiologically linked areas (regionally) or farms that source replacement animals (at-risk strains).<sup>10</sup> Prescription platform vaccines have shorter production times compared with commercial vaccines and can provide more closely matched strains, which is critical for successful stabilization or elimination.

The commercially licensed SEQUIVITY® IAV-S NA vaccine contains genes from four different NA sequences that represent the genetic diversity circulating in swine. The NA is a more conserved gene than the HA, offering broader cross-protection and maintaining its relevance over longer periods of time. Antibodies to NA prevent the virus from being released from the host cell, and research has demonstrated NA vaccines reduce lung lesions, viral shedding and clinical signs associated with IAV-S infection.<sup>11</sup> As an NA-only vaccine, there is no interference from antibodies, either active or passively derived, following vaccination with the HA vaccine only.

# INFLUENZA VACCINE PLATFORMS

# VACCINATION STRATEGIES

Vaccine Type	Pros	Cons	Antibodies Generated
<b>Prescription Platform HA*</b>	Vaccine antigens are matched to farm strains; high homology and key site match to farm strains; virus isolate is not required; does not generate antibodies to NA or NP.	Time lag for production; requires regular herd monitoring to maintain relevant vaccine antigens.	HA
<b>SEQUIVITY® IAV-S NA Commercial</b>	Readily available DIVA capable vaccine; does not generate antibodies to HA or NP; broad cross protection between different strains.	Additional storage and handling requirements.	NA
<b>Commercial Whole Inactivated Virus</b>	Readily available.	Limited number of antigens; risk of variable homology or poor key site match to farm strains; risk of VAERD.	HA NA NP
<b>Autogenous Whole Inactivated Virus</b>	Vaccine antigens are matched to farm strains; high homology and key site match to farm strain.	Requires viral isolate; time lag for production; requires regular herd monitoring to maintain relevant vaccine antigens; risk of VAERD.	HA NA NP

\*This product is a prescription platform veterinary biologic to be used under the supervision of a licensed veterinarian.

Vaccination Strategy	Animals Included	Frequency*	Considerations*
<b>Whole-Herd Mass Vaccination</b>	Sows, Gilts, Boars	2-4x a year	Initial mass vaccination event should include a booster in 3-4 weeks then maintain quarterly (booster vaccination required following vaccine strain updates). Frequency is dependent on several factors (including whether +/- in combination with pre-farrow strategy).
<b>Pre-Farrow Vaccination</b>	Gestating Animals (+ Boars)	1-2x during gestation	+/- in combination with mass vaccination. Ideally a minimum of 3 weeks apart (e.g., 6 and 3 weeks pre-farrow) and fits with timing of other pre-farrow vaccine(s). Will need to vaccinate boars on farm periodically (e.g., 2-4x a year).
<b>Gilt Vaccination</b>	Gilts	2x before breeding/entry onto sow farm	In combination with one or both strategies listed above. Ideally a minimum of 3 weeks apart, with second vaccination given 2 weeks before sow farm entry.

\*The recommendations within this table are from Merck Animal Health. Please consult your veterinarian on the appropriate vaccination program for your breeding herd.

## WHOLE HERD MASS VACCINATION

Whole herd mass vaccination produced with relevant herd strains is the starting point for IAV-S stabilization. In a breeding herd, this typically entails vaccinating all mature animals in the sow farm along with all gilts in the GDU over a 1-3 day period. Following two mass vaccinations of the entire farm (administered 3-4 weeks apart), maintenance vaccines should be administered to all breeding stock quarterly, semiannually or 3-4 weeks pre-farrow or a combination thereof.

The benefits of mass vaccination include all animals generating/stimulating immunity at the same time and virus shedding is reduced throughout the whole herd. With repeated vaccination, of the breeding herd, sows and gilts passively transfer high levels of antibodies to their piglets. These maternally derived antibodies protect the piglets while in the farrowing house. Breeding herd vaccination, coupled with on farm biosecurity, can reduce viral circulation in parts of the herd or the entire site.

After a mass-vaccination event, it is critical to continue to vaccinate replacement animals with vaccines containing the recipient farm's strains of IAV-S. Ideally, these animals will receive two doses, 3-4 weeks apart before arrival to the farm. Often, logistics prevent this possibility, and they often are vaccinated immediately upon arrival. Replacement gilts and boars in the GDU should be boosted with IAV-S vaccine at least 2 weeks before entry into the breeding area if they received their second IAV-S vaccine injection before 20 weeks of age.

