Sample Sizes for Surveillance and Suspected Contamination Sampling

Surveillance is the routine collection of information used to characterize risk with clearly established intervention points used to monitor and maintain animal health. Surveillance also uses thresholds to dictate further action either through sampling, implementing mitigation strategies, or a combination of both. If surveillance thresholds have been met or exceeded, it is time to transition to suspected contamination sampling. Pre-determined samples sizes for surveillance sampling with thresholds (Table 1) and suspected contamination sampling with return to surveillance sampling thresholds can be found at the end of this resource (Table 2). This resource will detail how to interpret those pre-determined sample sizes for surveillance and suspected contamination. To calculate sample sizes yourself, refer to "Calculating Sample Sizes and Thresholds" or to coordinate the transition to suspected contamination sampling, refer to "Transitioning from Surveillance Sampling to Suspected Contamination Sampling."

The pre-determined sample sizes depend on two factors, 1) the probability of feed serving as a source for pathogen of interest and 2) the severity of the pathogen in regards to species of interest. There are spectrums within these two factors that will determine sample size.

Probability of feed serving as a source for pathogen of interest

The probability of feed serving as a source for pathogen of interest takes in account the potential feed ingredients and mitigation strategies already implemented at the feed mill.

- **High probability**
  - High probability indicates that there is immediate danger that the hazard will occur.
  - If there are no mitigation techniques in place at a feed mill, then this is the proper designation.
- **Medium probability**
  - Medium probability indicates that the hazard will probably occur if not controlled.
  - If a feed mill utilizes only point-in-time mitigation techniques, this is the appropriate designation.
    - Examples of point-in-time mitigation techniques include quarantining or holding ingredients, thermally processing feed, implementing feed batch sequencing, or implementing flushes after manufacturing certain diets.
    - These techniques can only guarantee that infectivity of the pathogen has been reduced, but doesn't prevent recontamination.
  - If a feed mill has or utilizes rendered ingredients for diets, this is the appropriate designation.
    - Rendered ingredients are manufactured at a temperature range of 240-290°F for at least 40-90 minutes which reduces pathogen contamination (Hamilton, 2006). However, this temperature range does not prevent recontamination during further feed manufacturing or delivery.
    - Transportation of these ingredients from rendering facilities is also a risk of pathogen introduction to a feed mill (Lowe et al., 2014) as these types of ingredients have been shown to better support pathogen survival when compared to plant based ingredients (Dee et al., 2018)
- **Low probability**
  - Low probability indicates that it's possible for hazard to occur if not controlled.
  - If a feed mill utilizes a chemical feed additive as a means to reduce pathogen contamination or infectivity, this is the appropriate designation.
    - Chemical feed additives reduce pathogens in feed at time of application and remain active throughout the feed supply chain (Stewart et al., 2020).

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Sample Sizes for Surveillance and Suspected Contamination Sampling.*

- **Very low probability**
  - Very low probability indicates that it's unlikely for the hazard to occur and an assumption that the hazard will not occur is warranted.
  - If a feed mill utilizes point-in-time mitigation techniques in combination with a chemical feed additive, this is the appropriate designation.

Severity of the pathogen of interest in regards to species of interest

The severity of the pathogen of interest in regards to species of interest is based on the short and long term consequences if it were to be introduced. The type of production system served by the feed mill and the production system's definition of mortality and morbidity will influence the designated severity.

- **High severity**
  - Pathogen of interest would cause high mortality and high morbidity if introduced into the production system.
- **Medium severity**
  - Pathogen of interest would cause high mortality and low morbidity if introduced into the production system.
- **Low severity**
  - Pathogen of interest would cause low mortality and high morbidity if introduced into the production system.
- **Very low severity**
  - Pathogen of interest would cause low mortality and low morbidity if introduced into the production system.

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Sample Sizes for Surveillance and Suspected Contamination Sampling.*

Table 1: Recommendations for surveillance sample size and thresholds based on severity of pathogen of interest and probability of pathogen being introduced through feed.

Severity / Probability	**HIGH** Pathogen of interest would cause high mortality and high morbidity	**MEDIUM** Pathogen of interest would cause high mortality and low morbidity	**LOW** Pathogen of interest would cause low mortality and high morbidity	**VERY LOW** Pathogen of interest would cause low mortality and low morbidity
HIGH Immediate danger that the hazard will occur.	75 samples/week: 10 feed samples 65 environmental samples **Threshold = 1 positive**	75 samples/week: 10 feed samples 65 environmental samples **Threshold = 1 positive**	15 samples/week: 5 feed samples 10 environmental samples **Threshold = 1 positive**	5 samples/week: 1 feed sample 4 environmental samples **Threshold = 1 positive**
MEDIUM Hazard will probably occur if not controlled.	75 samples/week: 5 feed samples 70 environmental samples **Threshold = 1 positive**	15 samples/week: 2 feed samples 13 environmental samples **Threshold = 2 positives**	8 samples/week: 2 feed samples 6 environmental samples **Threshold = 2 positives**	5 samples/week: 0 feed samples 5 environmental samples **Threshold = 2 positives**
LOW It's possible for hazard to occur if not controlled.	25 samples/week: 1 feed sample 24 environmental samples **Threshold = 1 positive**	15 samples/week: 1 feed sample 14 environmental samples **Threshold = 2 positives**	8 samples/week: 1 feed sample 7 environmental samples **Threshold = 3 positives**	4 samples/week: 0 feed samples 4 environmental samples **Threshold = 2 positives**
VERY LOW It's unlikely for the hazard to occur and can assume that hazard will not occur.	25 samples/week: 0 feed samples 25 environmental samples **Threshold = 1 positive**	15 samples/week: 0 feed samples 15 environmental samples **Threshold = 2 positives**	8 samples/week: 0 feed samples 8 environmental samples **Threshold = 3 positives**	3 samples/week: 0 feed samples 3 environmental samples **Threshold = 2 positives**

