SUPPLEMENTAL TABLES AND FIGURES

TABLE S1. Mean OD readings for each serum and HRP-conjugated anti-fallow deer IgG dilution.

Antigen	Conjugate	Serum dilution	Serum [a]					
			89	108	138	136	137	120
A7	Anti-fallow deer IgG 1:250	1:20	3.174	3.245	3.231	2.759	1.979	1.395
		1.40	3.006	3.175	3.209	2.124	1.513	1.302
	Anti-fallow deer IgG 1:500	1:20	3.019	3.144	3.159	1.937	1.225	0.748
		1:40	2.653	2.978	3.183	1.525	1.094	0.734
	Anti-fallow deer IgG 1:1000	1:20	2.859	3.073	3.171	1.168	0.812	0.504
		1:40	2.255	2.717	3.151	0.976	0.716	0.496
	Anti-fallow deer IgG 1:1500	1:20	2.715	2.875	2.975	0.714	0.312	0.187
		1:40	1.987	2.122	2.298	0.678	0.267	0.121
PPA-3	Anti-fallow deer IgG 1:250	1:20	2.955	2.542	3.132	1.094	0.875	0.692
		1:40	1.324	1.515	2.531	0.579	0.482	0.607
	Anti-fallow deer IgG 1:500	1:20	1.785	1.782	2.607	0.599	0.587	0.356
		1:40	1.234	1.259	2.081	0.503	0.344	0.347
	Anti-fallow deer IgG 1:1000	1:20	1.556	1.314	2.229	0.482	0.297	0.144
		1:40	1.383	0.928	1.710	0.356	0.231	0.123
	Anti-fallow deer IgG 1:1500	1:20	1.234	0.667	1.579	0.256	0.145	0.046
		1:40	0.978	0.543	0.712	0.123	0.121	0.044

[a] Serum samples 89, 108, 138 were collected from three fallow deer with diffuse multibacillary lesions in the gut. Samples 136, 137 and 120 were collected from three animals without lesions in gut tissues.

FIG. S1. Optimization of the concentrations of serum samples and anti-fallow deer IgG conjugate that provide the best signal-to-noise ratios. Serum samples from three animals with histopathological lesions consistent with JD (89, 108, 138) and from three animals without lesions (136, 137, 120) were selected as positive and negative controls and tested at 1:20 and 1:40 dilutions in an ELISA using Antigen-A7 or PPA-3 as coating-antigens. Four concentrations of the HRP-conjugated anti-fallow deer IgG antibody were tested (1:250, 1.500, 1:1000 and 1:1500). The best discrimination between positive and negative sera samples was obtained when a 1:20 dilution of sera and 1:1500 dilutions of the conjugate anti-fallow deer IgG were used.