One of the immediate impacts of vaccinating gestating females will be high antibody levels against the IAV-S strains circulating at the farm, which will induce high levels of antibodies in their colostrum. This results in passive transfer of high levels of maternally derived antibodies to their suckling piglets. While high maternally derived IAV antibodies in piglets confer protection in the farrowing house and early nursery period, these antibodies may hinder effective vaccination against IAV-S until they have declined to sufficient levels to allow induction of a primary immune response. This restriction on active immunization of piglets due to high levels of MDAs is called maternal antibody interference.

To successfully vaccinate these piglets, there are two options:

### OPTION 1

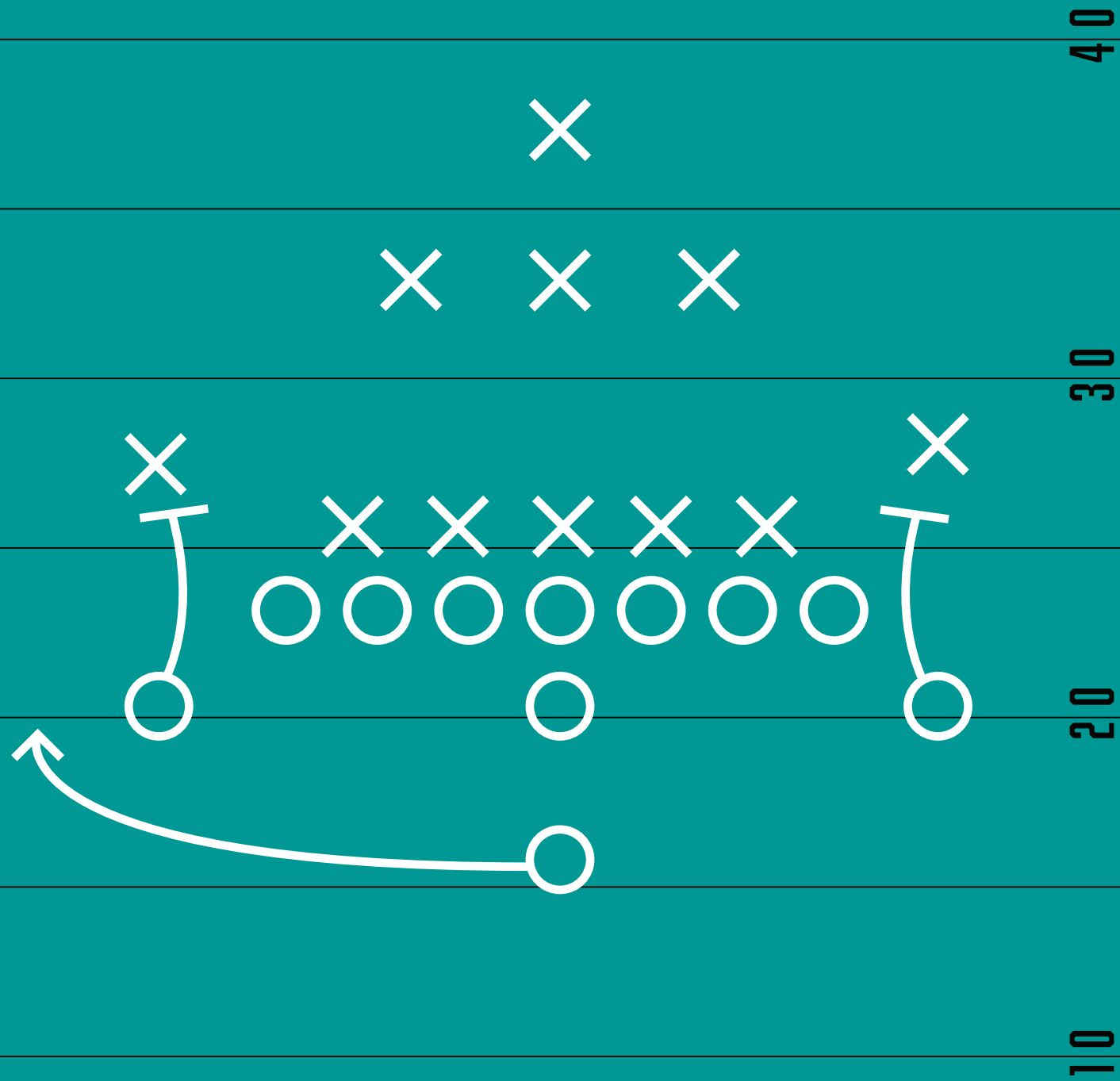
Utilize a subunit vaccine where different protective IAV-S antigens are used in sows and piglets. If the breeding herd uses a prescription IAV-S HA vaccine in sows and gilts, only HA antibodies will be generated (and elevated) and passed onto the piglet. The offspring can then be successfully vaccinated with a different IAV-S protein such as the commercially available NA vaccine without the risk of maternal antibody interference. This is a key advantage of using subunit vaccines in an elimination strategy.

