IDEXX CSFV Ag Serum Test

Validation Data Report

Glossary

Repeatability

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Test With Confidence™

The IDEXX CSFV Ag Serum Test is designed for the detection of classical swine fever virus (CSFV) antigens in swine serum or plasma.

This antigen-capture ELISA is performed in a microtiter well coated with monoclonal antibodies for CSFV (E^{rns}). After the assay protocol is completed, the absorbance of the generated color is measured using a spectrophotometer. Results are calculated by subtracting the negative control mean absorbance from the sample mean absorbance, which results in a corrected OD value, S – N. Samples with an S – N less than 0.30 are considered negative, while samples with an S – N greater than or equal to 0.30 are considered positive.

The IDEXX CSFV Ag Serum Test is a screening test and is not specific for CSFV. Since other pestiviral antigens can be detected using this ELISA, any positive results should be confirmed by virus isolation or CSFV-specific PCR.

I: Glossary of Terms

The following definitions have been taken from the Glossary of Terms section of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (World Organization for Animal Health, 2008) and may be used to describe the assay's performance characteristics in this validation report.

Repeatability—Level of agreement between replicates of a sample both within and between runs of the same test method in a given laboratory.

Reproducibility—Ability of a test method to provide consistent results when applied to aliquots of the same sample tested by the same method in different laboratories.

Sensitivity (analytical)—Synonymous with "Limit of Detection," smallest detectable amount of analyte that can be measured with a defined certainty; analyte may include antibodies, antigens, nucleic acids or live organisms.

Sensitivity (diagnostic)—Proportion of known infected reference animals that test positive in the assay; infected animals that test negative are considered to have false negative results.

Sensitivity (relative)—Proportion of reference animals defined as positive by one or a combination of test methods that also test positive in the assay being compared.

Specificity (analytical)—Degree to which the assay distinguishes between the target analyte and other components in the sample matrix; the higher the analytical specificity, the lower the level of false-positives.

Specificity (diagnostic)—Proportion of known uninfected reference animals that test negative in the assay; uninfected reference animals that test positive are considered to have false-positive results.

Specificity (relative)—Proportion of reference animals defined as negative by one or a combination of test methods that also test negative in the assay being compared.

II: Repeatability

1. Intraplate Variation

Purpose: To assess the intraplate variability of the IDEXX CSFV Ag Serum Test.

Procedure: One sample was tested on three plates, across all 96 wells, according to the standard assay short protocol.

Results/ Conclusions: Microtiter plate percent coefficient of variation (%CV) for this sample is shown in Figure 1. Percent CVs for the sample tested on three plates were 5.49%, 6.48%, and 4.73%. No significant trends were noted on a row-to-row or column-to-column analysis. These values indicate good intraplate repeatability for the IDEXX CSFV Ag Serum Test.

Figure 1. %CV

	1	2	3	4	5	6	7	8	9	10	11	12	ROWS MEAN	S.D.	%CV
A	1.150	1.114	1.058	1.029	1.015	1.032	1.020	1.000	1.022	0.993	0.999	1.123	1.046	0.053	5.10
B	1.102	1.062	1.011	0.995	0.966	0.960	0.974	0.981	0.975	1.013	0.996	1.098	1.011	0.050	4.92
C	1.060	1.002	0.994	0.953	0.948	0.923	0.921	0.953	0.935	0.957	0.956	1.013	0.968	0.041	4.28
D	1.052	0.988	0.951	0.933	0.918	0.924	0.941	0.949	0.952	0.926	0.921	0.996	0.954	0.040	4.14
E	1.045	1.015	0.988	1.000	0.944	0.935	0.944	0.974	0.952	0.972	0.992	1.066	0.986	0.041	4.17
F	1.054	0.999	0.989	0.975	0.959	0.945	0.967	0.965	0.969	0.978	1.006	1.055	0.988	0.035	3.56
G	1.088	1.025	1.018	1.012	0.972	0.988	0.988	1.017	1.003	1.015	1.024	1.083	1.019	0.035	3.43
H	1.139	1.109	1.079	1.075	1.044	1.044	1.024	1.055	1.043	1.057	1.075	1.135	1.073	0.037	3.44
Columns															
Mean	1.086	1.039	1.011	0.996	0.971	0.969	0.972	0.986	0.981	0.989	0.996	1.071	**Overall**	1.006	0.055	5.49
S.D.	0.041	0.050	0.041	0.044	0.041	0.047	0.037	0.036	0.038	0.040	0.045	0.049			
%CV	3.75	4.78	4.03	4.45	4.19	4.90	3.82	3.64	3.85	4.07	4.54	4.60			

