



A European interlaboratory evaluation of PCR and ELISA methods for *Mycoplasma bovis* diagnostics

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A European interlaboratory evaluation of PCR and ELISA methods for *Mycoplasma bovis* diagnostics

Mycoplasma bovis is known worldwide as a major bovine pathogen. Increasing prevalence has been recently reported in Northern Europe. Control of *M. bovis* infections in cattle herds is difficult as increasing antimicrobial resistance is reported, and commercial vaccines are not available or not effective. Therefore, preventive measures such as high biosecurity standards guided by results of a highly specific and sensitive diagnosis are essential.

A consortium of six European national veterinary institutes was established to evaluate the performance of PCR and ELISA diagnostic methods currently used by these institutes. For serodiagnosis two commercial ELISA test kits were used: the Bio K302 ELISA (Bio-X Diagnostics, Rochefort, Belgium) and the ID Screen® *Mycoplasma bovis* ELISA (IDvet, Grabels, France). These two tests were compared to an in-house Western blot method as to be able to use a latent class analysis statistical approach. A sample panel (n=180) was compiled of sera from cattle from five countries with high and low *M. bovis* prevalence. Sera were distributed to the six laboratories and tested as recommended by the suppliers of the test kits. Using latent class analysis, we found that the diagnostic sensitivities of the Western blot, the ID Screen® *Mycoplasma bovis* and the Bio K302 ELISA were 96.6 %, 99.6 % and 51.0 % respectively, and the diagnostic specificities were 99.8 %, 99.0 % and 85.7 % respectively (Table 1) (Andersson et al., manuscript in preparation).

For evaluation of PCR diagnostics, a sample panel was tested using five different DNA extraction methods, seven different real-time and/or end-point PCR methods targeting four different genes and six different real-time PCR platforms. Only one commercial kit was assessed (MPBO50 kit from ThermoFisher Scientific), all other were in-house PCR tests. Three different assays were conducted to assess the specificity, sensitivity and comparability of the PCRs. The sensitivity and comparability assays were conducted using (n=21) bronchoalveolar fluids of veal calves, artificially contaminated or naturally infected. With a few exceptions, all methods run routinely in the participating laboratories showed comparable performance (Figure 1) (Wisselink et al., manuscript submitted for publication).

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	Informative priors		Uniform priors	
	Median	95% PCI	Median	95% PCI
Sensitivity & specificity				
Sensitivity WB	0.930	[0.889; 0.966]	0.933	[0.887; 0.969]
Specificity WB	0.998	[0.990; 1.00]	0.999	[0.993; 1.00]
Sensitivity ID Screen	0.956	[0.916; 0.990]	0.958	[0.914; 0.994]
Specificity ID Screen	0.993	[0.983; 0.998]	0.994	[0.985; 0.999]
Sensitivity BIO K302	0.488	[0.444; 0.532]	0.488	[0.443; 0.533]
Specificity BIO K302	0.883	[0.854; 0.908]	0.880	[0.851; 0.906]
Covariances				
CoV _{Seq} (WB*IDScreen)	0.037	[0.001; 0.071]	0.035	[0.003; 0.072]
CoV _{Sp} (WB*IDScreen)	0.001	[0.000; 0.006]	0.001	[0.000; 0.004]

Table 1. Posterior median and 95% posterior credibility interval (95% PCI) of sensitivity and specificity for Western blot and the two ELISA systems obtained from latent class analysis, assuming conditional independence between tests and using informative or uniform priors.