If over time the IAV-S strains on the stable sow farm evolve or a new strain is introduced into a provisionally negative or negative/negative-vaccinated herd, the vaccines administered to the breeding herd must be updated to match the most relevant strains on the farm. It is critical to perform regular IAV-S surveillance/monitoring of the sow farm and sequence the HA and NA genes to drive vaccine updates.

### OPTION 2

Wait until maternal antibodies have waned (typically occurs by 10-12 weeks of age).





# DIAGNOSTIC PRINCIPLES

Diagnostic testing for IAV-S is important to classify the breeding herd, determine the appropriate vaccine for the stabilization program and monitor and manage the stabilization timeline. This section will provide helpful guidelines on appropriate sampling strategies depending on what the farm’s testing goal is and a review of common diagnostic samples and testing options.

# VIRUS DETECTION

**Sampling for IAV-S is used to determine the presence and prevalence of the virus, the subtype(s), the genetic sequence(s) and to obtain viable virus for vaccine production.**

If the goal is to determine if a group of pigs is actively circulating virus, oral fluids are a great tool in older animals housed in pens (>4-5 weeks of age), while udder wipes are an easy diagnostic method to utilize in farrowing rooms. If an IAV-S sequence and/or virus isolate is desired, nasal swabs are a common starting point. Lung samples are excellent but are a postmortem strategy. Antemortem sampling options are more commonly employed on farms for surveillance than postmortem samples because of the increased sensitivity that can be reached by higher sample numbers. The cycle time (Ct) value of the tested sample is helpful to discern which sample is best for sequencing and/or virus isolation, with lower Ct values more likely to be successful.

## Success rate of testing options based on farm goals

Testing goal:	Individual Animal Samples			Population Samples	
	Lungs	Nasal swabs	Snout wipes	Oral fluids	Udder wipes
Determine if farm/room/pen is positive	High success rate	High success rate	Moderate success rate	High success rate	High success rate
Determine prevalence in a farm/room/pen	Low success rate	High success rate	Moderate success rate	Moderate success rate	High success rate
Obtain sequence*	28	28	30	28	30
Isolate virus*	28	28	25	20	25

■ High success rate of testing goal     
 ■ Moderate success rate     
 ■ Low success rate

\*Maximum Cycle Time (Ct) for greater than 90% success rate of obtaining a sequence or live isolate. Ct recommendation per Iowa State University's Veterinary Diagnostic Lab.

## Animal Selection

Proper animal selection is key for successful virus sampling. Ideal candidates are acutely sick animals (recently infected). They can be identified by observing pigs with clear, serous bilateral nasal and/or ocular discharge, fever, increased respiration rate, lethargy and anorexia with low gut fill. Fever is defined as a rectal temperature above 104°F.

In contrast, animals in the later phase of the disease progress may exhibit a cough and mucopurulent nasal discharge. A coughing pig is often not actively shedding virus but is later in the disease progression and may be clearing the mucus from its lungs. Due to the short duration of viral shedding (typically 3-7 days), these animals are not ideal for successful virus sampling.

Many IAV-S infections are subclinical due to the presence of antibodies. Detecting IAV-S virus from suckling or newly weaned piglets can be challenging due to the presence of maternal antibodies. Maternal antibodies may mitigate clinical disease and reduce the level of virus shedding and may require more intensive sampling methods to ensure successful virus detection. This may include collecting nasal swabs or lungs from more pigs expected to be infected with virus.

## Nasal Swabs

Nasal swabs can be collected from any age of animal exhibiting acute clinical signs or that are included in a group of pigs exhibiting clinical signs. Insert a polyester or Dacron swab deep into the nose and slightly rotate to thoroughly sample the mucosal surface (perform in both nares with 1 swab). Animals can be manually restrained or snared for this procedure.

The best transport media to use is Dulbecco's Modified Eagle Medium (DMEM), which can be preplaced into Falcon tubes. After collection, the swab can be inserted into the Falcon tube and the shaft broken at a point that allows placement of the cap on the Falcon tube. Alternatively, commercial viral transport systems can be purchased. Individual samples can be pooled by three for initial screening. Positive pools can be tested individually to identify samples with low Ct values for isolation or sequencing.

