Collection and Submission Guidelines for Johne’s Fecal PCR Testing at NDSU-VDL

SAMPLE SUBMISSION:
Use the NDSU-VDL electronic herd submission form and include animal identification numbers. Age, sex and breed can be included if desired. If you are unable to use the electronic herd submission form the NDSU serology form can be used if necessary. **You must indicate on the form if you want the samples pooled or run individually, otherwise they will be pooled if more than 5 samples are submitted. For valid results samples must be pooled by the laboratory.** Samples will be pooled in groups of 5 as listed on the submission form. If you have a sample that you would like tested separately, please place the sample at the end of the list and clearly indicate that it is to be tested individually.

**Electronic herd submission form directions:**
1) Enter sample information or copy and paste from an existing file.
2) Print one copy and enclose with samples.
3) Email the electronic form (do not scan or fax) to ndsu.vetlab@ndsu.edu using the owner’s name as the subject.

SAMPLE COLLECTION:
The Johne’s PCR assay is extremely sensitive and therefore **it is very important that samples are not cross contaminated during collection.** Collect a minimum of 5 grams (1/2 ounce or 1 tablespoon) into a clean container (rectal sleeves*, Ziploc bags, Whirlpacks, examination gloves, any wide-mouthed screw-capped plastic jars – No blood tubes). *Clean, dry rectal sleeves are preferred.

Collect fecal samples directly from rectum. **Do not collect ground or floor samples.** Use a separate, clean, dry glove to collect individual sample (moisture and lube may adversely affect testing). Gloves should be kept in a clean environment away from dust to prevent cross contamination from different locations. For practitioner ease and to avoid cross contamination of samples before they get to the lab, collect the sample in a dry sleeve while palpating for pregnancy (do not use lube), turn the sleeve inside out, milk the fecal material into the fingers/finger, tie a knot close to the hand end (to keep the fecal material somewhat confined) and again towards the top so that any material that was on the outside is confined to the sleeve. **There should be no fecal material on the outside of the sleeve or container.** If such exists, please wipe it off or place the sleeve it into a separate, individual ziplock bag. Once the fecal material dries it easily cross contaminates other samples.

LABELING:
Label the samples with the sample number at hand end of the sleeve using permanent ink; allow ink to dry so that it does not smudge. You may place a card stock note with sample ID in between the two knots of the sleeve or use masking tape labeled with ID.

STORAGE AND SHIPMENT:
Fecal samples need to be chilled quickly and kept cold. When collecting during warmer months, place fecal samples in a cooler with cold packs; transfer samples to refrigerator ASAP. Fecal samples can be collected and stored at 4ºC for up to 1 week or freeze for longer storage up to 1 month before shipping to the lab. Include ice packs to keep samples cool; this also prevents containers from bursting open during shipment.

RESULT INTERPRETATION/SHEDDING STATUS:
The Johne’s PCR assay is extremely sensitive and has been shown to detect a single colony forming unit per gram of feces; therefore it is very important that samples are not cross contaminated during collection. The PCR assay Ct value and shedding status interpretation is determined based upon the yearly Johne’s
Fecal Proficiency Panels sponsored by USDA/APHIS/NVSL. The panels consist of samples with varying infection levels from 0 up to several thousand of *Mycobacterium avium* subsp. *paratuberculosis* (Johne’s) bacterial colony counts. Based upon our assay performance and Ct values corresponding to infection levels, we determine an approximate shedding status for the animal (high, moderate or low shedder).

If you have questions, please contact the laboratory at 701-231-8307 or 701-231-7527 or visit the VDL website at [www.vdl.ndsu.edu](http://www.vdl.ndsu.edu).

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