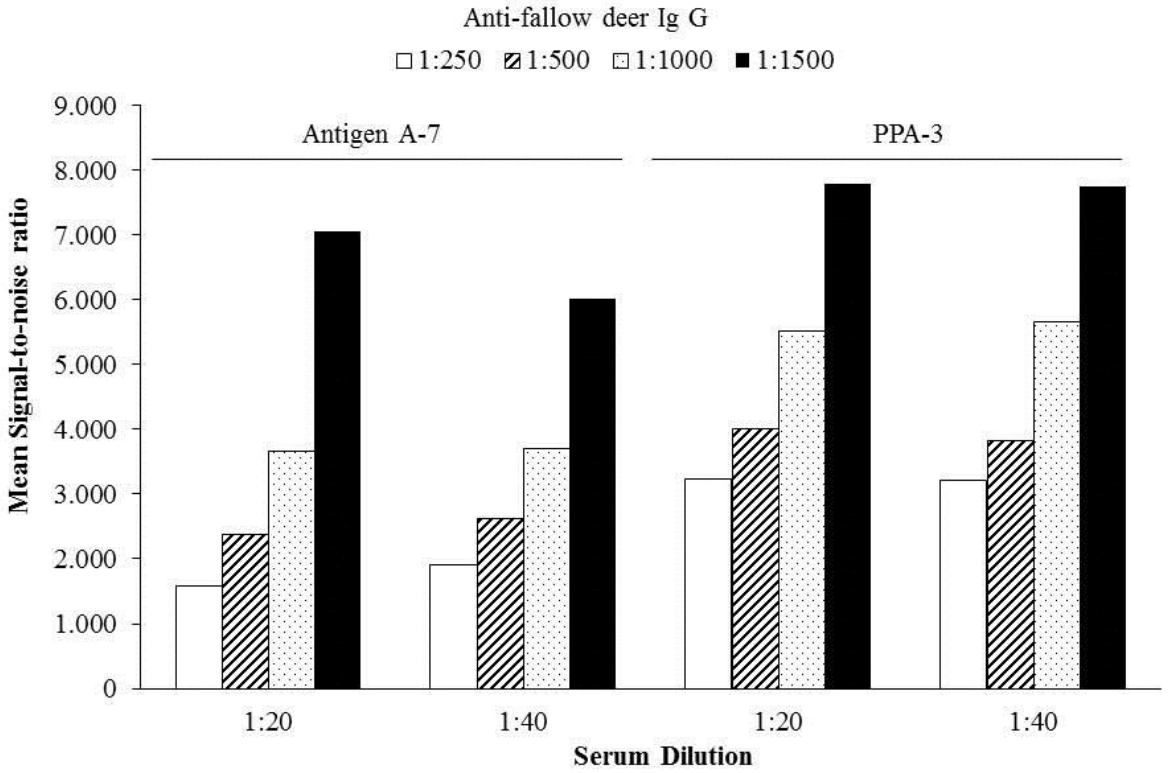
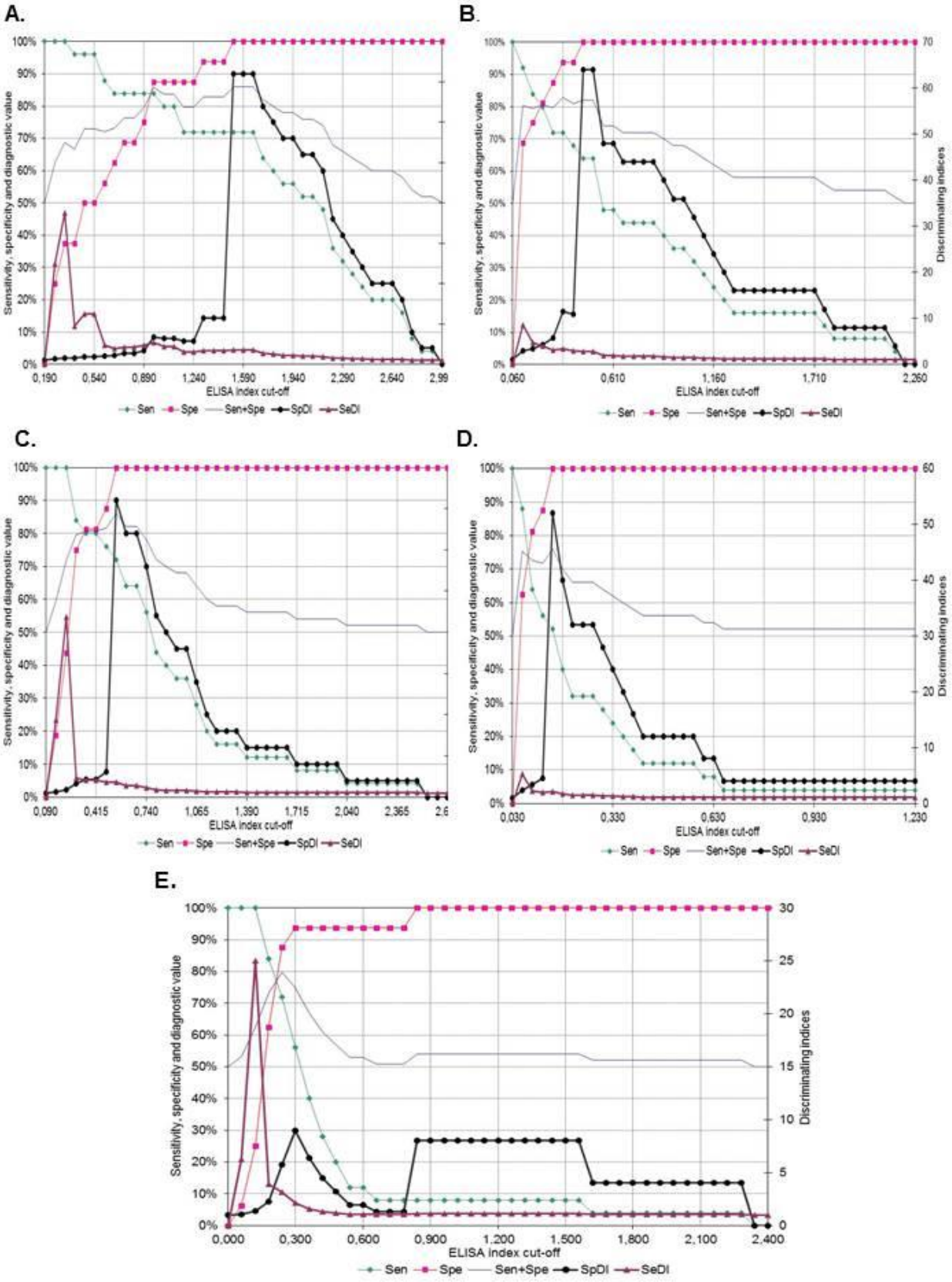


FIG. S2. Dynamics of sensitivity, specificity, semi-sum of sensitivity and specificity or diagnostic value, specificity discriminating index and sensitivity discriminating index of the ELISAs A, B, C, D and E. Serum samples from 25 fallow deer with confirmed *M. avium* subsp. *paratuberculosis* infection by histopathology and immunohistochemistry, as well as 16 serum samples obtained from fallow deer without histopathological lesions were assayed with the ELISAs A, B, C, D and E. Data from the five ELISAs were subjected to four-graph receiver operating characteristic (FG-ROC) analysis which is a plot of the test sensitivity (Sen) and specificity (Spe) for each threshold (cut-off) value. The plot also calculates the semi-sum of sensitivity and specificity (Sen+Spe) and the ratios of specificity to sensitivity (specificity discrimination index- SpDI) and of sensitivity to specificity (sensitivity discrimination index-SeDI).


A.
B.
C.
D.
E.
Sensitivity, specificity and diagnostic value
Discriminating indices
ELISA index cut-off
Sen
Spe
Sen+Spe
SpDI
SeDI