Multilocus Sequence Typing, Leukotoxin Identification, and 16S rDNA Biodiversity Determination of *Mannheimia haemolytica*, *Bibersteinia trehalosi*, *Pasteurella multocida*, and *Mycoplasma ovipneumoniae*. A Single Assay Using Multiplex PCR, Short-Read Sequencing, and Automated Bioinformatics.

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ABSTRACT: Multilocus sequence typing (MLST) characterizes bacteria based on genetic variability in constitutive (housekeeping) genes, and allows comparisons of bacteria beyond the species designation. This approach has been used to trace outbreaks of diseases, and an MLST approach has been used to examine *Mycoplasma ovipneumoniae* (Cassirer et al. 2017) in bighorn sheep. However, bighorn sheep respiratory disease is a polymicrobial concern, and focus on a single pathogen limits diagnostic and management strategies. To create a broader approach to bighorn sheep respiratory diagnostics, we created a single MLST assay to characterize *Mannheimia haemolytica, Bibersteinia trehalosi, Pasteurella multocida, and Mycoplasma ovipneumoniae*. The assay also assesses the *Pasteurellaceae* leukotoxin A gene (*lkt A*), and broadly assesses the bacterial composition of each sample based on 16S rDNA sequences. The assay is based on a three-step approach: 1) Multiplex PCR to probe samples for targets including four to eight housekeeping genes for each species, the *Pasteurellaceae lkt A* gene, and the 16S rDNA gene 2) Next generation sequencing to determine the genetic sequences of each target, and 3) Bioinformatics in the form of automated software to analyze genetic sequences. This assay was originally designed to assess possible transfer of pathogens from domestic to bighorn sheep in the event of a bighorn sheep mortality from respiratory disease. However, the assay could be useful for many applications in bighorn sheep respiratory disease research and management.