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Sample Sizes for Surveillance and Suspected Contamination Sampling.*

Table 2: Recommendations for suspected contamination sample size and when to return to surveillance sampling based on severity of pathogen of interest and probability of pathogen of interest introduced through feed.

<table>
<tr><th>Severity
Probability</th><th>HIGH
Pathogen of interest would cause high mortality and high morbidity</th><th>MEDIUM
Pathogen of interest would cause high mortality and low morbidity</th><th>LOW
Pathogen of interest would cause low mortality and high morbidity</th><th>VERY LOW
Pathogen of interest would cause low mortality and low morbidity</th></tr>
<tr><td>HIGH
Immediate danger that the hazard will occur.</td><td colspan="2" rowspan="2">300 samples:
102 feed samples
198 environmental samples
Return to surveillance: no more than 3 positive samples</td><td colspan="2" rowspan="2">100 samples:
25 feed samples
75 environmental samples
Return to surveillance: no more than 3 positive sample</td></tr>
<tr><td>MEDIUM
Hazard will probably occur if not controlled.</td></tr>
<tr><td>LOW
It’s possible for hazard to occur if not controlled.</td><td colspan="2" rowspan="2">100 samples:
15 feed sample
85 environmental samples
Return to surveillance: no more than 3 positive sample</td><td colspan="2" rowspan="2">60 samples:
5 feed samples
55 environmental samples
Return to surveillance: no more than 3 positive sample</td></tr>
<tr><td>VERY LOW
It’s unlikely for the hazard to occur and can assume that hazard will not occur.</td></tr>
</table>

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Sample Sizes for Surveillance and Suspected Contamination Sampling.*

References

Dee., S., F. Bauermann, M. Niederwerder, A. Singrey, T. Clement, M. DeLima, C. Long, G. 427 Patterson, M. Shehan, A. Stoian, V. Petrovan, C.K. Jones, J. De Jong, J. Ji., G Spronk, J. 428 Hennings, J. Zimmerman, B. Rowland, E. Nelson, P. Sundberg, D. Diel, and L. Minion. 2018. 429 Survival of viral pathogens in animal feed ingredients under transboundary shipping models. 430 PLoS ONE. doi: 10.1371/journal.pone.0194509.

Hamiliton CR. (2006). An Overview of the Rendering Industry. *Essential Rendering:* 1-16. Accessed 12 April 2022. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.457.5553&rep=rep1&type=pdf#page=12

Lowe, J., Gauger, P., Harmon, K., Zhang, J., Connor, J., Yeske, P., Loula, T., Levis, I., Dufresne, L., and Main, R. (2014). Role of transportation in spread of porcine epidemic diarrhea virus infection, United States. *Emerg Infect Dis. 20(5):*872-874. doi:10.3201/eid2005.131628

Stewart, S.C., Dritz, S.S., Woodworth, J.C., Paulk, C., and Jones, C.K. (2020). A review of strategies to impact swine feed biosecurity. *Anim Health Research Reviews. 21:*61-68. doi:10.1017/S14662523190015X

Calculating Sample Sizes and Thresholds

A challenge when trying to implement sampling programs within a feed mill is defining proper calculation of the necessary sample size to detect pathogens of interest. If interested in pre-determined sample size recommendations and thresholds, refer to "Sample Size for Surveillance and Suspected Contamination." However, if a production system or feed mill has a general idea of prevalence rate for the pathogen of interest and would like to defer from the general recommendations, this resource aims to explain how to calculate sample size, basis for sample size, and how to set thresholds for sampling feed mills. If interested in how to transition from surveillance to suspected contamination sampling, refer "Transitioning from Surveillance Sampling to Suspected Contamination Sampling."