## Udder Wipes

Udder wipes are performed by preparing Ziploc® or Whirl-Pak® bags with two or three 4x4 gauze pads soaked in DMEM. Wipe each teat starting cranially with the moistened gauze and replace into the bag and change gloves when sample collection is complete. Use a separate bag for each litter. The best litters to sample are those closer to weaning age when virus prevalence is typically higher. (See **Appendix C** for a detailed udder wipe protocol.)

# VIRUS DETECTION (CONTINUED)

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## Snout Wipes

Snout wipes are performed by preparing Ziploc® or Whirl-Pak® bags with three or four 4x4 gauze pads soaked in DMEM. Any age pig can be sampled, targeting those with clear nasal discharge or exhibiting clinical signs. Wipe the exterior of the nares with the moistened gauze and replace into the bag when sample collection is complete. Wipes can be pooled into sets of three at the laboratory, and positive pools can be tested individually to identify samples with low Ct values for isolation or sequencing. Pigs may be restrained manually or with a snare.

## Oral Fluids

Oral fluids are used for pen-based sampling. Pooling is not recommended. Hang a cotton rope at shoulder-length height of the pigs and allow them to chew on the rope for 15-20 minutes (variable based on age and interest of the pigs). Squeeze the oral fluid from the rope into a conical tube or Whirl-Pak® and seal for submission. Young animals (<5 weeks of age) are often reluctant to chew on ropes for oral fluids.

## Lung Tissues and Bronchoalveolar Lavage

Lung tissues and bronchoalveolar lavage are excellent samples for virus detection, sequencing and virus isolation, provided that the correct pig is selected. The disadvantage is that this requires a postmortem procedure. Submit whole fresh lungs from young piglets or sections of fresh lungs containing airways from older animals. Formalin, fixed samples that include the airways, are also useful to confirm lesions consistent with influenza. Before sectioning the lung, PBS can be injected into the trachea to fill the lung airways; the lung is gently massaged to distribute the fluid, which can be poured into a conical tube, Ziploc® or Whirl-Pak® bag for submission.

## Sampling Handling & Storage

Use aseptic technique as much as possible during sample collection, preparation and prepping for storage or shipment to a diagnostic lab. This includes wearing and changing gloves to avoid cross-contaminating samples. Samples should be stored in the refrigerator or on ice as soon as possible after collection.

Samples should be labeled with information such as pig/sow ID, date, room/location, parity and sample ID. To minimize temperature variations, keep samples cold and ship samples overnight on ice packs to the diagnostic lab. Ideally, samples should be shipped Monday through Thursday with overnight delivery to avoid long transit times and to ensure the samples arrive cold and are tested as soon as possible.

# DIAGNOSTIC TESTS FOR IAV-S

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## PCR Tests

There are two commonly utilized PCR tests for influenza detection and characterization: the screening PCR (based on the matrix gene) and the subtype PCR (based on the HA or NA gene). Cycle times (Cts) are reported for both the screening PCR and subtyping PCR, which aid in selecting samples appropriate for successful sequencing or virus isolation.

The screening PCR is the most sensitive test available for detection of IAV-S. Screening the PCR targets the matrix gene since it is a well-conserved gene in all IAV-S regardless of species. It will detect any strain and/or subtype of influenza regardless of the species sampled or the strain present.

The subtyping PCR is designed to target the HA and NA genes and will characterize the subtype of the virus detected with the screening real-time PCR. Commonly circulating swine subtypes include H1, H3, N1 and N2. The subtype PCR can detect one or more of the subtypes, which may include a mixed subtype present in the sample, or more than one strain is circulating in the population that was sampled.

## Virus Isolation

Virus isolation (VI) is required to harvest viable virus for propagation during WIV vaccine production. Samples need to be free of major contaminants, which negatively impact the cell culture systems used to grow the virus. Samples also need to have large amounts of virus (low Cts) if virus isolation is expected to be successful. Samples that are typically less successful for VI are oral fluids and udder wipes and snout wipes, and samples more likely to be successful are BAL, lung tissues and nasal swabs.

## Sequencing

There are two sequencing options for influenza: Sanger or gene sequencing and Next-Generation Sequencing (NGS). Sanger sequencing is the most common method used for generating HA and NA sequences. NGS is used to sequence the whole genome of the virus, which includes eight gene segments and is frequently used to identify mixed infections with multiple strains. Disadvantages of NGS include cost and longer turnaround times.

## USDA Swine Influenza Surveillance Program

USDA Swine Influenza Surveillance Program is a valuable program for the industry that aims to identify new influenza viruses circulating in swine that could impact public health. That provides valuable information needed to improve vaccines.<sup>12</sup> Samples submitted under this program receive financial assistance with IAV-S sequencing.