	1	2	3	4	5	6	7	8	9	10	11	12	MEAN	S.D.	%CV
A	1.088	1.017	0.967	0.946	0.931	0.899	0.924	0.927	0.920	0.926	0.925	0.968	0.953	0.052	5.50
B	1.058	0.997	0.947	0.927	0.877	0.866	0.903	0.910	0.914	0.936	0.964	0.962	0.938	0.053	5.64
C	1.016	0.950	0.901	0.845	0.844	0.832	0.822	0.841	0.864	0.893	0.878	0.935	0.885	0.058	6.52
D	1.001	0.936	0.908	0.857	0.851	0.857	0.840	0.818	0.867	0.876	0.876	0.929	0.885	0.051	5.74
E	1.008	0.967	0.924	0.881	0.878	0.879	0.865	0.856	0.866	0.889	0.908	0.964	0.907	0.049	5.37
F	1.022	0.971	0.933	0.897	0.878	0.867	0.870	0.872	0.870	0.880	0.926	0.939	0.910	0.049	5.40
G	1.046	1.004	0.973	0.945	0.926	0.919	0.911	0.893	0.927	0.951	0.918	0.975	0.949	0.044	4.62
H	1.077	1.060	1.006	0.995	0.976	0.989	0.960	0.983	0.985	0.964	0.991	1.013	1.000	0.036	3.57
Columns															
Mean	1.040	0.988	0.945	0.912	0.895	0.889	0.887	0.887	0.902	0.914	0.923	0.961	**Overall**	0.928	0.060	6.48
S.D.	0.033	0.040	0.036	0.051	0.045	0.048	0.046	0.053	0.043	0.034	0.039	0.027			
%CV	3.14	4.05	3.77	5.56	5.05	5.43	5.19	5.95	4.79	3.74	4.24	2.84			

	1	2	3	4	5	6	7	8	9	10	11	12	MEAN	S.D.	%CV
A	1.049	1.051	1.018	1.000	0.995	0.980	1.002	1.004	0.996	1.017	1.027	1.086	1.019	0.030	2.96
B	1.061	1.054	1.015	1.001	0.988	0.993	0.994	0.993	0.991	1.022	1.029	1.049	1.016	0.027	2.65
C	1.029	1.001	0.988	0.945	0.913	0.948	0.951	0.934	0.929	0.930	0.946	1.007	0.960	0.037	3.82
D	0.959	0.962	0.961	0.942	0.907	0.906	0.924	0.916	0.924	0.924	0.939	0.986	0.937	0.025	2.67
E	1.034	0.984	0.984	0.972	0.955	0.959	0.926	0.946	0.959	0.938	1.004	1.043	0.975	0.036	3.73
F	1.075	1.037	1.018	0.981	0.968	0.956	0.963	0.990	0.978	0.954	1.008	1.019	0.996	0.037	3.68
G	1.079	1.067	1.065	1.019	1.021	1.001	0.999	0.988	0.987	1.022	1.020	1.044	1.026	0.031	3.03
H	1.096	1.076	1.057	1.077	1.047	1.052	1.040	1.032	1.053	1.041	1.036	1.077	1.057	0.020	1.91
Columns															
Mean	1.048	1.029	1.013	0.992	0.974	0.974	0.975	0.975	0.977	0.981	1.001	1.039	**Overall**	0.998	0.047	4.73
S.D.	0.042	0.042	0.036	0.044	0.049	0.043	0.041	0.039	0.041	0.049	0.038	0.034			
%CV	4.05	4.05	3.51	4.40	5.03	4.43	4.17	4.03	4.20	4.98	3.76	3.28			

IDEXX

2. Interplate Variation

Purpose: To assess the interplate variability of the IDEXX CSFV Ag Serum Test.

Procedure: Five serum samples were tested on multiple batches of the IDEXX CSFV Ag Serum Test, according to the standard assay short protocol.

Results/ Conclusions: The %CVs for these five samples are shown in Figure 2. The %CVs for the samples were 4.1%, 4.3%, 5.2%, 5.8%, and 8.7%. These values indicate good interplate and lot-to-lot repeatability for the IDEXX CSFV Ag Serum Test.