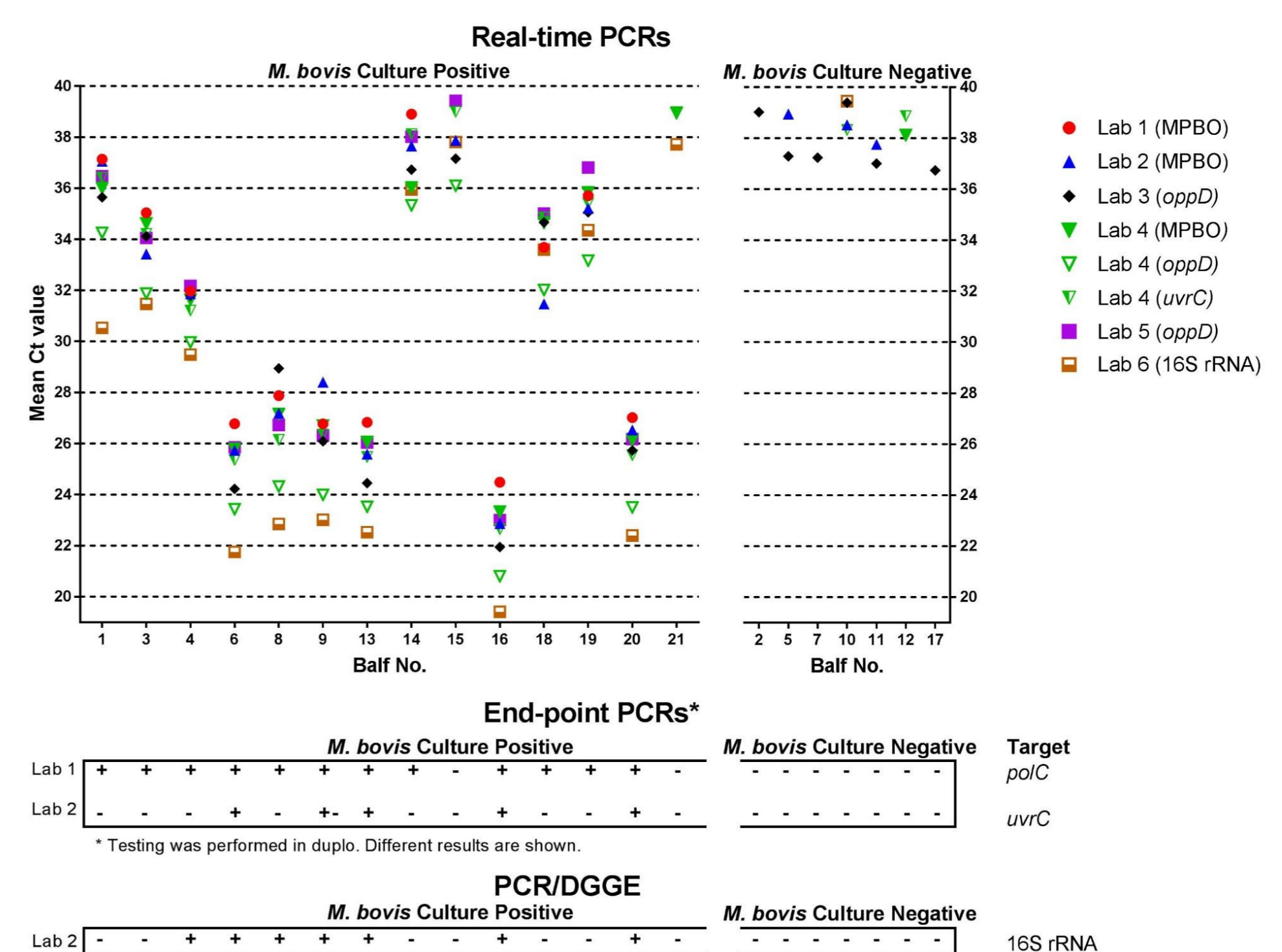


Figure 1. Comparability of results of various PCR methods in use by six laboratories (Lab 1 to Lab 6) on bronchoalveolar lavage fluid (BALF) samples (n=21) from veal calves from farms with bovine respiratory disease (BRD). BALF samples were tested twice by the PCR methods and the mean Ct values were calculated from the real-time PCRs. Viability of *M. bovis* in the BALF samples was verified with culture. On the basis of the culture results, the figure was split in two parts, either with BALF samples negative or positive for *M. bovis* by culture.

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