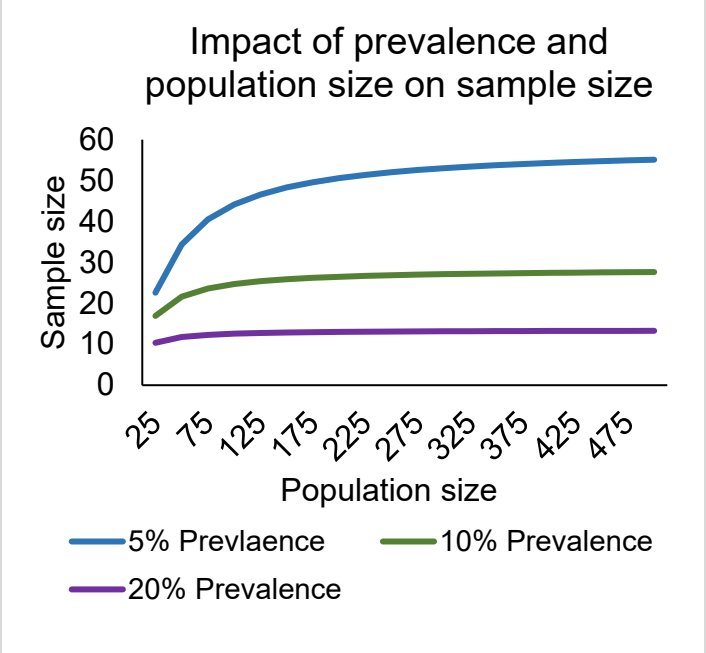
Sample size formula

The formula to determine sample size is the same as that which is used for sampling to detect disease in animals (Dahoo et al., 2014). This formula is used to determine the sample size necessary to have confidence in the outcome while minimizing interpretation error. It takes in to account the number of animals from within a population that must be sampled from a population to have a given level of confidence that at least one sample would be positive based on a given prevalence level. The sample size, n, is determined by the confidence interval (α = 1 – confidence level), the population size (N), and estimated minimum number of diseases animals in the group (D = estimated prevalence population size):

$$n = \left[\left(1 - \alpha^{1/D}\right) \times \left(N - \frac{D-1}{2}\right)\right]$$

However, when considering feed mills and the presence of a pathogen of interest, the feed mill is more concerned about detecting the pathogen of interest within feed or feed mill environment and not clinical disease within an animal. So in this instance, the sample size could be thought of as the number of total samples to take at the feed mill, the population could be thought of as the possible number of samples to take either in feed or in the environment, and the prevalence as the perceived prevalence of the pathogen of interest within the feed mill. When interpreting the formula in a scenario like this, it can be inferred that the number of possible samples to be collected approaches infinity because samples could be taken per ton, per pound, or per gram.

As shown in the graph on the right, when utilizing the sampling to detect disease formula, the sample size for a desired prevalence level will plateau. This is due to there being minimal changes within the sample sizes as the population size increases.



Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Calculating Sample Sizes and Thresholds*.

So if you are looking for a desired prevalence level within a population, identify where the sample size plateaus for the prevalence level, and utilize the sample size corresponding with the start of the plateau. As illustrated in the graph on the previous page, as prevalence rate increases, the sample size decreases which is because the pathogen of interest is present at higher percentages and thus, requires a smaller sample size to detect it within the population. When consulting the pre-determined sample sizes for surveillance, the basis for sample size recommendations was based on this same principle.

Sample size references

When trying to set sample sizes for feed and environmental samples, there are some peer-reviewed works to reference (Table 1). These sources evaluated the prevalence, or the number of samples containing detectable pathogen of interest divided by the total number of samples collected, for some pathogens within feed mills. Feed samples are defined as samples pulled directly from the source (either from feed ingredients or complete feed) while environmental samples are samples from surfaces. The data from these published works give reference ranges for prevalence of pathogens and can help guide surveillance sampling.

Thresholds

Thresholds designate action because the prevalence rate has increased during surveillance sampling. Thresholds can be adjusted to be stricter or more lenient with increased prevalence rates depending upon the production system and how risk averse they choose to be.

References

Dahoo, I., Martin, W., and Stryhm H. 2014. Sampling. In: S Marget McPike, editor, Veterinary Epidemiologic Research. VER Inc. Charlottetown, Prince Edward Island, Canada. p. 33-56.

Table 1. Prevalence rates from available published data where pathogens were naturally present in feed ingredients, complete feed, or feed mill environments.

Item	Reference doi	Pathogen[1]	Prevalence, %	Total Number of Samples Collected
Environmental Samples:				
Elijah et al., 2022	10.54846/jshap/1250	PDCoV	2.33	86
Elijah et al., 2022	10.54846/jshap/1250	PEDV	2.33	86
Garrido-Mantillo et al., 2022	10.1111/tbed.14354	PEDV	37.50	8
Gebhardt et al., 2021	10.1111/tbed.14335	ASFV	0.73	2186
Magossi et al., 2019	10.1002/mbo3.711	Salmonella sp.	66.24	237
Magossi et al., 2019	10.1002/mbo3.711	*Salmonella enterica*	19.75	157
Feed Samples:				
Gebhardt et al., 2021	10.1111/tbed.14335	ASFV	0.70	142
Leme et al., 2019	10.111/tbed.13215	SVA	25.93	27
Wu el al., 2021	10.1111/tbed.14209	PEDV	14.29	77

Environmental sample summary:	**Feed sample summary:**
Minimum prevalence: 0.73% Average prevalence: 21.48% Maximum prevalence: 66.24%	Minimum prevalence: 0.70% Average prevalence: 13.64% Maximum prevalence: 25.93%

[1]Abbreviations defined as: African swine fever virus (ASFV), Porcine deltacoronavirus (PDCoV), Porcine epidemic diarrhea virus (PEDV), Seneca valley virus (SVA)

Transitioning from Surveillance Sampling to Suspected Contamination Sampling

When surveillance sampling results meet or exceed pre-established thresholds, it's time to transition into suspected contamination sampling. The purpose of suspected contamination sampling is to identify areas within the feed mill or feed delivery that contribute to the increased prevalence of the pathogen of interest. Feed mills can also implement mitigation strategies while undergoing suspected contamination sampling to gauge how successful these mitigation strategies are for the pathogen of interest. This resource focuses on how to transition from surveillance to suspected contamination sampling through changes in sample size and sampling frequency. If there are questions regarding sample size and thresholds, refer to the additional resource titled "Calculating Sample Sizes and Thresholds." If there are questions regarding areas of focus, refer to the additional resource titled "Interpreting Sample Results."