# SAMPLING STRATEGIES

To set up a sampling strategy, a presumed prevalence of the virus should be estimated. Low prevalence of virus will require the collection of more samples. Conversely, populations with expected high virus prevalence require fewer samples to achieve detection.

To assign the initial classification to the herd, all production phases should be sampled for presence of virus (suckling piglet, nursery, finisher, GDU, gestation, lactation, farrowing). In the table below, high prevalence is assigned at 10% and low prevalence is at 5%. The sample sizes are based on a 95% confidence of finding one positive in a population of 10,000 head.<sup>13</sup>

High prevalence rate of 10% was used to reflect prevalence rates typical for endemic (chronically infected) herds. Of course, recent IAV-S introductions in a herd (epidemic/acute) may result in prevalence levels >10% and thus the numbers provided below may be adjusted lower in that circumstance.

Herd Category	Expected Prevalence
Unknown	Low
Unstable	High if epidemic Low if endemic
Stable	Low
Provisionally Negative	Low
Negative-Vaccinated	Low during maintenance High if new introduction
Negative-Unvaccinated/Naive	Low during maintenance High if new introduction

## Sampling scheme by sample type and expected prevalence

Location	Sample Type	Sample Numbers/Details
Farrowing room*	Udder wipes	High prevalence-20 litters Low prevalence-30 litters
	Nasal swabs	High prevalence-30 piglets Low prevalence-60 piglets
Pen gestation	Oral fluids	6 ropes per air space
Stall gestation	Nasal swabs	High prevalence-20 head Low prevalence-30 head Target sampling of clinical animals, heat check boars, newly entered gilts and animals located near newly entered gilts
Onsite nursery-aged pigs (3-12 weeks of age)	Oral fluids**	Single airspace with multiple ages: Sample every two weeks of age, minimum 6 ropes Multiple air spaces: 6 ropes per airspace
GDU (13 weeks of age and older)	Oral fluids**	6 ropes per air space: Sample every 3 weeks of age
Weaned-pig holding room	Nasal swabs	High prevalence-30 piglets Low prevalence-60 piglets
Nursery Off-site age pigs (4-10 weeks of age)***	Oral fluids**	6 ropes per air space: Sample every 2 weeks of age

\*Target piglets with clinical signs or due-to-wean pigs across multiple rooms. Off-site nursery needs to be tested as preliminary site surveillance and after restocking with weaned pigs after Phase 2.

\*\*Oral fluids are used to detect presence of IAV-S within the population, but follow-up sampling for sequencing or virus isolation may be required.

\*\*\*Off-site nursery needs to be tested as preliminary site surveillance and after restocking with weaned pigs after Phase 2.



# IAV-S SEROLOGY

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There are serological tests that detect IAV-S, which is a key component to understanding the extent of viral circulation, occurrence of historical infection and vaccine response.

Serology can be especially important for differentiating infected from vaccinated animals (DIVA) with compatible vaccines. The SEQUIVITY line of vaccines offers two separate vaccine options: the prescription platform (exclusively targeting HA) and the commercial SEQUIVITY NA (exclusively targeting NA). While WIV vaccines or natural infection contain HA, NA and NP proteins, the SEQUIVITY platform vaccines contain either HA or NA only, making it a DIVA capable vaccine. This provides the option of strategically using serological assays, such as the nucleoprotein (NP ELISA), to determine if an animal has been naturally infected with IAV-S or administered a WIV vaccine.

## Collection of Serum

Collecting blood from swine is challenging and requires some expertise to help reduce the risk of hemolysis that may occur during sample collection. Hemolyzed serum can interfere with the performance of serological assays. Because multiple serological assays may be performed on a sample, at least 3ml of blood per animal is recommended. Collect blood in a serum separator tube with the appropriate syringe and needle.

For example, a 1-inch, 21 or 22g needle with a 5-7ml serum separator tube or 3-6cc syringe is appropriate for piglets less than 6 weeks of age. If too large of a vacutainer or syringe is used in young piglets (<3 weeks of age), the vein frequently collapses and blood is difficult to collect. Once the piglet is weaned, it is recommended to increase the needle length to at least 1.5 inches. For sows, it is recommended to use either a 4-inch needle with a 12ml syringe, or vacutainer collection can be used, although it may be difficult in larger sows.

Once blood is obtained, it must be processed and stored correctly to help eliminate inaccurate test results. Once blood is collected, it should be allowed to sit at room temperature for 1 hour to generate a well-formed clot, and then it should be separated using a centrifuge to obtain serum. Serum should then be poured off into Falcon tubes and stored in the refrigerator (short-term = days) or frozen (long-term).