Figure 2. Repeatability: IDEXX CSFV Ag Serum Test

	S-N				
Batch Number	**#1**	**#2**	**#3**	**#4**	**#5**
R671	1.58	0.93	0.51	0.24	0.13
S441	1.49	0.86	0.44	0.22	0.11
R551	1.68	0.97	0.48	0.25	0.13
R831	1.58	0.91	0.48	0.23	0.11
Mean	1.58	0.92	0.48	0.23	0.12
SD	0.07	0.04	0.02	0.01	0.01
%CV	4.1%	4.3%	5.2%	5.8%	8.7%

III. Sensitivity

1. Relative Sensitivity

Purpose: To assess the relative sensitivity of the IDEXX CSFV Ag Serum Test using serum and plasma samples from experimentally infected swine.

Procedure: A total of 199 samples (90 serum samples and 109 plasma samples) from pigs infected with various strains of classical swine fever virus were obtained by the European Community Reference Laboratory (CRL) in Hannover, Germany. The animals were bled daily from day 3 through day 21 postinfection, and also on day 24 postinfection. These samples were tested on the IDEXX CSFV Ag Serum Test according to the standard assay short protocol.

All samples tested positive for the CSFV genome in real-time PCR using a CSFV-specific, 5' NTR assay with TaqMan® probe[1] according to the standard operating procedure at the CRL. A cycle threshold (Ct) value of <39 cycles was considered positive.

Results/ Conclusions: Figure 3a shows the ELISA results for this entire population. The relative sensitivity of the ELISA compared to PCR was 69% for plasma and 84% for serum samples.

Figure 3b shows ELISA results for samples with Ct values <30. Samples with Ct values <30 (i.e., samples with a higher viral RNA load) were detected more often by the IDEXX CSFV Ag Serum Test. The relative sensitivity values increased to 91% for plasma and 96% for serum samples.

This data shows good sensitivity for the IDEXX CSFV Ag Serum Test.

[1] Hoffmann B, Beer M, Schelp C, Schirrmeier H, Depner K. Validation of a real-time RT-PCR assay for sensitive and specific detection of classical swine fever. *J Virol Meth.* 2005; 130:36–44.

Figure 3a. All CSFV Experimentally Infected Animals

ELISA Results

	Total	Positive	Negative	Relative Sensitivity
Plasma	109	75	34	**69%**
Serum	90	76	14	**84%**

Figure 3b. CSFV Experimentally Infected Animals with PCR Ct Values <30

ELISA Results

	Total	Positive	Negative	Relative Sensitivity
Plasma	80	73	7	**91%**
Serum	72	69	3	**96%**

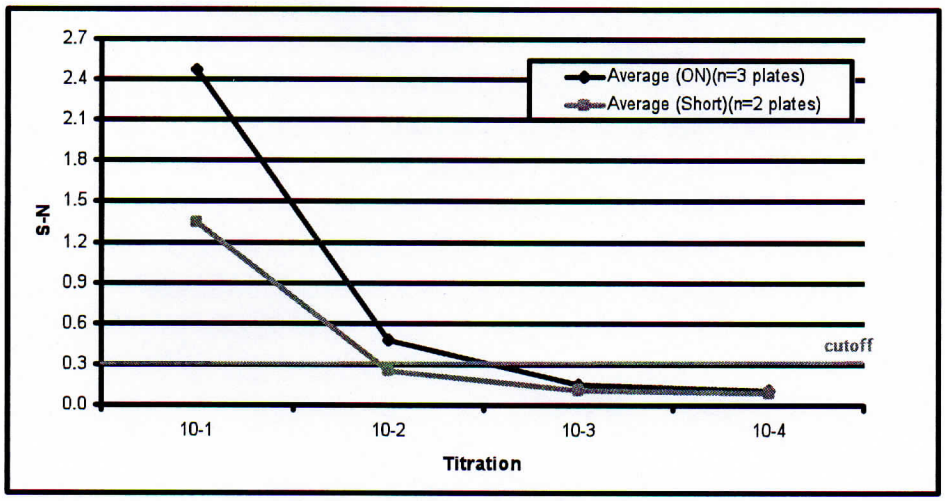
2. Analytical Sensitivity

Purpose: To observe the limit of detection of the IDEXX CSFV Ag Serum Test.

