

Supplementary file

Table S1. Serum samples collected in Italy from 2005 to 2017.

Year	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	
Samples	99	101	82	95	101	83	101	102	100	100	101	115	101	
Age groups	18-30	10	13	7	10	16	7	14	16	7	9	15	10	10
	31-40	19	14	14	18	17	14	18	19	16	15	16	16	17
	41-50	14	14	14	14	15	14	13	11	19	24	15	27	25
	51-60	18	15	15	16	18	16	16	18	18	16	12	20	19
	61-70	18	13	14	17	13	15	14	12	20	13	18	18	12
	71-80	14	14	11	14	14	11	16	14	14	10	17	14	10
	≥81	6	18	7	6	8	6	10	12	6	13	8	10	8
Sex	M	50	52	41	48	55	43	49	52	50	50	51	50	51
	F	49	49	41	47	46	40	52	50	50	50	50	65	50

Table S2 Results of the multiple proportion test among years.

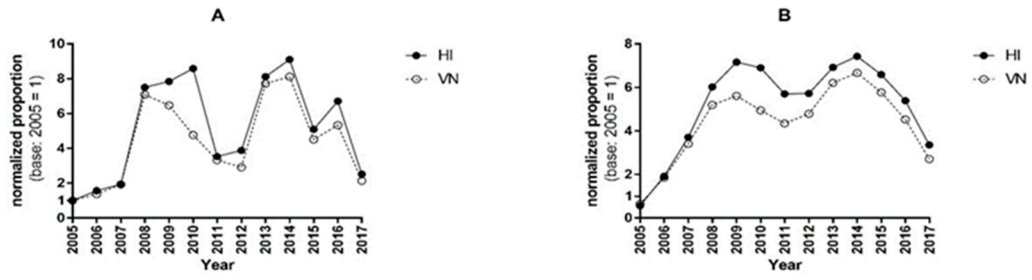
Assay	Titre class	Chi-squared	df	p value
HI	negative	131.22	12	< 0.0001
	≥1:10	131.22	12	< 0.0001
	≥1:20	86.31	12	< 0.0001
	≥1:40	34.95	12	0.0005
VN	≥1:10	105.78	12	< 0.0001
	≥1:20	86.32	12	< 0.0001
	≥1:40	23.32	12	0.02

Table S3. Multiple comparisons of proportions of positive titres ($\geq 1:10$, $\geq 1:20$) for HI and VN assays along the period 2005-2017.

HI positive titres ($\geq 1:10$)			HI positive titres ($\geq 1:20$)			VN positive titres ($\geq 1:10$)			VN positive titres ($\geq 1:20$)		
<i>value</i>	<i>CR</i>	<i>contrast</i>	<i>value</i>	<i>CR</i>	<i>contrast</i>	<i>value</i>	<i>CR</i>	<i>contrast</i>	<i>value</i>	<i>CR</i>	<i>contrast</i>
0.33	0.25	2005-2008	0.29	0.24	2005-2008	0.31	0.25	2005-2008	0.31	0.24	2005-2008
0.35	0.24	2005-2009	0.23	0.22	2005-2009	0.28	0.24	2005-2009	0.25	0.23	2005-2013
0.38	0.27	2005-2010	0.27	0.23	2005-2013	0.34	0.25	2005-2013	0.31	0.24	2005-2014
0.36	0.25	2005-2013	0.26	0.23	2005-2014	0.36	0.25	2005-2014	0.28	0.25	2006-2008
0.41	0.25	2005-2014	0.31	0.23	2007-2008	0.22	0.21	2005-2016	0.28	0.25	2006-2014
0.29	0.23	2005-2016	0.26	0.21	2007-2009	0.29	0.25	2006-2008	0.28	0.25	2008-2017
0.30	0.26	2006-2008	0.30	0.22	2007-2013	0.26	0.24	2006-2009	0.28	0.25	2014-2017
0.32	0.25	2006-2009	0.29	0.22	2007-2014	0.32	0.25	2006-2013			
0.35	0.28	2006-2010	0.22	0.19	2007-2016	0.34	0.25	2006-2014			
0.33	0.26	2006-2013				0.29	0.27	2007-2013			
0.38	0.26	2006-2014				0.31	0.27	2007-2014			
0.26	0.24	2006-2016				0.28	0.26	2013-2017			
0.28	0.27	2007-2008				0.30	0.27	2014-2017			
0.30	0.27	2007-2009									
0.34	0.29	2007-2010									
0.31	0.27	2007-2013									
0.36	0.27	2007-2014									
0.31	0.29	2010-2017									
0.28	0.27	2013-2017									
0.33	0.27	2014-2017									

Table shows differences in proportions, the corresponding critical value from the Marascuilo procedure (CR), and years compared. A difference greater than the CR indicates a statistically significant result. Only the significant differences for each class of positive titres in both assays are reported.

Figure S1. VN vs. HI: normalized proportions and trend lines for the titre class $\geq 1:10$.



Panel A: Time series of the normalized proportions of positive ($\geq 1:10$) titres of VN assay (empty black dots) compared to the time series of the HI assay (black filled dots).

Panel B: Trend curves related to the normalized proportions of positive ($\geq 1:10$) titres for the VN assay (empty black dots) and the HI assay (black filled dots).