

# Evaluation of a new multiplex immunoassay for bovine respiratory syncytial virus and bovine coronavirus using latent class analysis



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Bovine respiratory syncytial virus (BRSV), and bovine coronavirus (BCV) are responsible for respiratory disease and diarrhea in cattle worldwide. Recently, a control program against these infections started in Norway using a new multiplex immunoassay (MVD-Enferplex BCV/BRSV multiplex) for bulk tank milk. The multiplex technology allows for antibody testing of both BRSV and BCV by the use of a single test.

The objective of this study was to estimate sensitivity and specificity across different antigen cut-offs for the MVD-Enferplex BCV/BRSV multiplex, by comparison to two commercially available ELISAs (SVANOVIR<sup>®</sup> BRSV-Ab and SVANOVIR<sup>®</sup> BCV-Ab). As none of the tests are considered perfect, estimation of test characteristics was performed using Bayesian latent class models.

## Materials & methods

- 360 herds in a low prevalence area, and 360 herds in a high prevalence area.
- Bulk tank milk samples tested with MVD-Enferplex BCV/BRSV multiplex.
- Multiplex technology: Indirect ELISA based on detection of luminescence by an imaging system.
- BRSV multiplex included four antigens, BCV multiplex included three antigens.
- *Two-test conditional independence model*.
- Models were fit using Bayesian LCA in the OpenBugs version 3.2.1 rev 781 software.
- Each LCA was performed with a range of different cut-offs for the multiplex.
- Non-informative priors in the shape of uniform distributions on the interval between zero and one, beta (1, 1) distribution for test properties and sub-population prevalences in all analyses.

## Results

Test parameters at the manufacturers' recommended cut-offs:

Parameter	BRSV multiplex				BRSV ELISA			
	Se		Sp		Se		Sp	
Cut-off alternative	Median	[95% PCI]	Median	[95% PCI]	Median	[95% PCI]	Median	[95% PCI]
2*	94.4	[89.8;98.7]	90.6	[85.5;94.4]	99.8	[98.7;100]	57.4	[50.5;64.4]
	BCV multiplex				BCV ELISA			
	Se		Sp		Se		Sp	
1*	99.9	[99.4;100]	77.3	[69.8;84.8]	99.0	[96.9;100]	99.5	[97.1;100]
8**	99.9	[99.3;100]	93.7	[88.8;97.8]	99.5	[98.1;100]	99.6	[97.6;100]

