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, the sample will still have to be cultured to increase the sensitivity of the PCR assay. 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 desiring to centrifuge environmental samples before submission, refer to the standard operating procedure titled “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

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.*

expensive if concerned about two different viral pathogens since the samples will have to be run twice for different tests.

Once deciding on the PCR assay for samples, ask the diagnostic laboratory to run 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

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