Procedure: CSFV Alfort 187 virus at an original titer of $10^{5.66}$ was obtained from CODA-CERVA in Brussels, Belgium. The virus was diluted ten-fold in multiple steps and tested at CODA-CERVA on the IDEXX CSFV Ag Serum Test using the short and overnight (ON) protocols.

Results/ Conclusions: Figure 4 shows the titration curve of the CSFV Alfort 187 virus. The IDEXX CSFV Ag Serum Test showed good sensitivity, detecting ~10^{366} copies of the Alfort strain.

Figure 4. CSFV Alfort 187 Titration



3. Relative Sensitivity

Purpose: To assess the performance of the IDEXX CSFV Ag Serum Test compared to virus isolation (VI) when using experimentally infected swine sera.

Procedure: Sera from five experimentally infected pigs from Europe were tested using the IDEXX CSFV Ag Serum Test and virus isolation. The animals were bled at 0, 3, 5, 7, 10, and 14 days postinfection. These samples were tested in duplicate on the IDEXX CSFV Ag Serum Test according to the standard assay short protocol and also by VI.

Results/ Conclusions: Figure 5 summarizes the results for this sample set for both ELISA and VI. Virus isolation detected positives at 3 and 5 days postinfection. The IDEXX CSFV Ag Serum Test detected all samples as positive after day 5 postinfection. The correlation of ELISA to virus isolation was 90%. This data shows good sensitivity when using the IDEXX CSFV Ag Serum Test.

Figure 5. IDEXX CSFV Ag Serum Test vs. Virus Isolation

ANIMAL	21		22		23		24		25	
	Virus Isolation	IDEXX	Virus Isolation	IDEXX	Virus Isolation	IDEXX	Virus Isolation	IDEXX	Virus Isolation	IDEXX
0 DPI	N.D.	0.0645	N.D.	0.0655	N.D.	0.0735	N.D.	0.076	N.D.	0.071
		-0.0095		-0.0085		-0.0005		0.002		-0.003
3 DPI	POS	0.3025	N.D.	0.1555	N.D.	0.2405	POS	0.3675	N.D.	0.248
		0.2285		0.0815		0.1665		0.2935		0.174
5 DPI	POS	3.853	POS	3.7695	POS	3.909	POS	3.8	POS	3.8545
		3.779		3.6955		3.835		3.726		3.7805
7 DPI	X	X	POS	3.844	POS	3.599	X	X	POS	3.642
				3.77		3.525				3.568
10 DPI	X	X	POS	3.0715	POS	3.2335	X	X	POS	3.612
				2.9975		3.1595				3.538
14 DPI	X	X	X	X	X	X	X	X	POS	2.7745
										2.7005

N.D. = Not detected
X = Expired
S – N cutoff = 0.30

IDEXX

4. Temporal Study

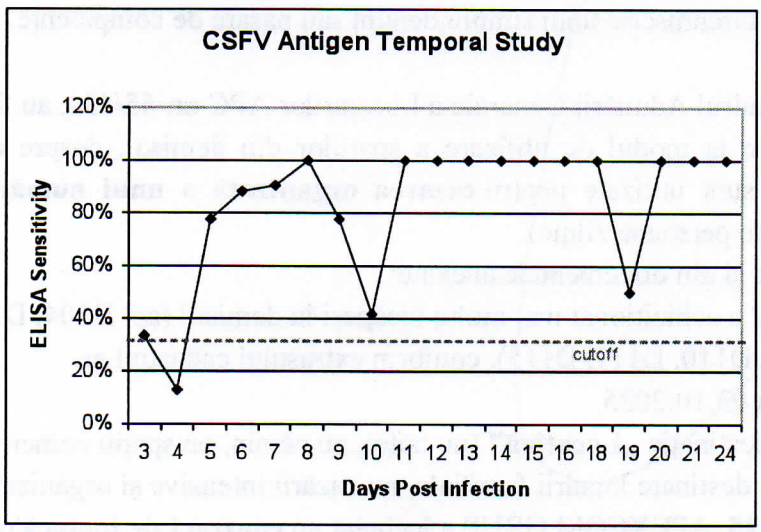
Purpose: To assess the performance of the IDEXX CSFV Ag Serum Test using a temporal study.