## Nucleoprotein ELISA

The Nucleoprotein (NP) ELISA is based on detection of antibody generated against the nucleoprotein of the IAV-S. The NP gene is well conserved and as a result, the protein does not alter its shape, making it a good target for detecting antibody against multiple disparate strains of IAV. The NP-ELISA is a commercial assay available at most veterinary diagnostic labs. An animal will be NP positive either from live virus exposure or whole virus vaccination. This includes maternally derived antibody passively transferred to piglets from naturally exposed WIV vaccinated dams. It is a critical tool when verifying animals that have been naturally infected versus vaccinated using a DIVA compatible vaccine.

## Hemagglutinin Inhibition Assay

The Hemagglutinin Inhibition (HI) Assay measures antibodies to the HA (hemagglutinin) glycoprotein, which is genetically diverse. The assay requires a test virus with functional HA glycoproteins. It is important to know if the test virus matches the IAV-S vaccine or the test virus. If an inappropriate test virus is used in the assay, it may produce a false negative result, which is misleading. It can be time-consuming and expensive to prepare new HI test virus thus many HIs used at diagnostic labs may not be relevant to herd strains. Therefore, it is necessary to inquire what is available and if it is appropriate to answer the diagnostic question. Merck Animal Health provides HI testing to over 20 strains of commonly circulating viruses in the United States but on a limited basis for research.

## Neuraminidase Inhibition Assay

The Neuraminidase Inhibition (NI) Assay measures antibodies against the NA (neuraminidase) protein and is also IAV-S strain specific. However, the NA is more conserved than the HA protein and thus has greater cross-reactivity, requiring fewer test antigens. Thus, the NI assay does not have to be matched as closely as the HA. It is not widely available at veterinary diagnostic labs, but Merck Animal Health provides this testing capability to support customers using SEQUIVITY vaccines.



# ANIMAL SELECTION

The two most likely areas of monitoring serologically during a stabilization or elimination program are **young replacement gilts** and **suckling piglets**.

Serology for replacement gilts is typically performed to monitor MDA levels to gauge at what age they can be successfully vaccinated with minimal/no MDA interference. Select gilts for sampling from a representative parity (P) distribution (e.g., 10-P1 (gilts), 10-P2/P3 and 10-P3+). Gilts can be serially bled every 3 weeks starting at 3 days of age until 12 weeks of age, or replacement gilts can be bled at one time and sampled at the same age time points described above.

Farrowing piglets can be bled during a stabilization program to gain understanding of the farm's response to the vaccine and/or gauge the level of virus circulation. This might be performed prior to initial mass vaccination, midstabilization (post second mass vaccination) and after successful elimination. Select 2 piglets per litter and tag for serial bleeding following the same parity distribution described above. Serially bleed these piglets starting at 3 days of age and every 3 weeks through their entire growing period.





# GLOSSARY OF TERMS

Terms	Definition
<b>AIAO (All-In-All-Out)</b>	An entire barn/site/room fills in a short period of time and is then closed until the pigs all leave. New pigs do not enter until all have left and the barn/site/room is washed and disinfected.
<b>Aseptic Technique</b>	Method of sample collection to limit cross-contamination (e.g., using and changing gloves between samples, changing needles, etc.)
<b>Ct (Cycle time)</b>	Cycle time is generated when using real-time PCR, which reports level of virus present in the tested sample. Low CTs indicate more virus but is not an indication if virus is infective.
<b>DIVA (Differentiating Infected from Vaccinated Animal)</b>	Typically, DIVA is achieved using a vaccine with only limited gene(s) from the target virus which allows for serology testing of other genes to identify infection. DIVA can also be achieved by using an unrelated gene/or marker which allows for testing of the marker to identify a vaccinated animal from infected.
<b>DTW (Due-to-Wean)</b>	Due-to-wean piglets
<b>HA (Hemagglutinin)</b>	A surface protein of the influenza virus responsible for binding the virus to the host cell for entry
<b>NA (Neuraminidase)</b>	A surface protein of the influenza virus that allows the virus to be released from the host cell

