OPTION 1: Direct PCR on smegma samples

- Collect smegma by scraping, brushing, washing or swab and transfer to a tube (red top tube, test tube) containing 1.5 ml PBS.
- Keep sample refrigerated at all times.
- Ship to LADDL on ice or ice pack overnight to be received within 48 hours of collection. Samples must be received cold.
- Smegma samples in PBS cannot be identified through culture.

OPTION 2: PCR Identification - InPouches or TF Transit Tubes Sent Directly to LADDL

- Inoculated InPouches/TF Transit Tubes are handled at ambient (not refrigerated) temperatures between 65-95°F.
- InPouches/TF Transit Tubes must be received at LADDL within **72 hours** of collection at ambient **(not refrigerated)** temperatures between 65-95°F.
- LADDL will incubate the pouches/tubes for 24 hours at 35-37°C (95-98.6°F) and then perform PCR (2-3 day turnaround).
- InPouches/TF Transit Tubes must be received at LADDL no later than Friday at noon.

OPTION 3: PCR Identification - InPouches or TF Transit Tubes Incubated at the Practitioner's Office

- If unable to make a Mon-Fri delivery, InPouches/TF Transit Tubes can be **incubated for 24 hours** at the practitioner's office at 35-37°C (95-98.6°F) and then **frozen**.
- Ship frozen InPouches/TF Transit Tubes overnight on ice or ice packs. Samples must be received cold or frozen.
- LADDL will perform PCR as soon as possible (1-2 working day turnaround).
- Previously incubated and frozen InPouches/TF Transit Tubes must be received no later than Friday at noon.
- Frozen InPouches/TF Transit Tubes cannot be identified by culture.

OPTION 4: Culture Identification (InPouch only)

- Inoculated InPouches are handled at ambient (not refrigerated) temperatures between 65-95°F.
- Inoculated InPouches must be received at LADDL within 48 hours of collection.
- InPouches must be shipped at ambient (not refrigerated) temperatures between 65-95°F.
- InPouches will be incubated at LADDL and microscopically examined daily for up to 6 days.

Pooling CANNOT be performed for official state regulatory testing.

<u>Pooling for non-regulatory purposes (herd diagnostics)</u>: Collect and send individual samples according to the guidelines above. The lab will pool up to 3 samples and test by PCR for the price of 1 sample (please indicate which samples are to be pooled). If a pooled sample is positive, the lab will individually test the samples for an additional fee if the practitioner agrees. If the practitioner does not want the individual samples tested, all the animals in the pool will be reported as positive.

SAMPLES THAT DEVIATE FROM THESE GUIDELINES WILL NOT BE TESTED. THIS INCLUDES EXPIRED INPOUCHES OR TF TRANSIT TUBES.

NOTICE: Poor sample quality can result in invalid results. Retesting of the bull is recommended.

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^{*}Guidelines based on "Current Data Suggest" Document of the AALVD Parasitology Committee, 2009

^{*}PCR reference: Summarell et al. 2018. Journal of Veterinary Diagnostic Investigation Vol. 30(4) 603–608.