

***Mycoplasma hyopneumoniae* culture and isolation**

Definition of services of culture or isolation of *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) offered by the Veterinary Diagnostic Laboratory.

Culture: Implies the attempt of growing *M. hyopneumoniae* in broth, using clinical samples as the starting material. The broth is checked to confirm the presence of *M. hyopneumoniae* and the absence of other microorganisms (including other swine Mycoplasmas) by PCR and by 16S sequencing. Results are reported ~4 weeks after sample submission.

Isolation: Implies the attempt of cloning *M. hyopneumoniae* from a single colony. The starting material is a culture of *M. hyopneumoniae*, and then this culture is plated and grown back in broth. This process involves several broth passages (~10) to adapt the isolate to grow faster under laboratory conditions before plating it to attempt the single colony cloning back into broth. Broth purity is re-checked to confirm the presence of *M. hyopneumoniae* and the absence of other microorganisms. There is a long a variable turnaround time for isolates depending on the inherent ability of each strain for growing under laboratory conditions. An isolate can be usually obtained ~8-10 weeks after being requested.

How to process the samples?

If you know ahead of time that a client is planning to submit samples for *M. hyopneumoniae* culture, please contact Maria Pieters. It is important to plan ahead of time because the media used to culture *M. hyopneumoniae* is enriched, expensive, and works best when fresh prepared.

The preferred sample to attempt *M. hyopneumoniae* culture is frozen lung tissue. Fresh lung tissue, bronchial swabs and BALF can also be used, but have a reported lower sensitivity.

Collect samples in Whirl-Pack® bags. If possible, collect 2 pieces of lung tissue from inside the lung that haven't been exposed to external contamination and don't include pleura. The ideal sample should include areas of lung consolidation and apparently unaffected areas. The two pieces should be about 2cm³ and should be placed in only one bag.

Place the samples in a freezer and e-mail me to let me know their location. The -20°C freezer by the dry ice chest (in the hallway) would be OK, so I can retrieve the samples any time. Culture and isolation are performed in room 322 (VMC/VDL). PCR and 16S are processed in the VDL.

Please contact me if you have questions or need clarifications.