Terms	Definition
<b>Naive</b>	A pig that has never been naturally infected with IAV-S nor vaccinated for IAV-S
<b>Negative</b>	A herd with no detectable virus for 1 year and with no detection of NP antibodies in recent naive introductions.
<b>Negative Vaccinated</b>	A herd with no detectable virus for 1 year and continues to vaccinate for IAV-S; vaxxinal antibodies are in recent introductions
<b>Provisionally Negative</b>	All areas of the farm are negative for IAV-S; the herd will likely have serum antibodies present from previous vaccines or previous virus infection
<b>Sentinel Gilt</b>	A replacement gilt who has never been infected or exposed to IAV-S via natural infection or vaccine and should be PCR and IAV-S seronegative. Sentinels are used to confirm no active IAV-S virus circulation within a IAV-S provisionally negative vaccinated herd that is seeking a negative classification
<b>Stable</b>	Herd continually tests PCR negative on DTW piglet sampling but may test positive in other areas of the farm
<b>Unstable</b>	Positive IAV-S circulating in the herd. A farm is unstable whether there is a recent introduction (epidemically/chronically) or a farm is endemically infected
<b>VAERD (Vaccine-Associated Enhanced Respiratory Disease)</b>	VAERD is an enhance pneumonia observed when swine vaccinated with a whole inactivated virus (WIV) vaccine are challenged with an antigenically mismatched, heterologous influenza virus within the same hemagglutinin (HA) subtype

# APPENDIX A: PLANNING DOCUMENT

## PART 1: GENERAL INFORMATION AND GOALS

What is the name of the farm?	
Who are the interested parties and decision makers?	
What is the specific issue you want to address?	
What are the production losses being incurred by its current IAV-S status?	
What is the 3 year history of IAV-S strains (subtypes and/or sequences/genetic clades) on the farm, and what stages of production were these detected?	



## PART 1: GENERAL INFORMATION AND GOALS

Where is IAV-S circulating on the farm (where are the IAV-S PCR-positive animals indicating active circulation)?*	
Which IAV-S classification is the goal of the herd?	
What productivity key performance indicators (KPIs) will be used to evaluate the impact/value of the change in classification?	
What are the concerns that could hinder this goal from being achieved?	

\*To answer this question, a thorough diagnostic workup may need to be performed where in the farm IAV-S is circulating and to obtain sequences or isolates for vaccine manufacture. This may need to be completed 12-16 weeks before implementing the control project to enable the proper timeline for vaccine manufacturing. It is important to conduct adequate surveillance across all ages of animals and air spaces within the farm with appropriate sample sizes to accurately identify where active IAV-S circulation might be occurring. Please work with your veterinarian to establish a robust diagnostic monitoring program for this purpose. Make sure to sequence IAV-S strains (both HA and NA) found in different areas of the farm so you know how many IAV-S strains based on genetic clades may be circulating. Refer to the diagnostic section of the playbook (**Section 9**) for more specific guidance on whole herd surveillance sampling strategies. Sequences from viruses isolated from the farm from the past three years should be compared to the present circulating strain(s). Ultimately, sequences must be obtained for all known IAV-S viruses currently circulating on the farm to guide vaccine options. Refer to Section 8 to help determine which IAV-S vaccine platform to select for the IAV-S stabilization program.

## PART 2: FARM LAYOUT AND FLOW

It is important to understand the farm layout and animal flow to determine if enhanced biosecurity steps are needed to reach the specific classification goal of the farm. The below list of questions will help identify any high-risk areas for IAV-S transmission that should be addressed prior to initiating a classification goal.

How many animals are on-site (sows, boars, gilts, piglets, nursery or finisher)?	
How many buildings/rooms/pens are there per production phase?	
What are the current biosecurity procedures on the farm?	
How many employees work on the farm?	
Is there crossover of employees between departments (if so, please describe)?	

## PART 2: FARM LAYOUT AND FLOW

What are typical employee traffic patterns per department?	
What is the employee sick-leave policy?	
What cleaning and sanitation practices are used in the office, farm hallways, medicine rooms and chutes?	
What is the cull animal schedule?	
Is there any equipment used on farm that is shared between departments or the GDU?	
Ease of employees to adhere to biosecurity changes on farm: High, Medium, Low?	

## PART 3A: FARROWING HOUSE QUESTIONS

What is the weaning age?	
What days of the week does weaning take place?	
Are complete rooms emptied at weaning?	
Is there a holding room for weaned pigs?	
If so, how frequently are pigs weaned out of it?	
What current diagnostic monitoring is conducted?	
What are the farm's procedures around animal movements (cross-fostering, fallbacks, nurse sows)?	
What are the clinical IAV-S picture of sows and piglets?	
Signs observed?	
Ages and percentage of piglets affected?	
What is the sanitation and disinfection program used?	
When are piglets vaccinated, processed, medicated?	

## PART 3B: GDU QUESTIONS

Are the gilt replacements raised onsite or brought from another source?	
Are gilt replacements quarantined in an off-site or on-site isolation?	
What ages are incoming replacement gilts?	
What is the IAV-S status of incoming replacement gilts?	
What current diagnostics are used to monitor the gilt population (incoming or resident gilts, frequency)?	
What is the average gilt group size?	
What is the vaccine schedule for replacement gilts?	