Changes in sampling size

Surveillance sample size is based on the probability of feed serving as a source of the pathogen of interest and the severity of the pathogen of interest. However in the case of suspected contamination, the thresholds have been met or exceeded, indicating that the pathogen of interest may be present more frequently or greater than originally perceived. Therefore, to maximize the ability to detect the pathogen of interest, increase the sample size. During suspected contamination sampling, sample sizes might be larger than the sample sizes commonly used for surveillance sampling. To accommodate the larger sampling size, adjust the threshold, or in this instance, the amount of samples that need to be negative in order to return back to surveillance sampling.

Changes in sampling frequency

Suspected contamination samplings need to occur more frequently than surveillance sampling if surveillance thresholds are met or exceeded. For example, the pre-determined surveillance sample sizes are based on monthly prevalence rates and divided across weeks to make sample collection more manageable. However, when thresholds are met or exceeded in surveillance sampling that is indicative that the set prevalence rates for a monthly basis have already been met or exceeded in a week. Therefore, to understand the source of suspected contamination, for the next week, the feed mill will transition to suspected contamination. The feed mill will transition back to the normal surveillance schedule if suspected contamination thresholds are not met or exceeded. If a production system or feed mill chooses to sample more or less frequently than suspected contamination sampling will need to be adjusted accordingly.

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Transitioning from Surveillance Sampling to Suspected Contamination Sampling*.

Types of Samples

There are two types of samples to take in a feed mill: feed or environmental samples. This resource will help explain the most current and scientifically proven methodology for sampling complete feed, feed ingredients, and feed mill environments. To access information on how to take feed or environmental samples, refer to “Collecting Feed Samples” and “Collecting Environmental Samples.” If requiring information on how to prepare for sampling, refer to “Assembling Materials for Environmental Sampling of Viral Pathogens.”

Feed samples

Sampling feed intended for livestock species can offer a way to assess potential contamination in either complete feed or feed ingredients. However, sampling feed is challenging since potential contamination may not be evenly distributed within the feed or ingredient, creating “hot spots” of contamination. To account for this type of distribution, the Association of American Feed Control Officials (AAFCO) feed inspector’s manual offers different solutions on how to sample feed: utilizing sleeved feed probes or single tube triers or sampling via cut stream (AAFCO, 2020). Sleeved feed probes are the only validated methodology for viral pathogens while the other methodologies have yet to be validated (Jones et al., 2020; Elijah et al., 2021, Dee et al., 2022). Table 1 offers a summary of the three methodologies for feed sampling.

All methodologies rely on collecting 10 subsamples per load or lot of complete feed or feed ingredients and combining the 10 subsamples for a single composite sample for submission. The AAFCO feed inspector’s manual recommends a minimum of 10 subsamples so that the sampling methodology can account for unevenly distributed contamination. If a feed mill is trying to identify potential contamination within a specific batch of feed, taking 10 subsamples within a single load of feed answers the question of potential contamination before delivery. However, if a feed mill is busier than normal, like during times of harvest, collecting 10 subsamples per truck load can be challenging. In this case, if the feed mill is interested in potential contamination throughout the day, each load of bulk ingredient could be considered a subsample, one subsample pulled from each load, and then 10 subsamples from 10 loads could be combined as a composite sample for the bulk ingredients received that day. Depending on the question, the minimum of 10 subsamples can be manipulated to account for different sampling scenarios.

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Type of Samples.*

Table 1. Methodologies for sampling feed ingredients or complete feed.

Methodology	Used When?	Materials Needed	Minimum number of sub-samples[1]
Sampling with Sleeved Probes[2]	• Can get an overview of the sampling container. • Sample container deep enough for the double tube feed probe. • Used for bulk feed ingredients or complete feed.	• Sleeved feed probe • Plastic storage bag • Permanent marker • Disinfectant wipes	10
Sampling via Cut Stream[3]	• If unable to sample feed with sleeved feed probes. • Used for bulk feed ingredients or complete feed.	• 8 ounce cup • Plastic storage bag • Permanent marker • Disinfectant wipes	10
Sampling with Single Tube Trier[4]	• Used for bagged feed ingredients or complete feed.	• Single tube trier • Plastic storage bag • Permanent marker • Disinfectant wipes	10

[1]Sub-samples refers to the number of samples, or pulls, from the intended sample container that will go into the composite sample.
[2]Sleeved feed probes have an internal and external compartment. Insert the sleeved probe with compartments closed, open compartments once probe is inserted into the feed ingredient or complete feed, shake the probe to fill, close the probe, then withdraw from feed ingredient or complete feed.
[3]Cut stream is the terminology used to describe when sampling relies on a stream of feed ingredients or complete feed and the sampling container passes through the stream and fills the sampling material to obtain a sub-sample.
[4]Single tube trier has an open sampling compartment with a handle. Single tube triers are rotated so sampling material is collected into the open compartment.

