

**Diagnostic guidelines to confirm elimination**

Modified from Sponheim, A., Munoz-Zanzi, C., Fano, E., Polson, D., Pieters, M., 2021.

Pooled-sample testing for detection of *Mycoplasma hyopneumoniae* during late experimental infection as a diagnostic tool for a herd eradication program. *Prev. Vet. Med* 189, 105313. <https://doi.org/10.1016/j.prevetmed.2021.105313>.

Compiled by Sponheim, Fano, and Pieters, 2023.

Number of individual pigs to sample and cost of sampling option based on the **mean sensitivity** of experimentally infected pigs when using deep tracheal secretions late during *M. hyopneumoniae* infection when tested individually or in pools of 3 or 5. Assumes 100% specificity, 95% population sensitivity, infinite population size, and a cost of 5 USD per sample collected by technician and 31 USD per PCR test. USD rounded to the nearest dollar. Divide number of individuals to sample by 3 or 5 to determine the respective number of pools for PCR.

	<b>Number of individuals to sample</b>					
	<b>Cost of sampling option (USD)</b>					
	<b>Prevalence estimate (% positive)</b>					
<b>Submission type</b>	<b>0.5</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
<b>Individual</b>	610	305	152	101	75	61
	\$21,960	\$10,980	\$5,472	\$3,636	\$2,700	\$2,196
<b>Pool of 3</b>	714	357	180	120	90	72
	\$10,948	\$5,474	\$2,760	\$1,840	\$1,380	\$1,104
<b>Pool of 5</b>	735	370	185	125	95	75
	\$8,232	\$4,144	\$2,072	\$1,400	\$1,064	\$840

For a lowest risk option, choose the number of individual pigs to sample and cost of sampling option based on **95% lower confidence limit (LCL) sensitivity** of experimentally infected pigs when using deep tracheal secretions late during *M. hyopneumoniae* infection when tested individually or in pools of 3 or 5. Assumes 100% specificity, 95% population sensitivity, infinite population size, and a cost of 5 USD per sample collected by technician and 31 USD per PCR test. USD rounded to the nearest dollar. Divide number of individuals to sample by 3 or 5 to determine the respective number of pools for PCR.

	<b>Number of individuals to sample</b>					
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	Cost of sampling option (USD)					
	Prevalence estimate (% positive)					
Submission type	0.5	1	2	3	4	5
Individual	672	336	167	111	83	66
	\$24,192	\$12,096	\$6,012	\$3,996	\$2,988	\$2,376
Pool of 3	846	423	213	141	108	87
	\$12,972	\$6,486	\$3,266	\$2,162	\$1,656	\$1,334
Pool of 5	870	440	220	150	110	90
	\$9,744	\$4,928	\$2,464	\$1,680	\$1,232	\$1,008

Looking for a different scenario? Use the link to calculate number of individuals to sample **without pooling**: [Epitools - Sample size to achieve specified population I ... \(ausvet.com.au\)](#)

Inputs:

Design prevalence = prevalence estimate (% positive, ie: 0.01 for 1%)

Unit (test) sensitivity = diagnostic sensitivity. For deep tracheal secretions use **98% for mean** or **89% for 95% LCL** (ie: for lowest risk use LCL of 0.89 for 89%)

Required population/herd sensitivity = 0.95 for 95%

Population size (if known) = select population size of interest or leave empty

**With pooling**, use the link to calculate number of pools to submit (multiply by pool size to determine number of individuals to sample): [Epitools - Sample size for demonstration of freedom \(det ... \(ausvet.com.au\)](#)

Inputs:

Size of pools = 3 or 5

Pool-level test sensitivity = pool sensitivity. For deep tracheal secretions **pool of 3 use 84% for mean** or **71% for 95% LCL**; **pool of 5 use 82% for mean** or **69% for 95% LCL** (ie: for pool of 3 lowest risk use LCL of 0.71 for 71%)

Desired cluster-sensitivity = 0.95 for 95%

Design (target) prevalence = prevalence estimate (% positive, ie: 0.01 for 1%)

## References:

Sergeant, ESG, 2018. Epitools epidemiological calculators. Ausvet Pty Ltd. Available at: <http://epitools.ausvet.com.au>.

Sponheim, A., Alvarez, J., Fano, E., Schmaling, E., Dee, S., Hanson, D., Wetzell, T., Pieters, M., 2020. Comparison of the sensitivity of laryngeal swabs and deep tracheal catheters for detection of *Mycoplasma hyopneumoniae* in experimentally and naturally infected pigs early and late after infection. *Vet. Microbiol.* 241, 108500. <https://doi.org/10.1016/j.vetmic.2019.108500>.

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