

Supplemental materials

Table S1. Positive and negative control sera for assay validation.

Serum	HI			Commercial ELISA				Western Blotting			
	NDV clone 30	AIV H5N3	AIV H7N7	IDEXX NDV NP	iD.Vet AIV NP	iD.Vet H5	iD.Vet H7	NDV rNP	IAV rNP	H5N3 rHA1	H7N7 rHA1
S82 (H5N1 ^a)	4	> 512	4	neg.	pos.	pos.	neg.	-	+	+	-
S304 (H7N3 ^a)	2	<2	> 512	neg.	pos.	neg.	pos.	-	+	-	+
S185 (ND- Ulster ¹)	> 512 ^b	<2	<2	pos.	neg.	neg.	neg.	+	-	-	-
SPF serum	4	<2	4	neg.	neg.	neg.	neg.	-	-	-	-

^a - Antigen used to raise the sera

^b - Bold-face values indicate specifically positive reactions. Western blotting reactions are also depicted in Figure 2.

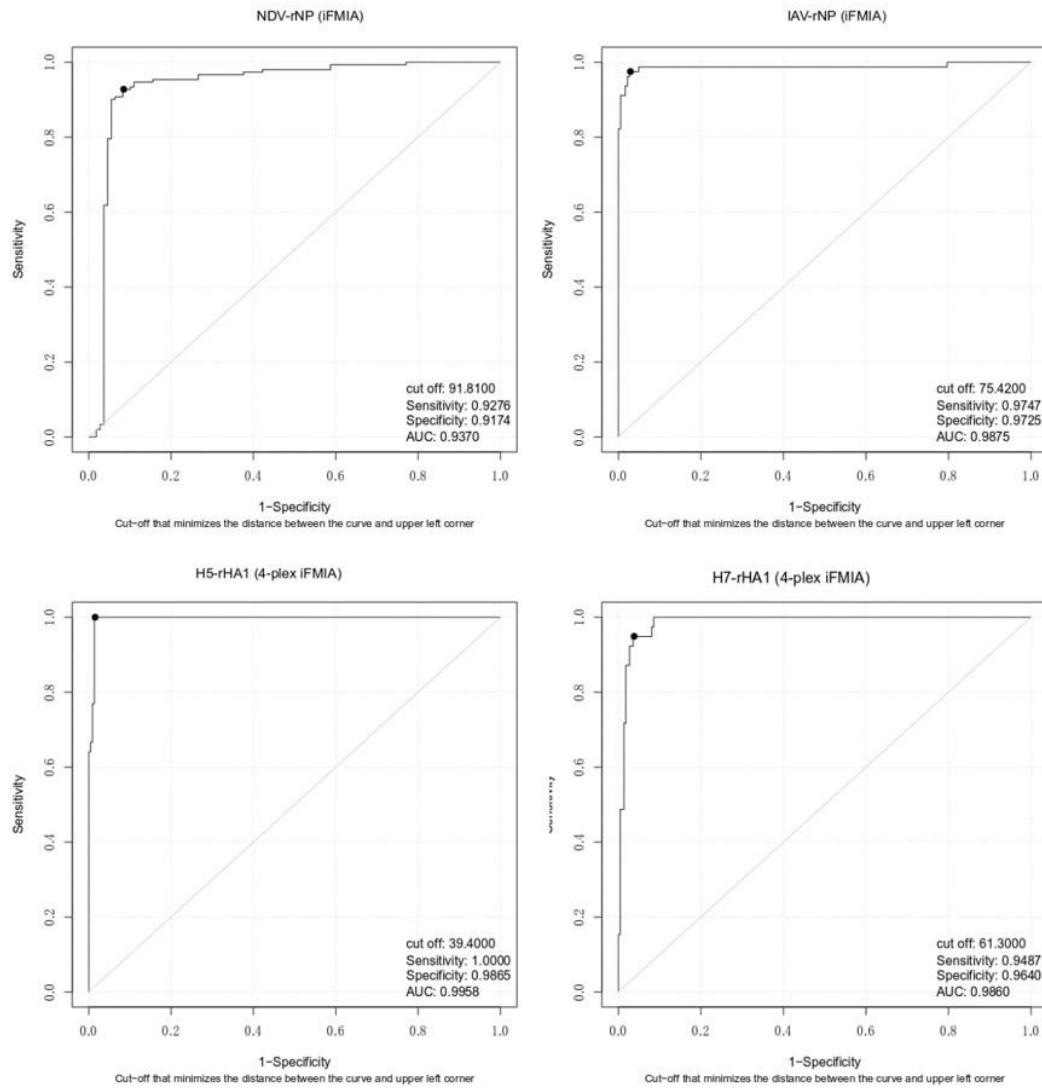


Figure S1. ROC analyses of the 4plex iFMA for performance characteristics in sera from experimentally infected and/or vaccinated galliform poultry. ROC curves of the 4plex iFMA reactivity to 4 recombinant proteins of NDV and AIV were compared with those of HI assay (n = 257).