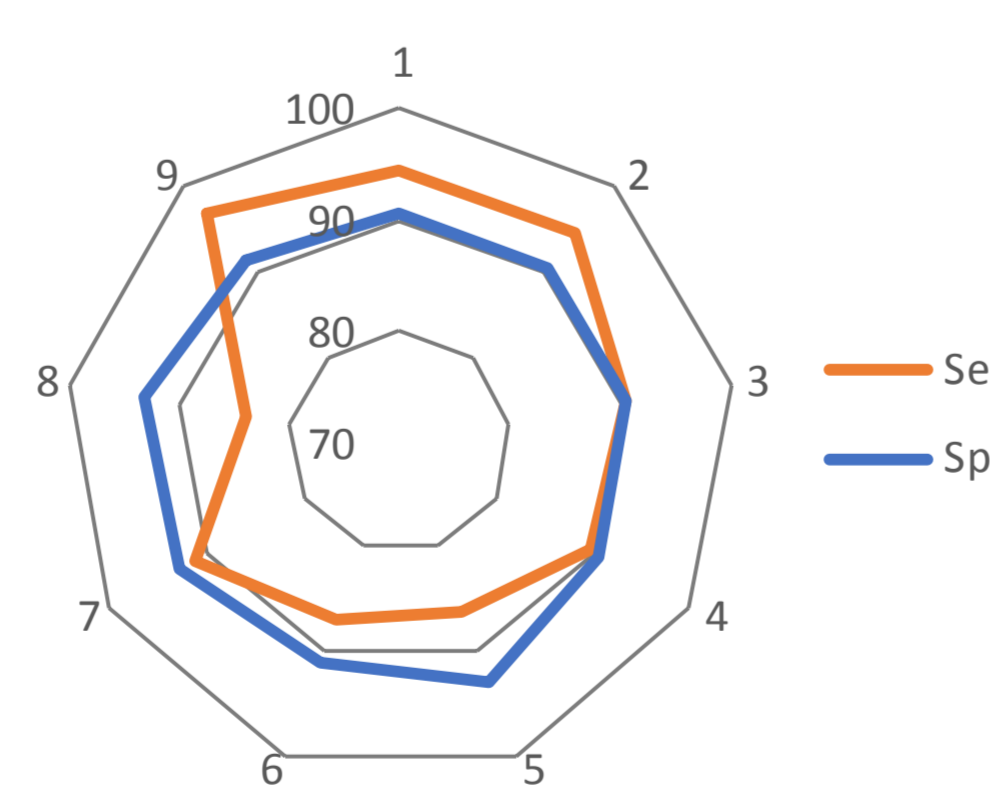
\*manufacturers recommended cut-off

\*\*Only BCV-A antigen included

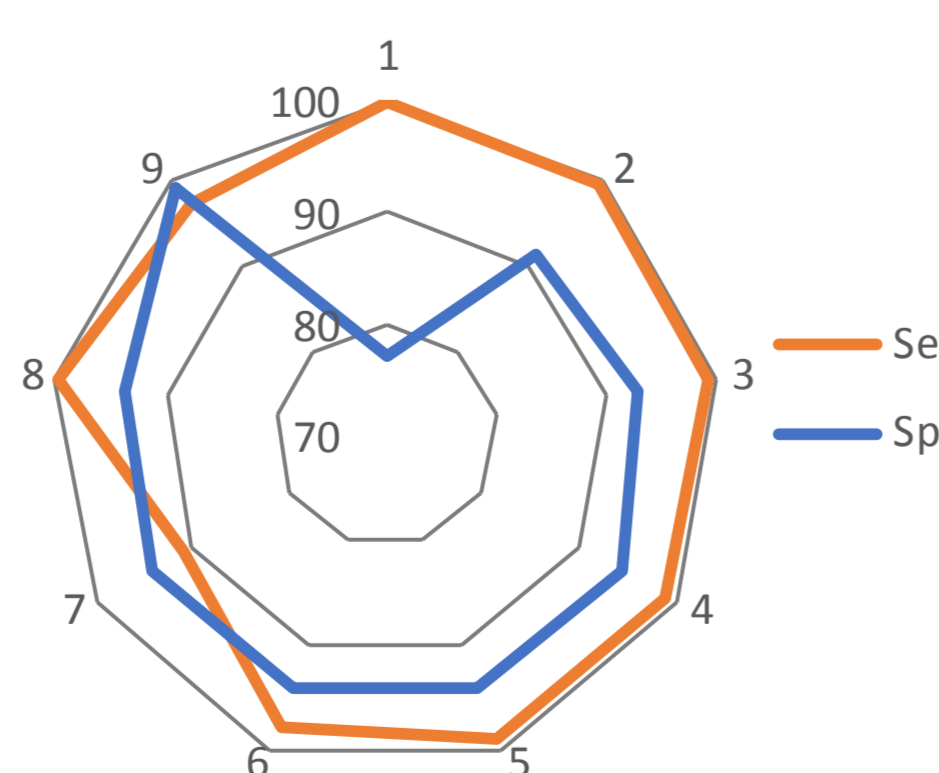
For the BCV multiplex we found that by using only one out of the three antigens included in the test, the specificity increased, without concurrent loss in sensitivity. The low Sp of the BRSV ELISA could be improved by increasing the cut-off for this test.

The different cut-offs that were assessed for the BRSV/BCV multiplex immunoassay:

Cut-off alternative	BRSV-A	BRSV-B	BRSV-C	BRSV-D
1	1000	4000	7000	1700
2	2000	4000	7000	1700
3	3000	4000	7000	1700
4	4000	4000	7000	1700
5	4000	6000	9000	2000
6	8000	4000	7000	1700
7	2000	-	-	-
8	4000	-	-	-
9	2000	4000	7000	1700



Cut-off alternative	BCV-A	BCV-B	BCV-C
1	10000	11000	30000
2	15000	15000	35000
3	20000	15000	35000
4	25000	15000	35000
5	30000	15000	35000
6	35000	15000	35000
7	40000	15000	35000
8	10000	-	-
9	35000	-	-



This study assessed the performance of a new multiplex immunoassay under field conditions in Norway. We conclude that the BRSV/BCV multiplex can be successfully used for detection of BRSV and BCV antibodies in bulk tank milk. Different cut-offs might be useful in different situations, and should be a subject to consideration by the user.

