Development of an epitope-blocking ELISA for detection of antibodies against Tembusu virus in domestic birds

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Running title: Blocking ELISA for Tembusu virus antibody detection

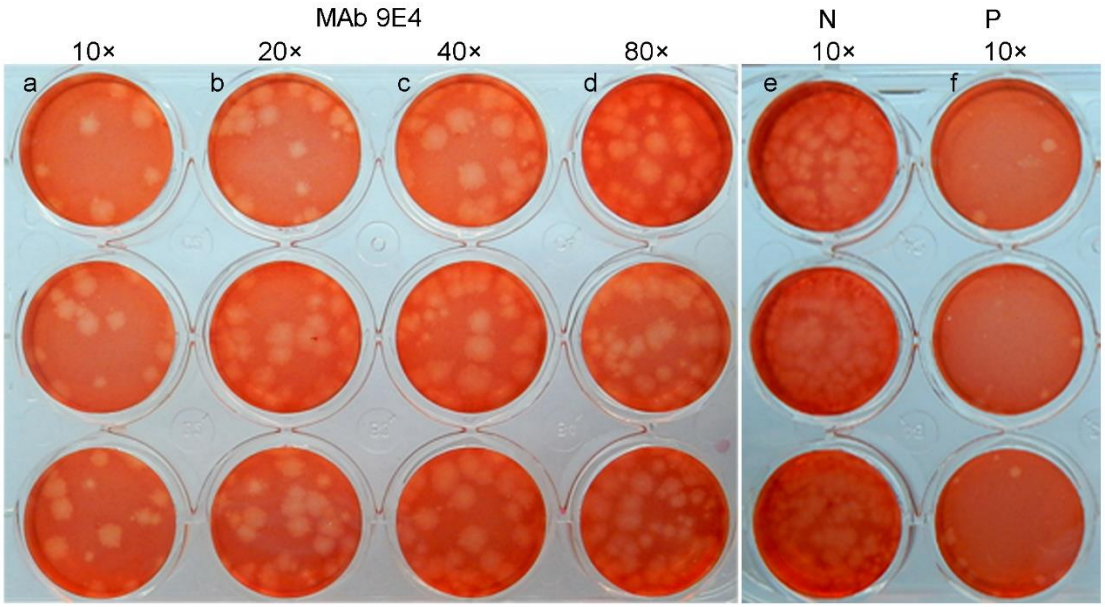

Fig. S3 Neutralizing activity of generated mAbs 9E4. (**a-d**) The mAb 9E4 was diluted 10-fold, 20-fold, 40-fold and 80-fold, respectively, to incubate with equal volume of DTMUV; (**e**) The mAb against Reovirus was diluted 10-fold to incubate with equal volume of DTMUV as negative control; (**f**) The antiserum against DTMUV was diluted 10-fold to incubate with equal volume of DTMUV as positive control

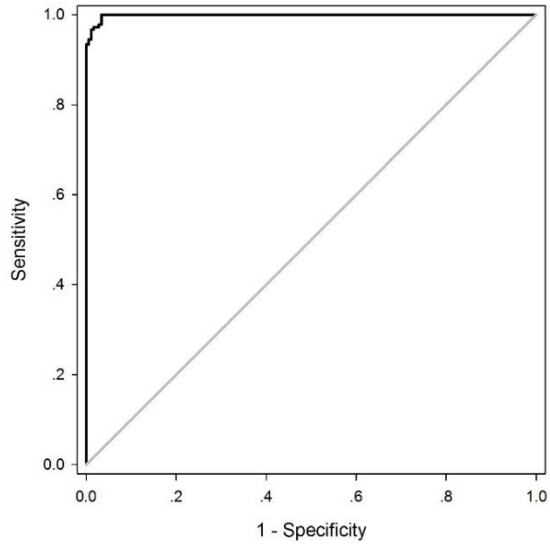

Fig. S4 ROC analysis curves for the blocking ELISA. The graph was calculated with 178 negative serum samples and 182 positive serum samples from ducks which comfirmed by PRNT. Points on the curve correspond to different cutoff values for the test. The area under the ROC curve was 0.9987

Table S2 Sensitivity and specificity of the blocking ELISA with different cut off value by ROC analysis

cut-off value	sensitivity	specificity
29.25%	100%	95.91%
29.95%	100%	96.07%
30.86%[a]	100%	96.63%
31.31%	99.45%	96.63%
31.61%	98.90%	96.63%

[a] The optimal cut-off value of the blocking ELISA was 30.86% according to the ROC analysis, yielding the maximal Yonden's index (sensitivity+specificity-1)