What age do gilts move into gestation?	
What age is selection occurring?	
What age are teaser boars introduced?	
Where are teaser boars housed?	
Where are cull gilts housed?	
What biosecurity procedures are in place?	
Where are the gilts bred?	
Separate gilt snake on farm or next to sows?	
Is any material used to acclimate gilts (i.e., feedback, mummies, stillborns, cull sows)?	

# APPENDIX B: STABILIZATION TIMELINE TEMPLATE

## STABILIZATION TIMELINE TEMPLATE

	Week	Event
Phase 1	1	Mass Vax 1
	2	
	3	
	4	Mass Vax 2
	5	
Phase 2	6	1st Fully Vax Piglets Farrow
	7	
	8	
	9	
Phase 3	10	1st Fully Vax Piglets Wean
	11	
	12	
	13+	
Stable/Provisionally Negative	22+	



# APPENDIX C: UDDER WIPE SAMPLING PROTOCOL

## UDDER WIPE SUPPLIES

<b>Gauze</b>	4x4 gauze pads (polyester may have better results than cotton) (2-3 pads per udder depending on thickness)
<b>Media (need media &amp; distilled water)</b>	We recommend Dulbecco's Modified Eagle Medium (DMEM) PBS, saline or minimal essential media
<b>Ziploc® Bags or Whirl-Pak®</b>	<ol style="list-style-type: none"> <li>1. Sandwich-or quart-size bags</li> <li>2. Gallon bags: To organize and keep your sample bags together to send to farm and to the diagnostic lab</li> </ol>
<b>Gloves</b>	Latex or nitrile (one pair of gloves per litter sampled)

# MAKING UDDER WIPE KITS

## STEP 1

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Prepare sample Ziploc® or Whirl-Pak® bags. Label bags (farm, parity, sow ID, room/crate, date). Record if each sow has a clinical or nonclinical litter.

Label Example:

Sow Farm: \_X\_

Date: \_\_00/00/00

Sow ID: \_\_\_\_\_

Room: \_\_ Crate: \_\_\_\_

Parity: P1 P2 P3 P4 P5 P6 P7

Clinical Litter?: Yes No

## STEP 2

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Wear clean gloves.

## STEP 3

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Add 2-3 4x4 gauze pads to each Ziploc® or Whirl-Pak® bag.

## STEP 4

---

Pour media liquid into each Ziploc® bag. Add enough to fully saturate the gauze, but avoid standing/free liquid in the bag. Make sure bags are fully sealed.

## STEP 5

---

Keep remaining media in the refrigerator (record mixing date on media container). Reconstituted DMEM can be stored in the refrigerator for 3-4 weeks. Discard if the media changes color.

# UDDER WIPE SAMPLE COLLECTION

## STEP 1

---

Encourage the sow to stand if she is not on her side nursing. It is okay to sample a sow if she is nursing on her side. The goal is to sample the majority of her teats.

## STEP 2

---

Don new gloves before pulling gauze out of bag.

## STEP 3

---

Open the Ziploc® or Whirl-Pak® bag and remove gauze.

## STEP 4

---

Wipe the teats in a sweeping motion from front to back, focusing on each individual teat. Start with the most cranial teats and work your way towards those closest to her hind end.

## STEP 5

---

Place the gauze back into the same Ziploc® or Whirl-Pak® bag from which it was removed, squeezing the fluid from the gauze into the Ziploc® or Whirl-Pak® bag.

## STEP 6

---

Seal the bag and complete the label.

## STEP 7

---

Collect remaining litters, making sure to change gloves before sampling the next litter.

## STEP 8

---

Chill the sample as soon as possible (on ice or in refrigerator).

## STEP 9

---

Submit samples to a veterinary diagnostic laboratory for IAV-S PCR testing via next-day shipping. Make sure to submit samples on enough ice to keep them cold during transit.

**For testing there are two options:**

1. Use the screening PCR and then select samples for subtype PCR, or
2. Go directly to subtype PCR (lower sensitivity)

The subtype PCR will help identify mixed infections and make the decision easier to break open pools, as well as determine how many samples should be sequenced.

**\*\*It is important to note that udder wipes are not a currently accepted sample type for the USDA surveillance program.\*\***

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# NOTES

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# EXECUTE THE PLAN PROTECT THE HERD

To learn more about protecting your operation from the economic impact of influenza and developing an effective stabilization strategy, contact your Merck Animal Health representative.

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