Procedure: A total of 199 samples (90 serum samples and 109 plasma samples) were obtained by the European Community Reference Laboratory (CRL) in Hannover, Germany, from pigs infected with various strains of classical swine fever virus. The animals were bled daily, from day 3 through day 21 postinfection, and also on day 24 postinfection. These samples were tested on the IDEXX CSFV Ag Serum Test according to the standard assay short protocol.

Results/ Conclusions: Figure 6 plots days postinfection vs. ELISA sensitivity for this population. All samples were detected as positive after day 4 postinfection. This data shows good sensitivity when using the IDEXX CSFV Ag Serum Test.

Note: The decrease of ELISA sensitivity at 10 and 19 days postinfection is most likely due to sample quality and/or the relatively low number of samples (10 at day 10 and 4 at day 19).

Figure 6. CSFV Antigen Temporal Study



IV. Specificity

1. Diagnostic Specificity: Negative Population

Purpose: To determine the distribution and characteristics of negative European serum populations tested on the IDEXX CSFV Ag Serum Test.

Procedure: A total of 459 CSFV-negative serum samples from France, Spain, Switzerland, and Hungary were tested on the IDEXX CSFV Ag Serum Test using the standard assay short protocol.

A total of 460 fresh swine samples submitted to a Belgian laboratory for routine Aujezsky virus testing were tested on the IDEXX CSFV Ag Serum Test using the overnight protocol.

Routine laboratory samples were tested in the Belgian CSFV reference lab in Brussels, Belgium, using the IDEXX CSFV Ag Serum Test. The overnight protocol was used for 225 samples; the short protocol was used for 150 samples.

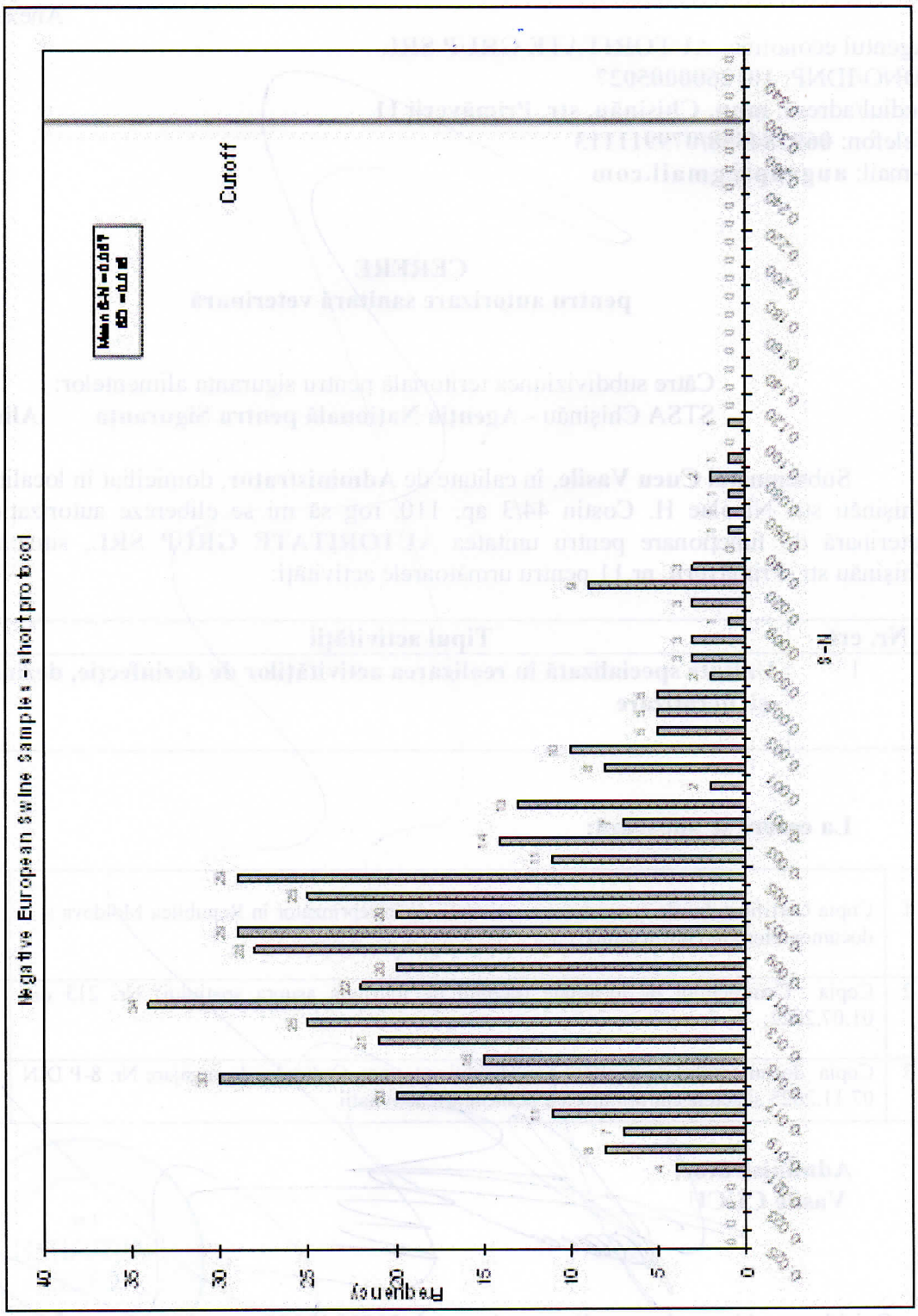
Results/ Conclusions: Figure 7a shows the distribution for the first negative population, representing samples from four European countries. This population had a specificity of 100% (459/459 = 100%).