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Type of Samples.*

Environmental Samples

Based on the pathogen of interest, three factors will influence how to take samples: 1) methodology, 2) sampling material, and 3) pre-moistening solution. Methodology is influenced by the accessibility of the sampling location. For viral pathogens, you can hand sample or use a paint roller to sample while for bacterial pathogens, you can only hand sample. Pathogen of interest will influence the sampling material. The 3M sponge sticks are the most effective for bacterial pathogens (Moore and Griffith, 2002; FDA, 2021) while cotton gauze is the most effective for viral pathogens (Stewart et al., 2019). For the paint roller methodology, material of choice is synthetic paint roller covers (Wu et al., 2021; Elijah et al., 2022) but if unable to acquire these, cotton paint roller covers are an acceptable substitute. Pathogen of interest will also influence the pre-moistening solution. By pre-moistening the sampling material, you maximize the ability of the sample material to pick up potential pathogens (Moore and Griffith, 2002). For gram negative bacteria, pre-moistening solution of choice is buffered peptone water. For viruses, pre-moistening solution of choice is phosphate buffered solution (1X concentration, pH=7.4) but recent research has shown that 0.9% NaCl sterile saline is an acceptable substitute (Rodino et al., 2020).

Sampling the environment of the feed mill can offer a way to understand the directionality, or spread, of pathogens of interest or monitor the biosecurity practices in place. To help with this, environmental samples are classified into zones based on the surface and what that surface comes into contact with.

Feed Contact Surfaces

Feed contact surfaces have direct contact with feed ingredients or complete feed. These surfaces are associated with feed manufacturing, storage, and delivery. If these surfaces are positive for pathogen of interest, its origin may have been from a contaminated feed ingredient.

Surfaces associated with the feed mill	Surfaces associated with feed delivery
Corn cleaner	Interior of feed truck compartments
Receiving pit grates	Interior of feed truck boom
Fat intake inlet or hose	
Interiors of feed bins	
Load out auger or sock	
Interiors of bucket elevators	

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Type of Samples.*

Non-Feed Contact Surfaces

Non-feed contact surfaces are surfaces with a fixed location that are close or next to feed contact surfaces. These surfaces are either covered by dust from feed manufacturing or have a lot of foot traffic. If these surfaces are positive for pathogen of interest, its origin may have been through a contaminated feed ingredient dust generated during feed manufacturing or transient surfaces spreading contamination.

Surfaces associated with the feed mill	Surfaces associated with feed delivery
Floor of load out bay	Exterior of feed truck compartments
Control room floor	Exterior of feed truck boom
Floor mat by main entrance	
Receiving floors	
Manufacturing floors ▪ Floors around hand add ports ▪ Areas near sample ports	
Warehouse floors	
Exterior of pellet mill	
Pellet mill air intake	
Inside dust collection system	

Transient Surfaces

Transient surfaces are surfaces with a non-fixed location and can move within the feed mill or during feed delivery. These surfaces have intermittent contact with other surfaces that could potentially have exposure to pathogen of interest. If these surfaces are positive for pathogen of interest, its origin may have been through employees introducing or spreading the contamination.

Surfaces associated with the feed mill	Surfaces associated with feed delivery
Fork lift tires	Feed truck steps
Broom	Feed truck floor mat and pedals
Shovels	Feed truck tires
Worker shoes	Workers shoes
Worker clothing	Workers clothing

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Type of Samples.*

Table 2. Methodologies for environmental sampling based on pathogen.

Pathogen	Methodology[1]	Sampling Material[2]	Pre-Moistening Solution[3]	Size of Sampling Area	Number of Passes of Sampling Area[4]
Bacteria	Hand Sampling	3M Sponge Sticks	Buffered Peptone Water	8 × 8 in.	10 horizontal pushes and pulls 10 vertical pushes and pulls
Virus	Hand Sampling	4 × 4 in. Cotton Gauze	Phosphate Buffered Solution 0.9% NaCl Sterile Saline	8 × 8 in.	10 horizontal pushes and pulls 10 vertical pushes and pulls
Virus	Extension Set Sampling	Synthetic Paint Roller Cover	Phosphate Buffered Solution 0.9% NaCl Sterile Saline	--	10 horizontal pushes and pulls 10 vertical pushes and pulls

[1]Methods of collecting environmental samples can rely on hand sampling or usage of an extension set to sample hard to reach areas for viral pathogens.
[2]Sampling material refers to the material that will pass over the surface of interest.
[3]Pre-moistening solution refers to the solution that moistens the material before sampling.
[4]Number of passes refers to the number of times the sampling material should pass over the sampling area to pick up the pathogen of interest.

