Type 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 backed methodology for sampling complete feed, feed ingredients, and feed mill environments. To access information on how to take feed or environmental samples, please refer to the standard operating procedures titled “Collecting Feed Samples” and “Collecting Environmental Samples.” If requiring information on how to prepare for sampling, please refer to the standard operating procedure titled “Preparing for 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, sometimes referred to as “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). Sampling with sleeved feed probes has been the only methodology to be validated for viral pathogens while the other methodologies have yet to be validated (Jones et al., 2020; Elijah et al., 2021, Dee et al., 2022). Sampling via cut stream could be a solution if the sampling person can’t get to a location to look down into the storage or transport container like what is done with sleeved feed probes. While sampling with single tube triers offers a solution if interested in sampling bagged feed ingredients or complete feed. 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 any potential of 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

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. Environmental samples can be classified into the following zones:

- Feed contact zones: these surfaces have direct contact with feed ingredients or complete feed.
- Non-feed contact zones: these surfaces have a fixed location and are close or next to feed contact zones.
- Transient zones: these surfaces do not have a fixed location and can move within the feed mill environment or feed delivery.

An example of these surfaces within the zones can be found in the “Sampling Locations” additional resource. Understanding which zone each sample was taken from can help guide strategies on how to reduce potential contamination.

Based on the pathogen of interest, there are methodologies that have been shown to maximize the potential of finding the pathogen on feed mill surfaces. Table 2 offers a summary of the methodologies based on pathogen.

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. The surface of interest will determine which mythology will work best. At this time, hand sampling is the only method for bacterial pathogens.
[2]Sampling material refers to the material that will pass over the surface of interest. The 3M sponge sticks have been shown to be 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). Synthetic paint roller covers is the material of choice and shown to work the best (Wu et al., 2021; Elijah et al., 2022) but if unable to acquire synthetic paint roller covers, cotton paint roller covers are an acceptable substitute given the data to support cotton as a suitable material for viral pathogens.
[3]Pre-moistening solution refers to the solution that moistens the material before sampling. By pre-moistening the material, the ability of the sample material to pick up potential pathogen is maximized (Moore and Griffith, 2002). For gram negative bacteria, buffered peptone water is the pre-mositening solution of choice. For viruses, phosphate buffered solution (1X concentration, pH=7.4) is the pre-moistening solution of choice but recent research has shown that 0.9% NaCl sterile saline is an acceptable solution if unable to acquire phosphate buffered solution (Rodino et al., 2020).
[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.

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.*

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