Figure 7b shows the distribution for the fresh swine serum samples tested using the overnight protocol. This population had a specificity of 99.8% (459/460 = 99.8%).

Figure 7c shows the summary table for the routine samples tested at the CSFV reference lab in Brussels, Belgium. The overnight protocol population resulted in a specificity of 98.7% (222/225 = 98.7%), and the short protocol population resulted in a specificity of 100% (150/150 = 100%)

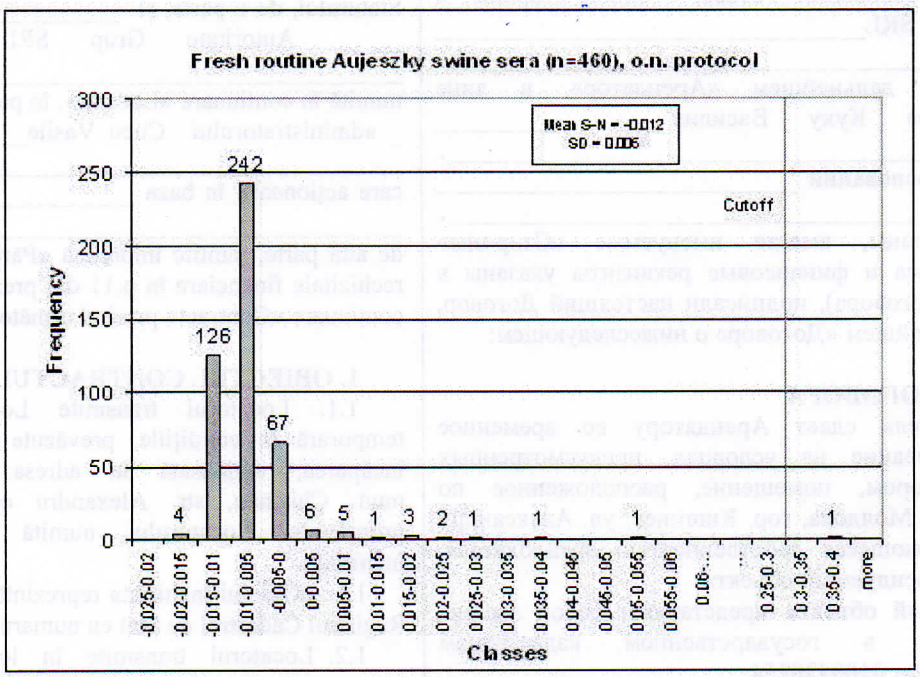
All populations showed an excellent level of specificity for the IDEXX CSFV Ag Serum Test.

Figure 7a. Negative European Swine Samples—Short Protocol



IDEXX

Figure 7b. Negative Fresh Swine Samples—Overnight Protocol



Note: Positive sample not included in mean and SD calculation.

Figure 7c. Negative Routine Swine Samples—Short and Overnight Protocol

	Over Night	Short
Number of Samples	225	150
Positive	3	0
Negative	222	150
Specificity	**98.7%**	**100%**
Average negative samples (S-N)	0.030	0.076
SD negative samples (S-N)	0.067	0.019