References

AAFCO - Association of American Feed Control Officials. (2020). Feed Inspector's Manual. https://www.aafco.org/Portals/0/SiteContent/Publications/AAFCO_Feed_Inspectors_Manual_8th_edition.pdf

Dee S, Shah A, Jones C, Singrey A, Hanson D, Edler R, Spronk G, Niederwerder M, and Nelson E. (2022). Evidence of viral survival in representative volumes of feed and feed ingredients during long-distance commercial transport across the continental United States. *Transbound and Emerg Dis. 69:*149-156. doi:10.1111/tbed.14057

Elijah CG, Trujillo JD, Jones CK, Kwon T, Stark CR, Cool KR, Paulk CB, Gaudreault NN, Woodworth JC, Morozov I, Gallardo C, Gebhardt JT, and Richt JA. (2021). Effect of mixing and feed batch sequencing on the prevalence and distribution of African swine fever virus in swine feed. *Trans. Emerg. Dis.* 1-6. doi:10.1111/tbed.14177.

Elijah CG, Harrison OL, Blomme AK, Woodworth JC, Jones CK, Paulk CB, and Gebhardt JT. (2022). Understanding the role of feed manufacturing and delivery within a series of porcine deltacoronavirus investigations. *J Swine Health Prod. 30(1):*17-23.

FDA – Food and Drug Administration Investigations Operations Manual. (2021). https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-references/investigations-operations-manual

Jones C, Stewart S, Woodworth J, Dritz S, and Paulk C. (2020). Validation of sampling methods in bulk feed ingredients for detection of swine viruses. *Transbound Emerg Dis. 67:*1-5. doi:10.1111/tbed.13326.

Moore G and Griffith C. (2002). Factors influencing recovery of micro-organisms from surfaces by use of traditional hygiene swabbing. *Dairy, Food, and Environ Sanit. 22(6):*410-421.

Rodino KG, Espy MJ, Buckwalter SP, Walchak RC, Gerner JJ, Fernholz E, Boerger A, Schuetz AN, Yao JD, and Binnincker MJ. (2020). Evaluation of saline, phosphate-buffere saline, and minimum essential medium as potential alternatives to vital transport media for SARS-CoV-2 testing. *J. Clin Microbiol:* 58:e00590-20. doi:10.1128/JCM.00590-20.

Stewart SC, Niederwerder MC, Woodworth JC, Dritz SS, and Jones CK. (2019). 350 – Effects of swab type on detection of PEDV from feed manufacturing surfaces. *J Anim Sci. 97(Supplement_2_July 2019):* 144-145. doi:10.1093/jas/skz122.256

Wu F, Cochrane R, Yaros J, Zhang C, Tsai SY, and Spronk G. (2021). Interventions to reduce porcine epidemic diarrhea virus prevalence in feed in a Chinese swine production system: A case study. *Transbound Emerg Dis 69:*57-65. doi:10.1111/tbed.14209

Diagnostic Tests for Samples

Once samples have been collected, the samples should be sent to a veterinary diagnostic laboratory for viral or bacterial analysis. Veterinary diagnostic laboratories can take a variety of sample types and have the personnel trained to appropriately handle the samples for analysis. A list of available accredited veterinary diagnostic laboratories can be found here. While the veterinary diagnostic laboratories have a wide variety of tests available, this resource will focus on the tests, assays, or processes that are typically conducted on environmental or feed samples.

Bacterial Pathogens

Before running any tests, instruct the diagnostic laboratory to enrich the sample. The purpose of the enrichment step is to grow the contamination present in the samples to detectable levels. Enriching samples increases the sensitivity of any intended tests to be run on the bacterial samples. If electing to run tests without enrichment, understand that there is the potential for the diagnostic tests to return as false negative since the contamination might not be present at detectable levels.

After enrichment, samples can be submitted to either bacterial culture or PCR analysis. Bacterial culture is considered the less expensive option but tends to be more time consuming and can identify genus of bacteria but not specific types of bacteria. While PCR analysis has the ability to identify specific bacterial genus and species, however most samples are cultured before PCR analysis. An example of when a production system or feed mill would elect PCR analysis over bacterial culture is if the production system or feed mill is concerned about *Salmonella* in the feed intended for livestock consumption. If concerned about *Salmonella* in feed for livestock species, consult the Food and Drug Administration guide on *Salmonella* in food for animals (FDA, 2013).

Another potential option for bacterial analysis is the use of near infrared spectroscopy (NIR). This is a relatively new technology to detect bacterial contamination within feed that offers a quick turnaround time and is less expensive than PCR (Tian et al., 2021). It offers the same results as PCR but since this is a more recently developed technology, the sample will still require bacterial culture and in some instances, NIR might be less precise than PCR assay (Tian et al., 2021). Consult with the diagnostic laboratory at time of sample submission if this is a test that they are offering and would recommend.

Viral Pathogens

Before running any tests, request that the diagnostic laboratory centrifuge all samples. Research suggests that centrifugation of samples before laboratory analysis increases the sensitivity of PCR (Elijah et al., 2021). Request the samples to be centrifuged at 4000 × g for 10 minutes (Khanal et al., 2022). If centrifuging environmental samples before submission, refer to “Centrifugation of Environmental Samples for Viral Pathogens” and notify the veterinary diagnostic laboratory that the environmental samples were centrifuged prior to submission.

Some diagnostic laboratories can offer multi-plex PCR assays that will look for multiple viruses within the sample at once. Generally, multi-plex assays are for enteric viral pathogens since it is difficult to distinguish the different enteric viruses based on clinical signs. If a production system or feed mill is concerned about enteric viral pathogens, then a multi-plex PCR assay offers the ability to test for multiple viral pathogens at once. Diagnostic laboratories also have single-plex PCR assays which look for one pathogen within the sample. There are a wide variety of options for evaluating for viral pathogens with single-plex PCR assays. However, single-plex PCR assays can be more expensive if concerned about two different viral pathogens since the samples will have to be run twice for different tests.

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Diagnostic Tests for Samples.*

Once deciding on the PCR assay for samples, consider running the PCR assay to maximum number of cycles of 45. By running the PCR assay to the maximum number of cycles, this practice increases the diagnostic sensitivity, or the ability to detect positive samples. However, by increasing the diagnostic sensitivity, this increases the risk of false positives thereby reducing the diagnostic specificity or the ability to detect negative samples. By running to the maximal number of cycles, the assay repeatability is also reduced. An example of when to run to the maximal number of cycles would be when the objective of the sampling is to identify lapses in biosecurity and want to maximize the sensitivity of the assay to detect the pathogen. While an example of when to utilize the lower cut off value would be when a feed mill is conducting confirmatory testing to prove pathogen presence.

References

Elijah C.G., Blomme A.K., Harrison O.L., Bai J., Woodworth J.C., Jones C.K., Poulsen-Porter E.G., Paulk C.B., and Gebhardt J.T. 2021. Evaluating the impact of presence of organic matter on environmental samples and sample processing technique on RNA detection of PEDV. *Kansas Agricultural. Experiment Station Research Reports 7(11).* doi:10.4148/2378-5977.8211

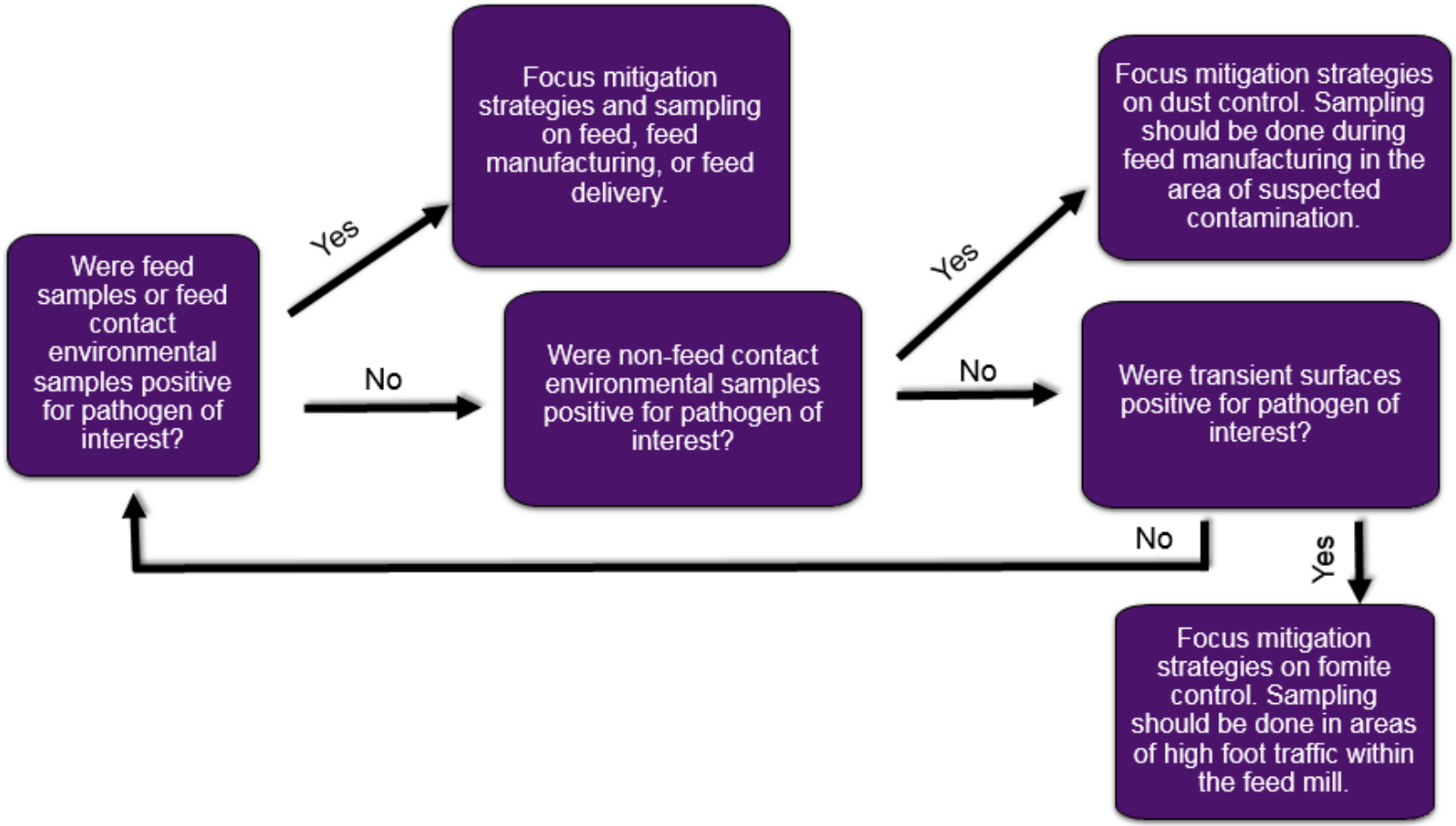
FDA – Food and Drug Administration Compliance Policy Guide Sec. 690.800 *Salmonella* in Food for Animals. 2013. https://www.fda.gov/media/86240/download

Khanal P., Olcha M., and Niederwerder M.C. 2022. Detection of African swine fever virus in feed dust collected from experimentally incoluated complete feed using quantitative PCR and virus titration assays. *Transbound and Emerg Dis. 69:*97-102. doi:10.1111/tbed.14176.

Tian Y., Gao X., Qi W.L., Wang Y., Wang X., Zhou J., Lu D., and Chen B. 2021. Advances in differentiation and identification of foodborne bacteria using near infrared spectroscopy. *Anal Methods 13:*2558-2566. doi:10.1039/d1ay00124h.

Interpreting Sample Results

After samples have been collected, submitted, and analyzed, communicating sample results to the appropriate groups is essential. Interpretation of the test resuults will guide future mitigation techniques but also areas of focus for sampling since these areas may be challenging to maintain or remain contamination free. Consult the flow chart below to understand how to interpret the sample results from surveillance or suspected contamination sampling.



Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Interpreting Sample Results.*

Strategies to Reduce Contamination

When samples from surveillance or suspected disease contamination come back as positive for the pathogen of interest, there are strategies that can be implemented to reduce contamination. These strategies can be implemented at any time that a feed mill or production system are concerned about potential pathogen contamination. This resource will discuss potential risk mitigation techniques and examples of these techniques.

- **Limiting entry of potential pathogens into the receiving pit.**
  - Utilizing receiving pit covers
    - Constructing a pit cover that lays flat while not receiving bulk ingredients but can be lifted up and constructed into a funnel shape to help reduce shrink during unloading of bulk feed ingredients.
    - Covering the receiving pit with a rubber mat, or something similar, when not in use.
  - Discarding spilled feed into the trash instead of adding it back into the receiving pit.

- **Chemical feed additives**
  - Addition of chemical feed additives to feed intended for livestock has been shown to potentially decrease the risk of cross-contamination during feed manufacturing or feed delivery.
    - Common chemical additives include organic acids, formaldehyde, essential oils, medium chain fatty acids, or dietary acidifiers (Huss et al., 2018).
    - More information on chemical feed additives can be found here.

- **Implementing point-in-time mitigation techniques.**
  - Point-in-time mitigation techniques are strategies implemented during a time point of feed manufacturing. These techniques do not prevent the possibility that feed may become contaminated again during further feed manufacturing or delivery.
    - Thermal Processing
      - Addition of heat to the feed manufacturing process to reduce potential infectivity of the pathogen of interest (Huss et al., 2018).
      - For livestock feed, pelleting is considered the traditional method of thermal processing.
    - Feed Batch Sequencing
      - Requires the order of production, storage, and distribution to be planned to reduce the carryover of high-risk ingredients to sensitive diets (Huss et al., 2018).
    - Flushing
      - Consists of running an ingredient, usually with abrasive material, through the system between batches to flush out any residual material (Huss et al., 2018).
    - Holding or quarantining feed ingredients
      - Consists of storing ingredients in a low foot traffic areas for a specified amount of time between manufacture and used to give an opportunity for viral contamination to naturally degrade so as not to be infectious.
      - More information on how to calculate holding times can be found here.

- **Zoning**
  - Restricting employees to certain locations within a feed mill to limit the spread of pathogen

Houston, Grace E., Gebhardt, Jordan T., Jones, Cassandra K., Woodworth, Jason C., Paulk, Chad B., and Dritz, Steve S. 2022. Kansas State University Feed Safety Sampling Resources: *Strategies to Reduce Contamination*.

- **Implementing downtime**
  - Require a specific amount of time that employees, after having recent contact with animals, must wait to return to the feed mill.

- **Limiting the amount of contaminated objects**
  - Requiring shoe covers for feed truck drivers and ensuring they wear and change them during deliveries.
  - Providing feed mill specific uniforms.
  - Scheduling or restricting deliveries to certain production sites on certain days.
  - Requiring showers before entering or exiting the feed mill.

- **Implementing usage of disinfectants**
  - Liquid or dry boot baths at the entrances or exits into the feed mill.
  - Applying disinfectants to semi-truck cabs after deliveries.
  - Combining disinfectant application with heat treatment like baking trailers after power washing with disinfectant.
  - More information regarding disinfectants can be found here.

References

Huss A., Cochrane R., Muckey M., and Jones C. 2018. Chapter 4: Animal Feed Mill Biosecurity: Prevention of Biological Hazards. Food and Feed Safety Systems and Analysis: 63-81. doi:10.1016/B978-0-12-811835-1.00004-X